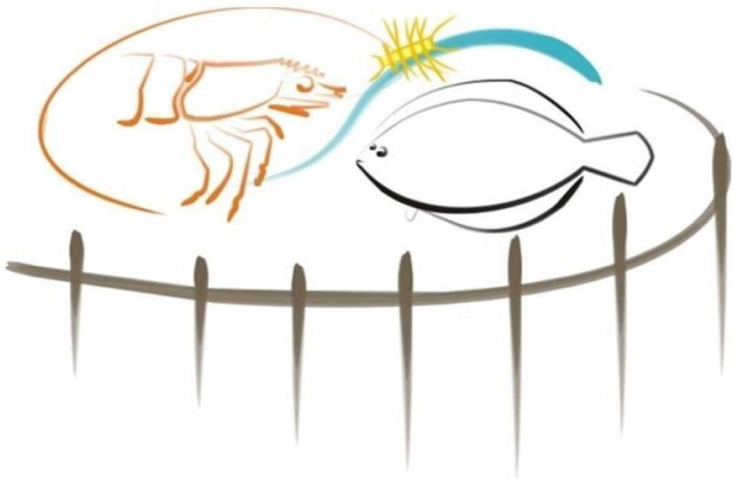
UNIVERSIDADE FEDERAL DO RIO GRANDE – FURG
INSTITUTO DE OCEANOGRAFIA - IO
PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA - PPGAqui



AÇAÍ (*Euterpe oleracea*) EM DIETAS PARA JUVENIS DE TAMBAQUI (*Colossoma macropomum*): PERFORMANCE DE CRESCIMENTO, STATUS REDOX, METABOLISMO ENERGÉTICO E EFEITO NEUROPROTETOR

THAMYRES VANESSA NASCIMENTO DA SILVA

FURG
RIO GRANDE – RS
2020

UNIVERSIDADE FEDERAL DO RIO GRANDE – FURG
INSTITUTO DE OCEANOGRAFIA – IO
PROGRAMA DE PÓS-GRADUAÇÃO EM AQUICULTURA – PPGAqui

AÇAÍ (*Euterpe oleracea*) EM DIETAS PARA JUVENIS DE TAMBAQUI (*Colossoma macropomum*): PERFORMANCE DE CRESCIMENTO, STATUS REDOX, METABOLISMO ENERGÉTICO E EFEITO NEUROPROTETOR

Thamyres Vanessa Nascimento da Silva

Tese apresentada ao Programa de Pós-Graduação em Aquicultura da Fundação Universidade Federal do Rio Grande - FURG, como requisito parcial à obtenção do título de Doutora.

Orientador: Prof. Dr. José María Monserrat (FURG)
Co-orientador: Prof. Dr. Luís André Nassr de Sampaio (FURG)

RIO GRANDE – RS
MARÇO, 2020

ÍNDICE

EPÍGRAFE

"Nenhuma sociedade que esquece a arte de questionar pode esperar encontrar respostas para os problemas que a afligem".

Zygmunt Bauman

DEDICATÓRIA

Dedico

Aos meus pais, Maria Zeneide e José Maria, grandes incentivadores e exemplos de amor, não medindo esforços para garantir minha educação.

A minha irmã Thayse Valéria, embora nossas diferenças, o amor que nos une é tão verdadeiro como a amizade que nos aproxima.

Aos meus avós João Honorato (in memoriam) e Oneide Nascimento, José Ferreira (in memoriam) e Maria Barbosa (in memoriam).

AGRADECIMENTOS

A Deus, por não me conceder o que lhe pedia, mas sim o que eu precisava.

Com prazer, agradeço a Fundação Universidade Federal do Rio Grande (FURG) pela oportunidade e excelente infraestrutura concedida para meu desenvolvimento no doutorado em aquicultura, bem como a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela bolsa de estudos. Tamanha é a felicidade por este projeto ter alcançado significantes resultados pelos quais eu gostaria de manifestar minha sincera gratidão aos meus orientadores.

Muito obrigada Dr. José María Monserrat pela confiança, comprometimento em orientar e apoiar; pelo incentivo e paciência nos momentos desafiadores; e pelo acolhimento que estendeu até meus familiares. Embora atarefados, você e o professor Dr. Luiz André Sampaio estiveram sempre disponíveis quando precisei de esclarecimentos e soluções sobre meu projeto de pesquisa, bem como de correções sobre a tese, o que sem dúvidas fez deste trabalho exitoso. A ambos, obrigada pela disposição durante esta jornada.

Gratidão ao Laboratório de Aquacultura de Espécies Tropicais (LAET), IFPA campus Castanhal pelo apoio e infraestrutura cedidos à realização das etapas experimentais. Meus sinceros agradecimentos ao Dr. Luís André Luz Barbas e ao Dr. Marcelo Ferreira Torres por serem fundamentais na minha caminhada pela pós-graduação e por pacientemente ajudarem e oferecerem assistência quando necessitei. Certamente vocês permitiram que meu trabalho progredisse com reveses mínimos. Obrigada Brenda da Costa, pela ajuda e presteza nas rotinas, análises de qualidade de água e coletas. Todas as amostragens do presente estudo foram possíveis pelo trabalho incansável e comprometido das alunas e alunos de iniciação científica do LAET, IFPA campus Castanhal.

O estudo de eletrofisiologia foi realizado no Laboratório de Farmacologia e Toxicologia de Produtos Naturais (LFTPN), UFPA, campus Belém, pelo qual agradeço ao Dr. Moisés Hamoy por ter assegurado extraordinária instalação experimental e por ter participado ativamente na execução do experimento, treinando-me pacientemente.

Também agradeço aos meus amigos Juan Rafael Ramírez, Robson Gomes, Grecica Colombo, Marcos Schmitz e Cléber Simião, por me motivarem especialmente em ocasiões difíceis, além de ajudarem e contribuírem em diversas coletas e determinações em laboratório, o que aliviou substancialmente a carga sobre meus ombros.

Ao querido Rodrigo Gomes, que embora tenha tomado caminho diferente, sempre cuidou e ajudou de todas as maneiras que estavam ao seu alcance. Com certeza sem você essa trilha teria sido mais árdua. Imensa é a gratidão pela ternura doada e evolução que a mim você proporcionou.

Um agradecimento especial é estendido a Beatriz Miranda, Priscila Nunes, Fernanda Nascimento e Lílian Cristianne Nascimento. Vocês, mesmo distantes se fizeram presentes e, mesmo atarefadas, me ampararam, foram gentis, solidárias, foram grandes amigas!

Agradeço a todos os professores do PPGAqui-EMA/FURG pela contribuição à minha formação, assim como aos funcionários das outras instituições de ensino por onde passei, por terem me ajudado de diferentes maneiras.

RESUMO GERAL

O presente estudo objetivou avaliar a inclusão dietética de açaí liofilizado *Euterpe oleracea* (LEO) como um promotor do crescimento para juvenis de tambaqui (*Colossoma macropomum*), determinando seus efeitos sobre a pigmentação da pele, status antioxidante, metabolismo energético, além das implicações de seu consumo sobre a resistência ao estresse de transporte e atividade neuroprotetora. Os peixes (0.92 ± 0.01 g) foram alimentados por 30 dias com seis dietas isoenergéticas e isoproteicas formuladas com 0,00; 0,63; 1,25; 2,50; 5,00; e 10.0% LEO (p/p). Um crescimento 7 vezes superior ao peso inicial foi alcançado ao final do teste alimentar. A inclusão de 5.00% e 10.0% LEO na dieta foram significativos em termos de ganho de peso e peso final corporal. A ingestão de 1.25% LEO também melhorou os parâmetros de eficiência alimentar, sendo efetiva sobre o aumento da taxa de crescimento específico (SGR). A capacidade antioxidante (DPPH), bem como o conteúdo de flavonoides e polifenóis totais não foram incrementados no músculo. Mas, com 5.00% de açaí na dieta, a intensidade da cor ciano na pele foi melhorada. A menor inclusão de açaí (0,63 %) na dieta resultou na maior competência antioxidante intestinal (39.57%), o que não foi observado para o fígado e músculo. Somente para o intestino, uma inclusão estimada em 5.47% LEO minimizou os níveis de peroxidação lipídica (TBARS). Desde o ponto de vista econômico, uma inclusão de até 1,25% LEO é viável (Capítulo 1). Foi também observada uma redução sobre a concentração de triglicerídeos (40%) no músculo pela ingestão de 0,63% LEO na dieta. Porém, não houveram alterações significativas sobre os níveis de colesterol, glicose, glicogênio e proteína total neste órgão. A atividade do sistema de transporte de elétrons (ETS) no músculo aumentou em 76,25% nos peixes alimentados com 1,25% LEO em relação ao controle, sendo que este parâmetro apresentou elevada correlação ($R^2 = 0,87$) com a SGR (Capítulo 2). A ingestão de LEO previamente ao estresse de transporte (3, 6, 12 e 24 h) resultou em um nível de oxigênio dissolvido 17.7% superior ao tratamento controle, após 24 h de transporte. O nível de glicose no sangue se manteve similar, independente das dietas ou dos tempos de transporte. A capacidade antioxidante total (ACAP) e o dano oxidativo lipídico (TBARS) foram mensurados nas brânquias, cérebro, fígado e músculo. Após transporte por 12 h, peixes tratados com 1,25% a 10,0% LEO exibiram maior competência antioxidante hepática (42% a 53%, respectivamente). Níveis dietéticos de 2,50% a 5,00% LEO desempenharam evidente proteção contra a peroxidação

lipídica no cérebro, fígado e brânquias até 12 h de transporte, reduzindo pontualmente o nível de TBARS no músculo (Capítulo 3). Convulsões induzidas por pentilenotetrazol (PTZ) foram reduzidas expressivamente por meio do LEO dietético. Os registros eletroencefalográficos indicaram menor excitabilidade e amplitude das ondas cerebrais nos peixes alimentados com 5,00% LEO na dieta. Essa redução foi 80% superior nos peixes tratados com 10,0% LEO comparado ao grupo controle. Os níveis de TBARS foram reduzidos em 60% no cérebro dos peixes alimentados com LEO na dieta. O efeito neuroprotetor do açaí transpareceu sobre o comportamento, reduzindo ou abreviando os sinais associados com crises convulsivas (Capítulo 4). De um modo geral, LEO dietético demonstra uma série de efeitos funcionais sobre os juvenis de tambaqui e, portanto, pode ser reconhecido como potencial aditivo alimentar para este peixe. Para promover o crescimento e o metabolismo energético muscular, sugere-se a inclusão dietética de 1,25%, pois é economicamente praticável com satisfatórios efeitos sobre a eficiência alimentar, crescimento e competência antioxidante intestinal. As inclusões de 2,50% a 5,00% LEO em dietas preparatórias para o transporte também são recomendadas, uma vez que melhora a qualidade da água após longo período de transporte, bem como aumenta a capacidade antioxidante do fígado e reduz a LPO em todos os órgãos avaliados dentro de até 12 h de transporte. Por fim, respostas anticonvulsivas e neuroprotetoras em juvenis de tambaqui são possíveis a partir da administração dietética de 5,00% LEO.

Palavras chave: fruta amazônica, desempenho zootécnico, estresse oxidativo, energia muscular, transporte, eletrofisiologia.

ABSTRACT

This study aimed to evaluate the dietary inclusion of lyophilized açai *Euterpe oleracea* (LEO) as a growth promoter for tambaqui juveniles (*Colossoma macropomum*), through the measurement of skin pigmentation, antioxidant status, energy metabolism, besides the implications of its intake on resistance to cope with transport stress and neuroprotective activity. The fish (0.92 ± 0.01 g) were fed for 30 days with six isoenergetic and isoprotein diets containing 0.00; 0.63; 1.25; 2.50; 5.00; and 10.0% LEO (w/w). A growth 7-fold higher than the initial weight was achieved at the end of the feeding trial. The inclusion of 5.00% and 10.0% LEO in the diet was significant in terms of weight gain and final body weight. The intake of 1.25% LEO by tambaqui also improved the parameters of feed efficiency, increasing the specific growth rate (SGR). The antioxidant capacity (DPPH), as well as the flavonoids and polyphenols content, were not increased in the muscle. However, dietary açai at 5.00%, intensify of the cyan color in the skin. The lowest açai inclusion level (0.63 %) resulted in higher intestinal antioxidant competence (39.57%), which was not observed in the liver and muscle. In the intestine, the estimated inclusion of 5.47% LEO minimized TBARS levels, while in the liver and muscle there were no significant differences. The inclusion of LEO in the diets showed to be economically feasible up to 1.25% LEO (Chapter 1). Reduction in the muscle triglyceride content (40%) in the muscle was obtained by the intake of 0.63% LEO. Despite this, there were no significant changes in cholesterol, glucose, glycogen and total protein levels in this organ. The electron transport system (ETS) activity in the muscle increased by 76.25% in fish fed with 1.25% LEO compared to the control, and this parameter showed a high correlation ($R^2 = 0.87$) with the SGR (Chapter 2). The intake of LEO before transport stress (3, 6, 12 and 24 h of duration) resulted in a dissolved oxygen level 17.7% higher than the observed in the control treatment, after 24 h of transport. The blood glucose level remained similar, regardless of feed treatments or transport times. Total antioxidant capacity (ACAP) and lipid peroxidation (TBARS) were measured in the gills, brain, liver, and muscle. After transport for 12 h, fish treated with 1.25% to 10.0% LEO exhibited higher hepatic antioxidant competence (42% to 53%, respectively). Dietary levels at 2.50% to 5.00% LEO played evident protection against lipid peroxidation in the brain, liver, and gills within a 12 h of transportation, punctually reducing the TBARS levels in the muscle (Chapter 3). Seizure-like state induced by pentylenetetrazole (PTZ) was significantly reduced by LEO intake. The electroencephalographic records show less excitability and

lower amplitudes in the cerebral wave tracings in fish fed with 5.00% LEO in the diet and exposed to PTZ. For tambaqui treated with 10.0% LEO, this reduction was 80% higher compared to the control group and TBARS levels were reduced by 60% in fish fed LEO in the diet. The neuroprotective effect of açaí was evident in the fish behavior, reducing or abbreviating the signs associated with seizures (Chapter 4). In general, dietary LEO demonstrates functional effects on tambaqui juveniles and, therefore, can be recognized as a potential food additive for this species. To promote fish growth and muscle energetic metabolism, we suggest a dietary inclusion of 1.25% LEO, because is economically feasible with satisfactory effects on food efficiency, growth, and intestinal antioxidant competence. Inclusions of 2.50% to 5.00% LEO in preparatory transport diets are also recommended because they improve water quality after a long transport period and also increase the antioxidant capacity of the liver and reduce lipid peroxidation in all evaluated organs within 12 h of transportation. Finally, anticonvulsant and neuroprotective responses in juvenile tambaqui are possible with the dietary administration from 5.00% LEO.

Keywords: Amazonian fruit, zootechnical performance, oxidative stress, muscle energy, transport, electrophysiology.

1. INTRODUÇÃO GERAL

1.1. Panorama aquícola mundial

A produção aquícola global tem aumentado velozmente ao longo das últimas três décadas, com significativa contribuição para o suprimento de pescado destinado ao consumo humano. A produção mundial da aquicultura em 2016 foi estimada em 110 milhões de toneladas (ton) em peso vivo (receita estimada em US$ 243 bilhões de dólares) (FAO, 2018). Esta produção foi resultado da soma de 54 milhões de toneladas de peixes, 17 milhões de ton de moluscos, 7,9 milhões de ton de crustáceos e 938 mil ton de outros animais aquáticos (FAO, 2018). Em comparação com a produção aquícola em 2014 – 73,8 milhões de ton contabilizando US$ 160,2 bilhões – (FAO, 2016), observa-se um importante aumento nos rendimentos deste setor, ao redor de 33%.

No ano de 2014, a produção aquícola mundial de peixes, crustáceos e moluscos em ambientes de água doce, salobra e salgada totalizaram, respectivamente, cerca de 46 milhões de ton (US$ 88 bilhões), 6 milhões de ton (US$ 24 bilhões) e 21 milhões de ton (US$ 46 bilhões). Entre as espécies mais reportadas na produção global da aquicultura estão as carpas capim (*Ctenopharyngodon idellus*), prateada (*Hypophthalmichthys molitrix*) e comum (*Cyprinus carpio*), com mais de 5 milhões ton/ano (US$ 7 bilhões) e 4 milhões ton/ano, respectivamente. Atualmente, estima-se que a aquicultura produz mais de 600 espécies em diferentes ambientes aquáticos e sistemas de produção (Naylor, 2016), distribuídos entre peixes (63%), crustáceos e moluscos (28%), algas (6.5%), anfíbios e répteis (2.5%). Já entre os crustáceos e moluscos, destacam-se o camarão branco do Pacífico (*Litopenaeus vannamei*) e o bivalve amêijoa chinesa (*Sinonovacula constricta*), os quais nesta ordem, perfazem cerca de 3 milhões ton/ano (US$ 18 bilhões) e 786 mil ton/ano (US$ 708 mil) (FAO, 2014).

O mercado internacional é fundamental à geração e movimentação de renda no setor da aquicultura. A China, além de maior produtora, lidera as exportações de peixes (US$ 20 milhões), sendo o terceiro país que mais importa (US$ 8 milhões), perdendo apenas para os Estados Unidos (US$ 20 milhões) e Japão (US$ 14 milhões) (FAO, 2018). A segunda e terceira posição é ocupada pela Noruega e Vietnã, os quais em ordem, acumularam US$ 10 milhões e US$ 8 milhões em exportações no ano de 2016 (FAO, 2018). Uma tendência que caracteriza esse comércio nos últimos 40 anos é a taxa de crescimento significativamente mais rápida nas exportações de países em desenvolvimento do que para países desenvolvidos (FAO, 2018).

Os números promissores da produção e do mercado aquícola, bem como a constante queda da produção pesqueira, vem levantando questões relevantes acerca da segurança alimentar do mundo (Natale et al., 2012). Conhecedores do setor predizem que a aquicultura garantirá o suprimento de alimentos à crescente demanda global de proteína animal - consumo per capita de 9,9 kg no ano de 1990 e estimado em mais de 20 kg em 2014-2015 (Bené et al., 2016, FAO, 2016). Até 2030 se estima que a aquicultura forneça 60% dos peixes disponíveis para consumo humano (FAO, 2018).

Por outro lado, os benefícios da segurança alimentar, particularmente providos por sistemas de aquicultura de alto valor, são dependentes da geração de empregos e da inclusão de produtores de baixa renda. Entretanto, o exemplo da Ásia (variando entre lugares desde a China a Bangladesh) com elevada produção de tilápias, carpas e bagres poderia ser tomada como um modelo de produção desenvolvida “pelos pobres e para os pobres” (Naylor, 2016). Neste caso, mesmo sendo comparável a Revolução Verde para grãos, torna-se essencial, na medida em que a aquicultura avança, desviar-se dos mesmos erros (danos ambientais) cometidos ao decorrer do aumento da intensificação da agricultura (Diana et al., 2013).

1.2. Aquicultura brasileira

A aquicultura vem gradativamente substituindo a pesca no Brasil em função do esgotamento dos estoques naturais de pescados. Mas, outros fatores como as maiores restrições ambientais à atividade pesqueira, crescente demanda por produtos padronizados e maior exigência pelo abastecimento regular no mercado também contribuem para a diminuição da participação da pesca na produção de pescado (Kato, Freitas, 2015). Atualmente, a aquicultura brasileira ocupa a 14º posição entre os principais países produtores, sendo considerado o segundo maior da América Latina (FAO, 2015, 2016). Apesar do Brasil apresentar aspectos naturais promissores, a aquicultura neste país somente disparou sobre as estatísticas de produção nas últimas duas décadas, passando de 159 mil ton no ano de 2000 para 562 mil ton em 2014 (Kato, Freitas, 2015; FAO, 2016; Saint-Paul, 2017). Segundo Saint-Paul (2017), 82% daquele total foram garantidos pela aquicultura de água doce.

A Pesquisa Pecuária Municipal (PPM) realizada pelo Instituto Brasileiro de Geografia e Estatística (IBGE) revelou que em 2017 a aquicultura produziu 547 mil ton de pescado. Esta soma é resultado da participação de 485,254 ton de peixes (R$ 3,07 bilhões), 40,967 ton de camarões (R$ 887 milhões) e 20,947 ton entre ostras, vieiras e mexilhões (R$ 83 milhões). No país, a tilápia (*Oreochromis niloticus*) liderou a produção de peixes com 58,3% (283 mil ton) apresentado um aumento de 18,5% em 2017 (IBGE, 2017). Em segundo lugar, o peixe amazônico tambaqui (*Colossoma macropomum*) contribuiu com 102,554.4 mil ton em 2018 (IBGE, 2020), representando no ano de 2017 18,2% da produção nacional (IBGE, 2017). Em seguida, destacaram-se os híbridos (tambacu e tambatinga), carpas, bagres (pintado, cachara, cachapira, surubim e etc) e outros peixes redondos como pacu e patinga (IBGE, 2017). Mesmo com estes números, o maior quantitativo de pescado consumido neste país ainda é suprido pela importação. Em 2014

foram importados cerca de 400 mil ton e, em contrapartida, exportou-se 34 mil ton de pescado, gerando um déficit 366 mil ton, isto é, US$ 1,3 bilhão na balança comercial (CONEPE, 2014).

As perspectivas futuras para a aquicultura brasileira são positivas, justamente pelos aspectos favoráveis que este país possui à pratica de distintas modalidades desta atividade, entre os quais se destacam: disponibilidade de água doce, salobra e salgada; clima tropical na maior parte do país; importante produção de grãos; e diversidade de espécies nativas com potencial zootécnico e mercadológico (MPA, 2011; Brabo et al, 2016). Por fim, o desenvolvimento de tecnologias também é essencial para o crescimento do setor e, entre as áreas prioritárias, a nutrição e alimentação de organismos aquáticos são considerados fatores chave para o desenvolvimento deste agronegócio no Brasil (Myre et al., 2017).

1.3. Tambaqui – *Colossoma macropomum*

O tambaqui (Cuvier, 1816) é um dos peixes mais importantes para o setor piscícola da América Latina. Este teleósteo está distribuído naturalmente nos rios Amazonas (a partir da foz do rio Xingu-PA), Orinoco (Venezuela) e seus afluentes, com águas abundantes em nutrientes e temperatura média entre 25°C e 34ºC. Este peixe pertencente a classe Actinopterygii, ordem Characiformes e família Serrasalminae, a qual abrange peixes

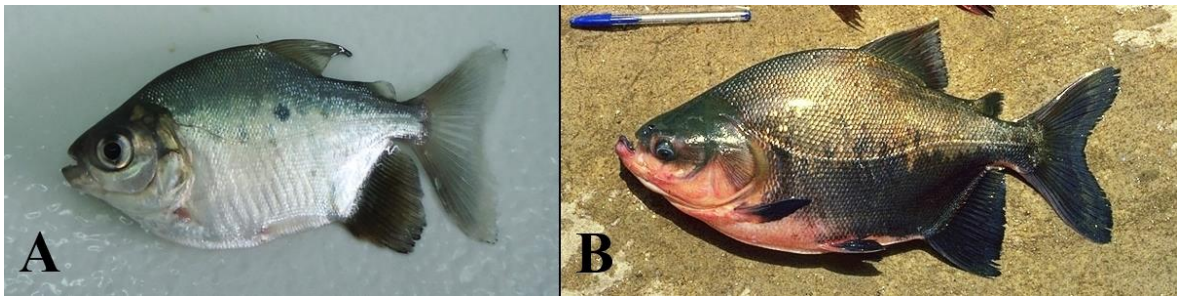


Figura 1. Exemplar de juvenil (A) e adulto (B) de tambaqui (*Colossoma macropomum*). Fonte: (A) autoria própria; (B) Lovshin, L. em FishBase.

como os pacus, pirapitinga e piranhas. Em outros países, o tambaqui é conhecido por gamitama (Peru), cachama (Venezuela e Colômbia) ou black pacu (Estados Unidos da

América) (Buckup et al., 2007; Gomes et al., 2010). Este peixe é um caracídeo redondo de corpo largo, que na fase adulta se torna mais alongado e levemente comprimido (Figura 1) (Morais, O´Sullivan, 2017). Além disso, é característica a coloração esverdeada na região dorsal e tons escuros na região ventral (Graça e Pavanelli, 2007).

Este peixe apresenta boca terminal com robustos dentes molariformes e incisiformes, além de quatro arcos branquiais com rastros longos e numerosos, que o permite se alimentar de zooplâncton, frutos e sementes que por ventura venham a cair nas águas dos rios nos períodos de cheia (Araújo-Lima, Goulding, 1998; Abelha et al., 2001; Santos et al., 2006, Morais, O´Sullivan, 2017). A aceitação de uma ampla variedade de alimentos o enquadra como uma espécie onívora, sendo zooplanctófago na fase jovem e preferencialmente frugívoro na fase adulta (Goulding, 1980; Claro-Jr et al., 2004), aproveitando ocasionalmente pequenos insetos, artrópodes, pequenos moluscos, folhas e caules moles nos períodos de cheia dos rios (Morais, O´Sullivan, 2017).

O tambaqui é produzido na maior parte dos estados brasileiros, mas em função do clima, sua produção se concentra na região Norte, Centro-Oeste, Nordeste e, em menor proporção, no Sudeste (Gomes et al., 2013). A sua difusão pelas pisciculturas nacionais e até mesmo internacional (Ásia) é explicada pelas potenciais características do espécime para a produção em cativeiro, que incluem rápido crescimento, alta produtividade, baixo custo de produção, rusticidade, flexibilidade à alimentação artificial, resistência a doenças e tolerância a água de baixa qualidade (Campos-Baca, Köhler, 2005; Guimarães, Martins, 2015; Saint-Paul, 2017). A produção pode ser realizada em sistema semi-intensivo utilizando viveiros escavados (recria: 3 a 12 peixes m^2; engorda: 0.42 a 1 peixe m^2), ou em sistema intensivo utilizando tanques-rede (recria: 300 peixes m^3; engorda: 20 peixes m^3) (Oliveira et al., 2013; da Silva, Fujimoto, 2015).

Devido ao seu hábito alimentar, o tambaqui possui grande capacidade de digerir proteína animal e vegetal (Oliveira et al., 2013). Entretanto, mesmo sendo necessárias mais investigações a respeito das exigências nutricionais deste peixe, concentrações de 50% e 30% de proteína bruta na dieta são reportadas para melhorar a retenção proteica e o crescimento em indivíduos nas fases iniciais e juvenis/adultos, respectivamente (Van der Meer, 1997). A exigência de aminoácidos essenciais (AAE) ainda é limitada, no entanto, segue o padrão dos 10 AAE para peixes de água doce como a tilápia (*O. niloticus*). Em adição, fontes de carboidratos complexos (até 30% de inclusão na dieta) e lipídeos são bem aproveitados, em função de adaptações morfológicas e bioquímicas do trato gastrointestinal deste peixe (Oliveira et al., 2013). Entre as enzimas digestivas detectadas, as proteases se destacam no estômago, além de lipases, amilases e enzimas exógenas em todo o trato. Uma das particularidades do tambaqui é a produção de amilase nos cecos pilóricos (Dairiki, da Silva, 2011).

Nas últimas décadas, estudos dose-resposta para níveis de fósforo (Araújo et al., 2017), níveis de vitamina C e ferro (Aride et al., 2010), relação proteína-energia na dieta (de Almeida et al. 2011), relação carboidrato-lipídio (Sandre et al., 2017), suplementação de probióticos (Dias et al., 2018), suplementação de imunoestimulante a partir de óleo essencial de hortelã-pimenta (*Mentha piperita*) (Ribeiro et al., 2018) e níveis de ácido graxos linolênico e linoleico na dieta (Paulino et al., 2018), por exemplo, têm sido realizados reportando resultados promissores. Embora muito ainda seja necessário para consolidar o protocolo nutricional do tambaqui, a busca pela formulação de dietas mais específicas, capazes de promover o crescimento e condicionar o organismo as diferentes situações de estresse - corriqueiras em sistemas de cultivo - é fundamental, uma vez que o sucesso de quaisquer modalidades de produção animal está atrelado sobretudo a nutrição.

1.4. Situações de estresse na piscicultura

No Brasil, cerca de 70% da produção de peixes provém da piscicultura intensiva (Tavares-Dias, Martins, 2017). Esta modalidade produtiva, exige altos investimentos com tecnologia e mão de obra especializada, uma vez que são praticadas altas densidades de estocagem (p. ex.150 kg/m³/ciclo, por exemplo) em sistema pobre em alimentos naturais (p. ex. tanques de alto fluxo – raceways, sistemas de recirculação de água - RAS) e altamente susceptível a flutuações na qualidade da água, demandando manejo intensivo sobre a biossegurança, monitoramento da qualidade da água, uso de profiláticos e rações de alta qualidade para garantir o bem-estar dos animais cultivados e prevenir problemas associados ao estresse (Lima et al., 2013).

Na literatura existe uma variedade de definições e discussões a respeito da palavra estresse (Schreck, Tort, 2016). Mas, uma definição que parece se ajustar a perspectiva da aquicultura é o estresse como "uma condição na qual o equilíbrio dinâmico do organismo, chamado homeostase, é ameaçado ou perturbado como resultado de estímulos intrínsecos ou extrínsecos que atuam como estressores" (Wendelaar-Bonga, 1997). Na piscicultura, a homeostase dos peixes pode ser afetada por uma série de fatores, tais como variações na temperatura, oxigênio dissolvido, pH, salinidade, amônia total, dióxido de carbono, sólidos suspensos (Boyd, 2017) e má nutrição; fatores sociais como superlotação, hierarquia ou territorialismo; e mesmo fatores decorrentes da ação humana, como manejo, manipulação e processos operacionais como transporte de peixes vivos (Bly et al., 1997).

Na tentativa de reestabelecer a homeostase e garantir a sobrevivência, os peixes desencadeiam ações comportamentais e processos fisiológicos (Mateus et al., 2017) de cunho compensatório ou conservatório de acordo com a natureza e o grau de estresse sofrido (Sokolova et al., 2012). Este é o ponto crítico, uma vez que a energia antes investida em processos anabólicos com elevado custo energético - como o crescimento,

583 reprodução (gametogênese e desova), atividade, ou mesmo manutenção em períodos de
584 restrição alimentar - passa a ser prioritariamente direcionada à compensação dos gastos
585 energéticos extras com atividades bioquímicas e fisiológicas que vão desde a aceleração da
586 ventilação, captação de oxigênio até a manutenção celular, reparo de danos e indução de
587 respostas detoxificadoras. Porém, em casos de estresse crônico/extremo, as atividades
588 gerais são desaceleradas e as taxas metabólicas são deprimidas, permitindo a conservação
589 de recursos metabolizáveis e o retardo dos efeitos deletérios de resíduos metabólicos ao
590 meio intracelular. Entretanto, a resposta conservatória a longo prazo é insustentável
591 quando as condições normais não são reestabelecidas, comprometendo não somente o
592 crescimento e a reprodução, mas também a sobrevivência (Sokolova et al., 2012).
593 A resposta ao estresse em peixes pode ser explicada através de um modelo
594 com três estágios categorizados como respostas primárias, secundárias e terciárias (Mateus
595 et al., 2017). Resumidamente, a reação primária consiste da detecção do estressor, com
596 indução de uma cascata neuroquímica envolvendo a ativação dos eixos do sistema nervoso
597 autônomo-tecido cromafim e eixo hipotálamo-hipófise-interrenal, seguida da respectiva
598 liberação e síntese de catecolaminas (sobretudo adrenalina e noradrenalina); e hormônio
599 corticosteroide (cortisol e compostos relacionados) (Baldisserotto, 2013; Mateus et al.,
600 2017). A resposta secundária é consequência das ações daqueles hormônicos para induzir
601 adaptações cardíacas e respiratórias e incrementar a distribuição de oxigênio e da energia.
602 Assim, os altos níveis de glicose circulante é resultado da ação do cortisol, o qual junto
603 com as catecolaminas permite o aumento da capacidade gliconeogênica (Urbinati et al.,
604 2014). Outro efeito é o da adrenalina sobre o aumento do fluxo sanguíneo e da
605 permeabilidade da membrana branquial, comprometendo o balanço hidromineral, com
606 consequente desequilíbrio osmorregulatório (perda de íons e redução de sódio e cloreto).

Alterações no hematócrito e número de linfócitos também podem ser verificados (Baldisserotto, 2013; Mateus et al., 2017).

Por fim, quando o animal perde essa capacidade adaptativa, instala-se um quadro de exaustão fisiológica denominada resposta terciária, com visíveis prejuízos sobre o crescimento, reprodução e sistema imune (Wendelaar-Bonga, 1997; Mommsen et al., 1999; Urbinati et al., 2014; Schreck, Tort, 2016). Tais consequências podem ser limitantes ao desenvolvimento e produtividade desse setor, sendo responsável por elevados prejuízos econômicos por ano tanto no Brasil como no mundo (Tavares-Dias, Martins, 2017). Por isso, a FAO (2018) reporta a urgência do desenvolvimento de tecnologias alternativas de controle na aquicultura, priorizando a prevenção ao invés da remediação.

1.4.1. Transporte de peixes vivos

O transporte de peixes vivos é uma operação logística corriqueira em atividades de estocagem de formas jovens, transferências de juvenis entre instalações, translado para comercialização e, mesmo, para fins de pesquisa. O processo de transporte pode ser variável, sendo determinado pelo sistema de transporte adotado, densidade de estocagem e duração do transporte (Sampaio, Freire, 2016). Os peixes podem ser transportados em grupo ou individualmente em sistema aberto (tanques) ou fechado (sacos plásticos).

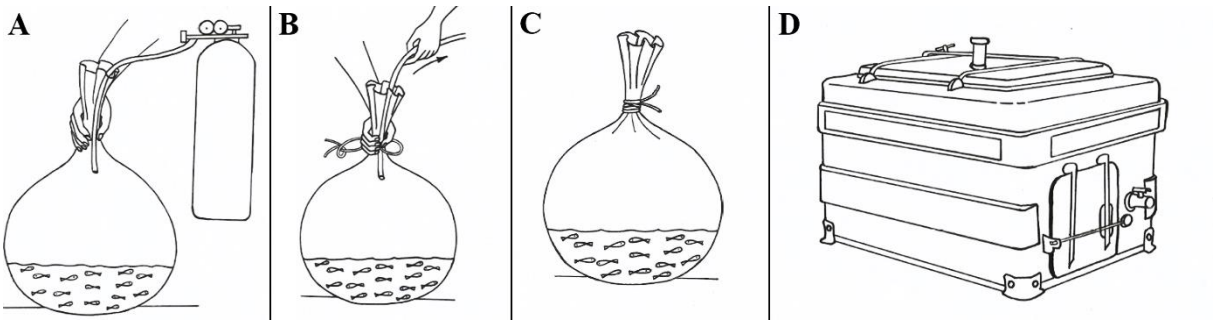


Figura 2. Sistema fechado de transporte para peixes vivos: injeção de oxigênio puro (A), remoção da mangueira injetora de oxigênio (B), embalagem lacrada e finalizada para transporte (C); sistema aberto de transporte (D). Fonte: Adaptado a partir de Berka (1986). Arte: Lílian Cristianne N. Barbosa.

Em sistemas abertos, são utilizadas caixas/tanques de material plástico, fibra ou alumínio com volume de 100 a 4000 L, equipadas com sistema de borbulhamento contínuo com oxigênio puro ou compressores de ar como fonte de oxigênio. Este sistema mantém melhores condições para controle e qualidade da água, no entanto exige um maior investimento (Figura 2D). Por outro lado, em sistema fechado utiliza-se sacos de polietileno (volume útil de 30 a 60L), preenchidos com um terço do volume útil com água e dois terços injetados com oxigênio puro. Embora menos oneroso, esta modalidade é altamente susceptível a deterioração da qualidade da água (Figura 2.A, B, C) (Berka, 1986; Sampaio, Freire, 2016).

Além da redução na qualidade da água, o manuseio prévio ao transporte, duração do translado, altas densidades de estocagem e dietas inadequadas contribuem para a promoção do estresse nos animais, que desencadeiam uma série de respostas fisiológicas primárias, secundárias e terciárias para restaurar a homeostase (Davis, 2006; Sampaio, Freire, 2016). A depender da duração do transporte, o acúmulo de níveis extremos de amônia total e dióxido de carbono (CO_2), além da redução da concentração de oxigênio dissolvido podem ocorrer (Berka, 1986; Gomes, 2003; Salbego et al., 2017). Altos níveis de CO_2 reduzem o pH, causando acidose da água. Como consequência, o pH plasmático baixa e o CO_2 plasmático eleva, prejudicando a capacidade de transporte de oxigênio no sangue em função da redução da afinidade da hemoglobina pelo oxigênio (efeito Bohr), mesmo em caso de saturados níveis de oxigênio na água, o que causa a hipercapnia plasmática (Baldisserotto, 2013). Por esta razão, satura-se a água com oxigênio puro. Entretanto, o pH ácido apesar de causar hipóxia funcional, protege os peixes dos efeitos tóxicos de elevados níveis de amônia (Sampaio, Freire, 2016).

A amônia também é um fator de alto risco no transporte e, sua nocividade é dependente da concentração de amônia total nitrogenada (TAN = NH_3 + NH_4^+), pH,

temperatura e oxigênio dissolvido. A determinação e controle da amônia não-ionizada (NH_3) é fundamental, não porque é extremamente tóxica, mas sim porque sua natureza lipofílica potencializa a sua permeabilidade através das biomembranas. Altas concentrações de amônia na água reduz a excreção deste composto pelo peixe, aumentando os níveis teciduais deste metabólito. Por conta do pH sanguíneo, boa parte de NH_3 é convertida em amônio (NH_4^+) (Baldisserotto, 2013).

Primariamente, a toxicidade aguda da amônia se deve a capacidade do NH_4^+ penetrar os neurônios através dos canais de potássio (K^+), despolarizando-os (Randall, Tsui, 2002; Baldisserotto, 2013). Quando isso ocorre, os íons Mg^+ são removidos, resultando na liberação demasiada do neurotransmissor excitatório glutamato, superativando receptores glutamato (excitotoxicidade), em particular o rececptor N-metil-D-aspartato (NMDA) no cérebro. Essa cascata promove o maior influxo de cálcio (Ca^{2+}), que por sua vez, ativa lipases e proteases que danificam a integridade da membrana celular e a homeostase iônica, resultando em inchaço e necrose neuronal (Ip, Chew, 2010; Baldisserotto, 2013).

Além disso, a amônia também reage com o α-cetoglutarato incorporando um grupo amina (aminação do α-cetoglutarato), incapacitando-o de participar do metabolismo oxidativo e processos subsequentes que levam a produção de ATP. Nesta condição, os neurônios do sistema nervoso central (SNC) têm suas funções severamente comprometidas pela falta de energia (Randall, Tsui, 2002; Ip, Chew, 2010; Baldisserotto, 2013). Em consequência, a disfunção neurológica provocada pela amônia pode ser visualmente verificada quando os peixes apresentam hiperatividade, natação irregular, aumento da taxa de ventilação e convulsões. Posteriormente, o peixe se torna letárgico, perde o equilíbrio e entra em coma, não reagindo a quaisquer estímulos externos (Sá, 2012).

Para amenizar o estresse durante o transporte, atenuar os efeitos deletérios de resíduos metabólicos e garantir o bem-estar, uma variedade de produtos e aditivos para aplicação direta na água têm sido recomendadas. Entre eles, adição de sal na água de transporte (Tacchi et al., 2015), anestésicos sintéticos (Barbas et al., 2017a), probióticos (Dhanasiri et al., 2011) e, mais recentemente, o uso de extrativos naturais como a erva amazônica jambu (*Spilanthes acmella*) e canela amarela (*Nectandra grandiflora*) para o transporte de tambaqui (Barbas et al., 2017b; 2019).

Alguns estudos, também reportam efeitos benéficos da nutrição na mitigação do estresse de transporte, mas poucas pesquisas estão concentradas nesta linha. A suplementação de vitamina C em dietas para pampo (*Pampus argenteus*) durante 9 semanas reduziu o cortisol sérico, níveis de glicose e mortalidade após 4 h de transporte em sacos plásticos (Peng et al., 2013). A administração de açafrão via dieta (*Curcuma longa*) por 60 dias prévios a 24h de transporte diminuiu a mortalidade, além dos níveis de lactato e glicose plasmática no lambari (*Astyanax aff. bimaculatus*) (Ferreira et al., 2017). Dez dias de administração de babosa (*Aloe vera*) em dietas para pacu (*Piaractus mesopotamicus*), seguido por um desafio com *Aeromonas hydrophila* durante 4h de transporte, resultou na diminuição dos níveis de cortisol nos peixes infectados, bem como, no aumento da explosão respiratória de leucócitos e da atividade hemolítica do sistema complemento após transporte (Zanuzzo et al., 2017). Portanto, a nutrição e o uso de aditivos alimentares como uma estratégia de condicionamento pode ser uma forma para preparar e aumentar a resistência dos peixes ao estresse de transporte (Vanderzwalmen et al., 2019).

1.5. Metabolismo oxidativo e sistema de defesa antioxidante

A milhões de anos atrás, cianobactérias causaram a saturação de oxigênio nos oceanos e consequentemente na atmosfera do planeta Terra, abrindo caminho para a

diversificação da vida que conhecemos hoje (Constantini, 2014). O aumento da capacidade metabólica e bioquímica dos organismos via respiração celular do oxigênio (O_2) permitiu a produção mais eficiente de energia (ATP) por meio da oxidação de combustíveis moleculares como a glicose e ácidos graxos (Lushchak, 2011; Halliwell, Gutteridge, 2015). Mas, a evolução para o metabolismo oxidativo não ficou livre de problemas, já que os organismos agora aeróbicos precisavam desenvolver mecanismos para prevenir e eliminar resíduos tóxicos da redução tetravalente do O_2, como radicais livres e espécies reativas não-radicais (Constantini, 2014).

Halliwell e Gutteridge (2015) definem radical livre como "qualquer espécie capaz de existência independente (daí o termo "livre") que contém um ou mais elétrons não emparelhados. Um elétron não emparelhado é aquele que ocupa um orbital atômico ou molecular por si só". Essa natureza, portanto, torna estas espécies instáveis e altamente reativas à outras biomoléculas, podendo causar danos oxidativos em diferentes tecidos (Constantini, 2014). Entretanto, estes danos não são exclusivamente provocados por radicais livres, mas também por outras espécies não-radicais, como peróxido de hidrogênio (H_2O_2), ácido hipocloroso (HOCl), peroxinitrito ($ONOO^-$) e oxigênio singlete ($^1O_2$). Desta forma, o termo 'espécies reativas' foi proposto por Halliwell e Gutteridge (2015) para englobar essa variedade de pró-oxidantes radicais e não-radicais, além de espécies reativas de oxigênio - ROS ($O_2^{\bullet-}$, radical ânion superóxido e $HO^{\bullet}$, radical hidroxila) ou espécies reativas derivadas de outros elementos, como as espécies reativas de nitrogênio. Estas duas categorias são frequentemente denominadas de RONS (sigla em inglês para "espécies reativas de oxigênio e nitrogênio).

Contudo, ROS são gerados durante o metabolismo celular normal, sendo as mitocôndrias as principais fontes *in vivo* dessas espécies reativas e, em concentrações fisiológicas, estão envolvidos em diversas atividades como transdução de sinais,

transcrição de genes, regulação da atividade de enzimas específicas, controle da ventilação respiratória, apoptose e, dentre outros, na ativação da resposta imunológica específica contra patógenos no processo inflamatório (Lushchak, 2011; Halliwell, Gutteridge, 2015; Saccol et al., 2017). De modo geral, a questão central é que fatores de estresse resultantes da intensificação da aquicultura ou de operações inerentes a esta atividade, estimulam a formação excessiva de ROS (ou RONS) nos animais em cultivo, com a consequente geração de estresse oxidativo quando sob perturbações severas. Estresse oxidativo é conceituado como o desbalanço entre oxidantes e antioxidantes a favor de pró-oxidantes, que induz a uma falha na sinalização redox, controle e/ou dano molecular (Jones, 2006; Sies e Jones, 2007).

Sob condição de estresse oxidativo, danos a lipídeos, proteínas e DNA podem ser provocados, desequilibrando a homeostase celular. A peroxidação lipídica (LPO) sobre as estruturas de fosfolipídios da membrana celular (oxidação de PUFA) compromete, por exemplo, a fluidez da membrana e danifica sua estrutura, afetando a função de enzimas, proteínas transportadoras e a função normal das biomembranas (Halliwell, Gutteridge, 2015). O retardo do crescimento é um dos efeitos práticos dos danos causados pela LPO em peixes (Oliva-Teles, 2012). Além disso, a oxidação de ácidos graxos (especialmente PUFA) com formação de peróxidos lipídicos no músculo pode prejudicar o sabor, cor, odor, qualidade e valor nutritivo da carne (Karami et al., 2011; Peixoto et al., 2019). Danos a proteínas causados por ROS estão associados a oxidação e diminuição de grupos sulfidrila (–SH), incluindo amino ácidos e pontes dissulfetos, promovendo também a formação de grupos carbonilas, um tipo de dano oxidativo irreversível que desencadeia a disfunção total ou parcial das proteínas. Nestas condições, danos secundários são provocados, tais como desativação de enzimas reparadoras de DNA, dentre outras (Halliwell, Gutteridge, 2015).

Por isso, organismos aeróbicos desenvolveram mecanismos antioxidantes baseados em defesa enzimática e não enzimática, com o papel de eliminar espécies reativas radicais e não-radicais, realizando sua detoxificação ou transformando-as em compostos menos reativos, ao mesmo tempo em que, também repara biomoléculas danificadas. Defesas antioxidantes enzimáticas são sintetizadas *in vivo*, sendo constituída pela superóxido dismutase (SOD), catalase (CAT), glutationa peroxidase (GPx), glutationa redutase (GR) e glutationa – S – transferase (GST). Antioxidantes obtidos a partir da dieta, compõem grande parte da defesa não enzimática, sendo capazes de atuar como co-fatores da atividade das defesas antioxidantes enzimáticas, quelando íons metálicos ou interceptando e degradando ROS. Nutrientes e micronutrientes como vitamina A, C e E; minerais (magnésio, selênio e zinco), aminoácidos (cisteína, histidina, isoleucina), extratos vegetais ou óleos essenciais (erva-cidreira, hortelã, orégano, tâmara, açaí), antioxidantes (p. ex. α–ácido lipóico, LA), dentre outros, podem desempenhar ação antioxidante *in vivo* (Constantini, 2014; Halliwell, Gutteridge, 2015; Saccol et al., 2017).

Nesse contexto, a inclusão ou suplementação de potenciais antioxidantes na dieta de peixes tem sido amplamente estudada pela sua capacidade de aumentar a resistência ao estresse frente a condições pró-oxidantes, bem como por favorecer o crescimento, melhorar a imunocompetência dos animais e ser uma alternativa ao uso de produtos quimioterápicos. A administração de LA via dieta, por exemplo aumenta a concentração do antioxidante não enzimático glutationa reduzida (GSH) em diferentes órgãos de carpa capim *Cyprinus carpio* (Enamorado et al., 2015) e reduz a peroxidação lipídica em pampo *Trachinotus marginatus* (Kütter et al., 2012). A spirulina (*Arthrospira platensis*) em dietas para tainha foi mais eficiente que o antioxidante β-caroteno, resultando em superior capacidade antioxidante total e maior supressão da peroxidação lipídica (Rosas et al., 2019). *C. carpio* alimentadas com gengibre na dieta e criadas em elevada densidade de

estocagem apresentaram reduzido estresse oxidativo, bem como menor imunossupressão (Fazelan et al., 2020).

1.6. Uso de plantas em dietas para peixes

1.6.1. Fitogênicos e fitoquímicos

Fitogênicos é o termo utilizado para designar uma classe de aditivos alimentares naturais (a base de plantas) promotores do crescimento, sendo assim uma alternativa ao uso de antibióticos promotores do crescimento na produção animal (Upadhaya e Kim, 2017). Fitoquímicos são compostos bioativos não-nutritivos encontrados em plantas e podem ser derivados a partir de sementes secas, galhos, raízes, cascas, caules, folhas, flores, frutos, ervas, especiarias ou extratos na forma de óleos essenciais (OE). A bioatividade dos fitogênicos se dá graças aos metabólitos secundários das plantas, tais como taninas, saponinas, terpenóides, alcaloides, glicosídeos, polifenóis, flavonoides, aminas, aminoácidos não proteicos e compostos organossulfurados não essenciais a mecanismos bioquímicos primários da planta, mas importantes para os mecanismos de defesa contra predadores, patógenos e estresse abiótico (Piasecka et al. 2015; Shahidi, Chandrasekara e Zhong, 2018).

Entre estes metabólitos vegetais os compostos fenólicos são os maiores grupos de fitoquímicos do reino vegetal, perfazendo mais de 8 mil dos compostos já isolados e descritos (Surai, 2014). Os polifenóis variam dentre moléculas simples (p. ex. ácido fenólico) a compostos mais polimerizados (p. ex. taninos). Geralmente são encontrados na forma conjugada com um ou mais resíduos de açúcar ligados a grupos OH, além de ligações diretas da molécula de açúcar com um átomo de carbono. Os açúcares podem ser monossacarídeos, dissacarídeos ou oligossacarídeos, sendo a glicose o açúcar mais comumente encontrado. Em adição, polifenóis são classificados de acordo com o número de anéis fenol e elementos estruturais que conectam estes anéis entre si (Chikezie et al.,

2015; Santana-Gálvez e Jacobo-Velázquez, 2018). Os principais grupos incluem os flavonoides, ácidos fenólicos, taninas, estilbenos e lignanas (Lopes et al., 2017) (Figura 3). Dentre estes, os flavonoides representam o maior grupo com mais 4 mil variedades reportadas e contribuem para a pigmentação e características sensoriais de vegetais e frutas (Chikezie et al., 2015; Santana-Gálvez e Jacobo-Velázquez, 2018).

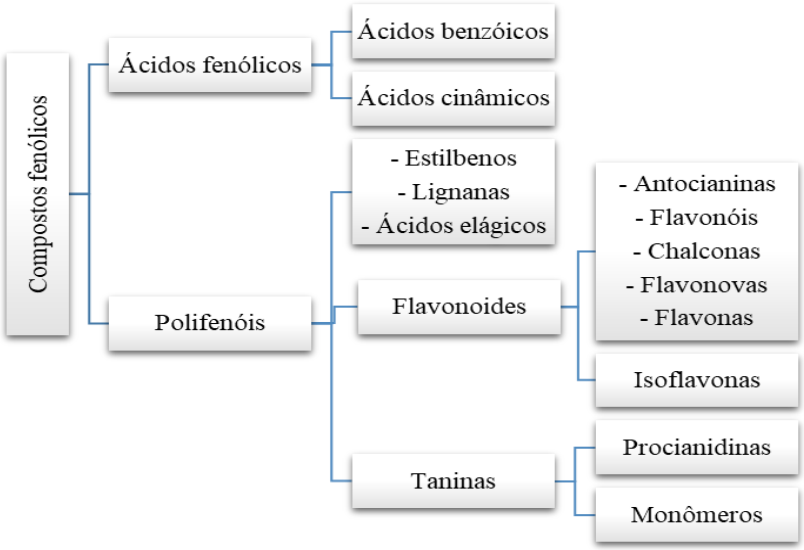


Figura 3. Principais grupos de compostos fenólicos. Fonte: adaptado a partir de Lopes et al. (2017).

As antocianinas são os pigmentos flavonoides mais diversificados nos tecidos do reino vegetal, incluindo as cores azul, púrpura, violeta e vermelho. A variação de cores depende de pequenas alterações químicas, bem como nível de oxigenação, ausência ou presença de grupos funcionais e sua disposição nos anéis aromáticos também influenciam os tons das cores (Faehnrich et al., 2015). O pH também pode afetar intensidade dos tons, como no caso da cor azul, que se torna mais intenso em pH elevado (He & Giusti, 2010). Na aquicultura, o fornecimento de pigmentos naturais via dieta é uma estratégia alternativa a pigmentos sintéticos para melhorar a coloração associada com o peixe, agregando valor sensorial ou ornamental (Amaya e Nickell, 2015; Vanegas-Espinoza et al., 2019).

1.6.2. Efeitos de fitogênicos sobre o crescimento em peixes

A preocupação em substituir quimioterapêuticos e produtos sintéticos de uso clássico na aquicultura tem motivado nas últimas décadas uma série de estudos acerca do uso de plantas medicinais e aditivos alimentares naturais em dietas para peixes (Abdel-Tawwab et al., 2015; Abdel-Tawwab, Abbass, 2017). Plantas são de fácil acesso, são mais biodegradáveis e, na maioria das vezes, são economicamente viáveis, o que aumenta os interesses sobre sua aplicação em larga escala na aquicultura para a promoção do crescimento e manutenção da saúde dos organismos cultivados. Isto porque, o uso de produtos sintéticos (antibióticos promotores do crescimento - AGP, desinfetantes, agentes antimicrobianos e etc.) em produções aquícolas comerciais apresenta restrições, uma vez que, de forma indiscriminada oferece altos riscos à saúde pública, pois podem desencadear resistência bacteriana à antibióticos, bem como aumentar a predisposição ao câncer (Ronquillo e Hernadez, 2016; Idowu e Sogbesan, 2017; Leal et al., 2018).

Por esse motivo, fitoquímicos têm sido testados para fins aquícolas tanto em sua forma bruta, como em extrato ou compostos ativos da planta. A Tabela 1 sumariza os mais recentes estudos realizados com peixes de água doce. A promoção do crescimento a partir de fitoquímicos está relacionada ao aumento de síntese de proteínas nas células; aumento da secreção de enzimas digestivas; preservação da saúde gástrica e intestinal; estimulação do apetite; eficiência alimentar; aumento da competência antioxidante; melhora no sistema imune; e mitigação do estresse (Reverter et al., 2014; Zeppenfeld et al., 2017; Amin et al., 2019).

Contudo, já se encontram alguns estudos que avaliam os mecanismos induzidos por compostos fitoquímicos sobre promoção do crescimento em peixes. Recentemente, verificou-se que fitoquímicos estimulam o crescimento, especificamente a hipertrofia muscular em pacu (*P. mesopotamicus*), modulando o eixo somatotrópico (p. ex. IGF-1) e,

também por esse motivo, podem ser chamados de fitogênicos. Adicionamente, melhoram o status das enzimas antioxidantes, otimizam o balanço energético para o crescimento; a integridade das vilosidades no intestino com consequente melhora na absorção de nutrientes; além de possivelmente influenciar mecanismos de detecção de nutrientes e energia celular regulando o apetite e processos anabólicos e catabólicos (Sokolova et al., 2012; Fuentes et al. 2013; Conde-Sieira, Soengas, 2017; Giampieri et al., 2017; Amin et al., 2019; Salomão et al., 2019).

Tabela 1. Efeitos da administração dietética de diferentes plantas com propriedades antioxidantes sobre a performance de crescimento de peixes de água doce.

Fitogênicos	**Peixe/Espécie**	**Dose**	**Experimento (dias)**	**Efeitos**	**Referências**
Extrato da tamareira (*Phoenix dactylifera*)	*Cyprinus carpio*	200mL kg	8 semanas	↑PFC ↑GP ↑TCE ↓TCA	Hoseinifar et al. (2015)
OE orégano (*Origanum onites*)	*Oncorhynchus mykiss*	0.125 1.50 2.50 3.0 ml kg	90 dias	↑PFC ↑TCE ↓TCA ↑PER	Diler et al. (2016)
OE casca de limão (*Citrus limon*)	*Oreochromis moçambique*	10.0 20.0 50.0 80.0 g kg	28 dias	↑PFC ↑TCE ↓TCA ↑PER	Njugi et al. (2016)
Extrato em pó de nespereira-europeia (*Mespilus germânica*)	*C. carpio*	1.00 2.50 5.00 g Kg	49 dias	↑PFC ↑GP ↑TCE ↓TCA	Hoseinifar et al. (2017a)
Açafrão em pó (*Curcuma longa*)	*C. carpio*	1 2.50 5.00 g kg	10 semanas	↑PFC ↑GP ↑TCE ↑CA	Abdel-Tawwab, Abbass (2017)
Extrato aquoso de folhas de goiaba (*Psidium guajava*)	*O. niloticus*	0.25 0.50 0.75 1.00%	84 dias	↑PFC ↑GP ↑TCE ↑CA ↓TCA ↑PER	Omitoyin et al. (2018)
OE casca de bergamota (*Citrus bergamia*)	*O. niloticus*	0.50 1.00 2.00%	60 dias	↑PFC ↑TCE ↑RGR ↓TCA	Kesbiç et al. (2019)
Folha em pó de tâmara chinesa (*Ziziphus mauritiana*)	*O. niloticus*	5.00 10.00 20.00 g kg	12 semanas	↑PFC ↑GP ↑TCE ↑CA	Amin et al. (2019)

Abreviações: CA; consumo alimentar; GP, ganho de peso; PER, taxa de eficiência proteica; PFC, peso final corporal; TCA, taxa de conversão alimentar; TCE, taxa de crescimento específico; RGR, taxa relativa de crescimento.

De fato, a fisiologia nutricional tem papel fundamental na regulação do crescimento em peixes e, boa parte dos estudos disponíveis ainda não exploraram completamente os mecanismos metabólicos ou os efeitos sobre o metabolismo energético induzidos por fitoquímicos nestes organismos. Desta forma, se recorre muitas vezes a literatura disponível que aborda efeitos de compostos fitoquímicos sobre gatilhos bioquímicos e metabólicos em animais monogástricos, particularmente ratos e seres humanos.

1.6.3. Efeitos antioxidantes dos fitogênicos em peixes

Compostos fenólicos representam um vasto grupo de antioxidantes naturais que têm sido extensivamente estudados por conta dos benefícios associados ao seu consumo, como por exemplo, efeitos antioxidantes, anti-inflamatórios, prevenção de doenças cardiovasculares, obesidade, potencial neuroprotetor, redução da diabetes e combate a alguns tipos de câncer (Shahidi, Chandrasekara e Zhong, 2018). As particularidades de suas estruturas moleculares os tornam capazes de estabilizar a ressonância (alteração da posição de elétrons sem mudar a posição dos átomos), interrompendo a reação em cadeia de radicais livres por meio da doação de átomos de hidrogênio ou elétrons de seus grupos OH. Os flavonoides em especial, são potenciais antioxidantes por sua habilidade em quelar metais, que está intrinsicamente relacionada com a localização dos grupos OH e carbonila ao redor da molécula (Domínguez-Avila et al., 2018).

Nesse contexto, além de promover o crescimento, fitogênicos e seus fitoquímicos constituem uma potencial linha de defesa antioxidante para peixes, bem como, regulam positivamente a expressão de genes relacionados com a defesa antioxidante enzimática (Guardiola et al., 2017) Omitoyin et al. (2019) verificaram aumentos na atividade de SOD, GPx, GST e GSH e atenuação da LPO no fígado e rim de tilápia (*O. niloticus*) alimentadas com dietas contendo 0.75% de inclusão de extrato de folhas de goiaba (*Psidium guajava*). Truta arco-íris (*O. mykiss*) também apresentaram aumento na atividade da SOD, GPx,

883 CAT e redução da LPO no fígado, rim e baço após 8 semanas de alimentação com dietas
884 contendo 1 a 4% de açafrão (*Curcuma longa*) (Yonar et al., 2019). Hoseinifar et al.
885 (2017a) reportaram aumento na expressão de gene relacionado com a atividade da enzima
886 GPx em *C. carpio* alimentadas com dietas contendo extrato de tamareira (200 mL kg^{-1})
887 (*Phoenix dactylifera* L.). Por fim, Safari et al. (2019), verificaram um importante aumento
888 na expressão do gene CAT no intestino de zebrafish (*Danio rerio*) alimentados com dietas
889 suplementadas com coentro *Coriandrum sativum* (20 g kg^{-1}) durante oito semanas.
890 É importante acrescentar que compostos dietéticos são capazes de produzir típicas
891 respostas horméticas, como ocorreu em pampos (*T. marginatus*) alimentados com LA
892 suplementado na dieta (Kütter et al., 2012). Hormese pode ser definida como um estímulo
893 para induzir respostas adaptativas no organismo através da ministração de baixas doses de
894 compostos considerados estressores ou tóxicos (p. ex. metabólitos secundários das
895 plantas), com possíveis efeitos benéficos (Calabrese et al., 2010; Constantini, 2014). Na
896 aquicultura o conceito de hormeses ainda não é abordado, mas é um processo fisiológico
897 fundamental para o condicionamento ao estresse e também fundamental para definir os
898 níveis de inclusão de diferentes tipos de suplementos que responderão de forma esperada.
899 Por outro lado, alguns estudos já reportaram ausência de respostas em alguns
900 órgãos após teste alimentar com fitogênicos. A administração dietética de casca de limão
901 desidratada (*Citrus limon*) para dourada (*Sparus aurata*), por exemplo, não melhorou a
902 atividade hepática das enzimas GR, SOD e CAT após quinze ou trinta dias sob teste
903 alimentar (Béltran et al., 2017). A suplementação de OE (óleos essenciais) de hortelã-
904 pimenta *Mentha piperita* durante trinta dias também não melhorou os níveis de SOD, GPx
905 e CAT e tampouco baixou os níveis de LPO em tambaqui (*C. colossoma*) (Ribeiro et al.,
906 2018). Desta forma, a efetividade de fitoquímicos sobre a modulação do estresse oxidativo
907 e defesas antioxidantes em peixes pode variar e, isso se deve a presença de outros

compostos; constituição da matriz alimentar (interações com outras macromoléculas da dieta); preparo e processamento dos extratos ou rações. Além disso, a concentração de fitoquímicos nas plantas é espécie-específica e, fatores bióticos e abióticos podem influenciar a produção de metabolitos secundários (Domínguez-Avila et al., 2018). Finalmente, e como mencionado anteriormente, o conceito de hormeses prediz que, efetivamente, haverá concentrações benéficas para o organismo e outras que podem induzir o efeito contrário ao desejado.

1.6.4. Fitogênicos e metabolismo energético

Em peixes, investigações sobre os efeitos de fitogênicos no metabolismo energético são escassos. Em animais monogástricos, por outro lado, a suplementação de fitogênicos tem apresentado efeito hipolipidêmico. A suplementação alimentar com uma mistura de ervas (caules e folhas de hortelã, tomilho e mandioca; sementes de pimenta e cominho preto; raízes de gengibre; e parte do bulbo de cebola e alho) diminuiu os níveis plasmáticos de triglicerídeos, colesterol total e glicose, com aumento do colesterol HDL em frangos de corte (Saleh et al., 2018). Haselgrübler et al. (2019) explicam que a proteína quinase ativada por AMP (AMPK) funciona como um sensor da homeostase energética celular e reduz processos anabólicos (p. ex. a síntese de lipídios), podendo ser ativada por fitoquímicos, inibindo assim, a lipogênese e estimulando a lipólise.

Compostos fenólicos (p. ex. polifenóis), por exemplo, são capazes de ativar a AMPK, estimulando o catabolismo da glicose e de estoques lipídicos (Herzig e Shaw, 2018; Thomson, 2018), gerando um aumento de fluxo de elétrons na cadeia respiratória (Cortassa et al., 2019). Além disso, a AMPK modula *Sirtuinas* (SIRT 1) que ativam o regulador chave do metabolismo energético PGC-1α e o fator transcricional Nrf1, resultando em um completo programa de biogênese mitocondrial (Hardie, Ross, Hawley, 2012; Giampieri et al., 2017). Zhang et al. (2017) reportaram que a suplementação de

resveratrol em dietas para frango de corte melhorou a mitocondriogênese e aumentou a atividade enzimática da citrato sintase no músculo peitoral, indicando melhora na atividade do ciclo de Krebs e sugerindo maior eficiência mitocondrial. Isto deveria redundar num aumento da produção energética, resposta que deveria influenciar positivamente na taxa de crescimento.

Embora a AMPK desative processos de síntese proteica (expressão de genes do crescimento como *mTOR*), a maior eficiência mitocondrial significa substancial produção de ATP, que por sua vez, aciona a enzima adenilato quinase para manter o AMP em níveis baixos, interrompendo os processos catabólicos desencadeados pela AMPK e favorecendo vias anabólicas (Craig, 2018). Um estudo recente suplementou resveratrol em dietas para pacu (*P. mesopotamicus*) submetidos ou não a exercícios e, benefícios sobre o crescimento muscular e o metabolismo foram encontrados como resultado do aumento da expressão de genes relacionados com a miogênese (*myod*), síntese proteica (*igf1* e *mTOR*) e metabolismo oxidativo (*sdha*), com simultânea diminuição da expressão de genes catabólicos (*murfla* e *floxo25*) (Salomão et al., 2019).

1.6.5. Fitogênicos anticonvulsivos

É consolidado em estudos da área biomédica que os receptores dos neurotransmissores GABA (ácido gama-aminobutírico) e glutamato podem ter participação substancial na iniciação, manutenção e interrupção de crises convulsivas. Em adição, a ativação em demasiada de receptores de aminoácidos excitatórios está relacionada com a produção de ROS e espécies reativas de nitrogênio (RNS), responsáveis pela origem de convulsões e morte celular relacionada (Souza Monteiro et al., 2015; Diniz et al., 2015).

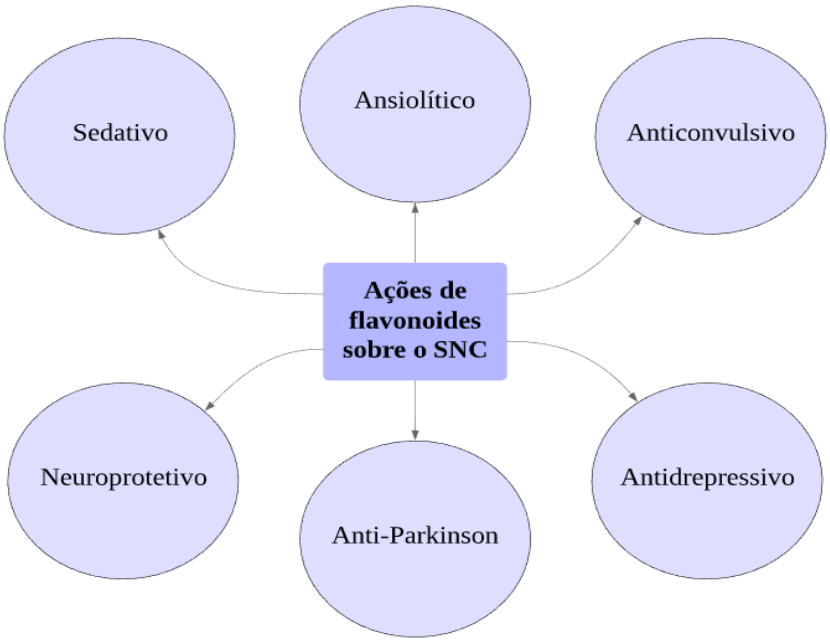


Figura 4. Atividades biológicas de flavonoides sobre o sistema nervoso central (SNC).

Desta forma, a administração de antioxidantes se apresenta como uma abordagem contra convulsões e seus efeitos deletérios. Entre as atividades biológicas de compostos fenólicos já apresentados, os flavonoides em particular, também têm atividade ansiolítica, neuroprotetora, anticonvulsiva, dentre outros (Figura 4) (Diniz et al., 2015). O efeito anticonvulsivo dos flavonoides se deve a sua capacidade de modular o canal complexo $GABA_A$ – Cl, pois são estruturalmente semelhantes aos benzodiazepínicos (Rabiei, 2017).

Extrato do fruto romã (*Tilia americana* var. *mexicana*), rico em flavonoides (p. ex. quercetina, rutina e isoquercitrina) amenizou significativamente as crises convulsivas induzidas por petilenotetrazol (PTZ), diminuindo os níveis de estresse oxidativo em camundongos (Cárdenas-Rodríguez et al., 2014). Estudos eletrofisiológicos a partir de registros eletroencefalográficos têm sido conduzidos para triar extrativos naturais com potencial anticonvulsivo, utilizando principalmente camundongos como modelo animal (Souza-Monteiro et al., 2015; González-Trujano et al., 2018). Em peixes, estudos desta natureza são inexistentes, havendo, no entanto, dois estudos que reportaram atividade

anestésica de OE de citronela (*Cymbopogon nardus*) e óleo de cravo induzindo sedação em tambaqui (Barbas et al., 2017c) e em outras três espécies de peixes amazônicos (*Paracheirodon axelrodi, Heros severus, Pterophyllum scalare*) (Fujimoto et al., 2018) por meio da avaliação de eletrocardiograma e ou eletromiograma.

1.7. Açaí – *Euterpe oleracea*

No noroeste do Brasil localiza-se a maior floresta equatorial do planeta, a Amazônia. Nesta floresta flui a maior bacia hídrica formada pelo rio Amazonas e seus afluentes, cercada por um bioma diverso. A palmeira açaizeiro (*Euterpe oleracea* Mart.) está distribuída naturalmente nesta floresta e nos estados do Amapá, Maranhão e Pará, com densas populações em áreas inundáveis, denominadas áreas de várzea alta, várzea baixa e igapó. O Pará é o maior produtor e extrativista (98,3% da produção nacional) de açaí (*E. oleracea*), sendo responsável pela produção de 1,278 milhões de ton em 2018 (Domingues et al., 2012; IBGE-PEVS, 2020). Este estado também explora a extração de palmito (Domingues et al., 2012). A *E. oleracea* pertence a classe Equisetopsida e a família Arecaceae, a qual inclui as palmeiras *Bactris gasipaes* (pupunha), *Butia capitata* (butiá) e *Cocos nucifera* (coqueiro).

Figura 5. Exemplares de açaizeiros (*Euterpe oleracea*). Fonte: autoria própria.

O açaizeiro é uma árvore cespitosa (Figura 5), suportando até 25 perfilhos por touceira em diferentes fases de desenvolvimento. Os troncos das plantas adultas alcançam entre 3 a 20 m de altura e diâmetro de 7 a 18 cm. As folhas são compostas com até 278 cm de comprimento com formação pinadas de aro espiralado e folíolos de 40 a 80 pares em intervalos regulares opostos ou subopostos (Henderson, 2000). O sistema radicular é fasciculado, com raízes brotando do estipe da planta adulta até 40 cm acima da superfície do solo (Yamaguchi et al., 2015).

O fruto é uma drupa globosa, com 1 a 2 cm de diâmetro e peso aproximado de 1,5 g. O amadurecimento do fruto ocorre em cerca de 175 dias, com cor variando entre verde e violeta. O mesocarpo polposo (1 mm de espessura) engloba o volumoso endocarpo. A parte comestível do fruto é formada pelo epicarpo e mesocarpo, constituindo 26,54 % do seu peso (Oliveira et al, 2007). Palmeiras maturas, podem produzir cerca de 1000 kg ou mais de açaí num período de 5 anos. A expectativa de produção ativa perfaz mais de 25 anos, demonstrando um alto estoque deste fruto nos mais de 11 milhões de hectares nas planícies de inundação da Amazônia onde estão distribuídos (Schauss, 2011).

Os estudos com *E. oleracea* são numerosos, por conta de seu perfil de fitoquímicos e seus efeitos benéficos à saúde (Yamaguchi et al., 2015). O açaí é rico em compostos fenólicos, especialmente ácidos fenólicos (ácido gálico, ácido 3,4-di-hidroxibenzóico, ácido 4-hidroxibenzóico, ácido gentísico, ácido síringico e ácido vanílico), flavonóis (quercetina), antocianinas (cianidina-3-*O*-glucosídeo e cianidina -3-*O*-rutinosídeo), carotenoides e ácidos hidroxicinâmicos (ácido clorogênico, ácido cafeico e ácido ferúlico) (Alqurashi et al., 2016) (Figura 6). Contém ainda abundância em ácidos graxos monossaturados e polinsaturados, especialmente ácido oleico, palmítico e linoleico (Del Pozo-Insfran et al., 2004; Heinrich et al., 2011; Alqurashi et al., 2016).

1020

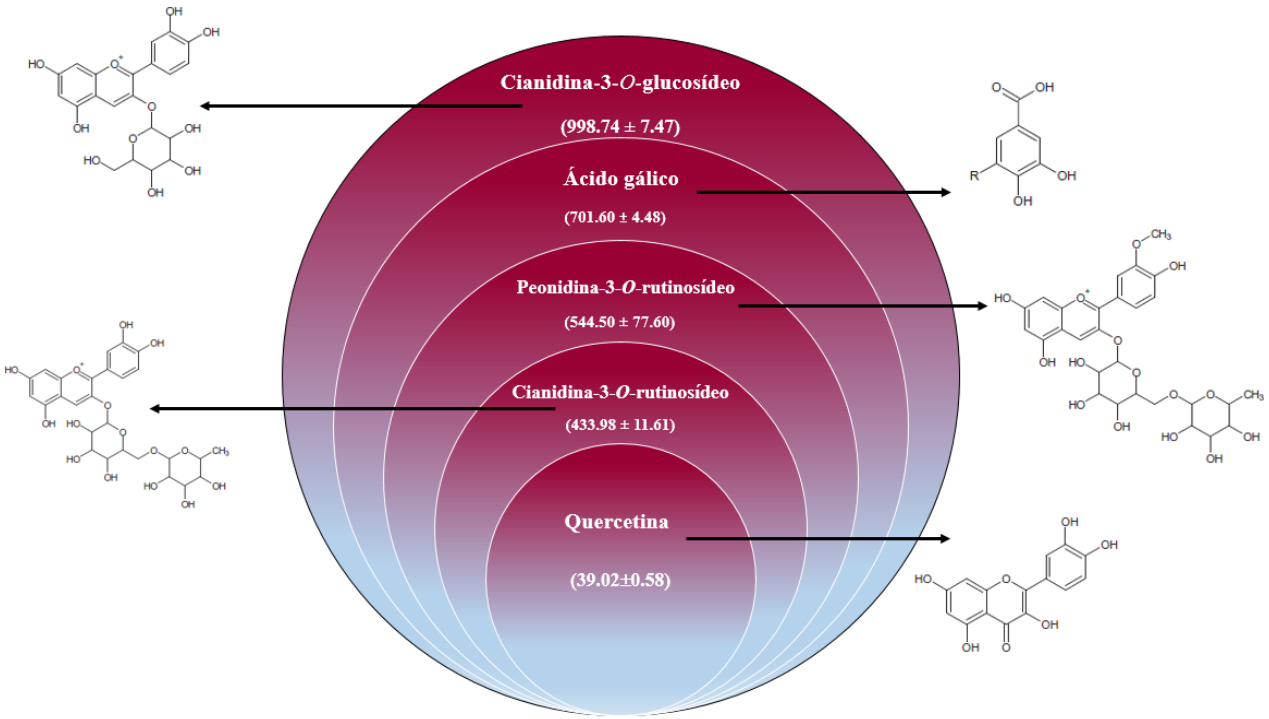


Figura 6. Compostos fenólicos majoritários do açaí (*Euterpe oleracea*). Adaptado de Alqurashi et al. (2016).

1021 A Tabela 2 reúne algumas das atividades biológicas relatadas para o consumo de
1022 açaí *E. oleracea* em diferentes modelos experimentais. A maioria de seus efeitos está
1023 relacionado a modulação de vias de resposta de resistência ao estresse (Keap1/Nrf2/ARE),
1024 induzindo a expressão de enzimas antioxidantes endógenas/desintoxicantes de fase II
1025 (Soares et al., 2017). Também foi relatado que o monoacilglicerol contendo ácido oleico
1026 exerce potentes efeitos antioxidantes (Cho et al., 2010). Além disso, é sugerido que a
1027 regulação negativa da via de sinalização NF-kB, bem como, da via da MAP-quinase são
1028 responsáveis pela atividade anti-inflamatória deste fruto (Aranha et al., 2019). Segundo
1029 Schauss (2016), o açaí regula negativamente a expressão de genes e proteínas pró-
1030 inflamatórias nas células CDD-18Co que participam de processos inflamatórios no trato
1031 gastrointestinal (Schauss, 2016).

Tabela 2. Atividade biológica do açaí (*Euterpe oleracea*) em diferentes modelos experimentais.

Açaí	**Modelo experimental**	**Efeitos**	**Referências**
Liofilizado	Camarão[1]	↑ Resistência ao estresse causado pela amônia; ↑ Competência antioxidante; ↓LPO; ↑ Proteção sobre as brânquias e hepatopâncreas contra toxicidade da amônia.	Colombo et al (2020)
Polpa congelada	Humanos	↓ Estresse oxidativo; ↑ Atividade anti-inflamatória em indivíduos com sobrepeso e dislipidemia.	Aranha et al. (2019)
Extrato da semente	Célula humana endotelial	↑ Proteção contra toxicidade endotelial do H_2O_2; ↑ modulação da via Nrf2 (genes CAT, GPx, NQO1 e HMOX1), ↑Expressão de enzimas antioxidantes.	Soares et al. (2017)
Suco	Camundongos	↑ Proteção anticonculsiva; ↓ LPO no córtex cerebral.	Souza Monteiro et al. (2015)
Extrato do fruto	Camundongos	↑ Proteção contra o desenvolvimento da hipertensão, disfunção endotelial e alterações estruturais vasculares.	da Costa et al. (2012)
Liofilizado	Peixe[2]	↓ Colesterol total sérico; ↓ glicose no sangue; ↓ CETP; ↓ estoque de gordura e inflamação no fígado; ↓ LPO hepático; e ↓ FRA.	Kim et al. (2012)
Polpa seca por pulverização	Camundongos	↓ Danos ao DNA causados por H_2O_2; ↑Atividade antiproliferativa e anticarcinogênica na bexiga urotelial.	Fragoso et al. (2012)
Polpa	Camundongos	Efeito hipocolesterolêmico; ↓ Níveis séricos de proteínas carboniladas e grupos sulfidrilas.	de Souza et al. (2010)
Suco	Humanos	↓ ROS; ↑ Antioxidantes séricos; e inibição da peroxidação lipídica.	Jensen et al. (2008)

Nota: 1, *Litopenaeus vannamei*; 2, *Danio rerio*. Abreviações: NQO1, quinona oxidoredutase-1; HMOX1, heme oxigenasse-1; CETP, proteína de transferência de éster colesteril; FRA, capacidade de redução férrica. LPO, peroxidação lipídica.

No contexto da aquicultura, estudos avaliando os efeitos do açaí em organismos aquáticos ainda são limitados. Para o melhor do nosso conhecimento, existem dois estudos publicados. Um deles é de caráter biomédico e relata efeito hipolipidêmico, redução da inflamação hepática e de espécies lipídicas oxidadas no fígado de peixe zebra (*D. rerio*) hipercolesterolêmico após consumo de 10.0% (w/w) açaí liofilizado (Kim et al., 2012). O segundo é o estudo pioneiro de Colombo et al. (2020), avaliando os efeitos da inclusão de açaí liofilizado (*E. oleracea*) em dietas para o camarão branco do Pacífico (*Litopenaeus vannamei*). Embora sem efeitos sobre o crescimento, após trinta e cinco dias de administração de açaí dietético, uma substancial proteção foi conferida contra danos histopatológicos em brânquias e hepatopâncreas de camarões desafiados com amônia, além de aumentar a competência antioxidante com incremento da atividade de GST neste último órgão. Melhora nas defesas antioxidantes também foram verificadas nas brânquias e músculo, com redução da peroxidação lipídica (LPO) neste último órgão.

Apesar da ausência de estudos prévios a respeito dos efeitos de *E. oleracea* na nutrição de peixes e, tomando por base as atividades biológicas já reportadas para o consumo de açaí em diferentes modelos animais, supõem-se que efeitos fisiológicos semelhantes possam ocorrer, mediando eficiência de utilização do alimento, metabolismo energético, sistema de defesa antioxidante e mecanismos de resistência ao estresse de modo a melhorar o crescimento. Adicionalmente, destaca-se a inexistência de investigações fisiológicas sobre fatores de estresse ambientais induzindo desordens neurológicas em organismos cultivados. Nesse sentido, a inclusão de açaí em dietas para peixes é justificável, especialmente por seu comprovado poder neuroprotetor mencionado previamente.

2. OBJETIVO GERAL

Determinar os efeitos da inclusão de açaí liofilizado *Euterpe oleracea* (LEO) em dietas para juvenis de tambaqui (*Colossoma macropomum*) sobre o desempenho de crescimento, parâmetros de estresse oxidativo e defesas antioxidantes, metabolismo energético e resistência ao estresse de transporte, avaliando também a atividade neuroprotetora deste fruto contra convulsões induzidas.

2.1. Objetivos específicos

- ✓ Avaliar os efeitos da inclusão de LEO em dietas sobre a performance de crescimento, coloração da pele e status antioxidante de juvenis de *C. macropomum*, considerando o benefício econômico associado as dietas e a performance dos peixes.
- ✓ Explorar a influência da administração dietética de LEO sobre o metabolismo energético muscular de juvenis de *C. macropomum*.
- ✓ Estudar a capacidade antiestresse de LEO dietético sobre os níveis de glicose, competência antioxidante e níveis de peroxidação lipídica em *C. macropomum* submetidos a diferentes tempos de transporte.
- ✓ Mensurar a atividade anticonvulsiva e neuroprotetora de LEO dietético em juvenis de *C. macropomum*.

3. REFERÊNCIAS

Abdel-Tawwab, M., Sharafeldin, K. M., Mosaad, M. N., Ismaiel, N. E., (2015). Coffee bean in common carp, *Cyprinus carpio* L. diets: effect on growth performance, biochemical status, and resistance to waterborne zinc toxicity. *Aquaculture*. 448, 207 – 213.

Abdel-Tawwab, M. (2017). Turmeric powder, *Curcuma longa* L., in common carp, *Cyprinus carpio* L., diets: growth performance, innate immunity, and challenge against pathogenic *Aeromonas hydrophila* infection. *Journal of the World Aquaculture Society*, *48*, 303 – 312.

Abelha, M. C. F.; Agostinho, A. A.; Goulart, E. (2001). Plasticidade trófica em peixes de água doce. Acta Scientiarum, Biological Sciences 23, 425 – 434.

Alqurashi, R. M., Commane, D. M., Rowland, I. R. (2016). Açai fruit as a source of bioactive phytochemicals. *Journal of Life Sciences*, *10*, 391 – 404.

Amaya, E., Nickell, D. (2015). Using feed to enhance the color quality of fish and crustaceans. In: *Feed and Feeding Practices in Aquaculture* (pp. 269 – 298). Woodhead Publishing.

Amin, A., El Aselyb, A., El-Nabyc, A. S. A., Samirc, F., El-Ashramd, A., Sudhakarane, R., Dawoodf, M. A. O. (2019). Growth performance, intestinal histomorphology and growth-related gene expression in response to dietary *Ziziphus mauritiana* in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *512*, 734301.

Aranha, L. N., Silva, M. G., Uehara, S. K., Luiz, R. R., Neto, J. F. N., Rosa, G., de Oliveira, G. M. M. (2019). Effects of a hypoenergetic diet associated with açaí (*Euterpe oleracea* Mart.) pulp consumption on antioxidant status, oxidative stress and inflammatory biomarkers in overweight, dyslipidemic individuals. *Clinical Nutrition*. Corrected proof.

Araújo-Lima, C. A. R. M.; Goulding, M. (1998). Os frutos do tambaqui: Ecologia, conservação e cultivo na Amazônia. Tefé: Sociedade Civil Mamirauá. Brasília: CNPq, 186p.

Araújo, J. G., Guimarães, I. G., Mota, C. S., De Paula, F. G., Café, M. B., Pádua, D. M. C. (2017). Dietary available phosphorus requirement for tambaqui, Colossoma

macropomum, juveniles based on growth, haematology and bone mineralization. *Aquaculture Nutrition*, *23*, 822 – 832.

Aride, P. H. R., Ferreira, M. S., Duarte, R. M., De Oliveira, A. M., De Freitas, D. V., Dos Santos, A. L. W., Nozawa, S. R., Val, A. L. (2010). Ascorbic Acid (Vitamin C) and iron concentration in tambaqui, *Colossoma macropomum*, iron absorption. *Journal of the World Aquaculture Society*, *41*, 291 – 297.

Baldisserotto, B. (2013). Fisiologia de peixes aplicada à piscicultura. Santa Maria, RS. 3° ed. UFSM Ed. 352 p.

Barbas, L. A. L., Pereira-Cardona, P. M., Maltez, L. C., Garcia, L. O., Monserrat, J. M., Sampaio, L. A. (2017a). Anaesthesia and transport of juvenile tambaqui *Colossoma macropomum* (Cuvier, 1818) with tricaine methane-sulphonate: Implications on secondary and oxidative stress responses. *Journal of Applied Ichthyology*, *33*, 720 – 730.

Barbas, L. A. L., Maltez, L. C., Stringhetta, G. R., de Oliveira Garcia, L., Monserrat, J. M., da Silva, D. T., Heinzmann, B. M., Sampaio, L. A. (2017b). Properties of two plant extractives as anaesthetics and antioxidants for juvenile tambaqui *Colossoma macropomum*. *Aquaculture*, *469*, 79 – 87.

Barbas, L. A. L., Hamoy, M., de Mello, V. J., Barbosa, R. P. M., de Lima, H. S. T., Torres, M. F., do Nascimento, L. A. S., da Silva, J. K. R., Andrade, E. H. A., Gomes, M. R. F. (2017). Essential oil of citronella modulates electrophysiological responses in tambaqui *Colossoma macropomum*: a new anaesthetic for use in fish. *Aquaculture*, *479*, 60 – 68.

Barbas, L. A. L., de Araújo, E. R. L., Torres, M. F., Maltez, L. C., de Oliveira Garcia, L., Heinzmann, B. M., Sampaio, L. A. (2019). Stress relieving potential of two plant-based sedatives in the transport of juvenile tambaqui *Colossoma macropomum*. *Aquaculture*, 734681.

Beltrán, J. M. G., Espinosa, C., Guardiola, F. A., Esteban, M. Á. (2017). Dietary dehydrated lemon peel improves the immune but not the antioxidant status of gilthead seabream (*Sparus aurata* L.). *Fish & Shellfish Immunology*, *64*, 426 – 436.

Béné, C., Arthur, R., Norbury, H., Allison, E. H., Beveridge, M., Bush, S., Campling, L., Leschen, W., Little, D., Squires, D., Thilsted, S. H., Troell, M., Thilsted, S. H.

(2016). Contribution of fisheries and aquaculture to food security and poverty reduction: assessing the current evidence. *World Development*, *79*, 177 – 196.

Berka, R. (1986). The transport of live fish: A review. EIFAC Tech. Pap. FAO. Rome, 48, 52.

Bly, J. E., Quiniou, S. M., Clem, L. W. (1997). Environmental effects on fish immune mechanisms. *Developmental Biology Standard*, *90*, 33 – 43.

Boyd, C. E. (2017). General relationship between water quality and aquaculture performance in Ponds. In: *Fish diseases*. Academic Press. 147 – 166.

Brabo, M. F., Pereira, L. F. S., Santana, J. V. M., Campelo, D. A. V., Veras, G. C. (2016). Cenário atual da produção de pescado no mundo, no Brasil e no estado do Pará: ênfase na aquicultura. *Acta Fisheries and Aquatic*, 4, 50 – 58.

Buckup, P. A., Menezes, N. A., Ghazzi, M. S. (2007). Catálogo das espécies de peixes de água doce do Brasil. Museu Nacional (eds). Rio de Janeiro. 195 p.

Calabrese, V., Cornelius, C., Dinkova-Kostova, A. T., Calabrese, E. J., Mattson, M. P. (2010). Cellular stress responses, the hormesis paradigm, and vitagenes: novel targets for therapeutic intervention in neurodegenerative disorders. *Antioxidants & Redox Signaling*, *13*, 1763 – 1811.

Campos-Baca, L., Köhler, C. C. (2005). Aquaculture of *Colossoma macropomum* and related species in Latin America. *American Fish Society Symposium Series*, *46*, 541 – 561.

Cárdenas-Rodríguez, N., González-Trujano, M. E., Aguirre-Hernández, E., Ruíz-García, M., Sampieri, A., Coballase-Urrutia, E., Carmona-Aparicio, L. (2014). Anticonvulsant and antioxidant effects of *Tilia americana var. mexicana* and flavonoids constituents in the pentylenetetrazole-induced seizures. *Oxidative Medicine and Cellular Longevity*, 2014, 1 – 10.

Chikezie, P.C., Ibegbulem, C.O., Mbagwu, F.N. (2015). Bioactive principles from medicinal plants. *Research Journal of Phytochemistry*, 9, 88 – 115.

Cho, K. H., Hong, J. H., Lee, K. T. (2010). Monoacylglycerol (MAG)-oleic acid has stronger antioxidant, anti-atherosclerotic, and protein glycation inhibitory activities than MAG-palmitic acid. *Journal of Medicinal Food*, 13, 99 – 107.

Colombo, G. M., dos Santos Simião, C., Schmitz, M. J., Pedrosa, V. F., Romano, L. A., Tesser, M. B., Ramos, P. B., Wasielesky, W., Monserrat, J. M. (2020). The role of açaí (*Euterpe oleracea* Mart. 1824) as a chemoprotective agent in the evaluation of antioxidant defence, oxidative damage and histology of juvenile shrimp *Litopenaeus vannamei* (BOONE, 1931) exposed to ammonia. *Aquaculture Research, 00, 1 – 16.*

Conde-Sieira, M., Soengas, J. L. (2017). Nutrient sensing systems in fish: impact on food intake regulation and energy homeostasis. *Frontiers in Neuroscience*, *10*, 1 – 21.

Conselho Nacional das Empresas de Pesca – CONEPE (2014). Estatística: Balança comercial de pescado até julho 2014. Brasília – DF.

Costantini, D. (2014). Oxidative stress and hormesis in evolutionary ecology and physiology – A marriage between mechanistic and evolutionary approaches. *Berlin and Heidelberg.* Springer. 359p.

Cortassa, S., Aon, M. A., Sollott, S. J. (2019). Control and regulation of substrate selection in cytoplasmic and mitochondrial catabolic networks. A systems biology analysis. *Frontiers in Physiology*, *10*, 201.

Craig, P. M., Moyes, C. D., LeMoine, C. M. (2018). Sensing and responding to energetic stress: evolution of the AMPK network. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *224*, 156 – 169.

da Costa, C. A., de Oliveira, P. R. B., de Bem, G. F., de Cavalho, L. C. R. M., Ognibene, D. T., da Silva, A. F. E., Valença, S. S., Pires, K. M. P., Sousa, P. J. C., de Moura, R. S., Resende, A. C. (2012). *Euterpe oleracea* Mart.-derived polyphenols prevent endothelial dysfunction and vascular structural changes in renovascular hypertensive rats: role of oxidative stress. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *385*, 1199 – 1209.

da Silva, C. A. D., Fujimoto, R. Y. (2015). Crescimento de tambaqui em resposta a densidade de estocagem em tanques-rede. *Acta Amazônia*, 323 – 332.

Dairiki, J. K., da Silva, T. B. A. (2011). Revisão de literatura: Exigências nutricionais do tambaqui – compilação de trabalhos, formulação de ração adequada e desafios futuros. Manaus: Embrapa Amazônia Ocidental. Documentos 91. 44 p.

Davis, K. B. (2006). Management of physiological stress in finfish aquaculture. *North American Journal of Aquaculture, 68*, 116 – 121.

de Almeida, L. C., Avilez, I. M., Honorato, C. A., Hori, T. S. F., Moraes, G. (2011). Growth and metabolic responses of tambaqui (*Colossoma macropomum*) fed different levels of protein and lipid. *Aquaculture Nutrition*, *17*, e253 – e262.

Del Pozo-Insfran, D., Brenes, C. H., Talcott, S. T. (2004). Phytochemical composition and pigment stability of açai (*Euterpe oleracea* Mart.). *Journal of Agricultural and Food Chemistry*, *52*, 1539 – 1545.

de Souza, M. O., Silva, M., Silva, M. E., de Paula Oliveira, R., Pedrosa, M. L. (2010). Diet supplementation with acai (*Euterpe oleracea* Mart.) pulp improves biomarkers of oxidative stress and the serum lipid profile in rats. *Nutrition, 26,* 804 – 810.

Diniz, T. C., Silva, J. C., Lima-Saraiva, S. R. G. D., Ribeiro, F. P. R. D. A., Pacheco, A. G. M., de Freitas, R. M., Quintans-Júnior, L. J., Quintans, J. S. S., Mendes, R. L., Almeida, J. R. G. D. S. (2015). The role of flavonoids on oxidative stress in epilepsy. *Oxidative Medicine and Cellular Longevity*. 2015: 1 – 9.

Dhanasiri, A. K. S., Kiron, V., Fernandes, J. M. O., Bergh, Ø., Powell, M. D. (2011). Novel application of nitrifying bacterial consortia to ease ammonia toxicity in ornamental fish transport units: trials with zebrafish. *Journal of Applied Microbiology, 111*, 278 – 292.

Diana, J. S., Egna, H. S., Chopin, T., Peterson, M. S., Cao, L., Pomeroy, R., Verdegem, M., Slack, W. T., Bondad-Reantaso, M. G., Cabello, F. (2013). Responsible aquaculture in 2050: valuing local conditions and human innovations will be key to success. *BioScience*, *63*, 255 – 262.

Dias, J. A., Abe, H. A., Sousa, N. C., Couto, M. V., Cordeiro, C. A., Meneses, J. O., Cunha, F. S., Mouriño, J. L. P., Martins, M. L., Barbas, L. A. L., Carneiro, P. C. F., Maria, A. N., Fujimoto, R. Y., Carneiro, P. C. (2018). Dietary supplementation with autochthonous *Bacillus cereus* improves growth performance and survival in tambaqui *Colossoma macropomum*. *Aquaculture Research*, *49*, 3063 – 3070.

Diler, O., Gormez, O., Diler, I., Metin, S. (2017). Effect of oregano (*Origanum onites* L.) essential oil on growth, lysozyme and antioxidant activity and resistance against

Lactococcus garvieae in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Nutrition*, *23*, 844 – 851.

Diniz, T. C., Silva, J. C., Lima-Saraiva, S. R. G. D., Ribeiro, F. P. R. D. A., Pacheco, A. G. M., de Freitas, R. M., Quintans-Júnior, L. J., Quintans, J. S. S., Mendes, R. L., Almeida, J. R. G. D. S. (2015). The role of flavonoids on oxidative stress in epilepsy. *Oxidative Medicine and Cellular Longevity*, 2015, 1 – 10.

Domingues, A. F. N., Emmi, D. T., Barroso, R. F. F., Mattietto, R. A. (2012). Pigmentos antociânicos do açaí (*Euterpe oleracea* Mart.) como evidenciadores de biofilme dental. In: Tecnologias para inovação nas cadeias euterpe. Ed. Pessoa, J. D. C., Teixeira, G. H. A. Embrapa. Brasília. 327 – 343.

Domínguez-Avila, J. A., Ruiz-Canizales, J., Pacheco-Ordaz, R., Villegas-Ochoa, M. A., González Aguilar, G. A. (2018). Antioxidant power. In: Phenolic compounds in food: Characterization and analysis. Nollet, L. M., Gutierrez-Uribe, J. A. (Eds.). CRC Press. Taylor & Francis Group. 261 – 277.

Enamorado, A. D., Martins, A. C., Flores, J. A., Tesser, M. B., Caldas, S. S., Primel, E. G., Monserrat, J. M., 2015. Biochemical responses over time in common carp *Cyprinus carpio* (Teleostei, Cyprinidae) during fed supplementation with α-lipoic acid. *Comparative Biochemistry and Physiology Part A*: *Molecular & Integrative Physiology*, *188*, 9 – 16.

Faehnrich, B., Lukas, B., Humer, E., Zebeli, Q. (2016). Phytogenic pigments in animal nutrition: potentials and risks. *Journal of the Science of Food and Agriculture*, *96*, 1420 – 1430.

Fazelan, Z., Vatnikov, Y. A., Kulikov, E. V., Plushikov, V. G., Yousefi, M. (2020). Effects of dietary ginger (*Zingiber officinale*) administration on growth performance and stress, immunological, and antioxidant responses of common carp (*Cyprinus carpio*) reared under high stocking density. *Aquaculture*, *518*, 734833.

Ferreira, P. D. M. F., Rocha, J. S., Gomes, J. R., Caldas, D. W., Martins, M. T. S., de Oliveira, J. M., Salaro, A. L., Zuanon, J. A. S. (2017). *Curcuma longa* supplementation in the diet of *Astyanax aff. bimaculatus* in preparation for transport. *Aquaculture Research*, 48, 4524 – 4532.

Food and Agriculture Organization of the United Nations – FAO (2014). The State of World Fisheries and Aquaculture. 243p.

Food and Agriculture Organization of the United Nations - FAO (2015). Brazil - National Aquaculture Sector Overview. (Retrieved April 27, 2016)

Food and Agriculture Organization of the United Nations – FAO (2016). The State of World Fisheries and Aquaculture – Contributing to food security and nutrition for all. Rome. 204p.

Food and Agriculture Organization of the United Nations - FAO (2018). The State of World Fisheries and Aquaculture – Meeting the sustainable development goals. Rome. 227p.

Fragoso, M. F., Prado, M. G., Barbosa, L., Rocha, N. S., Barbisan, L. F. (2012). Inhibition of mouse urinary bladder carcinogenesis by açai fruit (*Euterpe oleraceae* Martius) intake. *Plant Foods for Human Nutrition*, *67*, 235 – 241.

Fuentes, E. N., Safian, D., Einarsdottir, I. E., Valdés, J. A., Elorza, A. A., Molina, A., Björnsson, B. T. (2013). Nutritional status modulates plasma leptin, AMPK and TOR activation, and mitochondrial biogenesis: implications for cell metabolism and growth in skeletal muscle of the fine flounder. *General and Comparative Endocrinology*, 186, 172 – 180.

Fujimoto, R. Y., Pereira, D. M., Silva, J. C. S., de Oliveira, L. C. A., Inoue, L. A. K. A., Hamoy, M., de Mello, V. J., Torres, M. F., Barbas, L. A. L. (2018). Clove oil induces anaesthesia and blunts muscle contraction power in three Amazon fish species. *Fish Physiology and Biochemistry*, *44*, 245 – 256.

Giampieri, F., Alvarez-Suarez, J. M., Cordero, M. D., Gasparrini, M., Forbes-Hernandez, T. Y., Afrin, S., Santos-Buelga, C., González-Paramás, A. M., Astolfi, P., Rubini, C., Zizzi, A., Tulipani, S., Quiles, J. L., Mezzetti, B., Battino, M. (2017). Strawberry consumption improves aging-associated impairments, mitochondrial biogenesis and functionality through the AMP-Activated Protein Kinase signaling cascade. *Food Chemistry*, *234*, 464 – 471.

Gomes, L. C. (2003). Protocolo para o transporte de tambaqui (*Colossoma macropomum*) vivo. Manaus: Embrapa Amazônia Ocidental. Documentos 27. 20p.

Gomes, L. C., Simões, L. N., & Araújo-Lima, C. A. R. M. (2010). Tambaqui (*Colossoma macropomum*). In: Baldisserotto, B., & Gomes, L.C. (Eds.), Espécies nativas para piscicultura no Brasil (2 ed.) (pp. 175 – 204). Santa Maria: Ed. UFSM.

González-Trujano, M. E., Martínez-González, C. L., Flores-Carrillo, M., Luna-Nophal, S. I., Contreras-Murillo, G., Magdaleno-Madrigal, V. M. (2018). Behavioral and electroencephalographic evaluation of the anticonvulsive activity of *Moringa oleifera* leaf non-polar extracts and one metabolite in PTZ-induced seizures. *Phytomedicine*, *39*, 1 – 9.

Graça, W. J., Pavanelli, C. S. (2007). Peixes da planície de inundação do alto Rio Paraná e áreas adjacentes. Maringá: EDUEM. 241p.

Guardiola, F. A., Bahi, A., Messina, C. M., Mahdhi, A., Santulli, A., Arena, R., Bakhrouf, A., Esteban, M. A. (2017). Quality and antioxidant response of gilthead seabream (*Sparus aurata* L.) to dietary supplements of fenugreek (*Trigonella foenum* graecum) alone or combined with probiotic strains. *Fish & Shellfish Immunology*, *63*, 277 – 284.

Guimarães, I. G., Martins, G. P. (2015). Nutritional requirement of two Amazonian aquacultured fish species, *Colossoma macropomum* (Cuvier, 1816) and *Piaractus brachypomus* (Cuvier, 1818): A mini review. *Journal of Applied Ichthyology*, *31*, 57 – 66.

Halliwell, B., & Gutteridge, J. M. C. (2015). Free radicals in biology and medicine. Oxford (5th ed). Oxford University Press. EUA.

D. G., Ross, F. A., Hawley, S. A. (2012). AMPK: A nutrient and energy sensor that maintains energy homeostasis. *Nature Reviews Molecular Cell Biology*, *13*, 251 – 262.

Haselgrübler, R., Lanzerstorfer, P., Röhrl, C., Stübl, F., Schurr, J., Schwarzinger, B., Brameshuber, M., Wieser, S., Winkler, S. M., Weghuber, J. (2019). Hypolipidemic effects of herbal extracts by reduction of adipocyte differentiation, intracellular neutral lipid content, lipolysis, fatty acid exchange and lipid droplet motility. *Scientific Reports*, *9*, 1 – 18.

He, J., Giusti, M. M. (2010). Anthocyanins: natural colorants with health-promoting properties. *Annual Review of Food Science and Technology*, *1*, 163 – 187.

Heinrich, M., Dhanji, T., Casselman, I. (2011). Açai (*Euterpe oleracea* Mart.) — A phytochemical and pharmacological assessment of the species' health claims. *Phytochemistry Letters*, *4*, 10 – 21.

Henderson, A. (2000). The genus Euterpe in Brazil. Sellowia, 52, 1 – 22.

Herzig, S., Shaw, R. J. (2018). AMPK: guardian of metabolism and mitochondrial homeostasis. *Nature Reviews Molecular Cell Biology*, *19*, 121 – 135.

Hoseinifar S. H., Khalili M., Rufchaei R., Raeisi M., Attar M., Cordero H., Esteban M. A. (2015). Effects of date palm fruit extracts on skin mucosal immunity, immune related genes expression and growth performance of common carp (*Cyprinus carpio*) fry. *Fish & Shellfish Immunology*, *47*, 706 – 711.

Hoseinifar, S. H., Zou, H. K., Miandare, H. K., Van Doan, H., Romano, N., Dadar, M. (2017a). Enrichment of common carp (*Cyprinus carpio*) diet with medlar (*Mespilus germanica*) leaf extract: Effects on skin mucosal immunity and growth performance. *Fish & shellfish Immunology*, *67*, 346 – 352.

Hoseinifar, S. H., Dadar, M., Khalili, M., Cerezuela, R., Esteban, M. A. (2017b). Effect of dietary supplementation of palm fruit extracts on the transcriptomes of growth, antioxidant enzyme and immune-related genes in common carp (*Cyprinus carpio*) fingerlings. *Aquaculture Research*, 48, 3684 – 3692.

IBGE (2017). Instituto Brasileiro de Geografia e Estatística. PPM - Produção Pecuária Municipal. Rio de Janeiro, Brasil.

IBGE. Instituto Brasileiro de Geografia e Estatística (2018). PEVS - Produção da Extração Vegetal e da Silvicultura. Acessado em: 21 de fevereiro de 2020. Disponível em: <https://sidra.ibge.gov.br/pesquisa/pevs/quadros/brasil/2018>

IBGE. Instituto Brasileiro de Geografia e Estatística (2018). Produção da aquicultura - Tambaqui. Acessado em: 18 de fevereiro de 2020. Disponível em: <https://sidra.ibge.gov.br/Tabela/ 3940#resultado>

Idowu, T. A., Sogbesan, O. A. (2017). Synthetic chemical application in aquaculture production. *International Journal Environmental & Agricultural Science*, *1*, 1 – 10.

Ip, A. Y., Chew, S. F. (2010). Ammonia production, excretion, toxicity, and defense in fish: a review. *Frontiers in Physiology*, *134*, 1 – 20.

Jensen, G. S., Wu, X., Patterson, K. M., Barnes, J., Carter, S. G., Scherwitz, L., Beaman, R., Endres, J. R., Schauss, A. G., Schauss, A. G. (2008). In vitro and in vivo antioxidant and anti-inflammatory capacities of an antioxidant-rich fruit and berry juice blend, placebo-controlled, crossover study. *Journal of Agricultural and Food Chemistry, 56*, 8326 – 8333.

Jones, D. P. (2006). Extracellular redox state: refining the definition of oxidative stress in aging. *Rejuvenation Research, 9*, 169 – 181.

Kato, H. C. A., Freitas, A. A. (2015). Panorama of the aquaculture expansion of aquaculture and the fish consumption in Brazil. *Embrapa Pesca e Aquicultura, 9*, 080 – 083.

Karami, M., Alimon, A.R., Sazili, A.Q., Goh, Y.M., Ivan, M. (2011). Effects of dietary antioxidants on the quality, fatty acid profile, and lipid oxidation of longissimus muscle in Kacang goat with aging time. *Meat Science, 88*, 102 – 108.

Kesbiç, O. S., Acar, Ü., Yilmaz, S., Aydin, Ö. D. (2019). Effects of bergamot (*Citrus bergamia*) peel oil-supplemented diets on growth performance, haematology and serum biochemical parameters of Nile tilapia (*Oreochromis niloticus*). *Fish Physiology and Biochemistry*, 1 – 8.

Kim, J. Y., Hong, J. H., Jung, H. K., Jeong, Y. S., Cho, K. H. (2012). Grape skin and loquat leaf extracts and acai puree have potent anti-atherosclerotic and anti-diabetic activity *in vitro* and *in vivo* in hypercholesterolemic zebrafish. *International Journal of Molecular Medicine, 30*, 606 – 614.

Kütter, M. T., Monserrat, J. M., Primel, E. G., Caldas, S. S., Tesser, M. B. (2012). Effects of dietary α-lipoic acid on growth, body composition and antioxidant status in the Plata pompano *Trachinotus marginatus* (Pisces, Carangidae). *Aquaculture, 368*, 29 – 35.

Leal, J. F., Santos, E. B., Esteves, V. I. (2019). Oxytetracycline in intensive aquaculture: water quality during and after its administration, environmental fate, toxicity and bacterial resistance. *Reviews in Aquaculture, 11*, 1176 – 1194.

Lima, A. F. (2013). Sistemas de produção de peixes. In: Piscicultura de água doce: Multiplicando conhecimentos. Rodrigues, A. P. O., ... [et al] (eds.). Brasília, DF: Embrapa. 97 – 108.

Lopes, D. B., de Queirós, L. D., de Ávila, A. R., Monteiro, N. E., Macedo, G. A. (2017). The importance of microbial and enzymatic bioconversions of isoflavones in bioactive compounds. In: *Food Bioconversion* (pp. 55 – 93). Academic Press.

Lushchak, V. I. (2011). Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology*, *101*, 13 – 30.

Mateus, A. P., Power, D. M., Canário, A. V. (2017). Stress and disease in fish. In: *Fish Diseases* (pp. 187-220). Academic Press. 187 – 220.

Mommsen, T. P., Vijayan, M. M., Moon, T. W. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Review in Fish Biology and Fisheries*, *9*, 211 – 268.

Morais, I. D. S., O'Sullivan, F. D. A. (2017). Biologia, habitat e cultivo do tambaqui *Colossoma macropomum* (CUVIER, 1816). Embrapa Amazônia Ocidental, *6*, 81 - 93.

Myre, P., Nunes, A. J., Suplicy, F. M., Vidal, E. A., Rombenso, A. N., Hayashi, L., Kanemoto, F. T., Matias, J. F. (2017). World food giant - Brazil aiming to be one of the top five aquaculture producers in 2020. *World Aquaculture*, *48*, 31 – 37.

Natale, F., Hofherr, J., Fiore, G., Virtanen, J. (2012). Interactions between aquaculture and fisheries. Marine Policy, *38*, 205e213.

Naylor, R. L. (2016). Oil crops, aquaculture, and the rising role of demand: A fresh perspective on food security. *Global Food Security*, *11*, 17 – 25.

Ngugi, C. C., Oyoo-Okoth, E., Muchiri, M. (2017). Effects of dietary levels of essential oil (EO) extract from bitter lemon (*Citrus limon*) fruit peels on growth, biochemical, haemato-immunological parameters and disease resistance in Juvenile *Labeo victorianus* fingerlings challenged with *Aeromonas hydrophila*. *Aquaculture Research*, *48*, 2253 – 2265.

Oliva-Teles, A. (2012). Nutrition and health of aquaculture fish. *Journal of Fish Diseases*, *35*, 83 – 108.

Oliveira, A. C. B., Miranda, E. C. M., Correa, R. Exigências nutricionais e alimentação do tambaqui. In: NutriAqua: Nutrição e alimentação de espécies de interesse para a

aquicultura brasileira. Fracalossi, D. M., Cyrino, J. E. P. (Eds). 1° Ed. Florianópolis: Sociedade Brasileira de Aquicultura e Biologia Aquática, 231 – 238.

Omitoyin, B. O., Ajani, E. K., Orisasona, O., Bassey, H. E., Kareem, K. O., Osho, F. E. (2019). Effect of guava *Psidium guajava* (L.) aqueous extract diet on growth performance, intestinal morphology, immune response and survival of *Oreochromis niloticus* challenged with *Aeromonas hydrophila*. *Aquaculture Research*, *50*, 1851 – 1861.

Paulino, R. R., Pereira, R. T., Fontes, T. V., Oliva-Teles, A., Peres, H., Carneiro, D. J., Rosa, P. V. (2018). Optimal dietary linoleic acid to linolenic acid ratio improved fatty acid profile of the juvenile tambaqui (*Colossoma macropomum*). *Aquaculture*, *488*, 9 – 16.

Peixoto, M. J., Magnoni, L., Gonçalves, J. F., Twijnstra, R. H., Kijjoa, A., Pereira, R., Palstra, A. P., Ozório, R. O. (2019). Effects of dietary supplementation of *Gracilaria* sp. extracts on fillet quality, oxidative stress, and immune responses in European seabass (*Dicentrarchus labrax*). *Journal of Applied Phycology*, *31*, 761 – 770.

Peng, S. M., Shi, Z. H., Fei, Y., Gao, Q. X., Sun P., Wang, J. G. (2013). Effect of high-dose vitamin C supplementation on growth, tissue ascorbic acid concentrations and physiological response to transportation stress in juvenile silver pomfret, *Pampus argenteus*. *Journal of Applied Ichthyology, 29*, 1337 – 1341.

Piasecka A., Jedrzejczak-Rey N., Bednarek, P. (2015). Secondary metabolites in plant innate immunity: conserved function of divergent chemicals. *New Phytologist*, *206*, 948 – 964.

Randall, D. J., Tsui, T. K. N. (2002). Ammonia toxicity in fish. *Marine Pollution Bulletin, 45*, 17–323.

Rabiei, Z. (2017). Anticonvulsant effects of medicinal plants with emphasis on mechanisms of action. *Asian Pacific Journal of Tropical Biomedicine*, *7*, 166 – 172.

Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., & Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. *Aquaculture, 433*, 50 – 61.

Ribeiro, S. C., Malheiros, D. F., Guilozki, I. C., Majolo, C., Chaves, F. C. M., Chagas, E. C., de Assis, H. C. S., Tavares-Dias, M., Yoshioka, E. T. O. (2018). Antioxidants effects and resistance against pathogens of *Colossoma macropomum* (Serassalmidae) fed *Mentha piperita* essential oil. *Aquaculture*, *490*, 29 – 34.

Ronquillo, M. G., Hernandez, J. C. A. (2017). Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods. *Food Control*, *72*, 255 – 267.

Rosas, V. T., Monserrat, J. M., Bessonart, M., Magnone, L., Romano, L. A., Tesser, M. B. (2019). Comparison of β-carotene and Spirulina (*Arthrospira platensis*) in mullet (*Mugil liza*) diets and effects on antioxidant performance and fillet colouration. *Journal of Applied Phycology*, *31*, 2391 – 2399.

Sá, M. V. C. (2012). Liminocultura: limnologia para aquicultura. Fortaleza: edições UFC. 218 p.

Saccol, E. M. H., Liesuy, S. F., Pavanato, M. A. (2017). Antioxidantes na aquicultura. In: Farmacologia aplicada à aquicultura. Baldisserotto, B., ... [et al]. Santa Maria, RS: ed. UFSM. 569 – 592.

Schauss, A. G. (2016). Advances in the study of the health benefits and mechanisms of action of the pulp and seed of the Amazonian palm fruit, *Euterpe oleracea* Mart., known as "Açai". In: Watson, R. R., & Preedy, V. R. (Eds.). Fruits, Vegetables, and Herbs (pp. 177 – 220). Oxford: Academic Press.

Safari, R., Hoseinifar, S. H., Dadar, M., Sattari, M., Rahbar, M. (2019). The effects of *Coriandrum sativum* L. as feed additive on mucosal immune parameters, antioxidant defence and, immune-related genes expression in zebrafish (*Danio rerio*). *Aquaculture Research*, *50*, 2621 – 2627.

Saint-Paul, U. (2017). Native fish species boosting Brazilian's aquaculture development. *Acta of Fisheries and Aquatic Resources*, *5*, 1 – 9.

Saleh, A. A., Ebeid, T. A., Abudabos, A. M. (2018). Effect of dietary phytogenics (herbal mixture) supplementation on growth performance, nutrient utilization, antioxidative properties, and immune response in broilers. *Environmental Science and Pollution Research*, *25*, 14606 – 14613.

Salbego, J., Simões, L. N., Baldisserotto, B. (2017). Aditivos no transporte de organismos aquáticos. In: Farmacologia aplicada à aquicultura. Baldisserotto, B., ... [et al]. Santa Maria, RS: ed. UFSM. 539 – 562.

Salomão, R. A. S., De Paula, T. G., Zanella, B. T. T., Carvalho, P. L. P. F., da Silva Duran, B. O., Valente, J. S., ... & Carvalho, R. F. (2019). The combination of resveratrol and exercise enhances muscle growth characteristics in pacu (*Piaractus mesopotamicus*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *235*, 46 – 55.

Sampaio, F. D., Freire, C. A. (2016). An overview of stress physiology of fish transport: changes in water quality as a function of transport duration. *Fish and Fisheries*, *17*, 1055 – 1072.

Sandre, L. C. G., Buzollo, H., Nascimento, T. M. T., Neira, L. M., Jomori, R. K., Carneiro, D. J. (2017). Productive performance and digestibility in the initial growth phase of tambaqui (*Colossoma macropomum*) fed diets with different carbohydrate and lipid levels. *Aquaculture Reports*, *6*, 28 – 34.

Santana-Gálvez, J., Jacobo-Velázquez, D. A. Phenolic compounds. In: Phenolic compounds in food: Characterization and analysis. Nollet, L. M., Gutierrez-Uribe, J. A. (Eds.). CRC Press. Taylor & Francis Group. 3 – 33.

Santos, G. M.; Ferreira, E. J. G.; Zuanon, J. A. S. (2006). Peixes comercias de Manaus. In: Geraldo (Ed.). – Manaus: Ibama/AM, ProVárzea. 144 p.

Schauss, A. G. (2011). Açai: An extraordinary antioxidant-rich palm fruit from the Amazon, third ed. Biosocial Publications, Tacoma, 33 – 40.

Schreck, C. B., Tort, L. (2016). The concept of stress in fish. In: *Fish physiology*. Academic Press, 35, 1 – 34.

Shahidi, F., Chandrasekara, A., & Zhong, Y. (2018). Bioactive Phytochemicals in Vegetables. In: Handbook of vegetables and vegetable processing. Siddiq, M., & Uebersax, M. (Eds.), 2nd ed. Hoboken, NJ: John Wiley & Sons. pp. 171.

Sies, H., Jones, D. P. (2007). *Encyclopaedia of Stress.* Fink, G. (ed.). Elsevier, San Diego, CA. 45 – 48.

Soares, E. R., Monteiro, E. B., Graziele, F., Inada, K. O., Torres, A. G., Perrone, D., Soulage, C. O, Monteiro, M . C., Resende, A. C., Moura-Nunes, N., Costa, C. A. (2017). Up-regulation of Nrf2-antioxidant signaling by Açaí (*Euterpe oleracea* Mart.) extract prevents oxidative stress in human endothelial cells. *Journal of Functional Foods, 37*, 107-115.

Sokolova, I. M., Frederich, M., Bagwe, R., Lannig, G., & Sukhotin, A. A. (2012). Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Marine Environmental Research*, *79*, 1 – 15.

Souza-Monteiro, J. R., Hamoy, M., Santana-Coelho, D., Arrifano, G. P., Paraense, R. S., Costa-Malaquias, A., Mendonça, J. R., da Silva, R. F., Monteiro, W. S. C., Rogez, H., de Oliveira, D. L., do Nascimento, J. L. M., de Oliveira, D. L. (2015). Anticonvulsant properties of *Euterpe oleracea* in mice. *Neurochemistry International*, *90*, 20 – 27.

Surai, P. F. (2014). Polyphenol compounds in the chicken/animal diet: from the past to the future. *Journal of Animal Physiology and Animal Nutrition*, *98*, 19 – 31.

Tacchi, L., Lowrey, L., Musharrafieh, R., Crossey, K., Larragoite, E. T., Salinas, I. (2015). Effects of transportation stress and addition of salt to transport water on the skin mucosal homeostasis of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *435*, 120 – 127.

Tavares-Dias, M., Martins, M. L. (2017). An overall estimation of losses caused by diseases in the Brazilian fish farms. *Journal of Parasitic Diseases*, *41*, 913 – 918.

Thomson, D. M. (2018). The role of AMPK in the regulation of skeletal muscle size, hypertrophy, and regeneration. *International Journal of Molecular Sciences*, *19*, 3125.

Upadhaya, S. D., Kim, I. H. (2017). Efficacy of phytogenic feed additive on performance, production and health status of monogastric animals–a review. *Annals of Animal Science*, *17*, 929 – 948.

Urbinati, E. C., Zanuzzo, F. S., Biller-Takahashi, J. D. (2014). Estresse e sistema immune em peixes. In: Biologia e fisiologia de peixes neotropicais de água doce. Baldisserotto, B., Cyrino, J. E. P., Urbinati, E. C. (Ed.). Jaboticabal: FUNEP; UNESP. 87 – 100.

Van Der Meer, M. B., Van Herwaarden, H., Verdegem, M. C. J. (1997). Effect of number of meals and frequency of feeding on voluntary feed intake of *Colossoma macropomum* (Cuvier). *Aquaculture Research*, *28*, 419 – 432.

Vanderzwalmen, M., Eaton, L., Mullen, C., Henriquez, F., Carey, P., Snellgrove, D., Sloman, K. A. (2019). The use of feed and water additives for live fish transport. *Reviews in Aquaculture*, *11*, 263 – 278.

Wendelaar-Bonga, S. E. (1977). The stress response in fish. *Physiological Reviews*. *77*, 591 – 625.

Vanegas-Espinoza, P. E., Pérez-Escalante, V., Aguirre-Guzman, G., Hoyos-Leyva, J. D., & Del Villar-Martínez, A. A. (2019). Microencapsulation of anthocyanins from roselle (*Hibiscus sabdariffa*) and its application on a pigment supplied diet to fantail goldfish (*Carassius auratus*). *Aquaculture International*, *27*, 1801 – 1811.

Yamaguchi, M. Y., Barreto, L. E. G. S., Igarashi, M. A. (2008). Estratégias para o desenvolvimento da aqüicultura no Brasil. UNOPAR Cient. Ciênc. Exatas Tecnol., Londrina, 7, 13 – 24.

Yonar, M. E., Yonar, S. M., İspir, Ü., Ural, M. Ş. (2019). Effects of curcumin on haematological values, immunity, antioxidant status and resistance of rainbow trout (*Oncorhynchus mykiss*) against *Aeromonas salmonicida* subsp. achromogenes. *Fish & Shellfish Immunology*, *89*, 83 – 90.

Zanuzzo, F. S., Sabioni, R. E., Montoya, L. N. F., Favero, G., Urbinati, E. C. (2017). *Aloe vera* enhances the innate immune response of pacu (*Piaractus mesopotamicus*) after transport stress and combined heat killed *Aeromonas hydrophila* infection. *Fish & Shellfish Immunology*, *65*, 198 – 205.

Zeppenfeld, C. C., Lopes, J. M., Baldisserotto, B. (2017). Promotores de crescimento na aquicultura. In: Farmacologia aplicada à aquicultura. Baldisserotto, B., ... [et al]. Santa Maria, RS: ed. UFSM. 435 – 455.

Zhang, C., Yang, L., Zhao, X., Chen, X., Wang, L., Geng, Z. (2018). Effect of dietary resveratrol supplementation on meat quality, muscle antioxidative capacity and mitochondrial biogenesis of broilers. *Journal of the Science of Food and Agriculture*, *98*, 1216 – 1221.

CAPÍTULO 1

Effects of dietary Amazonian açai (*Euterpe oleracea* Martius) fruit on growth performance, skin coloration and antioxidant status of *Colossoma macropomum*

Thamyres Vanessa N. da Silva[1*] , Camila F. dos Santos[2], Marcelo F. Torres[2], Luis André L. Barbas[2], Luís A. Sampaio[3], Pablo E. Verde[4], Marcelo B. Tesser[5], José M. Monserrat[1,6]

[1] Functional Biochemistry of Aquatic Organisms Laboratory (BiFOA), Center for Biotechnology and Diseases of Aquatic Organisms (CBD). Federal University of Rio Grande (FURG), Institute of Oceanography (IO), Rio Grande, RS, Brazil.

[2] Tropical Species Aquaculture Laboratory (LAET), Federal Institute of Pará (IFPA), Castanhal, PA, Brazil.

[3] Estuarine and Marine Fish Laboratory (LAPEM), Federal University of Rio Grande (FURG), Institute of Oceanography (IO), Rio Grande, RS, Brazil.

[4] Coordination Center for Clinical Trials, Heinrich-Heine-University, University Hospital Düsseldorf, Düsseldorf, Germany.

[5] Nutrition of Aquatic Organisms Laboratory (LANOA), CBD, Federal University of Rio Grande (FURG), Institute of Oceanography (IO), Rio Grande, RS, Brazil.

[6] Institute of Biological Sciences (ICB), Federal University of Rio Grande (FURG), Rio Grande, RS, Brazil.

* Corresponding author at:
Thamyres V. N. da Silva, Laboratory of Functional Biochemistry of Aquatic Organisms (BIFOA), Federal University of Rio Grande - FURG, Institute of Oceanography (IO), CP 96201-900, Rio Grande, RS, Brazil.
E-mail: thamyrestvns@gmail.com

Manuscrito submetido à revista *Aquaculture Nutrition*.
Formatado conforme as normas da revista.

Abstract

A 30-day feeding trial was carried to evaluate the effects of dietary lyophilized *Euterpe oleracea* (LEO) on growth performance, skin coloration, antioxidant status, and feed cost of tambaqui *Colossoma macropomum*. Six isoenergetic and isoproteic diets were formulated with 0.00, 0.63, 1.25, 2.50, 5.00, and 10.0% (w/w) of LEO, respectively. Growth performance and biochemical parameters in the intestine, liver, and muscle were assessed. The results showed that the zootechnical indexes were optimized in groups fed with diets 1.25% to 10.0% LEO ($p < 0.05$). Muscle showed no increase in flavonoid and polyphenols content, neither *in vitro* antioxidant capacity (DPPH). Fish fed with 5.00% to 10.0% LEO had an increase in cyan color ($p < 0.05$). Administration from 0.63% LEO increases by 39.57% the intestinal antioxidant capacity total (ACAP) ($p < 0.05$). An estimated inclusion of 5.47 % LEO minimizes intestinal TBARS levels ($p < 0.05$). Higher levels of LEO inclusion increase the feed cost of tambaqui, but inclusion up to 1.25% LEO is economically feasible. Therefore, the inclusion of up to 1.25% LEO in the diet is recommended because growth and feed utilization are satisfactory, with higher intestinal antioxidant capacity, and viable feed cost.

Key words: Amazonian fruit; feed additive; tambaqui; zootechnical indexes; antioxidant activity; economic efficiency.

1. INTRODUCTION

Inclusion of plants rich in phytochemical compounds in fish feed has demonstrated positive effects on colors, appetite, and growth (Awad & Awaad, 2017; Shaluei, Nematollahi, Farsani-Naderi, Rahimi, & Katadj, 2017; Amin et al., 2019; Vanegas-Espinoza, Pérez-Escalante, Aguirre-Guzman, Hoyos-Leyva, & Villar-Martínez, 2020), as well on the health status of these animals (Reverter, Bontemps, Lecchini, Banaigs, & Sasal, 2014; Abdel-Tawwab & Abbass, 2017; Bahi et al., 2017; Tan et al., 2017). Hence, in the last decade, many studies have focused on the development of functional feed for aquaculture purposes, attracting attention from aquatic feed industry (Chakraborty et al., 2013; Bulfon et al., 2017; Sutili, Gatlin III, Heinzmann, & Baldisserotto, 2018).

Besides, worldwide aquaculture is developing into new trends that intensify and diversify production (Chakraborty et al., 2013). Aquatic organisms reared in intensive systems are vulnerable to a series of biotic or abiotic stressors that can result in important economic losses (Bulfon, Volpatti, & Galeotti, 2015). Factors such as fluctuations in oxygen levels, temperature, pH, nitrogen compounds; or transportation, malnutrition, handling, and overcrowding can induce to stress and influence directly or indirectly the generation of reactive oxygen species - ROS (Souza et al., 2016; Birnie-Gauvin, Costantini, Cooke, & Willmore, 2017; da Silva, Barbas, Torres, Sampaio, & Monserrat, 2017; Maltez et al., 2017; Maltez et al., 2018).

Various biochemical processes generate ROS during normal cell metabolism. However, when in excess, they affect several biological processes leading to oxidative stress (Lushchak, 2014; Sies, 2015). Lipid peroxidation (LPO), oxidation of proteins and nucleic acids (DNA) are direct damages caused by ROS (Jones, 2006; Lushchak, 2011; Halliwell & Gutteridge, 2015). In fish, the increased LPO can affect cell membrane phospholipid structures (polyunsaturated - PUFA and highly unsaturated fatty acids -

HUFA), which affect the physiological parameters (Oliva-Teles, 2012) and reduce growth (Lu et al., 2016). To prevent and combat the oxidative stress, aerobic organisms developed the antioxidant system based on enzymatic and non-enzymatic defense. Superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and peroxidase (GPx) are the main enzymatic antioxidant systems (Lushchak, 2011; Halliwell & Gutteridge, 2015). These enzymes are the first defense line against the biomolecules oxidation. Therefore, the use of oxidative stress biomarkers such as the total antioxidant capacity and the LPO levels can reveal the state of equilibrium of the fish redox system (Díaz-Jaramillo et al., 2010).

The non-enzymatic system includes nutrients or micronutrients obtained from the diet, such as vitamins (e.g. A, C, and E), α-lipoic acid (LA), minerals (e.g. manganese, selenium, and zinc) and a variety of compounds such as flavonoids, phenolic acids, pigments and essential oils (Kütter, Monserrat, Santos, & Tesser, 2013; Hoseinifar, Dadar, Khalili, Cerezuela, & Esteban, 2017a; Sallam, Mansour, Srour, & Goda, 2017; Takahashi et al., 2017; Wu et al., 2017). For this reason, it is recommended the administration of antioxidants through the feed to increase stress resistance against pro-oxidant conditions and favor the growth of farmed animals (Chakraborty & Hancz, 2011; Chakraborty et al., 2013; Vanderzwalmen et al., 2019). Hoseinifar et al. (2017b) demonstrated that supplementation of date palm fruit (*Phoenix dactylifera* L. Arecaceae) in the diet increased the antioxidant activity in common carp (*Cyprinus carpio*) at earlier growth stages. LA and curcumin fed-supplemented improved the antioxidant system and decreased lipid peroxidation in the juvenile pompano (*Trachinotus marginatus*) and crucian carp (*Carassius auratus*), respectively (Kütter, Monserrat, Primel, Caldas, & Tesser, 2012; Jiang et al., 2016). *Ziziphus mauritiana* leaf powder in the diet improved growth and the feed utilization efficiency of Nile tilapia played positive effects on gastric and intestinal health (Amin et al., 2019).

Açai (*Euterpe oleracea*), a native fruit from the Amazon region, has a set of bioactive compounds, such as phenolic acids, flavonols (quercetin), anthocyanins (mainly cyanidin 3-glucoside and cyanidin 3-rutinoside), carotenoids (lutein, α-carotene, and β-carotene) and hydroxycinnamic acids (chlorogenic acid, caffeic acid, and ferulic acid) (Alqurashi, Commane, & Rowland, 2016), with high antioxidant potential. Because of these aspects, this fruit has been used in studies involving different *in vivo* models and has proved to be beneficial, showing neuroprotective activity, decreasing protein damage and increasing the stress resistance (Peixoto, Roxo, Krstin, Wang, & Wink, 2016). It is also known to be antihypertensive (da Costa et al., 2012; de Bem et al., 2014), reducing hydrogen peroxide (H_2O_2) - induced damage to DNA, presenting an antiproliferative and anticancer potential in the bladder urothelial tissue (Fragoso, Prado, Barbosa, Rocha, & Barbisan, 2012). Attenuation of total triacylglycerol content in the liver and hepatic steatosis were also reported (da Costa Guerra et al., 2015). Moreover, increased antioxidant capacity and inhibition of lipid peroxidation in blood samples were observed in humans (Jensen et al., 2008).

The high concentration of phenolic pigments and carotenoids in *E. oleracea* are responsible for their attractive red, purple and blue colors (Alqurashi, Commane, & Rowland, 2016). Fish are not able to synthesize pigments via *de novo*, and therefore, depend on dietary pigments to maintain its natural skin color (Amaya & Nickell, 2015). In aquaculture, supply natural pigments instead of synthetic pigments in the diet is a practical strategy to enhance the natural color of fish and to produce the sensory features desired by costumers (Amaya & Nickell, 2015; Yesilayer, Mutlu & Yidirim, 2020). From a commercial point of view, the skin natural pigmentation is a criterion for consumer acceptance or rejection, and determinant for the product final price (Amaya & Nickell, 2015). For these reasons, Vanegas-Espinoza et al. (2019) successfully tested the

anthocyanins microencapsulation from roselle (*Hibiscus sabdariffa*) on pigment intensification to *C. auratus*. Moreover, supplementation of nettle (*Urtica spp*.), marigold (*Tagetes erecta*), and alfalfa (*Medicago sativa*) extracts are effective on pigmentation on skin of electric yellow cichlids (*Labidochromis caeruleus*) (Yeşilayer, Mutlu & Yidirim, 2020).

Many fish species in the Amazon region eat fruits and seeds (Goulding, 1980; Claro-Jr, Ferreira, Zuanon, & Araújo-Lima, 2004), as in the case of *Colossoma macropomum* (Cuvier, 1818) (Guimarães & Martins, 2015), popularly known as tambaqui (Actinopterygii class and Characiformes order). This omnivorous fish, exhibits a natural distribution in the Amazon and Orinoco river basins (Gomes, Simões, & Araújo-Lima, 2010), being the second largest fish species (reaching up to 30 kg in the natural environment) after *Arapaima gigas* (Osteoglossidae) in the Amazonian region (Valladão, Gallani, & Pilarski, 2018). Tambaqui is one of the most important farmed fish in Latin America because of its growth potential, high productivity, hardiness, low production cost, acceptance of artificial foods, resistance to diseases, handling, and tolerance to low water quality. Additionality it has high acceptance by the consumers and can be traded as an ornamental fish (Campos-Baca & Köhler, 2005; Guimarães & Martins, 2015; Saint-Paul, 2017).

In the Brazil, the tambaqui production reached 102,554.4 tons in 2018 (IBGE, 2020). While the *C. macropomum* industry is expanding, many researchers and producers have increased their efforts to find technological solutions, aiming to promote faster growth, better-feed utilization, health and welfare of tambaqui, with practicable production costs (Paulino et al., 2018; Ribeiro et al., 2018; da Costa et al., 2019). To the best of our knowledge, no information is available in the literature on the effects of the inclusion of açai in diets for farmed fish. It was hypothesized that açai would enhance the zootechnical

indexes and skin color, as well as optimize antioxidant defense system, and reduce the oxidative damage. Therefore, the aim was to evaluate the effects of dietary lyophilized *E. oleracea* (LEO) on growth; total antioxidant capacity and levels of lipid peroxidation in the intestine, liver, and muscle; DPPH scavenging activity and flavonoids and polyphenols content in the muscle of *C. macropomum* juveniles, assessing the feed cost associated to the use of this Amazonian fruit.

2. MATERIAL AND METHODS

2.1. Experimental diets

Lyophilized açai (*E. oleracea*) was purchased from the Company Amazon Comércio de Açaí Liofilizado e Exportação LTDA, located in Belém, Pará, Brazil. The formula and centesimal composition of the experimental diets are shown in Table 1. Six diets isoenergetic (9% of lipid) and isoproteic (40% of crude protein) were formulated with different levels of inclusion of açai (w/w): 0 g of açai Kg^{-1} (Ctr), 6 g of açai Kg^{-1} (0.63%), 13 g of açai Kg^{-1} (1.25%), 25 g of açai Kg^{-1} (2.50%), 50 g of açai Kg^{-1} (5.00%), and 100 g of açai Kg^{-1} (10.0%). All ingredients were ground into a powder, mixed, and added with warm water to produce consistency and soft dough. The dough was pelleted using an automatic pellet-producing machine and dried for 24 hr in a drying oven at 65°C. After that, diets were crushed to a small diameter (1.0 – 2.0 mm), packed in double plastic bags and conserved at – 20°C until use.

2.2. Feeding trial and sampling

The experiments were conducted in the Laboratory of Aquaculture of Tropical Species (LAET) – IFPA (1°17'52.9"S 47°57'03.6"W). The tambaqui juveniles were obtained from a local hatchery (Pará, Brazil). Fish were acclimated in a semi-static water

system (200 L tanks) for ten days. During this period, juveniles were fed three times a day (09:00, 13:00, and 17:00 h) with the control diet (0.00% LEO) at 10% of body weight. Before the feeding trial, fish were fasted for 24 hr and then weighed (fresh fish) and measured (total length) (n = 540). Fish (900) with an initial body weight of 0.92 ± 0.01 g were randomly distributed into 18 200L - tanks at a density of 50 fish per tank. Fish were hand fed until apparent satiation four times daily (08:00, 11:30, 14:00, and 17:30 h, respectively) for 30 days. When apparent satiety was verified, the feed was interrupted and the leftover was reserved for later weighing in a precision analytical balance (± 0.0001 g, EEQ9003F-B, EDUTEC) to calculate daily feed intake. The amount of feed administered was adjusted every five days considering a feed conversion ratio of 1.2:1. All diets were tested with three repetitions. Treatments were carried on semi-static aerated water conditions with a daily water renewal rate of 50%. Water parameters were maintained at the temperature of 27.29 ± 0.11 °C; dissolved oxygen 7.37 ± 0.01 mg L^{-1}; pH 6.33 ± 0.11; conductivity 49.16 ± 0.84 μS cm^{-1}; and alkalinity 17.22 ± 0.58 mg $CaCO_3$ L^{-1}. The photoperiod was adjusted to 12 hr light:12 hr dark.

At the end of the rearing trial, the fish were fasted for 24 hr. Then, the fish (n = 30/tank) were anesthetized with eugenol at 50 ppm (Roubach, Gomes, Leão Fonseca, & Val, 2005), weighed, measured and counted to determine the following zootechnical indexes. Thirty fish per treatment were euthanized (500 ppm of eugenol via immersion). From these fish, were collected intestine, liver and muscle to determine oxidative stress parameters (n = 15 per treatment). Another fish were collected to determine the muscle bioactive constitution (n = 15 per treatment). Carcasses (n = 9 per treatment) were frozen at −20 °C and reserved for determination of the centesimal composition. Immediately organs were stored at – 80°C until biochemical analyses.

The zootechnical variables calculated were:

- Weight gain (WG) = final body weight (g) – initial body weight (g);
- Specific growth rate (SRG) (% day^{-1}) = [(ln Wtf – ln Wt_i) / (t)] x 100,

Where Wt_i and Wt_f correspond to the initial and final weights (g), respectively, within the considered period (t, in days).

- Feed conversion ratio (FCR) = dry weight of feed consumed (g) / weight gain of fish (g);
- Protein efficiency ratio (PER) = weight gain (g) / protein consumed (g);
- Condition factor (K) = 100 × body weight (g) / body length $(cm)^3$
- Survival (S %) = 100 x final number/initial fish number;
- Feed intake (FI) = [mean daily consumption (g) / mean fish biomass] x 100;

Where: mean fish mass = (fish biomass at t_2 + fish biomass at t_1) / 2; and fish biomass = mean body weight × fish number. Here t_1 and t_2 represent the initial (0) and final (30 days) times of the weight measurements, respectively.

2.3. Composition analysis

Contents of crude protein, crude lipid, moisture and ash in experimental diets (n = 3 per LEO level) and carcass were measured following the methodology of the Association of Official Analytical Chemists (AOAC, 1990).

2.4. DPPH assay, total flavonoids, and polyphenols content

The experimental diets (n = 5 per diet) and muscle (n = 15) were homogenized in methanol (0.5:3 w/v), at constant agitation for three hours and centrifuged at 2,500 × g for five minutes at 4 °C and the supernatants stored at -20 °C. For all analyses, only the supernatants of diets were previously diluted to 30% in methanol. The ability of diets and muscle to scavenge the DPPH radical was measured using the method of Brand-Williams,

Cuvelier & Berset (1995). Briefly, the homogenized (50 µL) were added to 200 µL of DPPH (2,2 – diphenyl – 1 – picrylhydrazyl) solution (0.08 mg mL^{-1} in methanol), and the absorbance was measured at 515 nm for 15 min (one reading per min) in a Synergy™ HT microplate reader (BioTek® Instruments, Inc). The results were in terms of antioxidant activity (%) by comparing the DPPH bleaching in the presence of the samples with the blank (no extracted samples added).

Total flavonoids content (TFC) was determined according to the method of Marques, Monteiro & Leão (2012). The diets or muscle extracts and quercetin standards (80 µL) were added in 55 µL methanol and 40 µL $AlCl_3$ (5% w/v). Then they were incubated in the dark for 30 min. Immediately, absorbance was measured at 415 nm in a Synergy™ HT microplate reader (BioTek® Instruments, Inc). The results were expressed as mg of quercetin per g of the sample. Total polyphenols content (TPC) was determined according to the method of Gajula et al. (2009). The diets or muscle extracts and quercetin standards (25 µL) were added in 625 µL Folin-Ciocalteu's phenol reagent (0.1 M) and after 5 minutes 500 µL Na_2SO_4 (7.5% w/v). Then they were incubated in the dark for 60 min. Thereafter, absorbance was measured at 740 nm in a microplate reader. The results were expressed as mg of polyphenols per g of the sample.

2.5. Fish color intensity

The quantification of the intensity of colors followed the method proposed by Rezende, Júnior, Andrade, Mendonça & Santos (2012) with adaptations, which from photographs of fish and support of Photoshop measures the desired colors. Photographs were done in a closed room. Two artificial illumination sources (60w fluorescent lamps) were installed at 80 cm above the surface where the fish were set for the photographs registers. A digital camera (Sony DSC-W530), with 14.1 megapixels resolution, positioned

at an angle of 60° and 50 cm from the fish to be photographed. The photos (jpg.) registered were submitted to color standardization, through the Adobe®Photoshop® software version 5, triggering the following commands: Menu [Image]–[Adjust]–[Automatic Levels] and Menu [Image]–[Mode]–[Colors CMYK]. Then, the command Menu [File]–[Save as] was used. All fish were photographed to obtain the colors indices from 5 random points in the dorsal region. The direct quotation provided indexes expressed in the percentile of the colors cyan and black. The percentile values were transformed in radians from the following formula:

$$Crad = archsin\sqrt{\frac{C\%}{100}}$$

Where: C% = percentile and *Crad* = value expressed in radians of the evaluated color.

2.6. Oxidative stress parameters

The intestine, liver and muscle (n = 15) were homogenized (1:5 w/v) in a Tris–HCl (100 mM, pH 7.75) buffer with EDTA (2 mM) and Mg^{2+} (5 mM) (Amado et al., 2011). Homogenates were centrifuged at 10,000 × g for 20 min at 4 °C and the supernatants stored at −80 °C. The total protein content was determined in triplicate by the Biuret method (Amado et al., 2011), using a commercial kit (Total Protein Kit Doles) and a microplate reader (BioTek® Instruments, Inc; wavelength: 550 nm).

Antioxidant capacity against peroxyl radicals (ACAP) was determined by quantifying ROS in the intestine, liver and muscle homogenates. The homogenates were previously diluted to 2.0 mg protein/mL using a homogenization buffer. Samples (10 µL) were added in six wells and 127.5 µL of reaction buffer (30 mM HEPES pH 7.2, 200 mM KCl, and 1 mM MgCl2). Three of the six wells per sample were treated with or without 2,2′- azobis-2-methylpropionamidine dihydrochloride (7.5 µL of ABAP at 20 µM) (produce peroxyl radicals via thermolysis). Fluorescence was generated by adding 10 µL of

2′,7′ dichlorofluorescein diacetate at 40 μM (H_2DCF-DA), being detected at wavelengths of 485 and 530 nm for excitation and emission, respectively, under 37 °C in a microplate reader (BioTek® Instruments, Inc). Thermal decomposition of ABAP (Sigma-Aldrich) and ROS production were monitored for 30 min (a reading every five minutes). Results are presented as a relative area (the difference between the area with and without ABAP divided by the area without ABAP). For interpretation purposes, a low relative area means high ACAP and vice-versa (Amado et al., 2009).

The lipid peroxidation followed the procedures of Oakes & Van Der Kraak (2003), which quantifies the concentration of thiobarbituric acid reactive substances (TBARS). This method promotes the reaction of the lipid peroxidation by-product (malondialdehyde - MDA) with thiobarbituric acid (TBA, from Baker) under high temperature and acidity, which generates a chromogen that can be quantified by fluorimetry. Homogenized extract of intestine (50 μL), liver (30 μL) or muscle (100 μL) were added to 20 μL butylated hydroxytoluene solution (BHT at 67 μM), 150 μL acetic acid (at 20% and pH 3.5), 150 μL TBA (0.8%), 50 μL Milli-Q water, and 20 μL sodium dodecyl sulfate (SDS at 8.1%). Samples were heated at 95 °C for 30 min. After being cooled for ten minutes, 100 μL Milli-Q water and 500 μL n-butanol were added to the samples. The final solution obtained was centrifuged (3000 ×g for ten minutes at 15 °C) to obtain the separation of the alcohol phase. The b-butanol phase (150 μL) was placed in a microplate reader to determine the fluorescence (excitation: 520 nm and emission: 580 nm). Results were expressed as the concentration of TBARS in nmol TMP/mg of protein, where TMP stands for tetramethoxypropane (TMP, Acros Organics) employed as a standard.

2.7. Economic evaluation

The experimental diets were economically assessed according to the method proposed by Khalil, Mansour, Goda, & Omar (2019). The cost of the feed was calculated in Brazilian currency Real (R$) needed to produce 1 kg of live weight of fish and 1 kg of protein. The formulas for the calculations are presented below:

- Feed cost kg^{-1} of live weight: WG = FCR × cost kg^{-1} feed
- Feed cost kg^{-1} protein production = Feed intake/protein gain x cost kg^{-1} feed

2.8. Water quality parameters

Dissolved oxygen (DO), temperature, pH and conductivity were measured daily using an oxygen meter (HANNA instruments®, HI 9828). Total nitrogen ammonia (TAN) and nitrite were determined every three days according to the methods presented by Unesco (1983) and Benderschneider & Robinson (1952), respectively. Total alkalinity was measured according to Eaton, Clesceri, Rice & Greenberg (2005) every three days.

2.9. Statistical analyses

Zootechnical and color intensity responses were evaluated by two different techniques. First, a dose-response to evaluate LEO inclusion was analyzed using a linear mixed-effects model. In this model, the different dose levels were defined as fixed effects and the different tanks employed at each dose as a random effect. The model was fitted using the restricted maximum likelihood method (REML). The main results are presented as estimated contrasts, which compare each dose level against the control. The second technique considered the fitting of zootechnical responses as function of LEO inclusion levels. Linear and non-linear models were statistically evaluated. Minimum of TBARS

values were estimated for muscle and liver fitting TBARS data to a second-order polynomial, where levels of LEO inclusion was the independent variable. Data of total antioxidant capacity were analyzed using the linear mixed-effect model described above. In all cases, descriptive statistics were performed by calculating the mean and the standard error (SE) for each tank and all data were evaluated for homogeneity and normality by Levene's and Shapiro-Wilk tests, respectively. The statistical significance level was defined as 5% (Searle, Casella, & McCulloch, 2008) in all cases. Statistical computations were performed using the statistical software R version 3.4.3. The mixed-effects model was calculated using the R's package lme4.

3. RESULTS

3.1. Water quality parameters

Most of the water parameters remained unchanged ($p > 0.05$) throughout the experimental period, except for the temperature. The temperature varied from 27 to 28 ° C, being higher in the control and 10.0% LEO treatments ($p < 0.05$). The mean values of pH and dissolved oxygen were 6.65 ± 0.0 and 5.08 ± 0.0 mg/L^{-1}, respectively. The mean values of the other parameters were: alkalinity 15.92 ± 0.10 mg/L^{-1} $CaCO_3$; conductivity 45.21 ± 0.05 $\mu S\ cm^{-1}$; nitrite 0.65 ± 0.02 mg/L^{-1}; and total nitrogen ammonia 1.89 ± 0.02 mg/L^{-1} (Table 2).

3.2. *In vivo* effects of *E. oleracea* administration

3.2.1. Growth performance

The results of growth performance are shown in Table 3. The administration of dietary LEO for 30 days resulted in a final weight seven times superior to the initial weight. Survival was 100% during the experimental period for all the treatments, except

for the control treatment (99.39%) ($p > 0.05$). All diets were well accepted by the fish (visual observation). The FBW was significantly increased ($p < 0.05$) by inclusion of 5.00% (8.00 ± 0.15 g) and 10.0% LEO (8.26 ± 0.53 g) in diets, compared to control group (6.93 ± 0.08 g). A significant positive linear model was found between dietary LEO level and FBW ($p < 0.05$). Similarly, WG showed the same response pattern as that of the FBW ($p < 0.05$) both in terms of mean comparison and in the fitted linear model. The fish fed from 1.25% LEO had the highest SGR (3.09 ± 0.08 % day^{-1}), being different from that from the control treatment (2.86 ± 0.02 % day^{-1}) ($p < 0.05$). There was a significant linear positive model between dietary LEO level and SGR of fish ($p < 0.05$). Concerning feed utilization, positive effects were observed in FCR, PER, and FI with the dietary inclusion from 1.25% to 10.0% LEO ($p < 0.05$). However, only FCR and FI showed a significant dose-response inhibition (67.0%) and a positive linear model, respectively, in respect to dietary LEO levels ($p < 0.05$). No significant linear and non-linear models were detected on dietary LEO level to PER ($p > 0.05$). There were no differences in the K among all feed groups ($p > 0.05$) and no significant linear and non-linear models were found for dietary LEO level to this variable ($p > 0.05$).

3.2.2. Composition analysis

Results on carcass centesimal composition are presented in Table 4. No alterations were observed in the content of moisture, crude lipid, crude protein, neither ash in the carcass among all the groups ($p > 0.05$).

3.2.3. DPPH assay, TFC, and TPC in the diets and muscle

Data of DPPH assay (AC %), TFC and TPC in the diets and muscle are shown in Table 5. The scavenging activity against DPPH (AC %) was significantly higher in the diet

10.0% LEO, without differing from the diet containing 5.00% LEO ($p > 0.05$). The TFC increased in the ration by the inclusion of LEO, especially in the diet containing 10.0% LEO. The TPC also was increased in the açai diets, with higher concentrations only in the diets with 5.00% and 10.0% LEO compared to the control diet ($p < 0.05$). However, the radical scavenging competence against DPPH in the muscle showed no significant differences among all the feed treatments ($p > 0.05$). TFC and TPC showed no increase in muscle regardless of the administered LEO levels.

3.2.4. Fish color intensity

Photographs of fish after a 30-days feeding trial with dietary LEO or control diet are presented in Figure 1. The values of fish coloring index are presented in Table 6. The effects of dietary LEO was significant only in the skin coloring index of cyan in the dorsal region ($p < 0.05$). A non-linear model explained 40.0% of the data variability for cyan color. No significant relationship was detected between dietary LEO levels and black color ($p > 0.05$).

3.2.5. Oxidative stress parameters

Responses of ACAP and TBARS are presented in Figures 2 and 3, respectively. Total antioxidant capacity in the intestine was shown to be 39.57% higher in fish fed with diets from 0.63% LEO inclusion compared to the control group ($p < 0.05$) (Fig. 2a). No statistical differences were found in the ACAP of the liver (Fig. 2b) and muscle (Fig. 2c) ($p > 0.05$). The MDA content in the tambaqui intestine fitted to a second-order polynomial model with LEO as independent variable and showed a strong correlation ($R^2 = 0.93$). With the fitted second-order polynomial it wat estimated that 5.47% LEO (54.7 g of açai Kg^{-1}) inclusion in the diet provides the minimum MDA levels in the intestine (Fig. 3a). In the

liver and muscle, no differences were found in the TBARS among the dietary treatments (Fig. 3b and c) ($p > 0.05$).

3.2.6. Economic evaluation

Data on economic evaluation of the experimental diets are presented in Table 7. The cost for the diets production depended on the average price (Brazilian Real currency) of each ingredient in the local market in 2017. Increased dietary inclusion of LEO elevated the final costs of the experimental diets. However, the feed cost kg^{-1} gain of the live weight of fish is feasible up to 1.25% LEO in the diet. The feed cost kg^{-1} protein gain of fish fed with up to 2.50% LEO in the diet showed no significant difference in respect to the control diet ($p > 0.05$).

4. DISCUSSION

Aquaculture is one of the food-producing sectors with a fundamental role in the humanity food security. For these reasons, the fish growth, health and welfare management is essential and must go beyond the use of antibiotics and chemotherapeutics (Abdel-Tawwab & Abbass, 2016). In this context, the use of based-plant feed additives should increase resistance to pro-oxidants conditions and promote growth. The present study demonstrates that dietary LEO played a positive effect on growth and feed utilization in the tambaqui. Although the feed intake has decreased, the optimization of the FCR and PER ratio should be some of the reasons for higher SGR in groups fed with LEO. Additionally, during the feeding trial, the water quality was maintained in acceptable ranges for the tambaqui (Gomes et al., 2013).

Although previous information on the effects of açai fruit added to the fish diet is scarce (Kim, Hong, Jung, Jeong, & Cho, 2012), improvement in growth performance could be explained by factors linked to nutritional aspects, including feed perception and

1985 acceptance. Several studies have reported the rich nutritional and functional composition of
1986 *E. oleracea*, which have vitamin A, flavonoids and polyphenols, as well as essential
1987 inorganic compounds (e.g. potassium, calcium, magnesium, phosphorus, sodium, and
1988 manganese, amongothers) (Schauss et al., 2006; Menezes, Torres, & Srur, 2008; Alqurashi
1989 et al., 2016). Açai is also rich in fatty acids (e.g. MUFA and PUFA) and its lipid fraction
1990 (49%) is considered equivalent to that of olive oil, mainly composed of oleic
1991 (~56.2%), palmitic (~24.1%) and linoleic (~12.5%) acids (Del Pozo-Insfran, Brenes, &
1992 Talcott, 2004; Heinrich, Dhanji, & Casselman, 2011; Alqurashi et al., 2016).

1993 The inclusion of 5.00% açai has higher incorporation of flavonoids and polyphenols
1994 in the diet, with the double of such compounds at 10.0% LEO, as well as higher radical
1995 scavenging competence. Phytochemicals and their metabolic products may play a
1996 prebiotic-like role, since they promote selective growth factors, assist fermentation
1997 substrates for beneficial gastrointestinal bacteria, and selectively inhibits deleterious
1998 intestinal bacteria (Zheng et al. 2009; Harikrishnan, Balasundaram, & Heo, 2011; Halliwell
1999 & Gutteridge, 2015). Alqurashi et al. (2017), simulating an *in vitro* gastrointestinal
2000 digestion of açai pulp (*E. oleracea*) found that this fruit can selectively inhibit harmful
2001 bacterial growth in the intestinal microflora, characterizing it as potentially prebiotic. Thus,
2002 it is possible that açai optimized the digestibility and availability of nutrients, allowing an
2003 efficient feed conversion, greater protein synthesis and higher growth (Citarasu, 2010;
2004 Reverter et al., 2014).

2005 Besides, the presence of nutrient detection mechanisms have been recently
2006 supported in some fish species, mainly in rainbow trout (*Oncorhynchus mykiss*). Feed
2007 intake regulation and fish metabolism are related to the aptitude to detect glucose and fatty
2008 acids levels. Hypothalamus is a signaling integratory center in a way that the detection of
2009 increased levels of nutrients results in the feed intake inhibition through modifications in

2010 the expression of anorexigenic and orexigenic neuropeptides (Conde-Sieira & Soengas,
2011 2017). In this context, presumable participation of nutrient-sensing mechanisms occurred
2012 for the feed intake regulation in the tambaqui fed with dietary LEO levels.

2013 The positive effects of the dietary LEO on feed utilization and growth needs further
2014 studies. However, a variety of phytochemicals common in plants and herbs, acting
2015 individually or together can trigger several beneficial effects (Hoseinifar et al., 2017a).
2016 There are kinases such as AMPK (AMP-activated protein kinase) that are known to control
2017 catabolic processes and mitochondrial biogenesis. Recent evidence indicates that AMPK
2018 regulation in fish behaves similarly to that already described for mammals (Bremer,
2019 Kocha, Snider, & Moyes et al., 2016). Dietary polyphenols can activate the AMPK
2020 signaling pathway, triggering cellular response against ROS-induced oxidative stress
2021 damage and the energy homeostasis regulation in skeletal muscle (Fuentes et al. 2013;
2022 Giampieri et al., 2017). For example, fruits with antioxidant properties (e.g. strawberry)
2023 may induce AMPK activity with positive effects on bioenergetics status (Giampieri et al.,
2024 2017). Resveratrol, a polyphenol found mainly in grape seeds, has induced muscle
2025 hypertrophy and higher body weight in pacu (*Piaractus mesopotamicus*) submitted or not
2026 to exercise (Salomão et al., 2019).

2027 LEO inclusion levels seem associated with the growth-promoting effect in the
2028 tambaqui. In line with the present study, the dietary administration of 200 mL kg^{-1} of palm
2029 fruit (*P. dactylifera L*); and 5 g kg^{-1} of ethanolic ginger (*Zingiber officinale*) extract
2030 resulted in an important effect on growth and feed utilization by common carp (*C. carpio*)
2031 and rainbow trout juveniles (*O. mykiss*), respectively (Hoseinifar et al., 2015; Shaluei et al.,
2032 2017). The same positive effect was reported on *C. carpio* fed with 0.25% medlar
2033 (*Mespilus germanica*) leaf extract via diet (Hoseinifar et al., 2017a). In tilapia (*O.*
2034 *niloticus*), the inclusion of 20 g kg^{-1} of *Z. mauritiana* leave powder in the diet optimized

growth rate and FCR (Amin et al., 2019). In this latest study, 20 g kg^{-1} of *Z. mauritiana* increases the total protein content, whereas reducing total lipids in tilapia. Dietary LEO did not significantly change the tambaqui carcass composition among all the treatments, with an average of 6.8% crude lipid content, classified as moderately fat (Jobling, 2001).

In the early stages of life, tambaqui presents a predominantly silver color, and gradually acquire a dark green color in the dorsal region and dark tones in the ventral region on adult lifetime. In fish, the cells chromatophores are responsible for the characteristic color through the store or synthesis of specific pigments (Goda & Fujii, 1995). However, the literature on skin pigmentation of *C. macropomum* is null. Kottler et al. (2014) postulate that melanophores contribute to ultrastructure (pigment cell distribution) of the blue color of the guppy (*Poecilia reticulata*), modulating a reflection of a layer of iridophores located parallel above them. Thus, it is speculated that dietary LEO increased the pigments in the melanophores and subjacent chromatophores, resulting in the increase of the cyan-silver color, by the influence of the pigment cell organization as proposed by Kottler et al. (2014).

Ingestion of phenolic compounds may result in direct or indirect antioxidant responses (Jensen et al., 2008; Mertens-Talcott et al., 2008). In vivo, direct effect occurs in the gastrointestinal tract, and unabsorbed phenols ingress the colon, where the gut flora intensively metabolized polyphenols to monophenols and other metabolites, being that some may enter the circulation. Indirectly, the response is involved in the activation of nuclear factor erythroid-2-related factor 2 (Nrf2), inducing the expression of antioxidant enzyme genes (for example, SOD, GSH-Px or GST) in the organs (Halliwell & Gutteridge, 2015; Soares et al. al., 2017). In the current study, ACAP in the intestine was 39.57% higher on tambaqui that received at least 0.63% inclusion of LEO (6 g of açai Kg^{-1}) in the diet. On the other hand, the liver and muscle of *C. macropomum* did not exhibit differences

in antioxidant competence compared to control treatment. This indicates that nutrients and phytochemicals are bioaccessible for direct absorption by the intestinal epithelium, allowing the most powerful LEO antioxidant activity in this organ (Halliwell, Rafter, & Jenner, 2005) than in liver or muscle.

Studies report that the molecular chemical structure of flavonoids substantially affect their bioavailability and, in some cases, bioactivity (Heim, Tagliaferro, & Bobilya, 2002; Manach, Williamson, Morand, Scalbert, & Rémésy, 2005; Gonzales et al., 2015). Flavonoids glycosides are better absorbed than flavonoids aglycones (Gonzales et al., 2015). Manach et al. (2005), Georgiades, Pudney, Rogers, Thornton, & Waigh (2014), and Gonzales et al. (2015) perform an excellent review and discussion on the bioavailability and intestinal absorption of flavonoids. The fact is that *E. oleracea* has a high amount of anthocyanins, having also flavonols such as quercetin (39.02 ± 0.58 mg/100 g) and phenolic acid such as gallic acid (701.60 ± 4.48 mg/100 g) (Alqurashi et al., 2016), being the latter two ones among the most bioaccessible antioxidant compounds. Schulz et al. (2017) observed a reduction of 64% and 78% in the bioaccessibility of phenolic compounds and in the DPPH scavenging, respectively after *in vitro* gastrointestinal digestion of juçara açai (*Euterpe edulis*). These results were associated with alterations in phenolic structures; metabolization to phenolic derivatives; phenols interaction with other components of the diet (e.g. iron, fibers, and proteins); pH changes; and others.

There is a single biomedical study available which reported a higher antioxidant capacity, lower hepatic inflammation and reduced LPO in the liver of hypercholesterolemic zebrafish (*Danio rerio*) fed with a commercial diet supplemented with an extract of LEO (final concentration of 10% w/w of powder/Tetrabit) (Kim et al., 2012). In the tambaqui intestine, TBARS levels were minimum in fish fed with 5.47% LEO inclusion (54.7 g of açai Kg^{-1}) in the diet, according to the fitted second-order

polynomial function. Certainly, this result is associated with increased antioxidant competence provided by direct protection of phenolic compounds from the diet on the gastrointestinal tract, scavenging ROS and chlorine-derived species (Halliwell et al., 2005). These results are in line with previous studies. Jiang et al., (2016) found that crucian carp (*C. auratus*) fed with curcumin (1 g kg^{-1} and 5 g kg^{-1}) has a reduction in intestinal MDA content. Gao et al. (2012) reported that Japanese sea bass decreased LPO in the intestine with increasing supplementation of dietary palm oil. In tambaqui muscle and liver, LEO did not change TBARS levels. In fact, previous studies showed that these organs did not show a response on TBARS levels after antioxidant supplements, as reported for LA administration in the *T. marginatus* (Kütter et al., 2013), and for dietary administration of essential oil of *M. piperita* in the tambaqui (Ribeiro et al., 2018).

From a perspective of preserving the homeostasis energy, the use of LEO in aquafeeds is especially justified by exerting antioxidant activity and stimulating the production of endogenous antioxidants (Schauss, 2016). The energy assimilated by the organism is intended for maintenance and primarily for growth, storage or reproduction. However, the energy allocation to cope with recurrent stresses in aquaculture may compromise the maintenance of these vital processes. In order, to tolerate and survive stress conditions, organisms perform compensatory mechanisms or, in extreme cases, conservation mechanisms. This latter long-term mechanism is considered energetically expensive and involves the expression/activation of proteins, such as antioxidant enzymes and DNA repair proteins (Sokolova, Frederich, Bagwe, Lannig, & Sukhotin, 2012). From this perspective, the use of LEO as a feed additive emerges as a strategy in growth-promoting and presumably as a stress-conditioning nutrition strategy for tambaqui fish.

The feeding corresponds to 50 – 80% of the total costs in the intensive aquaculture. The economic evaluation depends on the cost of the consumed feed, as well as the use of

diets efficiency by the fish (Khalil et al., 2019). The results herein demonstrate that the experimental inclusion of up to 1.25% LEO (13 g of açai Kg^{-1}) in the diet is economically feasible, providing fish production efficiency from a natural product. Moreover, there is a growing demand for products based on natural compounds, likely in part motivated by increasing consumers' awareness (either of food fish or pet fish) to purchase products free of synthetic chemicals (Vanderzwalmen et al., 2019). Thus, LEO represents an opportunity in the aquaculture industry. In the Brazil, for example, it is estimated that 123,065.3 tons of feed are used to achieve the annual production of tambaqui (base FCR of 1.2: 1). It is estimated that 1,538.3 tons of LEO would be necessary considering the percentage of 1.25% inclusion of this fruit. This corresponds to approximately 16,541.5 tons of fresh açai fruit to produce the annual amount of feed. In this country, the state of Pará is the largest producer mainly from extraction on native forests (98.3% of national production), making up around 1.278 million tons of this fruit in 2018 (IBGE-PEVS, 2020).

In conclusion, LEO inclusion levels had benefited most of the measured variables, confirming its potential as a growth promotor and effective natural pigment, reducing also the intestinal oxidative stress. Moreover, LEO also optimizes the FCR and, therefore, this implies lower feed intake to produce the same biomass. Noteworthy, therefore, the possibility to decrease the environmental impacts and ameliorate the support capacity of the tambaqui production system. Based on the results, the use of 1.25% LEO as a feed additive is suggested for tambaqui juveniles when the purpose is to provide a functional diet as well as promote growth with feasible feed cost. Further studies are encouraged to understand the determinants factors for the LEO bioavailability and bioactive in other organs, its effects on fish metabolism, and their effectiveness in the conditioning to stress resistance in farmed fish.

ACKNOWLEDGEMENTS

da Silva T.V.N. acknowledges Brazilian CAPES for the graduate scholarship (2016-2020) provided. The authors would like to thank de Mendonça, A.J.C., Rosas, V.T., Ramos, P.B. (# 150718/2017-9), Simião, C., Colombo, G.M., Schmitz, M.J., Ramírez, J.R.B., and Lopes, J.M., for their assistance with the biochemical analyses and/or technical support. We are thankful to the staff of the Tropical Species Aquaculture Laboratory -LAET/IFPA Castanhal for the support provided. Monserrat J. M. (# 308539/2016-8), Sampaio L. A. (# 307445/2016-0) and Tesser, M. B. (# 304119/2017-2) are research fellows of the Brazilian CNPq. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

CONFLICTS OF INTEREST

The authors have no actual or potential conflicts of interest.

ETHICS STATEMENT

This study followed the ethical standards established by the 'Conselho Nacional de Controle de Experimentação Animal' – CONCEA (Brasília, Brazil). The ethical and legal approval was obtained previously to the start of experiments by the Ethics Committee on Experimental Animals of the Instituto Federal do Pará – IFPA (Protocol number CEUA nº 3095220419).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.11897928.v1, reference number 25.02.2020.

AUTHOR'S CONTRIBUTIONS

T.V.N.S, L.A.L.B, L.A.S, and J.M.M. designed the study, with intellectual contribution from all authors. C.F.S and M.F.T contributed with the maintenance of the fish, analyses and technical support. P.E.V and J.M.M carried out the statistical analyses and the application of the linear mixed effects model. M.B.T contributed with the experimental diet formulations as well as technical support. T.V.N.S and J.M.M. drafted the manuscript and all authors contributed equally to the writing of the final version of the manuscript.

REFERENCES

Abdel-Tawwab, M. (2017). Turmeric powder, *Curcuma longa* L., in common carp, *Cyprinus carpio* L., diets: growth performance, innate immunity, and challenge against pathogenic *Aeromonas hydrophila* infection. *Journal of the World Aquaculture Society*, 48, 303 – 312. https://doi.org/10.1111/jwas.12349

Amado, L. L., Ramos, P. B., Freitas, R. F., Zafalon, B., Ferreira, J. L., Yunes, J. S., & Monserrat, J. M. (2009). A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: application to evaluate microcystins toxicity. *Science of the Total Environment*, 407, 2115 – 2123. https://doi.org/10.1016/j.scitotenv.2008.11.038

Amado, L. L., Garcia, M. L., Pereira, T. C., Yunes, J. S., Bogo, M. B., & Monserrat, J. M. (2011). Chemoprotection of lipoic acid against microcystin-induced toxicosis in common carp (*Cyprinus carpio*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 154, 146 – 153. https://doi.org/10.1016/j.cbpc.2011.04.007

Amaya, E., & Nickell, D. (2015). Using feed to enhance the color quality of fish and crustaceans. In: *Feed and feeding practices in aquaculture* (pp. 269 – 298). Woodhead Publishing. https://doi.org/10.1016/B978-0-08-100506-4.00011-8

Alqurashi, R. M., Commane, D. M., & Rowland, I. R. (2016). Açai fruit as a source of bioactive phytochemicals. *Journal of Life Sciences*, 10, 391 – 404. https://doi.org/10.17265/1934-7391/2016.08.005

Alqurashi, R. M., Alarifi, S. N., Walton, G. E., Costabile, A. F., Rowland, I. R., & Commane, D. M. (2017). *In vitro* approaches to assess the effects of açai (*Euterpe oleracea*) digestion on polyphenol availability and the subsequent impact on the

faecal microbiota. *Food Chemistry*, 234, 190 – 198. https://doi.org/10.1016/j.foodchem.2017.04.164.

Amin, A., El Aselyb, A., El-Nabyc, A. S. A., Samirc, F., El-Ashramd, A., Sudhakarane, R., & Dawoodf, M. A. O. (2019). Growth performance, intestinal histomorphology and growth-related gene expression in response to dietary *Ziziphus mauritiana* in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *512*, 734301. https://doi.org/10.1016/j.aquaculture.2019.734301

AOAC (Association of Official Analytical Chemists) (1990). The official methods of analysis of the association of official analytical chemists (15th ed.). Arlington, Virginia.

Awad, E., & Awaad, A. (2017). Role of medicinal plants on growth performance and immune status in fish. *Fish & Shellfish Immunology*, 67, 40 – 54. https://doi.org/10.1016/j.fsi.2017.05.034

Bahi, A., Guardiola, F. A., Messina, C., Mahdhi, A., Cerezuela, R., Santulli, A., ... & Esteban, M. A. (2017). Effects of dietary administration of fenugreek seeds, alone or in combination with probiotics, on growth performance parameters, humoral immune response and gene expression of gilthead seabream (*Sparus aurata* L.). *Fish & Shellfish Immunology*, 60, 50 – 58. https://doi.org/10.1016/j.fsi.2016.11.039

Benderschneider, K., & Robinson, R. J. (1952). A new spectrophotometric method for determination of nitrate in seawater. *Journal of Marine Research*, 1, 69 – 87.

Birnie-Gauvin, K, Costantini, D., Cooke, S. J, & Willmore, W. G. (2017). A comparative and evolutionary approach to oxidative stress in fish: A review. *Fish and Fisheries*, 18, 928 – 942. https://doi.org/10.1111/faf.12215

Brand-Williams, C., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28, 25 – 30. https://doi.org/10.1590/1983-084X/12_165

Bremer, K., Kocha, K. M., Snider, T., & Moyes, C. D. (2016). Sensing and responding to energetic stress: the role of the AMPK-PGC1α-NRF1 axis in control of mitochondrial biogenesis in fish. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *199*, 4 – 12. http://dx.doi.org/10.1016/j.cbpb.2015.09.005

Bulfon, C., Volpatti, D., & Galeotti, M. (2015). Current research on the use of plant-derived products in farmed fish. *Aquaculture Research*, 46, 513 – 551. https://doi.org/10.1111/are.12238

Bulfon, C., Bongiorno, T., Messina, M., Volpatti, D., Tibaldi, E., & Tulli, F. (2017). Effects of *Panax ginseng* extract in practical diets for rainbow trout (*Oncorhynchus mykiss*) on growth performance, immune response and resistance to *Yersinia ruckeri*. *Aquaculture Research*, 48, 2369 – 2379. https://doi.org/10.1111/are.13072

Campos-Baca, L., & Köhler, C. C. (2005). Aquaculture of *Colossoma macropomum* and related species in Latin America. *American Fish Society Symposium Series*, 46, 541 – 561.

Citarasu, T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. *Aquaculture International*, 18, 403 – 414. https://doi.org/10.1007/s10499-009-9253-7

Claro-Jr, L., Ferreira, E., Zuanon, J., & Araújo-Lima, C. (2004). O efeito da floresta alagada na alimentação de três espécies de peixes onívoros em lagos de várzea da Amazônia Central, Brasil. *Acta Amazônica*, 34, 133 – 137. https://*doi*.org/10.1590/S0044-59672004000100018

Chakraborty, S. B., & Hancz C. (2011). Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. *Reviews in Aquaculture*, 3, 103 – 119. https://*doi*.org/10.1111/j.1753-5131.2011.01048.x

Chakraborty, S. B., Horn, P., & Hancz, C. (2013). Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. *Reviews in Aquaculture*, 5: 1 – 19. https://*doi*.org/10.1111/raq.12021

Conde-Sieira, M., & Soengas, J. L. (2017). Nutrient sensing systems in fish: impact on food intake regulation and energy homeostasis. *Frontiers in Neuroscience*, 10, 1 – 21. https://*doi*.org/10.3389/fnins.2016.00603

da Costa, C. A., de Oliveira, P. R. B., de Bem, G. F., de Cavalho, L. C. R. M., Ognibene, D. T., da Silva, A. F. E., ... , & Resende, A. C. (2012). *Euterpe oleracea* Mart.-derived polyphenols prevent endothelial dysfunction and vascular structural changes in renovascular hypertensive rats: role of oxidative stress. *Naunyn-Schmiedeberg's Archives of Pharmacology*, 385, 1199 – 1209. https://*doi*.org/10.1007/s00210-012-0798-z

da Costa, O. T. F., Dias, L. C., Malmann, C. S. Y., de Lima Ferreira, C. A., do Carmo, I. B., Wischneski, A. G., ... , & Dos-Santos, M. C. (2019). The effects of stocking density on the hematology, plasma protein profile and immunoglobulin production of juvenile tambaqui (*Colossoma macropomum*) farmed in Brazil. *Aquaculture*, 499, 260 – 268. https://doi.org/10.1016/j.aquaculture.2018.09.040

da Costa Guerra, J. F., Maciel, P. S., e Abreu, I. C. M. E., Pereira, R. R., Silva, M., de Morais Cardoso, L., ... , & Pedrosa, M. L. (2015). Dietary açai attenuates hepatic steatosis via adiponectin-mediated effects on lipid metabolism in high-fat diet

mice. *Journal of Functional Foods*, 14, 192 – 202. https://doi.org/10.1016/j.jff.2015.01.025

da Silva, T. V. N., Barbas, L. A. L., Torres, M. F., Sampaio, L. A., & Monserrat, J. M. (2017). Lipid peroxidation and antioxidant capacity in *Peckoltia oligospila* (Günther, 1864) submitted to transport under different concentration of dissolved oxygen. *Aquaculture*, 481, 72 – 78. https://doi.org/10.1016/j.aquaculture.2017.08.024

de Bem, G. F., Costa, C. A., Oliveira, P. R. B., Cordeiro, V. S. C., Santos, I. B., Carvalho, L. C. R. M., ... , & de Moura, R. S. (2014). Protective effect of *Euterpe oleracea* Mart (acai) extract on programmed changes in the adult rat offspring caused by maternal protein restriction during pregnancy. *Journal of Pharmacy and Pharmacology*, 66, 1328 – 1338. https://doi.org/10.1111/jphp.12258

Del Pozo-Insfran, D., Brenes, C. H., & Talcott, S. T. (2004). Phytochemical composition and pigment stability of açai (*Euterpe oleracea* Mart.). *Journal of Agricultural and Food Chemistry*, 52, 1539 – 1545. https://doi.org/10.1021/jf035189n

Díaz-Jaramillo, M., Ferreira, J. L., Amado, L. L., Ventura-Lima, J., Martins, A., Retamal, ..., & Monserrat, J. M. (2010). Biomonitoring of antioxidant and oxidative stress responses in *Perinereis gualpensis* (Polychaeta: Nereididae) in Chilean estuarine regions under different anthropogenic pressure. *Ecotoxicology and Environmental Safety*, *73*, 515 – 523. https://doi.org/10.1016/j.ecoenv.2009.12.004

Eaton, A. D., Clesceri, L. S., Rice, E. W., & Greenberg, A. E. (2005). Standard methods for the examination of water and wastewater, (21st ed). American Public Health Association, Springfield.

Fragoso, M. F., Prado, M. G., Barbosa, L., Rocha, N. S., & Barbisan, L. F. (2012). Inhibition of mouse urinary bladder carcinogenesis by açai fruit (*Euterpe oleraceae* Martius) intake. *Plant Foods for Human Nutrition*, 67, 235 – 241. https://doi.org/10.1007/s11130-012-0308-y

Fuentes, E. N., Safian, D., Einarsdottir, I. E., Valdés, J. A., Elorza, A. A., Molina, A., & Björnsson, B. T. (2013). Nutritional status modulates plasma leptin, AMPK and TOR activation, and mitochondrial biogenesis: implications for cell metabolism and growth in skeletal muscle of the fine flounder. *General and Comparative Endocrinology*, 186, 172 – 180. https://doi.org/10.1016/j.ygcen.2013.02.009

Gajula, D., Verghese, M., Boateng, J., Walker, L. T., Shackelford, L., Mentreddy, S. R., & Cedric, S. (2009). Determination of total phenolics, flavonoids and antioxidant and chemopreventive potential of Basil (*Ocimum basilicum* L. and *Ocimum tenuiflorum* L.). *International Journal of Cancer Research*, 5, 130 – 143. https://doi.org/10.3923/ijcr.2009.130.143

Gao, J., Koshio, S., Ishikawa, M., Yokoyama, S., Ren, T., Komilus, C.F., & Han, Y. (2012). Effects of dietary palm oil supplements with oxidized and non-oxidized fish oil on growth performances and fatty acid compositions of juvenile japanese sea bass, *Lateolabrax japonicus*. *Aquaculture*, 324, 97 – 103. https://doi.org/10.1016/j.aquaculture.2011.10.031

Georgiades, P., Pudney, P. D., Rogers, S., Thornton, D. J., & Waigh, T. A. (2014). Tea derived galloylated polyphenols cross-link purified gastrointestinal mucins. *PLoS One*, 9, e105302. https://doi.org/10.1371/journal.pone.0105302

Giampieri, F., Alvarez-Suarez, J. M., Cordero, M. D., Gasparrini, M., Forbes-Hernandez, T. Y., Afrin, S., ..., & Battino, M. (2017). Strawberry consumption improves aging-associated impairments, mitochondrial biogenesis and functionality through the

AMP-Activated protein kinase signaling cascade. *Food Chemistry*, 234, 464 – 471. https://doi.org/10.1016/j.foodchem.2017.05.017

Goda, M., & Fujii, R. (1995). Blue chromatophores in two species of callionymid fish. *Zoological science*, *12*, 811 – 813. https://doi.org/10.2108/zsj.12.811

Gomes, L. C., Simões, L. N., & Araújo-Lima, C. A. R. M. (2013). Tambaqui (*Colossoma macropomum*). In: Baldisserotto, B., & Gomes, L.C. (Eds.), Espécies nativas para piscicultura no Brasil (2 ed.) (pp. 175 – 204). Santa Maria: Ed. UFSM.

Gonzales, G. B., Smagghe, G., Grootaert, C., Zotti, M., Raes, K., & Camp, J. V. (2015). Flavonoid interactions during digestion, absorption, distribution and metabolism: a sequential structure–activity/property relationship-based approach in the study of bioavailability and bioactivity. *Drug Metabolism Reviews*, 47, 175 – 190. https://doi.org/10.3109/03602532.2014.1003649

Goulding, M. (1980). The fishes and the forest: Explorations in Amazonian natural history. University of California Press (pp. 280). London.

Guimarães, I. G., & Martins, G. P. (2015). Nutritional requirement of two Amazonian aquacultured fish species, *Colossoma macropomum* (Cuvier, 1816) and *Piaractus brachypomus* (Cuvier, 1818): A mini review. *Journal of Applied Ichthyology*, 31, 57 – 66. https://doi.org/10.1111/jai.12976

Halliwell, B., Rafter, J., & Jenner, A. (2005). Health promotion by flavonoids, tocopherols, tocotrienols and other phenols: direct or indirect effects? Antioxidant or not? *The American Journal of Clinical Nutrition*, 81, 268S – 276S. https://doi.org/10.1093/ajcn/81.1.268S

Halliwell, B., & Gutteridge, J. M. C. (2015). Free radicals in biology and medicine. Oxford (5th ed). Oxford University Press. EUA.

Harikrishnan, R., Balasundaram, C., & Heo, M. S. (2011). Diet enriched with mushroom *Phellinus linteus* extract enhances the growth, innate immune response, and disease resistance of kelp grouper, *Epinephelus bruneus* against vibriosis. *Fish & Shellfish Immunology*, 30, 128 – 134. https://doi.org/10.1016/j.fsi.2010.09.013

Heinrich, M., Dhanji, T., & Casselman, I. (2011). Açai (*Euterpe oleracea* Mart.) — A phytochemical and pharmacological assessment of the species' health claims. *Phytochemistry Letters*, 4, 10 – 21. https://doi.org/10.1016/j.phytol.2010.11.005

Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry*, 13, 572 – 584. https://doi.org/10.1016/S0955-2863(02)00208-5

Hoseinifar S. H., Khalili M., Rufchaei R., Raeisi M., Attar M., Cordero H., & Esteban M. A. (2015). Effects of date palm fruit extracts on skin mucosal immunity, immune related genes expression and growth performance of common carp (*Cyprinus carpio*) fry. *Fish & Shellfish Immunology*, 47, 706 – 711. https://doi.org/10.1016/j.fsi.2015.09.046

Hoseinifar, S. H., Zou, H. K., Miandare, H. K., Van Doan, H., Romano, N., & Dadar, M. (2017a). Enrichment of common carp (*Cyprinus carpio*) diet with medlar (*Mespilus germanica*) leaf extract: Effects on skin mucosal immunity and growth performance. *Fish & Shellfish Immunology*, 67, 346 – 352. https://doi.org/10.1016/j.fsi.2017.06.023

Hoseinifar, S. H., Dadar, M., Khalili, M., Cerezuela, R., & Esteban, M. A. (2017b). Effect of dietary supplementation of palm fruit extracts on the transcriptomes of growth, antioxidant enzyme and immune-related genes in common carp (*Cyprinus carpio*)

fingerlings. *Aquaculture Research*, 48, 3684 – 3692. https://doi.org/10.1111/are.13192

IBGE. Instituto Brasileiro de Geografia e Estatística (2018). PEVS - Produção da Extração Vegetal e da Silvicultura. Accessed: February 21, 2020. In: <https://sidra.ibge.gov.br/pesquisa/pevs/quadros/brasil/2018>

IBGE. Instituto Brasileiro de Geografia e Estatística (2018). Produção da aquicultura - Tambaqui. Accessed: February 18, 2020. <https://sidra.ibge.gov.br/Tabela/3940#resultado>

Jensen, G. S., Wu, X., Patterson, K. M., Barnes, J., Carter, S. G., Scherwitz, L., …, & Schauss, A. G. (2008). *In vitro* and *in vivo* antioxidant and anti-inflammatory capacities of an antioxidant-rich fruit and berry juice blend, placebo-controlled, crossover study. *Journal of Agricultural and Food Chemistry*, 56, 8326 – 8333. https://doi.org/10.1021/jf8016157

Jiang, J., Wu, X. Y., Zhou, X. Q., Feng, L., Liu, Y., Jiang, W. D., …, & Zhao, Y. (2016). Effects of dietary curcumin supplementation on growth performance, intestinal digestive enzyme activities and antioxidant capacity of crucian carp *Carassius auratus*. *Aquaculture*, 463, 174 – 180. https://doi.org/10.1016/j.aquaculture.2016.05.040

Jobling, M. (2001). Nutrient repartitioning and the influence of feed composition on body composition. In: Houlihan D., Boujard T., & Jobling, M. (Eds). Food Intake in Fish (pp. 354 – 375). Blackwell Publishing, Oxford.

Jones, D. P. (2006). Extracellular redox state: refining the definition of oxidative stress in aging. *Rejuvenation Research*, 9, 169 – 181. https://doi.org/10.1089/rej.2006.9.169

Kim, J. Y., Hong, J. H., Jung, H. K., Jeong, Y. S., & Cho, K. H. (2012). Grape skin and loquat leaf extracts and acai puree have potent anti-atherosclerotic and anti-diabetic

activity in vitro and in vivo in hypercholesterolemic zebrafish. *International Journal of Molecular Medicine*, 30, 606 – 614. https://doi.org/10.3892/ijmm.2012.1045

Khalil, H. S., Mansour, A. T., Goda, A. M. A., & Omar, E. A. (2019). Effect of selenium yeast supplementation on growth performance, feed utilization, lipid profile, liver and intestine histological changes, and economic benefit in meagre, *Argyrosomus regius*, fingerlings. *Aquaculture*, 501, 135 – 143. https://doi.org/10.1016/j.aquaculture.2018.11.018

Kütter, M. T., Monserrat, J. M., Primel, E. G., Caldas, S. S., & Tesser, M. B. (2012). Effects of dietary α-lipoic acid on growth, body composition and antioxidant status in the Plata pompano *Trachinotus marginatus* (Pisces, Carangidae). *Aquaculture*, 368, 29 – 35. https://doi.org/10.1016/j.aquaculture.2012.09.010

Kütter, M. T., Monserrat, J. M., Santos, R. A., & Tesser, M. B. (2013). Dose-response effects of the antioxidante α-lipoic acid in the liver and brain of pompano *Trachinotus marginatus* (Pisces, Carangidae). *Journal of Applied Ichthyology*, 29, 1123 – 1128. https://doi.org/10.1111/jai.12137

Kottler, V. A., Koch, I., Flötenmeyer, M., Hashimoto, H., Weigel, D., & Dreyer, C. (2014). Multiple pigment cell types contribute to the black, blue, and orange ornaments of male guppies (*Poecilia reticulata*). *PloS one*, *9*, e85647 – e85647. https://doi.org/10.1371/journal.pone.0085647

Lu, Y., Liang, X. P., Jin, M., Sun, P., Ma, H. N., Yuan, Y., & Zhou, Q. C. (2016). Effects of dietary vitamin E on the growth performance, antioxidant status and innate immune response in juvenile yellow catfish (*Pelteobagrus fulvidraco*). *Aquaculture*, 464, 609 – 617. https://doi.org/10.1016/j.aquaculture.2016.08.009

Lushchak, V. I. (2011). Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology*, 101, 13 – 30. https://doi.org/10.1016/j.aquatox.2010.10.006

Lushchak, V. I. (2014). Free radicals, reactive oxygen species, oxidative stress and its classification. *Chemical-Biological Interactions*, 224, 164 – 175. https://doi.org/10.1016/j.cbi.2014.10.016

Maltez, L. C., Stringhetta, G. R., Enamorado, A. L., Okamoto, M. H., Romano, L. A., Monserrat, J. M., ..., & Garcia, L. (2017). Ammonia exposure and subsequent recovery trigger oxidative stress responses in juveniles of Brazilian flounder *Paralichthys orbignyanus*. *Fish Physiology and Biochemistry*, 43, 1747 – 1759. https://doi.org/ 10.1007/s10695-017-0406-8

Maltez, L. C., Barbas, L. A. L., Nitz, L. F., Pellegrin, L., Okamoto, M. H., Sampaio, L. A., ..., & Garcia, L. (2018). Oxidative stress and antioxidant responses in juvenile Brazilian flounder *Paralichthys orbignyanus* exposed to sublethal levels of nitrite. *Fish Physiology and Biochemistry*, *44*, 1349 – 1362. https://doi.org/10.1007/s10695-018-0526-9

Manach, C., Williamson, G., Morand, C., Scalbert, A., & Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American Journal of Clinical Nutrition*, 81, 230S – 242S. https://doi.org/10.1093/ajcn/81.1.230S

Marques, G. S., Monteiro, R. P. M., & Leão, W. F. (2012). Avaliação de procedimentos para quantificação espectrofotométrica de flavonoides totais em folhas de *Bauhinia forficata* Link. *Química Nova*, 35, 517 – 522.

Menezes, E. M. S.; Torres, A. T., & Srur, A. U. S. (2008). Valor nutricional da polpa de açaí (*Euterpe oleracea* Mart) liofilizada. *Acta Amazônica*, 38, 311 – 316.

2443 Mertens-Talcott, S. U., Rios, J., Jilma-Stohlawetz, P., Pacheco-Palencia, L. A., Meibohm,
2444 B., Talcott, S. T., & Derendorf, H. (2008). Pharmacokinetics of anthocyanins and
2445 antioxidant effects after consumption of anthocyanin-rich acai juice and pulp
2446 (*Euterpe oleracea* Mart.) in human healthy volunteers. *Journal of Agricultural and*
2447 *Food Chemistry*, 56, 7796 – 7802. https://doi.org/10.1021/jf8007037
2448 Monserrat, J. M., da Silva, T. V. N. (2020). Effects of dietary Amazonian açai (*Euterpe*
2449 *oleracea* Martius) fruit on growth performance, skin coloration and antioxidant
2450 status of *Colossoma macropomum*. Figshare.
2451 Dataset. https://doi.org/10.6084/m9.figshare.11897928.v1
2452 Oliva-Teles, A. (2012). Nutrition and health of aquaculture fish. *Journal of Fish Diseases*,
2453 35, 83 – 108. https://doi.org/10.1111/j.1365-2761.2011.01333.x
2454 Oakes, K. D., & Van Der Kraak, G. J. V. (2003). Utility of the TBARS assay in detecting
2455 oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to
2456 pulp mill effluent. *Aquatic Toxicology*, 63, 447 – 463.
2457 https://doi.org/10.1016/S0166-445X(02)00204-7
2458 Packer, L., Witt, E. H., & Tritschler, H. J. (1995). Alpha-lipoic acid as a biological
2459 antioxidant. *Free Radical Biology & Medicine*, 19, 227 – 250.
2460 https://doi.org/10.1016/0891-5849(95)00017-R
2461 Paulino, R. R., Pereira, R. T., Fontes, T. V., Oliva-Teles, A., Peres, H., Carneiro, D. J., &
2462 Rosa, P. V. (2018). Optimal dietary linoleic acid to linolenic acid ratio improved
2463 fatty acid profile of the juvenile tambaqui (*Colossoma*
2464 *macropomum*). *Aquaculture*, 488, 9 – 16.
2465 https://doi.org/10.1016/j.aquaculture.2018.01.014
2466 Peixoto, H., Roxo, M., Krstin, S., Wang, X., & Wink, M. (2016). Anthocyanin-rich extract
2467 of Acai (*Euterpe precatoria* Mart.) mediates neuroprotective activities in

Caenorhabditis elegans. *Journal of Functional Foods*, 26, 385 – 393. https://doi.org/10.1016/j.jff.2016.08.012

Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., & Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. *Aquaculture*, 433, 50 – 61. https://doi.org/10.1016/j.aquaculture.2014.05.048

Rezende, F. P., Júnior, M. V. V., da Andrade, D. R., & Mendonça, P. P. (2012). Characterization of a new methodology based on the intensity of skin staining of ornamental fish with applications in nutrition. *Journal of Agricultural Science and Technology*, *B 2*, 606 – 613.

Ribeiro, S. C., Malheiros, D. F., Guilozki, I. C., Majolo, C., Chaves, F. C. M., Chagas, E. C., ..., Yoshioka, E. T. O. (2018). Antioxidants effects and resistance against pathogens of *Colossoma macropomum* (Serassalmidae) fed *Mentha piperita* essential oil. *Aquaculture*, 490, 29 – 34. https://doi.org/10.1016/j.aquaculture.2018.02.024

Roubach, R., Gomes, L. C., Leão Fonseca, F. A., & Val, A. L. (2005). Eugenol as an efficacious anaesthetic for tambaqui, *Colossoma macropomum* (Cuvier). *Aquaculture Research*, 36, 1056 – 1061. https://doi.org/10.1111/j.1365-2109.2005.01319.x

Saint-Paul, U. (2017). Native fish species boosting Brazilian's aquaculture development. *Acta of Fisheries and Aquatic Resources*, 5, 1 – 9. https://doi.org/10.2312/ActaFish.2017.5.1.1-9

Sallam, A. E., Mansour, A. T., Srour, T. M., & Goda, A. M. A. (2017). Effects of different carotenoid supplementation sources with or without sodium taurocholate on growth, feed utilization, carotenoid content and antioxidant status in fry of the

European seabass, *Dicentrarchus labrax*. *Aquaculture Research*, 48, 3848 – 3858. https://doi.org/10.1111/are.13212

Salomão, R. A. S., De Paula, T. G., Zanella, B. T. T., Carvalho, P. L. P. F., da Silva Duran, B. O., Valente, J. S., ... & Carvalho, R. F. (2019). The combination of resveratrol and exercise enhances muscle growth characteristics in pacu (*Piaractus mesopotamicus*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *235*, 46 – 55. https://doi.org/10.1016/j.cbpa.2019.05.002

Schauss, A. G., Wu, X., Prior, R. L., Ou, B., Huang, D., Owens, J., …, & Shanbro, E. (2006). Antioxidant capacity and other bioactivities of the freeze-dried Amazonian palm berry, *Euterpe oleraceae* Mart. (acai). *Journal of Agricultural and Food Chemistry*, 54, 8604 – 8610. https://doi.org/10.1021/jf0609779

Schauss, A. G. (2016). Advances in the study of the health benefits and mechanisms of action of the pulp and seed of the Amazonian palm fruit, *Euterpe oleracea* Mart., known as "Açai". In: Watson, R. R., & Preedy, V. R. (Eds.). Fruits, Vegetables, and Herbs (pp. 177 – 220). Oxford: Academic Press.

Schulz, M., Biluca, F. C., Gonzaga, L. V., Borges, G. D. S. C., Vitali, L., Micke, G. A., ..., & Fett, R. (2017). Bioaccessibility of bioactive compounds and antioxidant potential of juçara fruits (*Euterpe edulis* Martius) subjected to in vitro gastrointestinal digestion. *Food Chemistry*, 228, 447 – 454. https://doi.org/10.1016/j.foodchem.2017.02.038

Searle, S. R., Casella, G., & McCulloch, C. E. (2008). Variance Components. John Wiley & Sons, Inc. Publication.

Shaluei, F., Nematollahi, A., Naderi-Farsani, H. R., Rahimi, R., & Kaboutari Katadj, J. (2017). Effect of ethanolic extract of *Zingiber officinale* on growth performance

and mucosal immune responses in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition,* 23, 814 – 821. https://doi.org/10.1111/anu.12448

Sies, H. (2015). Oxidative stress: a concept in redox biology and medicine. *Redox Biology*, 4, 180 – 183. https://doi.org/10.1016/j.redox.2015.01.002

Soares, E. R., Monteiro, E. B., Graziele, F., Inada, K. O., Torres, A. G., Perrone, D., ..., & Daleprane, J. B. (2017). Up-regulation of Nrf2-antioxidant signaling by Açaí (*Euterpe oleracea* Mart.) extract prevents oxidative stress in human endothelial cells. *Journal of Functional Foods*, 37, 107 – 115. https://doi.org/10.1016/j.jff.2017.07.035

Sokolova, I. M., Frederich, M., Bagwe, R., Lannig, G., & Sukhotin, A. A. (2012). Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Marine Environmental Research*, 79, 1 – 15. https://doi.org/10.1016/j.jff.2017.07.035

Souza, D. M., Borges, V. D., Furtado, P., Romano, L. A., Wasielesky Jr, W., Monserrat, J. M., & Garcia, L. O. (2016). Antioxidant enzyme activities and immunological system analysis of *Litopenaeus vannamei* reared in biofloc technology (BFT) at different water temperatures. *Aquaculture*, 451, 436 – 443. https://doi.org/10.1016/j.aquaculture.2015.10.006

Sutili, F. J., Gatlin III, D. M., Heinzmann, B. M., & Baldisserotto, B. (2018). Plant essential oils as fish diet additives: benefits on fish health and stability in feed. *Reviews in Aquaculture,* 10, 716 – 726. https://doi.org/10.1111/raq.12197

Tan, X., Sun, Z., Chen, S., Chen, S., Huang, Z., Zhou, C., …, & Wang, A. (2017). Effects of dietary dandelion extracts on growth performance, body composition, plasma biochemical parameters, immune responses and disease resistance of juvenile

golden pompano *Trachinotus ovatus*. *Fish & Shellfish Immunology*, 66, 198 – 206. https://doi.org/10.1016/j.fsi.2017.05.028

Takahashi, L. S., Biller-Takahashi, J. D., Mansano, C. F. M., Urbinati, E. C., Gimbo, R. Y., & Saita, M. V. (2017). Long-term organic selenium supplementation overcomes the trade-off between immune and antioxidant systems in pacu (*Piaractus mesopotamicus*). *Fish & Shellfish Immunology*, 60, 311 – 317. https://doi.org/10.1016/j.fsi.2016.11.060

Unesco (1983). Chemical methods for use in marine environmental monitoring. Manual and Guides 12. Intergovernamental Oceanographic Commissiony, Paris, France.

Vanderzwalmen, M., Eaton, L., Mullen, C., Henriquez, F., Carey, P., Snellgrove, D., & Sloman, K. A. (2019). The use of feed and water additives for live fish transport. *Reviews in Aquaculture*, *11*, 263 – 278. https://doi.org/10.1111/raq.12239

Vanegas-Espinoza, P. E., Pérez-Escalante, V., Aguirre-Guzman, G., Hoyos-Leyva, J. D., & Del Villar-Martínez, A. A. (2019). Microencapsulation of anthocyanins from roselle (*Hibiscus sabdariffa*) and its application on a pigment supplied diet to fantail goldfish (*Carassius auratus*). *Aquaculture International*, *27*, 1801 – 1811. https://doi.org/10.1007/s10499-019-00430-1

Wu, F., Jiang, M., Wen, H., Liu, W., Tian, J., Yang, C. G., & Huang, F. (2017). Dietary vitamin E effects on growth, fillet textural parameters, and antioxidant capacity of genetically improved farmed tilapia (GIFT), *Oreochromis niloticus*. *Aquaculture International*, 25, 991 – 1003. https://doi.org/10.1007/s10499-016-0089-7

Yeşilayer, N., Mutlu, G., & Yıldırım, A. (2020). Effect of nettle (*Urtica* spp.), marigold (*Tagetes erecta*), alfalfa (*Medicago sativa*) extracts and synthetic xanthophyll (zeaxanthin) carotenoid supplementations into diets on skin pigmentation and

growth parameters of electric yellow cichlid (*Labidochromis caeruleus*). *Aquaculture*, 734964. https://doi.org/10.1016/j.aquaculture.2020.734964

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, 214 – 218. https://doi.org/10.1016/j.aquaculture.2009.04.025

TABLES

TABLE 1. Formula and centesimal composition of the experimental diets (n = 3) tested with different levels of inclusion of lyophilized *Euterpe oleracea* - LEO.

	LEO (%)					
Ingredients (g Kg^{-1})	Ctr.	0.63	1.25	2.50	5.00	10.0
Fish meal	271.5	271.5	271.5	271.5	271.5	271.5
Soy oil	11	10	9	8	7	6
Soybean meal	330	330	330	330	330	330
Euterpe oleracea (EO)	0	6.3	12.5	25	50	100
Coconut meal	104	98.7	93.5	82	58	9
Corn	243.5	243.5	243.5	243.5	243.5	243.5
CMC[a]	30	30	30	30	30	30
Min. and vit. mixture[b]	10	10	10	10	10	10
Centesimal composition						
Dry matter (% DM)	98.67	98.96	99.05	97.70	96.67	97.82
Crude protein (%DM)	40.09	40.68	40.37	40.53	40.39	40.39
Crude lipid (%DM)	9.35	9.66	9.73	9.9	9.88	9.22
NFE (%DM)[c]	38.91	37.44	37.35	37.51	37.84	37.66
Fibers (%DM)	3.59	4.02	4.32	3.91	3.85	4.45
Ash (%DM)	8.06	8.19	8.23	8.15	8.04	8.29
Gross energy (Kcal/g^{-1})	4891.24	4911.68	4908.79	4924.07	4924.66	4879.56

[a] Cellulose.

[b] Premix M. Cassab, SP, Brazil (Vitamin A (500000 UI kg^{-1}), Vit. D3 (250 000 UI kg^{-1}), Vit. E (5000 mg kg^{-1}), Vit. K3 (500 mg kg^{-1}),Vit. B1 (1000 mg kg^{-1}), Vit. B2 (1000 mg kg^{-1}), Vit. B6 (1000 mg kg^{-1}), Vit. B12 (2000 mcg kg^{-1}), Niacin (2500 mg kg^{-1}), Calcium pantothenate (4000 mg kg^{-1}), Folic acid (500 mg kg^{-1}), Biotin (10 mg kg^{-1}), Vit. C (10 000 mg kg^{-1}), Choline (100 000 mg kg^{-1}), Inositol (1000 mg kg^{-1}). Trace elements: Selenium (30 mg kg^{-1}), Iron (5000 mg kg^{-1}), Copper (1000 mg kg^{-1}), Manganese (5000 mg kg^{-1}), Zinc (9000 mg kg^{-1}), Cobalt (50 mg kg^{-1}), Iodine (200 mg kg^{-1}).

[c] Nitrogen-free extract (NFE): calculated by difference (100 – crude protein – crude lipid – ash – fibre).

TABLE 2. Physicochemical parameters of water in the experimental tanks of *Colossoma macropomum* after a 30-days fed with diets containing different levels of inclusion of lyophilized *Euterpe oleracea* - LEO.

	LEO (%)					
	Ctr.	0.63	1.25	2.50	5.00	10.0
Parameters						
Temperature (° C)	28.36 ± 0.07[a]	27.56 ± 0.10[b]	27.77 ± 0.10[b]	27.59 ± 0.13[b]	27.58 ± 0.12[b]	28.34 ± 0.11[a]
pH	6.65 ± 0.10[a]	6.65 ± 0.10[a]	6.67 ± 0.10[a]	6.64 ± 0.11[a]	6.61 ± 0.09[a]	6.67 ± 0.10[a]
DO (mg L^{-1})	5.34 ± 0.22[a]	4.94 ± 0.20[a]	5.43 ± 0.18[a]	5.07 ± 0.21[a]	4.86 ± 0.21[a]	4.81 ± 0.23[a]
Alkalinity (mg L^{-1})	14.83 ± 0.97[a]	14.66 ± 1.04[a]	15.83 ± 1.09[a]	16.16 ± 1.92[a]	17.83 ± 1.59[a]	16.16 ± 1.49[a]
Conductivity (µS cm^{-1})	44.04 ± 0.62[a]	45.78 ± 0.33[a]	44.80 ± 0.69[a]	46.16 ± 0.49[a]	45.04 ± 0.53[a]	45.42 ± 0.61[a]
TAN (mg L^{-1})	1.97 ± 0.57[a]	1.98 ± 0.46[a]	2.00 ± 0.45[a]	1.89 ± 0.55[a]	1.55 ± 0.39[a]	1.92 ± 0.51[a]
Nitrite (mg L^{-1})	0.80 ± 0.31[a]	0.74 ± 0.30[a]	0.55 ± 0.20[a]	0.56 ± 0.16[a]	0.62 ± 0.24[a]	0.61 ± 0.19[a]

Note: Data are presented as means and standard errors of three tanks. Means with superscripts in the same line are significantly different ($p < 0.05$; n = 3 per treatment). Abbreviations: DO, dissolved oxygen; TAN, total ammonia nitrogen.

TABLE 3. Effect of increasing levels of dietary lyophilized *Euterpe oleracea* (LEO) on the zootechnical performance of juvenile *Colossoma macropomum* after a 30-days feeding trial.

	LEO (%) (*x*)						Model	*p*-value	R^2
	Ctr.	0.63	1.25	2.50	5.00	10.0			
Index (*y*)									
IBW (g)	0.91 ± 0.02	0.95 ± 0.08	0.87 ± 0.02	0.92 ± 0.03	0.91 ± 0.02	0.90 ± 0.02	-	-	-
FBW (g) †	6.93 ± 0.08	7.31 ± 0.08	7.58 ± 0.28	7.61 ± 0.02	8.00 ± 0.15*	8.26 ± 0.53*	Linear	0.0020	0.48
WG (g) ‡	6.02 ± 0.07	6.36 ± 0.14	6.71 ± 0.29	6.70 ± 0.02	7.09 ± 0.14*	7.47 ± 0.61*	Linear	0.0015	0.48
SGR (% day^{-1}) §	2.86 ± 0.02	3.00 ± 0.08	3.09 ± 0.08*	3.06 ± 0.01	3.13 ± 0.04*	3.19 ± 0.07*	Linear	0.0055	0.41
FCR¶	0.97 ± 0.04	0.83 ± 0.05	0.77 ± 0.05*	0.78 ± 0.0*	0.73 ± 0.02*	0.76 ± 0.01*	Dose-response inhibition	0.007	0.67
PER	2.98 ± 0.03	3.22 ± 0.22	3.48 ± 0.18*	3.32 ± 0.02	3.48 ± 0.09*	3.44 ± 0.06*	-	ns	-
FI♦	4.63 ± 0.08	4.21 ± 0.21	3.94 ± 0.18*	4.01 ± 0.02*	3.85 ± 0.08*	3.98 ± 0.09*	Linear	0.0493	0.23
K	1.68 ± 0.02	164 ± 0.01	1.68 ± 0.03	1.72 ± 0.0	1.61 ± 0.02	1.71 ± 0.02	-	ns	-
S (%)	99.39 ± 0.33	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	-	-	-

Note: Data are presented as means and standard errors of three tanks per treatment. Means with an asterisk in the same line are significantly different from the control ($p < 0.05$; $n = 90$ per treatment). Abbreviations: IBW, initial body weight; FBW, final body weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; FI, feed intake; K, condition factor; S, survival; ns, not significant.

†$FBW = 0.1260x + 7.220$

‡$WG = 0.1318x + 6.264$

§$SGR = 0.02784x + 2.948$

¶$FCR = 0.7527 + (0.9771 – 0.7527) / (1 + 10 \wedge (0.7766 – x) * (- 2.317))$

♦$FI = – 0.05004x + 4.316$

TABLE 4. Effects of increasing levels of the dietary lyophilized *Euterpe oleracea* (LEO) on carcass composition of juvenile *Colossoma macropomum* after a 30-days feeding trial.

	LEO (%)					
Carcass composition (wet weight %)	Ctr.	0.63	1.25	2.50	5.00	10.0
Moisture	74.90 ± 0.96[a]	74.48 ± 0.43[a]	75.69 ± 0.40[a]	75.06 ± 0.18[a]	75.31 ± 0.03[a]	73.62 ± 1.06[a]
Crude lipid	6.98 ± 0.21[a]	7.24 ± 0.32[a]	6.51 ± 0.22[a]	6.93 ± 0.11[a]	6.52 ± 0.08[a]	7.08 ± 0.47[a]
Crude protein	12.50 ± 0.73[ab]	13.18 ± 0.08[b]	11.05 ± 0.35[a]	11.60 ± 0.35[ab]	10.87 ± 0.14[a]	12.02 ± 0.38[ab]
Ash	3.46 ± 0.15[a]	3.56 ± 0.04[a]	3.40 ± 0.10[a]	3.40 ± 0.06[a]	3.52 ± 0.05[a]	3.77 ± 0.18[a]

Note: Data are presented as means and standard errors of three replicates per treatment. Means with different superscripts in the same line are significantly different ($p < 0.05$; $n = 9$ per treatment).

TABLE 5. Bioactive constitution and radical scavenging activity of experimental diets and muscle of juvenile *Colossoma macropomum* after a 30-days feeding trial with dietary lyophilized *Euterpe oleracea* - LEO.

	LEO (%)					
	Ctr.	0.63	1.25	2.50	5.00	10.0
Diets						
AC (%)	2.4 ± 0.4^{a}	1.8 ± 0.8^{a}	1.1 ± 0.7^{a}	2.8 ± 0.6^{a}	2.5 ± 0.6^{ab}	4.4 ± 0.2^{b}
TFC (mg g^{-1})	5.39 ± 0.22^{c}	8.16 ± 0.84^{a}	8.57 ± 0.77^{a}	10.77 ± 1.21^{ab}	12.85 ± 0.80^{b}	28.69 ± 3.89^{d}
TPC (mg g^{-1})	0.068 ± 0.013^{a}	0.071 ± 0.007^{ab}	0.092 ± 0.006^{ab}	0.120 ± 0.006^{ab}	0.154 ± 0.010^{bc}	0.200 ± 0.010^{c}
Muscle						
AC (%)	5.38 ± 0.28^{a}	5.47 ± 0.32^{a}	6.23 ± 0.32^{a}	4.45 ± 0.21^{a}	4.57 ± 0.20^{a}	5.38 ± 0.22^{a}
TFC (mg g^{-1})	6.58 ± 0.81^{a}	5.66 ± 0.58^{a}	6.85 ± 0.53^{a}	5.92 ±0.55^{a}	5.74 ± 0.68^{a}	6.09 ± 0.55^{a}
TPC (mg g^{-1})	0.0103 ± 0.0008^{a}	0.0093 ± 0.0012^{a}	0.0097 ± 0.0009^{a}	0.0099± 0.0016^{a}	0.0094 ± 0.0023^{a}	0.008 ± 0.0007^{a}

Note: Data are presented as means and standard errors of three replicates per treatment. Means with different letter superscripts in the same line are significantly different ($p < 0.05$; diets n = 5; muscle n = 15 per treatment). Abbreviations: AC, antioxidant capacity against DPPH; TFC, total flavonoid content; TPC, total polyphenols content.

TABLE 6. Intensity of color cyan and black expressed in radians from the dorsal region of juvenile *Colossoma macropomum* after a 30-days feeding trial with dietary lyophilized *Euterpe oleracea* - LEO.

	LEO (%) (*x*)								
							Model	*p*-value	R^2
Color (*y*)	Ctr.	0.63	1.25	2.50	5.00	10.0			
Cyan [†]	0.623 ± 0.009^{a}	0.616 ± 0.006^{a}	0.653 ± 0.006^{a}	0.648 ± 0.011^{a}	0.670 ± 0.007^{b}	0.650 ± 0.009^{b}	Non-linear	na	0.40
Black	0.482 ± 0.029^{a}	0.457 ± 0.023^{a}	0.419 ± 0.015^{a}	0.448 ± 0.030^{a}	0.456 ± 0.013^{a}	0.414 ± 0.014^{a}	Linear	ns	-

Note: Data are presented as means and standard errors of three replicates. Means with superscripts in the same line are significantly different ($p < 0.05$; n = 15 per treatment). Abbreviations: ns, not significant; na, not applicable.

[†]$\text{Cyan} = 0.6173 + (0.6585 - 0.6173) * (1^{-\exp(-0.7151 * x)})$

TABLE 7. Feed costs for production of juvenile *Colossoma macropomum* reared for 30-days on diets with increasing levels of lyophilized *Euterpe oleracea* – LEO in the diet.

	LEO (%)					
	Ctr.	0.63	1.25	2.50	5.00	10.0
Cost (R$)*	6.53	7.42	8.30	10.06	13.57	20.58
Relative to control	100.00	113.50	127.02	153.93	207.65	314.98
Decrease in feed cost	0.00	−13.50	−27.02	−53.93	−107.65	−214.98
Feed cost kg^{-1} again (R$)	6.64 ± 0.32^{a}	6.03 ± 0.19^{a}	5.87 ± 0.15^{a}	7.82 ± 0.23^{b}	9.69 ± 0.18^{c}	14.88 ± 0.31^{d}
Relative to control	100.00	91.22	88.40	117.77	145.93	224.09
Decrease in feed cost kg^{-1} again	0.00	8.78	11.60	−17.77	−45.93	−124.09
Feed cost kg^{-1} protein again (R$)	14.17 ± 1^{ab}	12.13 ± 0.66^{a}	10.35 ± 0.63^{a}	11.64 ± 0.50^{ab}	14.62 ± 0.40^{b}	23.22 ± 0.81^{c}
Relative to control	100.00	85.60	73.04	82.14	103.17	163.86
Decrease in feed cost kg^{-1} protein gain	0.00	14.40	26.96	17.86	−3.17	−63.86

Note: Values are presented as means and standard errors of three replicates per experimental diet. Means with different letter superscripts in the same line are significantly different ($p < 0.05$; $n = 90$ per treatment). *The costs of feed ingredients were fishmeal (R$ 6.58 per Kg), soy oil (R$ 2.97 per L), soybean meal (R$ 1.80 per Kg), lyophilized *Euterpe oleracea* (R$ 150.00 per Kg), coconut meal (R$ 9.90 per Kg), corn (R$ 0.50 per Kg), cellulose - CMC (R$ 97.00 per Kg), mineral and vitamin mixture (R$ 5.86 per Kg). R$= Brazilian Real; $1 US equals R$ 3.76.

FIGURE CAPTIONS

FIGURE 1. Photographs of fish after a 30-days feeding trial with dietary control (Ctr.) or lyophilized *Euterpe oleracea* – LEO levels. The pictures were submitted to color balance to measure the percentiles of cyan and black color, using a photo editing Adobe®Photoshop® software version 5 (n = 15 per treatment).

FIGURE 2. Total antioxidant capacity against peroxyl radicals – ACAP (relative area) in the intestine (a), liver (b), and muscle (c) of juvenile *Colossoma macropomum* reared per 30 days with diets containing different levels of lyophilized *Euterpe oleracea* (LEO). Data are presented as means and standard errors of three tanks per treatment. The bars assigned with different letters denote significant differences between treatments ($p < 0.05$; n = 15 per treatment).

FIGURE 3. Thiobarbituric acid reactive substances – TBARS content (nmol TMP/mg of protein) in (a) intestine, adjusted to a polynomial second order regression model with dietary levels of lyophilized *Euterpe oleracea* (LEO); (b) liver and (c) muscle of juvenile *Colossoma macropomum* reared per 30 days with diets containing different levels of lyophilized *Euterpe oleracea* (LEO). Data are presented as means and standard errors of three tanks per treatment. The bars assigned with different letters denote significant differences between treatments ($p < 0.05$; n = 15 per treatment).

FIGURE 1

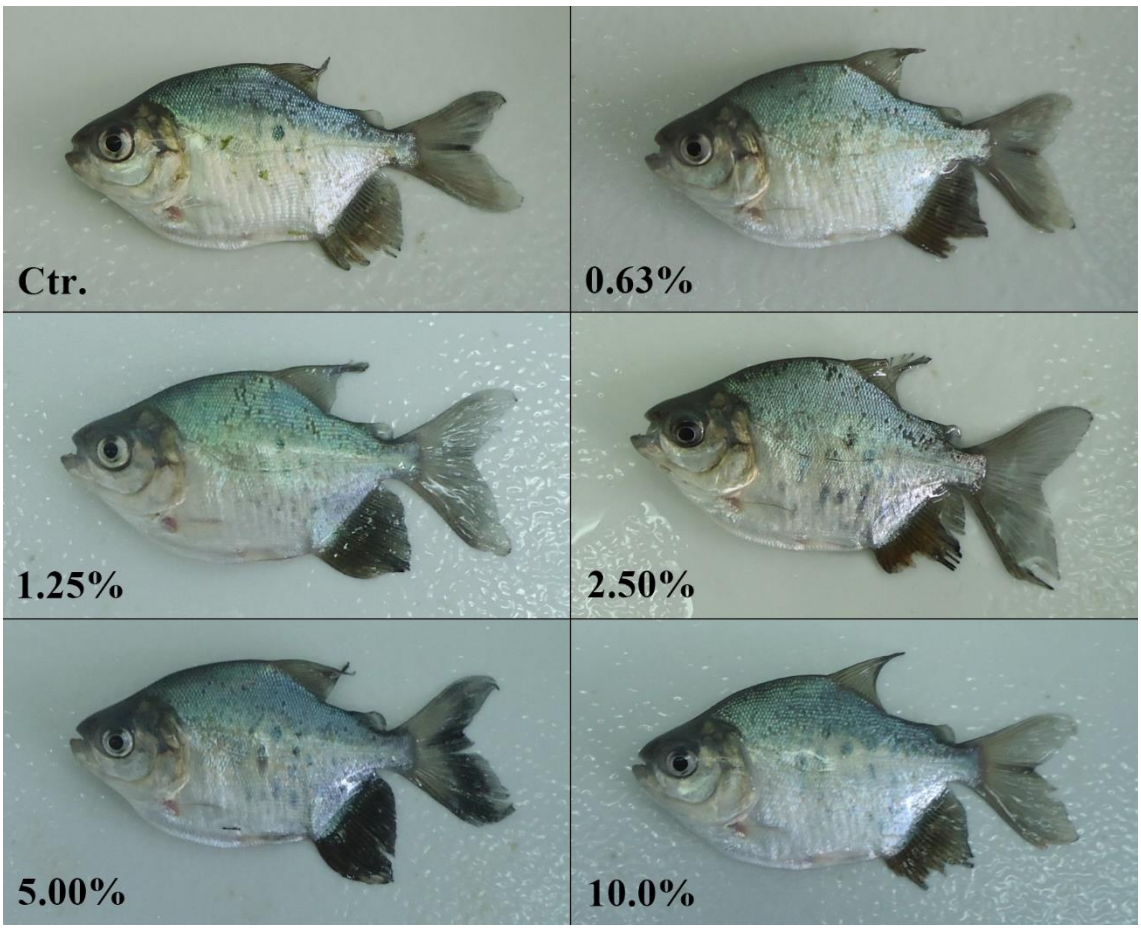


FIGURE 2

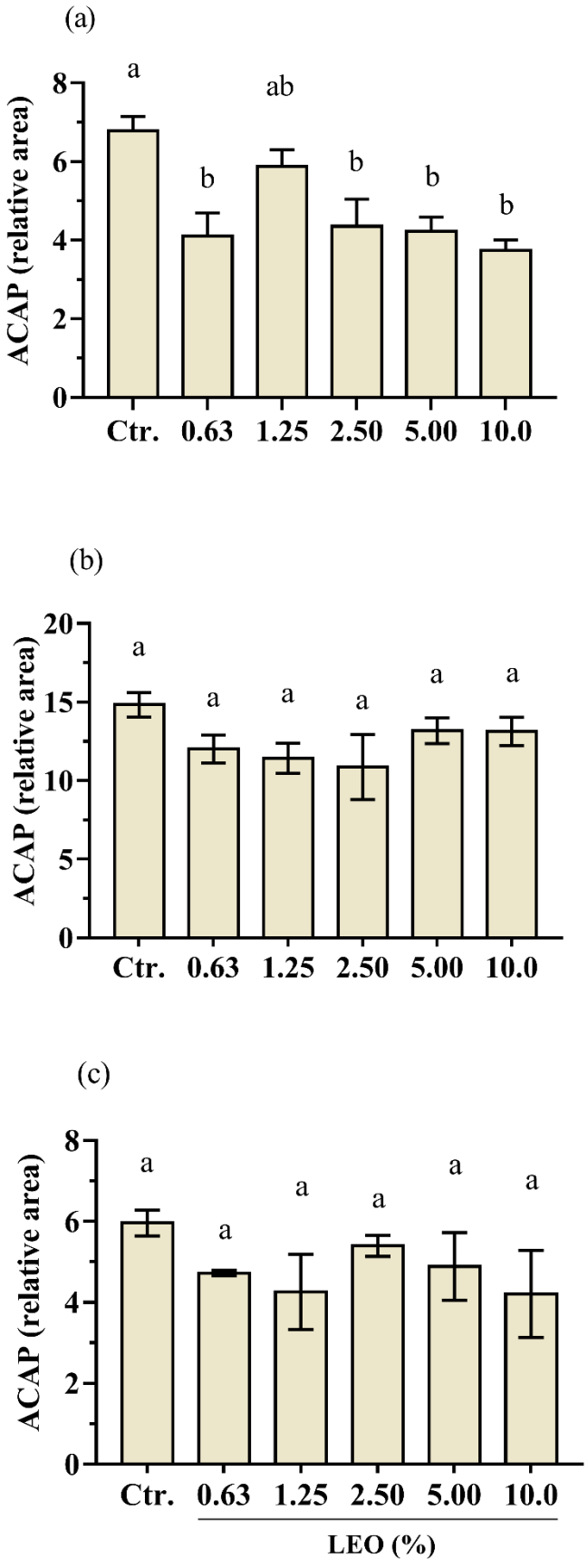
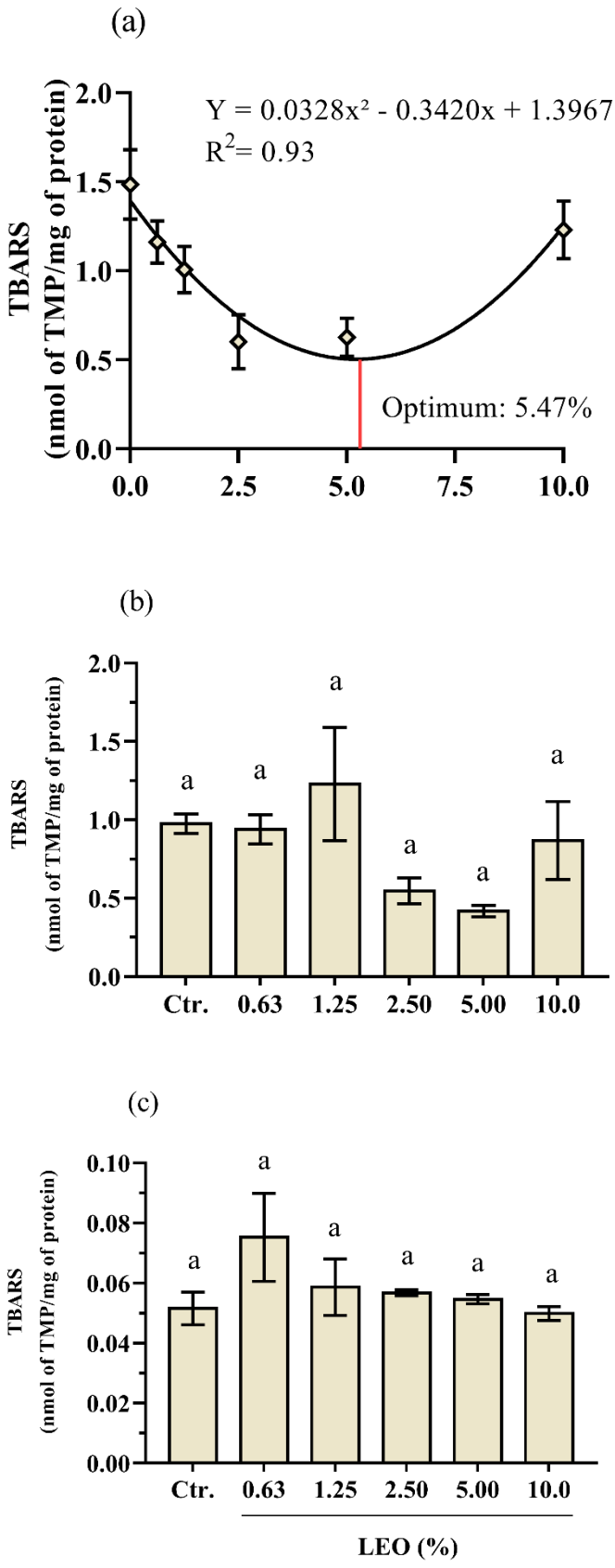

(a)
ACAP (relative area)
a
b
ab
b
b
b
Ctr.
0.63
1.25
2.50
5.00
10.0
(b)
ACAP (relative area)
a
a
a
a
a
a
Ctr.
0.63
1.25
2.50
5.00
10.0
(c)
ACAP (relative area)
a
a
a
a
a
a
Ctr.
0.63
1.25
2.50
5.00
10.0
LEO (%)

FIGURE 3


(a)
Y = 0.0328x² - 0.3420x + 1.3967
R²= 0.93
TBARS (nmol of TMP/mg of protein)
Optimum: 5.47%
(b)
TBARS (nmol of TMP/mg of protein)
Ctr.
0.63
1.25
2.50
5.00
10.0
(c)
TBARS (nmol of TMP/mg of protein)
LEO (%)

CAPÍTULO 2

Energetic metabolism in the muscle of tambaqui juveniles (*Colossoma macropomum*) fed with dietary açai (*Euterpe oleracea* Mart).

Thamyres V. N. da Silva[1,5*]; Jessica M. L. dos Santos[2]; Glaycilane G. de Deus[1]; Juan R. B. Ramírez[1,5]; Marcelo F. Torres[2]; Luis A. L. Barbas[2]; Luís A. Sampaio[3,5]; José M. Monserrat[1,4,5]

[1] Functional Biochemistry of Aquatic Organisms Laboratory (BIFOA), Center for Biotechnology and Diseases of Aquatic Organisms (CBD), Federal University of Rio Grande (FURG), Institute of Oceanography (IO), Rio Grande, RS, Brazil.

[2] Tropical Species Aquaculture Laboratory (LAET), Federal Institute of Pará (IFPA), Castanhal, PA, Brazil.

[3] Estuarine and Marine Fish Laboratory (LAPEM), Federal University of Rio Grande (FURG), Institute of Oceanography (IO), Rio Grande, RS, Brazil.

[4] Institute of Biological Sciences (ICB), Federal University of Rio Grande (FURG), Rio Grande, RS, Brazil.

[5] *Postgraduate program in Aquaculture,* Federal University of Rio Grande (FURG), Institute of Oceanography (IO), Rio Grande, RS, Brazil.

* Corresponding author at:
Thamyres V. N. da Silva, Laboratory of Functional Biochemistry of Aquatic Organisms (BIFOA), Federal University of Rio Grande - FURG, Institute of Oceanography (IO), CP 96201-900, Rio Grande, RS, Brazil.
E-mail: thamyrestvns@gmail.com

Manuscrito submetido à revista *Fish Physiology and Biochemistry*.
Formatado conforme as normas da revista.

Abstract

Açai, *Euterpe oleracea*, is an Amazonian fruit constituted for a noble profile of phytochemicals that coming recently studied in feed for aquaculture. The present study evaluated the hypotheses that in tambaqui juveniles, *Colossoma macropomum*, the lyophilized *E. oleracea* (LEO) should increase the muscle energetic potential from the optimization of energy fuels and reserve mobilization. These effects were predicted by higher growth performance and optimization feed utilization in a previous study. Fuels energy and reserves, besides the electron transport system (ETS) were measured in the muscle of tambaqui. Fish were fed a control diet (0.00% LEO) or LEO diets with containing 0.63, 1.25, 2.50, 5.00, and 10.0% (w/w) of inclusion for 30 days. Fish fed from dietary 0.63% LEO had a significant reduction in triglyceride levels in the muscle when compared with the control group. LEO did not alter the content of cholesterol, glucose, glycogen and total protein in the muscle. However, there was an increment of ETS activity to 76.25% in tambaqui fed with the diet 1.25% LEO compared to the control group, indicating an important improvement in the muscle metabolic potential. These results suggest that açai has positive effects on the energy metabolism of tambaqui due to its phytochemical properties that probably derived energy from food and additionally from lipolysis (triglyceride), promoting a muscular energy state that favor growth.

Keyword: Amazonian fruit, phytochemical, feed additive, muscle energy, electron transport system, teleost.

1. Introduction

Globally, fish farming is developing into new trends, intensifying and diversifying the fish production (Chakraborty, Horn and Hancz, 2014). For this reason, nutritional strategies directed for the use of phytochemicals as modulators of growth, as well as promoters of antioxidant and antistress responses have attracted interest in aquaculture (Sutili et al., 2018). Phytochemicals correspond to a large group of secondary metabolites of plants commonly found in fruits, seeds, vegetables and other plant-derived products (Sutili et al., 2018; Valenzuela-Grijalva et al., 2017, Simião et al., 2020). Depending on their chemical structure, these natural compounds are generally categorized into alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids and essential oils (Chakraborty, Horn and Hancz, 2014).

Among the beneficial effects of phytochemicals, are reported: anabolic induction with consequent animal growth and optimization of feed utilization; antimicrobial action; maintenance of redox status; action about gut health and integrity; decrease the serum cholesterol and triglyceride levels; and improvement of immune responses and disease resistance (Gisbert et al., 2017; Valenzuela-Grijalva et al., 2017; Al-Sagheer et al., 2018; Abdel-Tawwab et al., 2018; Hoseini et al., 2018; da Silva et al., 2020; Li et al., 2019). The use of phytochemicals in animal nutrition present advantages because it is healthier and biodegradable, and therefore, more biosecure sustainable than antibiotics and hormone growth-promoters, whose use in animal feed has been recommended with caution by various international organizations such as the World Health Organization (Yang et al., 2015; Vélez et al., 2017; Reverter et al., 2017; Tang et al., 2017).

The açai palm tree (*Euterpe oleracea* Martius), a native plant of the Amazon Region of Brazil, generates a reddish-purple berry known as açai or açai berry (Schauss, 2016). This plant and its fruit are rich in polyphenols, such as anthocyanins (cyanidin-3-

glucoside and cyanidin 3-rutinoside), quercetin, and hydroxycinnamic acids; besides fatty acids, mainly oleic acid (MUFA) and linoleic acid (PUFA); fibers; and phytosterols (Alqurashi et al., 2016; Aranha et al., 2019). These phytochemicals have aroused interest and increased efforts in studies focused on the biomedical area (Kim et al., 2012; Souza Monteiro et al., 2015; Fragoso et al., 2018) and, more recently, for application in aquaculture (Colombo et al., 2020). Among the biological activities of açai has reported antioxidant activity in fish (da Silva et al., 2020); chemoprotection against ammonia toxicity in shrimp (Colombo et al., 2020); anti-inflammatory and potential antitumor activity (Fragoso et al., 2018; Machado et al., 2019); and prevention of hepatic steatosis through the increase of cholesterol excretion (da Silva et al., 2018) in other animal models.

Regarding the promotion of growth, phytochemicals can modulate gene expression related to growth (GH and IGF-1) and so they can be dubbed phytogenics (Hoseinifar et al., 2017; Safari et al., 2017). This effect suggests that ATP supply via aerobic metabolism is enough high to satisfy the maintenance costs, as well as energy costs associated with other organism functions, including growth (Sokolova et al., 2012). The intake of phenolic compounds can modulate the capacity of mitochondrial biogenesis and several of their functions, including membrane potential, electron transfer chain activity, and ATP synthesis (Lagouge et al., 2006; Skemiene, Liobikas and Borutaite, 2015). In eukaryotes, mitochondrial biogenesis is stimulated by the modulation of the ATP/ADP ratio, activation of AMP-activated protein kinase (AMPK) pathway, and the subsequent expression of peroxisomal proliferator activator receptor γ co-activator 1α (PGC-1α) and nuclear respiratory factor-1 (Nrf1) transcription factors (Hardie, Ross and Hawley, 2012; Giampieri et al., 2017).

Recently, dietary polyphenols have been considered as activators of the AMPK signaling pathway – an intracellular sensor of ATP consumption - that is an important

cellular cascade involved in the response against oxidative damage and the regulation of energy homeostasis in skeletal muscle (Giampieri et al., 2017; Salomão et al., 2019). When under cell stress, the AMPK also can be activated by ATP depletion with an increase in the ratio of AMP:ATP (Lu et al., 2018). Thus, anabolic processes are blocked - such as lipogenesis (via inhibition of the pacemaker enzyme acetyl-CoA carboxylase); synthesis of glycogen (via glycogen synthetase kinase); gluconeogenesis (via glucose 6-phosphatase, PEP carboxykinase) and protein synthesis (via a mechanistic target rapamycin, complex 1 - mTORC1). To restore energy homeostasis, catabolic processes are increased, resulting in higher glucose utilization, mobilization of lipid stores and a turnover of macromolecules by autophagy (Herzig and Shaw, 2018; Thomson, 2018).

The main mechanism of action of plant-based fish growth promoters is associated with raise cellular protein synthesis (Fernández-Navarro et al., 2008; Domínguez-Vara et al., 2009; Baldisserotto et al., 2017). This process is a high ATP consumption event, since the cell growth and protein translation involve the modulation of the master regulator of growth mTOR (target of rapamycin) (Herzig and Shaw, 2018). The mTOR is relevant in regulating muscle size, integrating three main growth inputs such as growth factors (e.g. IGF-I), nutrients (e.g. amino acids), and energy status (i.e. AMP/ATP ratio via AMPK) (Loewith and Hall, 2011; Thomson, 2018). At this point, the favoring of anabolic processes probably correlates with the dynamic effects of the metabolic cascades (e.g. AMPK) stimulated by phytochemicals, which can potentiate the energy load and trigger processes such as protein synthesis with consequent skeletal muscle growth (Domínguez-Vara et al., 2009; Giampieri et al., 2017; Valenzuela-Grijalva et al., 2017; Martínez-Antonio, Racotta, Ruvalcaba-Márquez and Magallón-Barajas, 2019).

Dietary phytochemicals widely distributed in the plant kingdom have contributed to up-regulate the expression of growth genes in *Cyprinus carpio* and *Danio rerio* fed with

supplemented-diets with palm fruit (*Phoenix dactylifera* L.) and myrtle (*Myrtus communis* L.), respectively (Hoseinifar et al., 2017; Safari et al., 2017). In adults of *D. rerio*, ferulic acid promoted hypertrophic growth of fast skeletal muscle by up-regulation in the expression levels of myogenic transcriptional factors, translation efficiency (higher phosphorylated level of TOR) and protein synthesis capacity (Wen and Ushio, 2017). Furthermore, in juveniles of pacu (*Piaractus mesopotamicus*) a diet supplemented with resveratrol - a polyphenol found mainly in grape seeds - resulted in muscle hypertrophy and better body weight in fish submitted or not to exercise (Salomão et al., 2019). These results are probably effects of a dynamic modulation of process anabolic and catabolic by resveratrol, increasing the gene expression related to myogenesis, protein synthesis (IGF-I and mTOR), and oxidative metabolism in skeletal muscle – inhibiting the genes related to muscle atrophy (atrogenes) and preventing the degradation and loss of muscle mass (Salomão et al., 2019).

Lyophilized *Euterpe oleracea* (LEO) has been tested in diets for tambaqui fish *Colossoma macropomum* juveniles (da Silva et al., 2020), an omnivorous Amazon freshwater fish that is receiving an increasing interest in Latin America aquaculture (Cambos-Baca and Kohler, 2013, Guimarães and Martins, 2015, Saint-Paul, 2017). The inclusion of 1.25% LEO in the diet for tambaqui enhances the feed utilization, protein efficiency ratio, and the intestinal antioxidant competence. The LEO also reduced feed intake, whereas improves growth in this fish, being suggested the participation of nutrient-sensing mechanisms induced the satiation and amelioration of energetic status, respectively (da Silva et al., 2020). In this context, it is pertinent to consider the hypothesis that the dietary administration of LEO assists the maintenance of energy homeostasis and optimizes oxidative metabolic processes, which favor the energetic status of the tambaqui with subsequent growth. Thus, this study aimed to determine the effect of dietetic inclusion

of LEO on the energy fuels and reserves, as well as on energy potential based on measurement of the electron transport system (ETS) activity in the muscle of *C. macropomum* juveniles.

2. Material and Methods

This study followed the ethical standards established by the 'Conselho Nacional de Controle de Experimentação Animal' – CONCEA (Brasília, Brazil). The ethical and legal approval was obtained previously to the start of experiments through the Ethics Committee on Experimental Animals of the Instituto Federal do Pará – IFPA (Protocol number CEUA nº 3095220419).

2.1. Fish culture and experimental design

This study was conducted in the Laboratory of Aquaculture of Tropical Species (LAET) – IFPA (1°17'52.9"S 47°57'03.6"W). Tambaqui juveniles (0.92 ± 0.01 g average initial weight) were obtained from a local hatchery (Pará, Brazil). Fish were acclimatized for ten days before the experiment, being fed three times a day (09:00, 13:00, and 17:00 h) with a control diet (0.00% of LEO inclusion) at 10% of body weight. Afterward, tambaqui juveniles were randomly stocked into 18 200L-tanks at a density of 50 fish per tank. For the feeding trial, six experimental diets isoenergetic (9% of lipid) and isoproteic (40% of crude protein) were formulated (w/w): 0 g Kg^{-1} (Ctr.), 6 g Kg^{-1} (0.63%), 13 g Kg^{-1} (1.25%), 25 g Kg^{-1} (2.50%), 50 g Kg^{-1} (5.00%), and 100 g of LEO Kg^{-1} (10.0%). For 30 days, the fish were hand-fed until apparent satiation four times daily (08:00, 11:30, 14:00, and 17:30 h). The feeding rate was adjusted every five days, seeing a feed conversion ratio of 1.2:1. All treatments were performed in triplicate and under semi-static water system maintained with constant aeration and 50% water renewal every day. A description of the measurement of water physicochemical parameters can be found in the da Silva et al.

(2020). The water quality was maintained within the recommended ranges for the species (see Table 2 of the manuscript cited) (Gomes et al., 2013).

2.2. Diets and sampling

The six experimental diets were prepared as described in a previous study by da Silva et al. (2020). The diet without the inclusion of LEO (0 g Kg^{-1}) was used as a control (Ctr.). Fifteen fish per feed-treatment were sampled after 30 days of the feeding trial. For sampling, fish were euthanized by an overdose of eugenol (500 ppm) via bath immersion. Then, fish were dissected to collect muscle, which soon stored at −80 °C until biochemical analyses.

2.3. Parameters related to energy fuels and reserves

Total protein, enzymatic triglycerides, and enzymatic glucose levels were determined in muscle by colorimetric methods using commercial kits (Doles Reagentes LTDA) and according to the protocols of the manufacturer. The samples were homogenized according to the method adopted by Zamora-Sillero et al. (2013). The frozen muscle was homogenized for 10 min by ultrasonic disruption with 7.5 volumes of cooled 6% perchloric acid. After the ultrasonic bath, the homogenates were neutralized with the same volume of 1M potassium bicarbonate. The homogenates were centrifuged at 10,000 × g for 30 min and supernatants reserved.

Enzymatic cholesterol was measured through kits Doles according to the protocols of the manufacturer. The lipid extraction from muscle was adapted according to a standard technique of partitioning into chloroform:methanol (Langan, Rust and Volpe 1988). Muscle were homogenized (1:4 w/v) in 0.15M NaCl. The extract obtained was added (1:2 w/v) in a mix of the chloroform:methanol (4:2 v/v), followed by centrifugation at 3.000 × g for 5 min at 4 °C. The organic phase was dried down under gaseous nitrogen and then re-

suspended (1:2 w/v) with KOH at 20% in methanol. Saponification of the samples (70°C for 30 min) is followed by three consecutive extractions into petroleum ether. The combined ether extracts were dried under gaseous nitrogen and re-suspended with isopropanol (1:1 w/v).

The Dubois colorimetric method was utilized to measure glycogen levels in muscle. The muscle (250 mg) was added in 250 µl of KOH 6N containing 5% of saturated Na_2SO_4. Samples were heated at 100 °C for 20 min to promote alkaline digestion. After cooling, an aliquot of the extract (125 µl) was added to 1.5 mL of ethanol at 95% and centrifuged at 3,500 × g for 5 min at 15 °C. The supernatant was discarded and the pellet re-suspended in 1.25 mL of distilled water (Van Handel, 1965). Then an aliquot of this mixture (50 µl) was added to 70 µl phenol at 3% and 200 µl sulfuric acid (Dubois, 1956). Aliquots (250 µl) were transferred to a microplate and determined at 540 nm using a microplate reader (BioTek® Instruments, Inc).

2.4. Electron transport system (ETS)

The ETS activity method was adapted from Gopalan et al. (1996). The muscle was homogenized (1:6 w/v) in ice-cold homogenization buffer (0.09 M sodium phosphate buffer pH = 8.5) containing Na_2HPO_4 (0.09 M), $KHPO_4$ (0.09 M), 250 µl $MgSO_4$ (5 mM), 0.45 mg/mL polyvinyl pyrrolidone, and 0.16 % (v/v) Triton-X-100. Homogenate was centrifuged at 2,500 × g for 10 min at 4 °C, and the supernatant was stored at −80 °C. The total protein content was determined in triplicate by the Biuret method, using a commercial kit (Total Protein Kit Doles) and a microplate reader (BioTek® Instruments, Inc; wavelength: 550 nm). Posteriorly, the samples were diluted to 0.5 mg protein/mL using the homogenization buffer.

In a microplate was added 12.5 µl of substrate solution (0.01% Tris-Base buffer, 30 mM NADH, and 2.2 mM NADPH), 183.3 µl reaction buffer (0.1 M sodium phosphate buffer pH = 8.5, with 0.1 M Na_2HPO_4, 0.1 M $KHPO_4$, 55 µl $MgSO_4$ at 5 mM, and 0.275 mg/mL polyvinyl pyrrolidone), besides 55.6 µl INT solution (2.5 mM 2-p-iodophenyl 3-p-nitrophenyl 5-phenyl tetrazolium chloride). The reaction was initiated by adding 12.5 µl of muscle homogenate. The reaction was monitored for 30 min (a reading per minute) in a microplate reader (BioTek® Instruments, Inc; wavelength: 490 nm). The result was converted to equivalent oxygen utilized per unit mass per hour.

2.5. Statistical analyses

All data were submitted for analysis of homogeneity and normality by Levene's and Shapiro-Wilk tests, respectively. Descriptive statistics were executed by calculating the mean and the standard error (SE) for each tank. Results are presented by ordering each dose in a graphical display of mean and SE values. To determine the dose effects, a linear mixed-effects model was applied. In this model, the different inclusion LEO levels were defined as fixed effects and the different tanks employed at each LEO level as a random effect. The model was fitted using the restricted maximum likelihood method (REML). The main results are presented as estimated contrasts, which compare each dose level against the control. The statistical significance level was defined as 5% (Searle, Casella, & McCulloch, 2008) in all cases. A linear model was fitted to identify the relationship between the ETS activity and specific growth rate – SGR (data obtained from the da Silva et al., 2020) of animals fed the different experimental diets.

3. Results

Fish promptly accepted diets and survival was not affected significantly by the experimental diets over a 30-day feeding trial. Growth indexes and physicochemical parameters of water quality are summarized and described by the da Silva et al. (2020).

3.1. Energy Reserve Responses

Muscle cholesterol levels remained equivalent regardless of dietary treatments ($p > 0.05$). Dietary LEO also did not alter the levels of glucose, glycogen and total proteins in this tissue ($p > 0.05$). However, muscle triglyceride levels were reduced in fish fed diets containing 0.63% to 10.0% LEO per kg^{-1} compared to the control group ($p < 0.05$). In the group that received 0.63% LEO in the diet, was observed a reduction of 40.52% in the triglyceride level when compared to the control group (Table 1).

3.2. Electron transport system (ETS)

The ETS activity in the muscle of tambaqui is presented in Figure 1. The administration of LEO increased the metabolic potential in the muscle of this fish. LEO triggered a major increase in muscle ETS activity in the fish fed with 1.25% to 10.0% of açai (13106.75 ± 1080.733 and 18133.15 ± 1458.98 mg of consumed O_2/h/mg of protein, respectively) in respect to control and 0.63% LEO (7436.13 ± 919.72 and 7067.47 ± 800.822 of consumed O_2/h/mg of protein, respectively). This increase in muscle metabolic potential reaches 76.25% from the dietary inclusion of 1.25% LEO compared to the control group. The improvement in mitochondrial activity of the tambaqui muscle had correlated with SGR data ($R^2 = 0.87$; $p < 0.05$) (da Silva et al., 2020) as showed in Figure 2.

4. Discussion

The future of aquaculture demands improvement of the practices to promote the growth of cultivated organisms and ensure quality for a final product in line with the biosafety of this activity (Vélez et al., 2017; Reverter et al., 2017). From this viewpoint, is relevant to develop nutritional strategies to promote muscle growth and for reaching higher weight gain (Yang et al., 2015; Salomão et al., 2019). The use of plant extracts as growth promoters in terrestrial animal farming has been extensively studied (Valenzuela-Grijalva et al., 2017). Although recent studies confirm similar responses of phytochemicals on growth in fish (Hoseinifar et al., 2017; Safari et al., 2017; Wen and Ushio, 2017; Salomão et al., 2019; da Silva et al., 2020) few studies focus for effects on bioenergetics state in the fish muscle. The present study indicates that the inclusion of LEO in diets promoted muscle oxidative metabolism since mobilized an energy reserve (triglycerides) and substantially improved the metabolic potential of this organ. Therefore, these results are in accordance with the effects reported for LEO diets on the zootechnical performance of the tambaqui (da Silva et al., 2020), explaining the higher growth despite the reduced feed intake in fish treated with 1.25% to 10.0% inclusion of this fruit.

Additionally, the reduction of the muscle triglyceride content in the tambaqui fed with dietary LEO suggests a dynamic activation of both catabolic (mediated by AMPK) and anabolic processes. The hypolipidemic effect has also been reported in Fischer rats after açai pulp intake, particularly by reducing cholesterol and triacylglycerol levels (de Souza et al., 2010). Moreover, the administration of dietary cineole (eucalyptol) from 0.5 to 1.00% and 0.25 to 1.00% also decreased serum cholesterol and triglyceride levels, respectively, in rainbow trout (*Oncorhynchus mykiss*) (Hoseini et al., 2018). According to a previous study, açai could reduce glucose levels via AMPK activation followed by up-

regulation of glucose transporter 4 (GLUT4), suppressing the gluconeogenesis (Udani et al. 2011). The glycogen is the primary intracellular storable form of glucose and its levels are an immediate effect of insulin activity (Shivana et al., 2013). The high glucose concentration causes secretion of insulin, which trigger glucose uptake in peripheral tissues including muscle, besides increases glycogen synthase activity and inhibiting glycogen phosphorylase in this organ (Jensen and Lai, 2009; Hanhineva et al., 2010). Thus, since glucose levels were similar among all feed treatments, the glycogenesis and glycogenolysis processes were possibly not altered.

The regulatory mechanisms underlying the use of phytochemical additives for animal growth promoters are not yet fully understood. Meantime, in a review, Valenzuela-Grijalva et al. (2017) summarize the main mechanisms that support physiological changes and growth improvement of terrestrial animals, of which we like to highlight: (1) optimization of food status and animal feed intake; (2) antioxidant intestinal action, with prebiotic effect that results in better digestion of the nutrients and their absorption; (3) direct and indirect regulation of anabolic activity on target tissues. In terrestrial animals, phytochemicals may respond by modulating the metabolism in a manner equivalent to the action of β-adrenergic agonist compounds. It is proposed the structural similarities between some compounds of plant origin (e.g., derivatives of hydroxycinnamic acid and phenylalanine amino acid) to catecholamine hormones (Valenzuela-Grijalva et al., 2017).

These hormones interact with β-adrenergic receptor agonists (β-ARs) to alter animal metabolism, resulting in higher protein synthesis and lipolysis with decreasing lipogenesis (Domínguez-Vara et al., 2009; Valenzuela-Grijalva et al., 2017). It is notable the similarity in the activity of phytochemicals (Giampieri et al., 2017) to β-ARs in adipose tissues to promote lipid catabolism through the activation of AMPK (Domínguez-Vara et

al., 2009). For the muscle of terrestrial animals, it is postulated that β-ARs increase blood perfusion to this tissue, incrementing the availability of energy and amino acids, leading to higher protein synthesis and retention, resulting in muscle hypertrophy (Domínguez-Vara et al., 2009). Although the total protein muscle content was not increased by LEO, da Silva et al. (2020) verified an increase in the protein-efficiency ratio (PER) in the tambaqui from the inclusion of 1.25% LEO. This is in line with Fernández-Navarro et al. (2008), where the intake of maslinic acid (25 and 250 mg kg^{-1}) increments the PER and protein-synthesis rate in the white muscle of the *O. mykiss*.

Vertebrates from fish to mammals share a high level of likeness on the issue of the adrenergic receptor system (Fabbri and Moon, 2016). In fish, the oral administration of drugs β2-Adrenergic agonists play a similar role to catecholamines and bind to β-ARs to trigger anabolic actions in the muscle (Salem et al., 2006; Vélez et al., 2019). The Ractopamine (a β-adrenergic agonist) in diets for pacu (*P. mesopotamicus*) for example, increased protein retention and suppressed lipid anabolism, improving sensory and qualitative aspects of the fillet (Oliveira et al., 2014). This approach based on dietary LEO seems to be promising for tambaqui, which is considered an intermediate to high fat-fish (de Almeida and Franco, 2006). Thus, we propose the hypothesis that phytochemicals present in LEO (abundant source of hydroxycinnamic acid and other bioactive agents) interact with β-ARs to modulate the tambaqui metabolism up to anabolic processes, deriving energy from food and additionally from the oxidation of triglycerides (lipolysis) for growth (protein synthesis). It is expected that these effects should increase mitochondrial ETS activity, such as verified in the present study. Moreover, the high ETS activity in the fish fed with dietary LEO is significantly correlated with the specific growth ratio (SGR).

The increment of 76.25% of ETS activity in fish fed with the dietary inclusion from 1.25% LEO compared to control treatment points that the phytochemical profile of açai (*E. oleracea*) may exert anabolic promoting activity in muscle. The findings proved that this increment on muscle metabolic potential is favored via promoting lipolysis by dietary LEO from up 0.63% inclusion, resulting in a lowering of triglyceride levels. In this way, 1.25% LEO is promising for use in feed for *C. macropomum* juveniles, since the improved muscular bioenergetics state promoted by this fruit can favor physiological functions in this tissue, such as growth. Further studies are needed to answer the gaps detected in this study, especially to elucidate the endocrine mechanisms induced by açai in the regulation of muscle metabolism and its possible effects on the quality and sensory aspects of the fish fillet.

Acknowledgements

da Silva T.V.N. acknowledges Brazilian CAPES for the graduate scholarship (2016-2020) provided. We are thankful to the staff of the Tropical Species Aquaculture Laboratory - LAET/IFPA Castanhal for the support provided. Monserrat J. M. (# 308539/2016-8) and Sampaio L. A. (# 307445/2016-0) are research fellows of the Brazilian CNPq. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Conflicts of Interest

The authors have no actual or potential conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.11897940.v1, reference number 25.02.2020.

Author's Contributions

Thamyres V. N. da Silva, Luís A. Sampaio, and José M. Monserrat designed the study, with intellectual contribution from all authors. Jessica M. L. dos Santos, Glaycilane G. de Deus, Luis A. L. Barbas, Marcelo F. Torres, and Juan R. B. Ramírez contributed with the maintenance of the fish, analyses and technical support. José M. Monserrat carried out the statistical analyses and the application of the linear mixed effects model. Thamyres V. N. da Silva and José M. Monserrat drafted the manuscript and all authors contributed equally to the writing of the final version of the manuscript.

References

Abdel-Tawwab M, Adeshina I, Jenyo-Oni A, Ajani EK, Emikpe BO (2018) Growth, physiological, antioxidants, and immune response of African catfish, *Clarias gariepinus* (B.), to dietary clove basil, *Ocimum gratissimum*, leaf extract and its susceptibility to *Listeria monocytogenes* infection Fish Shellfish Immunol *78*: 346 – 354. http://doi.org/10.1016/j.fsi.2018.04.057

Alqurashi RM, Commane DM, Rowland IR (2016) Açai fruit as a source of bioactive phytochemicals *J Life Sci* 10: 391 – 404. http://doi.org/10.17265/1934-7391/2016.08.005

Al-Sagheer AA, Mahmoud HK, Reda FM, Mahgoub SA, Ayyat MS (2018) 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 immune activities *Aquac Nutr 24:* 1006 – 1014. https://doi.org/10.1111/anu.12637

Aranha LN, Silva MG, Uehara SK, Luiz RR, Neto JFN, Rosa G, de Oliveira GMM (2019) Effects of a hypoenergetic diet associated with açaí (*Euterpe oleracea* Mart.) pulp consumption on antioxidant status, oxidative stress and inflammatory biomarkers in overweight, dyslipidemic individuals *Clin Nutr* 00: 1 – 6. https://doi.org/10.1016/j.clnu.2019.06.008

da Silva RC, Batista A, da Costa DCF, Moura-Nunes N, Koury JC, da Costa CA, Resende A, Daleprane JB (2018) Açai (*Euterpe oleracea* Mart.) seed flour prevents obesity-induced hepatic steatosis regulating lipid metabolism by increasing cholesterol excretion in high-fat diet-fed mice *Food Res Int* 111: 408 – 415. https://doi.org/10.1016 / j.foodres.2018.05.043

da Silva TVN, dos Santos CF, Torres MF, Barbas LAL, Sampaio LA, Verde PE, Tesser MB, Monserrat JM (2020) Effects of dietary Amazonian açai (*Euterpe oleracea* Martius) fruit on growth performance, skin coloration and antioxidant status of *Colossoma macropomum*. Submitted to *Aquaculture Nutrition.*

de Almeida NM, Franco MRB (2006) Determination of essential fatty acids in captured and farmed tambaqui (*Colossoma macropomum*) from the Brazilian Amazonian area *J Amer Oil Chem Soc* 83*:* 707 – 711. https://doi.org/ 10.1007/s11746-006-5027-9

Campos-Baca L, Köhler CC (2005) Aquaculture of *Colossoma macropomum* and related species in Latin America. *Am Fish Soc Sym Ser*, 46, 541 – 561.

Chakraborty SB, Horn P, Hancz C (2014) Application of phytochemicals as growth-promoters and endocrine modulators in fish culture Rev Aquac 6: 1 – 19. https://doi.org/10.1111/raq.12021

Colombo GM, dos Santos SC, Schmitz MJ, Pedrosa VF, Romano LA, Tesser MB, Ramos PB, Wasielesky W, Monserrat JM (2020) The role of açaí (*Euterpe oleracea* Mart. 1824) as a chemoprotective agent in the evaluation of antioxidant defence, oxidative damage and histology of juvenile shrimp *Litopenaeus vannamei* (BOONE, 1931) exposed to ammonia *Aquac Res* 00: 1 – 16. https://doi.org/10.1111/are.14503

de Souza MO, Silva M, Silva ME, de Paula Oliveira R, Pedrosa ML (2010) Diet supplementation with acai (*Euterpe oleracea* Mart.) pulp improves biomarkers of oxidative stress and the serum lipid profile in rats *Nutrition* 26: 804 – 810. https://doi.org/10.1016/j.nut.2009.09.007

Domínguez-Vara IA, Mondragón-Ancelmo J, Ronquillo MG, Salazar-García F, Bórquez-Gastelum JL, Aragón-Martínez A (2009) Los β-agonistas adrenérgicos como modificadores metabólicos y su efecto en la producción, calidad e inocuidad de la

carne de bovinos y ovinos: una revisión *CIENCIA Ergo Sum Rev Cient Mult Prosp* 16: 278 – 284.

Dubois M, Gilles KA, Hamilton JK, Rebers PT, Smith F (1956) Colorimetric method for determination of sugars and related substances *Anal Chem* 28: 350 – 356. https://doi.org/10.1021/ac60111a017

Fabbri E, Moon TW (2016) Adrenergic signaling in teleost fish liver, a challenging path *Comp Biochem Physiol B Biochem Mol Biol* 199: 74 – 86. https://doi.org/10.1016/j.cbpb.2015.10.002

Fernández-Navarro M, Peragón J, Amores V, De La Higuera M, Lupiáñez JA (2008) Maslinic acid added to the diet increases growth and protein-turnover rates in the white muscle of rainbow trout (*Oncorhynchus mykiss*) *Comp Biochem and Physiol C Toxcicol Pharmacol* 147: 158 – 167. https://doi.org/10.1016/j.cbpc.2007.09.010

Fragoso MF, Prado MG, Barbosa L, Rocha NS, Barbisan LF (2012) Inhibition of mouse urinary bladder carcinogenesis by açai fruit (*Euterpe oleraceae* Martius) intake Plant Foods Hum Nutr 67: 235 – 241. https://doi.org/10.1007/s11130-012-0308-y

Giampieri F, Alvarez-Suarez JM, Cordero MD et al (2017). Strawberry consumption improves aging-associated impairments, mitochondrial biogenesis and functionality through the AMP-Activated Protein Kinase signaling cascade *Food Chem*, 234: 464 – 471. https://doi.org/10.1016/j.foodchem.2017.05.017

Gisbert E, Andree KB, Quintela JC, Calduch-Giner JA, Ipharraguerre IR, Pérez-Sánchez, J (2017) Olive oil bioactive compounds increase body weight, and improve gut health and integrity in gilthead sea bream (*Sparus aurata*) *Br J Nutr*, 117: 351 – 363. https://doi.org/10.1017/S0007114517000228

Gomes LC, Simões LN, Araújo-Lima CARM. (2013) Tambaqui (*Colossoma macropomum*). In: Baldisserotto B, Gomes LC (eds.), Espécies nativas para piscicultura no Brasil (2nd ed.). Santa Maria: Ed. UFSM, pp. 175 – 204.

Gopalan G, Madon SP, Culver DA, Pappas PW (1996) Measurement of metabolism in free ranging juvenile fishes using electron transport system (ETS) enzyme asssays. In: MacKinlay D, Nelson J (eds) *High Performance Fish, II Symposium Proceedings, International Congress on the Biology of Fishes* (pp. 21-30).

Guimarães IG, Martins GP (2015) Nutritional requirement of two Amazonian aquacultured fish species, *Colossoma macropomum* (Cuvier, 1816) and *Piaractus brachypomus* (Cuvier, 1818): A mini review J Appl Ichthyol 31: 57 – 66. https://doi.org/10.1111/jai.12976

Hanhineva K, Törrönen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkänen H, Poutanen K (2010) Impact of dietary polyphenols on carbohydrate metabolism *Int J Mol Sci*, 11, 1365 – 1402. https://doi.org/10.3390/ijms11041365

Hardie DG, Ross FA, Hawley SA (2012) AMPK: a nutrient and energy sensor that maintains energy homeostasis *Nat Rev Mol Cell Biol* 13: 251 – 262. https://doi.org/10.1038/nrm3311

Herzig S, Shaw RJ (2018) AMPK: guardian of metabolism and mitochondrial homeostasis *Nat Rev Mol Cell Biol* 19: 121. https://doi.org/10.1038/nrm.2017.95

Hoseini SM, Mirghaed AT, Iri Y, Ghelichpour M (2018) Effects of dietary cineole administration on growth performance, hematological and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*) *Aquaculture* 495: 766 – 772. https://doi.org/10.1016/j.aquaculture.2018.06.073

Hoseinifar SH, Dadar M, Khalili M, Cerezuela R, Esteban MA (2017) Effect of dietary supplementation of palm fruit extracts on the transcriptomes of growth, antioxidant

enzyme and immune-related genes in common carp (*Cyprinus carpio*) fingerlings Aquac Res 48: 3684 – 3692. https://doi.org/10.1111/are.13192

Jensen J, Lai YC (2009) Regulation of muscle glycogen synthase phosphorylation and kinetic properties by insulin, exercise, adrenaline and role in insulin resistance *Arch Physiol Piochem* 115: 13 – 21. https://doi.org/10.1080/13813450902778171

Kim JY, Hong JH, Jung HK, Jeong YS, Cho KH (2012) Grape skin and loquat leaf extracts and acai puree have potent anti-atherosclerotic and anti-diabetic activity *in vitro* and *in vivo* in hypercholesterolemic zebrafish Int J Mol Med 30: 606 – 614. https://doi.org/10.3892/ijmm.2012

Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1[alpha] *Cell* 12: 1109 – 1122. https://doi.org/10.1016/j.cell.2006.11.013

Langan TJ, Rust RS, Volpe JJ (1988) Steroids and related isoprenoids In: Alan A, Baker GB (eds.) Neuromethods 7 – Lipids and Related Compounds. Humana Press, Cliftion, New Jersey, pp 111 – 140

Li M, Zhu X, Tian J, Liu M, Wang G (2019) Dietary flavonoids from *Allium mongolicum* Regel promotes growth, improves immune, antioxidant status, immune-related signaling molecules and disease resistance in juvenile northern snakehead fish (*Channa argus*) *Aquaculture* 501: 473 – 481. https://doi.org/10.1016/j.aquaculture.2018.12.011

Loewith R, Hall MN (2011) Target of rapamycin (TOR) in nutrient signaling and growth control *Genetics* 189: 1177 – 1201. https://doi.org/10.1016/10.1534/genetics.111.133363

Lu Y, Nie M, Wang L et al (2018) Energy response and modulation of AMPK pathway of the olive flounder *Paralichthys olivaceus* in low-temperature challenged *Aquaculture* 484: 205 – 213. https://doi.org/10.1016/j.aquaculture.2017.11.031

Machado AK, Cadoná FC, Assmann CE et al (2019). Açaí (*Euterpe oleracea* Mart.) has anti-inflammatory potential through NLRP3-inflammasome modulation *J Funct Foods* 56: 364 – 371. https://doi.org/10.1016/j.jff.2019.03.034

Martínez-Antonio EM, Racotta IS, Ruvalcaba-Márquez JC, Magallón-Barajas F (2019) Modulation of stress response and productive performance of *Litopenaeus vannamei* through diet. *PeerJ 7*: e6850. https://doi.org/10.7717/peerj.6850

Moon TW (2001) Glucose intolerance in teleost fish: fact or fiction? *Comp Biochem Physiol B Biochem Mol Biol* 129: 243 – 249. https://doi.org/10.1016 / s1096-4959 (01) 00316-5

Monserrat JM, da Silva TVN (2020) Energetic metabolism in muscle of tambaqui juveniles (*Colossoma macropomum*) fed with dietary açai (*Euterpe oleracea* Mart). Figshare. Dataset. https://doi.org/10.6084/m9.figshare.11897940.v1

Oliveira LMFS, Leal RS, Mesquita TC, Pimenta MESG, Zangeronimo MG, Sousa RV, Alvarenga, RR (2014) Effect of ractopamine on the chemical and physical characteristics of pacu (*Piaractus mesopotamicus*) steaks *Arq Bras Med Veter Zoot* 66: 185 – 194. https://doi.org/10.1590/S0102-09352014000100026

Reverter M, Tapissier-Bontemps N, Sasal P, Saulnier D (2017) Use of medicinal plants in aquaculture. In: Austin B. Newaj-Fyzul A (eds.) Diagnosis and Control of Diseases of Fish and Shellfish. JohnWiley & Sons Ltd, New Jersey, pp 223–261

Safari R, Hoseinifar SH, Van Doan H, Dadar M (2017) The effects of dietary Myrtle (*Myrtus communis*) on skin mucus immune parameters and mRNA levels of

growth, antioxidant and immune related genes in zebrafish (*Danio rerio*) *Fish & Shellfish Immunol* 66: 264 – 269. https://doi.org/10.1016/j.fsi.2017.05.007

Saint-Paul U (2017) Native fish species boosting Brazilian's aquaculture development *Acta Fish Aquat Res* 5: 1 – 9. https://doi.org/10.2312/ActaFish.2017.5.1.1-9

Salem M, Levesque H, Moon TW, Rexroad CE, Yao J (2006) Anabolic effects of feeding β2-adrenergic agonists on rainbow trout muscle proteases and proteins *Comp Biochem Physiol A Mol Integr Physiol* 144: 145 – 154. https://doi.org/10.1016/j.cbpa.2006.02.021

Salomão RAS, De Paula TG, Zanella BTT et al (2019) The combination of resveratrol and exercise enhances muscle growth characteristics in pacu (*Piaractus mesopotamicus*). *Comp Biochem Physiol A Mol Integr Physiol 235*: 46 – 55. https://doi.org/10.1016/j.cbpa.2019.05.002

Schauss AG (2016) Advances in the study of the health benefits and mechanisms of action of the pulp and seed of the Amazonian palm fruit, *Euterpe oleracea* Mart., known as "Açai". In: Watson RR, Preedy VR (eds.) Fruits, Vegetables, and Herb, Oxford: Academic Press, pp 177 – 220

Searle SR, Casella G, McCulloch, CE (2008) Variance Components. John Wiley & Sons, Inc. Publication.

Shivanna N, Naika M, Khanum F, Kaul VK (2013) Antioxidant, anti-diabetic and renal protective properties of *Stevia rebaudiana J Diabetes Complications* 27: 103 – 113. https://doi.org/10.1016 / j.jdiacomp.2012.10.001

Simião CDS, Colombo GM, Schmitz MJ, Ramos PB, Tesser MB, Wasielesky Jr W, Monserrat JM (2020) Inclusion of Amazonian *Mauritia flexuosa* fruit pulp as functional feed in the diet for the juvenile Pacific white shrimp *Litopenaeus vannamei Aquac Res* 00: 1 – 12. https://doi.org/10.1111/are.14520

Skemiene K, Liobikas J, Borutaite V (2015) Anthocyanins as substrates for mitochondrial complex I - Protective effect against heart ischemic injury. *FEBS J* 282: 963 – 71. https://doi.org/10.1111/febs.13195

Sokolova IM, Frederich M, Bagwe R, Lannig G, Sukhotin AA (2012) Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Mar Environ Res* 79: 1 – 15. https://doi.org/10.1016/j.marenvres.2012.04.003

Souza-Monteiro JR, Hamoy M, Santana-Coelho D et al (2015) Anticonvulsant properties of *Euterpe oleracea* in mice. *Neurochem Int* 90: 20 – 27. https://doi.org/10.1016/j.neuint.2015.06.014

Sutili FJ, Gatlin III DM, Heinzmann BM, Baldisserotto B (2018) Plant essential oils as fish diet additives: benefits on fish health and stability in feed. *Rev Aquac* 10: 716 – 726. https://doi.org/10.1111/raq.12197

Tang KL, Caffrey NP, Nóbrega DB et al (2017) Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis *The Lancet Planetary Health* 1: e316 – e327. https://doi.org/10.1016/S2542-5196(17)30141-9

Thomson DM (2018) The role of AMPK in the regulation of skeletal muscle size, hypertrophy, and regeneration. *Int J Mol Sci* 19: 3125. https://doi.org/10.3390 / ijms19103125

Udani JK, Singh BB, Singh VJ, Barrett ML (2011) Effects of açai (*Euterpe oleracea* Mart.) berry preparation on metabolic parameters in a healthy overweight population: A pilot study. *Nutr J* 10: 45. https://doi.org/10.1186/1475-2891-10-45

Valenzuela-Grijalva NV, Pinelli-Saavedra A, Muhlia-Almazan A, Domínguez-Díaz D, González-Ríos H (2017) Dietary inclusion effects of phytochemicals as growth

promoters in animal production *J Anim Sci Technol* 59: 8. https://doi.org/10.1186 / s40781-017-0133-9

Van Handel E (1965) Estimation of glycogen in small amounts of tissue. *Anal Biochem* 11: 256 – 265. https://doi.org/10.1016 / 0003-2697 (65) 90013-8

Vélez EJ, Lutfi E, Azizi S et al (2017). Understanding fish muscle growth regulation to optimize aquaculture production. *Aquaculture* 467: 28 – 40. https://doi.org/10.1016/j.aquaculture.2016.07.004

Vélez EJ, Balbuena-Pecino S, Capilla E, Navarro I, Gutiérrez J, Riera-Codina M (2019) Effects of β2-adrenoceptor agonists on gilthead sea bream (*Sparus aurata*) cultured muscle cells *Comp Biochem Physiol A Mol Integr Physiol* 227: 179 – 193. https://doi.org/10.1016 / j.cbpa.2018.10.015

Wen Y, Ushio H (2017) Ferulic acid promotes hypertrophic growth of fast skeletal muscle in zebrafish model. *Nutrients* 9: 1066. https://doi.org/10.3390/nu9101066

Willis LB, Omar WSW, Sambanthamurthi R, Sinskey AJ (2008) Non-radioactive assay for acetyl-CoA carboxylase activity *J Oil Palm Res* 2: 30 – 36.

Yang C, Chowdhury MA, Huo Y, Gong J (2015) Phytogenic compounds as alternatives to in-feed antibiotics: potentials and challenges in application *Pathogens* 4: 137 – 156. https://doi.org/10.3390/pathogens4010137

Zamora-Sillero J, Ramos LRV, Romano LA, Monserrat JM, Tesser MB (2013) Effect of dietary dextrin levels on the growth performance, blood chemistry, body composition, hepatic triglycerides and glycogen of Lebranche mullet juveniles (*Mugil liza* Valenciennes 1836, Mugilidae) *J Appl Ichthyol* 29: 1342 – 1347. https://doi.org/10.1111/jai.12255

Zeppenfeld CC, Lopes JM, Baldisserotto B (2017) Promotores de crescimento na aquicultura. In: Baldisserotto B et al (eds) Farmacologia aplicada à Aquicultura. (Eds.), Ed. UFSM, Santa Maria, Rio Grande do Sul, pp 435 – 455.

Table 1. Fuels and energy reserves of muscle tissue of fish *Colossoma macropomum* after a 30-days feeding trial with dietary lyophilized *Euterpe oleracea* - LEO.

Parameters	LEO (%)					
	Ctr.	0.63	1.25	2.50	5.00	10.0
Cholesterol ($mg.dL^{-1}$)	22.8 ± 1.7^{ab}	24.9 ± 1.4^{a}	21.6 ± 1.4^{ab}	20.2 ± 1.0^{b}	22.1 ± 1.3^{ab}	19.6 ± 0.9^{b}
Glucose ($mg.dL^{-1}$)	17.7 ± 1.0^{ab}	19.1 ± 1.4^{a}	17.9 ± 1.2^{ab}	13.0 ± 1.2^{ab}	11.3 ± 0.9^{b}	13.1 ± 0.09^{ab}
Glycogen ($mg.mL^{-1}$)	0.79 ± 0.1^{a}	1.08 ± 0.1^{a}	0.82 ± 0.1^{a}	0.98 ± 0.1^{a}	1.34 ± 0.3^{a}	1.64 ± 0.3^{a}
Total protein ($mg.mL^{-1}$)	19.9 ± 1.0^{a}	14.1 ± 0.9^{b}	18.4 ± 0.8^{ab}	18.0 ± 0.9^{ab}	18.0 ± 1.2^{ab}	19.6 ± 1.1^{a}
Triglycerides ($mg.dL^{-1}$)	29.5 ± 1.6^{a}	17.5 ± 1.3^{b}	16.3 ± 0.6^{b}	19.8 ± 1.1^{b}	18.1 ± 1.0^{b}	15.0 ± 0.9^{b}

Note: Data are presented as means and standard errors of three replicates per treatment. Means with different superscripts in the same line are significantly different ($p < 0.05$; $n = 15$ per treatment).

Figure 1. Electron transport system (ETS) activity (mg of consumed O_2/h/mg of protein) of muscle tissue of fish *Colossoma macropomum* fed with diets with different levels of lyophilized *Euterpe oleracea* (LEO) for 30 days. Data are presented as means and standard errors of three tanks for treatment. Treatments with different letter denote significant differences between them ($p < 0.05$; $n = 15$ per treatment).

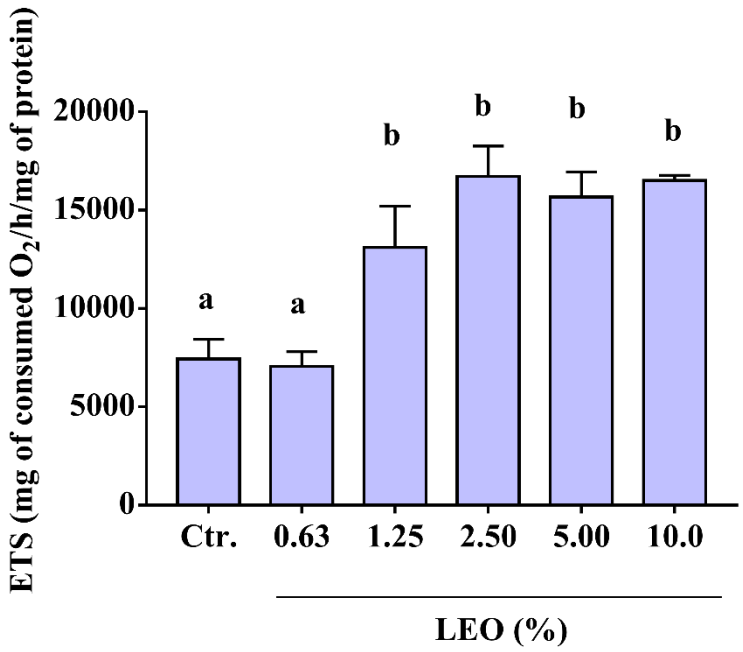
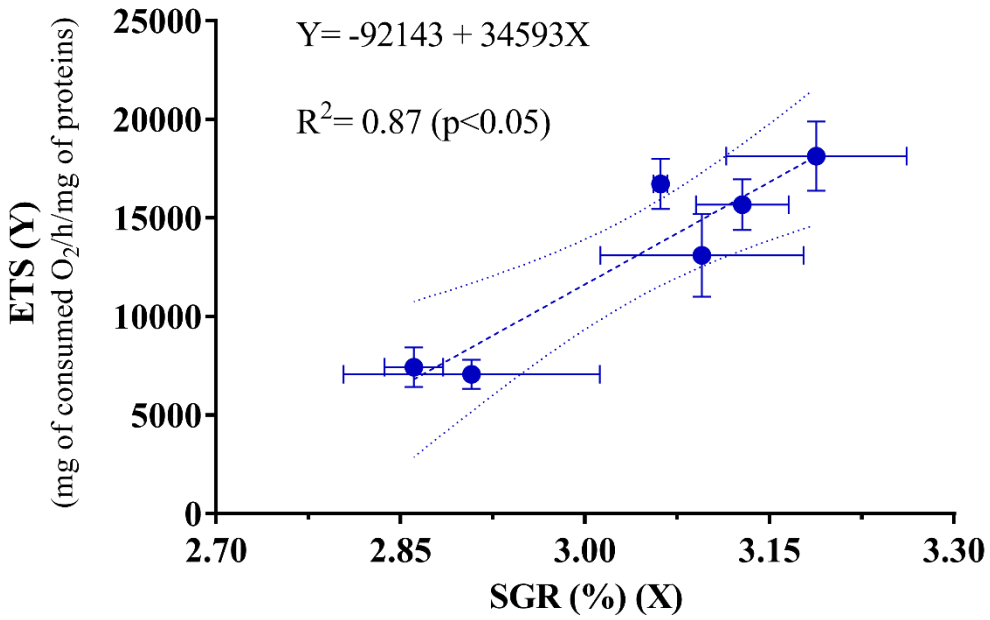


Figure 2. Linear regression describing relationships between electron transport system (ETS) activity and specific growth ratio (SGR) (da Silva et al., 2020) of fish *Colossoma macropomum* fed with diets with different levels of lyophilized *Euterpe oleracea* (LEO) for 30 days ($p < 0.05$; n = 15 per treatment).



CAPÍTULO 3

Effects of dietary Amazonian lyophilized fruit *Euterpe oleracea* Mart. on fish *Colossoma macropomum* submitted to transport stress

Thamyres Vanessa Nascimento da Silva[1,5*]; Robson Matheus Marreiro Gomes[1,5]; Marcelo Ferreira Torres[2]; Luis André Luz Barbas[2]; Luís André Sampaio[3,5]; José Maria Monserrat[1,4,5]

[1] Functional Biochemistry of Aquatic Organisms Laboratory (BiFOA), Center for Biotechnology and Diseases of Aquatic Organisms (CBD), Federal University of Rio Grande (FURG), Institute of Oceanography (IO), Rio Grande, RS, Brazil.

[2] Tropical Species Aquaculture Laboratory (LAET), Federal Institute of Pará (IFPA), Castanhal, PA, Brazil.

[3] Estuarine and Marine Fish Laboratory (LAPEM), Federal University of Rio Grande (FURG), Institute of Oceanography (IO), Rio Grande, RS, Brazil.

[4] Institute of Biological Sciences (ICB), Federal University of Rio Grande (FURG), Rio Grande, RS, Brazil.

[5] *Postgraduate program in Aquaculture*, Federal University of Rio Grande (FURG), Institute of Oceanography (IO), Rio Grande, RS, Brazil.

* Corresponding author at:
Thamyres V. N. da Silva, Laboratory of Functional Biochemistry of Aquatic Organisms (BIFOA), Federal University of Rio Grande - FURG, Institute of Oceanography (IO), CP 96201-900, Rio Grande, RS, Brazil.
E-mail: thamyrestvns@gmail.com

Manuscrito submetido à revista *Aquaculture*.
Formatado conforme as normas da revista.

Abstract

In this study, a 30-day feeding experiment was carried to evaluate the effects of the intake of lyophilized *E. oleracea* (LEO) by tambaqui fish *Colossoma macropomum* juveniles after shipping stress. Six experimental diets containing 0.00, 0.63, 1.25, 2.50, 5.00, and 10.0% (w/w) of LEO inclusion were considered. In plastic bags, a simulated transport was performed for up to 3, 6, 12 and 24 h. Dietary LEO prepares the tambaqui for long transport, particularly improving the availability of oxygen in the water. The dietary açai did not change the glucose levels after transport. However, 1.25% and 10.0% LEO promoted a better hepatic antioxidant capacity, being higher 42% and 53%, respectively, than the control when transported for up to 12 h. Nevertheless, the transport time was masterful to the impairment of antioxidant competence in the other organs studied (gills, brain, and muscle). In contrast, LEO counterbalanced the lipid peroxidation on brain, gills, liver and muscle of the tambaqui in up to 12 h, although still effective in the gills within 24 h. Thus, advantages are notable of LEO intake by tambaqui prior to transport. The inclusion of 2.50% to 5.00% LEO should be considered because provided a higher resistance against lipid damage in all organs evaluated and improved the water quality conditions for tambaqui juveniles transported in short to medium duration.

Keywords: dietary açai; tambaqui; water quality; oxidative stress; antioxidant capacity; lipid damage.

1. Introduction

Various managements in aquaculture, such as handling, biometrics, confinement, fertilization, transportation, and other operations can compromise the welfare of cultivated organisms at different stages of life (Sampaio and Freire, 2016; Vanderzwalmen et al., 2019). The shipping of live fish is usually carried out in closed systems (e.g. plastic bags) filled with a third of its volume with cold water and two-thirds with pure oxygen (Berka, 1986; Sampaio and Freire, 2016). Even so, this process is stressful and can induce mortality, being a decisive operation for the productivity and economic success of the sector (Vanderzwalmen et al., 2019).

The abrupt occurrence of disease and high mortality rates during and after this operation is associated with handling before transport, and especially, physiological injury induced by water deterioration due to accumulation of metabolites, such as ammonia and carbon dioxide (Sampaio and Freire, 2016). In an attempt to manage imbalances created by the stressor and restore the homeostatic state, primary stress responses are triggered, including secretion of corticosteroids and catecholamines. Secondarily are triggered hyperglycemia, gluconeogenesis and lipolysis. Cardiac and respiratory functions are also regulated by catecholamines, increasing the blood flux in the gills, brain and muscle, as well as of gill permeability. In freshwater fish, these responses to stress can promote a hydromineral disturbance, impairing osmoregulation. However, if exposure to stress is chronic, the growth and metabolic aptitude are impaired, affecting the reproduction, stress tolerance and survival - signals of stress tertiary response in fish (Barton and Iwama, 1991; Mommsen et al. 1999; Barton, 2002; da Silva et al., 2017; Souza et al., 2019).

Oxidative stress is the result of redox imbalance promoted by excessive pro-oxidants accumulation - including reactive oxygen species (ROS) and free radicals -

against antioxidants, induced by stress factors or xenobiotics (Jones, 2006; Halliwell and Gutteridge, 2015). These pro-oxidants can cause dysfunction of redox signaling/control resulted in oxidation of proteins, lipids, and DNA, with reversible or non-reversible cellular damage (Jones, 2006; Lushchak, 2011; Halliwell and Gutteridge, 2015). Typical stresses in the transport of live fish, such as hypoxia, hyperoxia, higher ammonia levels, and high stocking density can cause oxidative stress (Lushchak, 2011; Barbas et al., 2017a; da Silva et al., 2017; Refaey and Li, 2018; Hoseini et al., 2019; Zhao et al., 2019). Cells intercept and scavenge ROS and repair oxidative damage to cope with oxidative stress through the antioxidant defense system, constituted by endogenous enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST); and also via non-enzymatic antioxidant defense system, which is represented by nutrients or micronutrients (e.g. minerals, vitamins, lipoic acid and phytochemicals) obtained from the diet or produced by cells as reduced glutathione (Kütter et al., 2014; Hoseinifar et al., 2017; Baldissera et al., 2019; Rosas et al., 2019).

Plant extracts with antioxidant properties, including phenolic compounds and alkaloids, are being recently studied in aquaculture (Reverter et al., 2014; Sutili et al., 2018; Yousefi et al., 2020). In fish, growth performance, antioxidant defense and immune system can also be modulated by endocrine pathways through phytochemicals presents in plants (Chakraborty, Horn and Hancz, 2014; Farsani et al., 2019; Hoseinifar et al., 2019; Rosas et al., 2019; da Silva et al., 2020). Foods containing biologically active compounds provide functional properties to the diet, with beneficial physiologic effects to health, being able to treat diseases and/or its symptoms (Martirosyan and Miller, 2018). Therefore, the administration of functional feed before transport emerges as a strategy to alleviate stress and ensure the welfare of fish.

Several transport protocols are applied to mitigate stress in fish (Sampaio and Freire, 2016) which includes direct application of the salt in water of shipping (Tacchi et al., 2015), synthetic anesthetics (Barbas et al. 2017a), adequate levels of pure oxygen (da Silva et al., 2017), or probiotics (Dhanasiri et al., 2011). Moreover, the use of natural extracts of Amazonian plants as "jambu" (*Spilanthes acmella*) and "canela-amarela" (*Nectandra grandiflora*) in the water to shipping tambaqui (*Colossoma macropomum*) (Barbas et al., 2017b; 2019) and extract of "I-Tiao Gung" (*Glycine tomentella*) to transport of the orange-spotted grouper (*Epinephelus coioides*) (Wu et al., 2020) has been efficiently.

On the other hand, there is little information about the use of additives in the diet as a conditioner scope for stress transport. Vanderzwalmen et al. (2019) compile in a review feed supplements that were evaluated in fish transport, reporting the few studies that tested glucan, probiotics, ascorbic acid, carotenoids, and herbal products for this purpose. Reduction of oxidative stress in the transporting live fish is one of the effects reported in the literature, as in the case of saffron *Curcuma longa* supplementation in the diet on *Astyanax aff. bimaculatus*, which increased SOD activity and reduced lipid peroxidation (LPO) (Ferreira et al., 2017).

In aquaculture, the Amazon fruit açai (*Euterpe oleracea*) is emerging as a potent phytochemicals provider (Colombo et al., 2020; da Silva et al., 2020). *E. oleracea* is a palm tree native to South America that grows in the areas flooded by the rivers of the Brazilian Amazon. The antioxidant power of this palm tree is related to their profile of phenolic compounds, highlighting anthocyanins, quercetin, hydroxycinnamic acids. *E. olaracea* also contains oleic acid (MUFA) and linoleic acid (PUFA), fibers, and phytosterols (Alqurashi et al., 2016; Aranha et al., 2019). Although few studies evaluated the effects of *E. oleracea* on diets for aquatic organisms, the reported effects include an

increase in the antioxidant competence, lower inflammation and hepatic LPO on hypercholesterolemic zebrafish *Danio rerio* (Kim et al., 2012); better growth performance, increase the antioxidant capacity and lower intestinal LPO, and enhanced muscle metabolic potential in *C. macropomum* juveniles (da Silva et al., 2020); and reduced histopathological and oxidative damage in the Pacific shrimp (*Litopenaeus vannamei*) challenged with ammonia (Colombo et al., 2020).

The tambaqui (*C. macropomum*) is a South American fish freshwater widely distributed in the Brazilian Amazon and Orinoco river basins in Venezuela (Gomes et al., 2013). This fish is one of the main produced by Brazilian fish farming (Guimarães and Martins, 2015; Saint-Paul, 2017), with important participation in the production of other South and Central American countries (FAO, 2014), also waking interest in Asia's aquaculture industry. According to the arguments presented above, we hypothesize that lyophilized *E. oleracea* (LEO) administrated via the diet to tambaqui juveniles before transport can attenuate oxidative stress and enhance resistance to transport stress. Therefore, the present study aimed to evaluate the effects of prior LEO intake by tambaqui after simulated transport for up to 24 h, determining the water quality conditions, blood glucose levels, besides antioxidant competence and LPO levels in the brain, gills, liver and muscle of this fish.

2. Material and methods

The experiment was conducted following ethical standards instituted by the 'Conselho Nacional de Controle de Experimentação Animal' – CONCEA (Brazil). The experimental procedures described were approved by the Ethics Committee on Experimental Animals of the Instituto Federal do Pará – IFPA (Protocol number CEUA nº 3095220419). Experiments were carried in the Laboratory of Aquaculture of Tropical Species (LAET) – IFPA (1°17'52.9"S 47°57'03.6"W).

2.1. Fish and feed conditioning

Tambaqui juveniles (0.92 ± 0.01 g) were acquired from commercial channel fish farms (Pará, Brazil). The feed treatments constituted of six isoenergetic (9% of lipid) and isoproteic (40% of crude protein) diets containing 0 g Kg^{-1} (Ctr), 6 g Kg^{-1} (0.63%), 13 g Kg^{-1} (1.25%), 25 g Kg^{-1} (2.50%), 50 g Kg^{-1} (5.00%), and 100 g of LEO Kg^{-1} (10.0%) (w/w). Previously, fish were acclimated for 10 days on the laboratory environment and fed three times a day (09:00, 13:00, and 17:00 h) with a control diet (0.00% of LEO inclusion) at 10% of the body weight. After this period, 50 fish per tank (4 fish L^{-1}) were randomly stocked into 18 200L-tanks, being hand-fed four times a day (08:00, 11:30, 14:00, and 17:30 h) with the six experimental diets at 10.0% of body weight for 30 days. The feed ration was adjusted every five days, considering a feed conversion ratio of 1.2:1. Tanks were maintained with water at a temperature of 27.29 ± 0.11 °C, pH 6.33 ± 0.11, dissolved oxygen (DO) and total nitrogen ammonia (TAN) levels at 7.37 ± 0.01 mg L^{-1} and 1.89 ± 0.18 mg L^{-1}, respectively. The photoperiod was 12 h light:12 h dark. The six feed treatments were conducted considering three replicates each, on semi-static water system controlled with constant aeration and 50% water renewal every day. The full description of the feeding trial and its effects are available in da Silva et al. (2020).

2.2. Transport and experimental design

After 30 days, 360 fish (final average weight 7.63 ± 0.15 g) were fasted for 24 h and distributed into four-time groups for the exposure to simulated transport (3h, 6h, 12h e 24h). Then the fish were placed into 72 plastic bags, containing 1 L of water (5 fish bag^{-1}) and approximately 2 L of pure oxygen each. The feed treatments per transport time were tested with three replicates, being each plastic bag an experimental unit. The transport simulation was carried alternating between movements and stationary periods.

2.3. Measurement of water quality

After each transport exposure time, the bags were opened for immediate analyses of water parameters and quantification of mortality. The water temperature, DO and pH were measured using a multiparameter HANNA instruments® (HI 9828). Total nitrogen ammonia (TAN) and non-ionized ammonia were measured following to UNESCO (1983) and estimated from Ostrensky et al. (1992), respectively. The total alkalinity was determined according to the method of Eaton et al. (2005). Carbon dioxide (CO_2) concentration was calculated using Salt® software (Timmons and Ebeling, 2010).

2.4. Sample collection and preparation

Fish were euthanized by immersion in water containing a lethal concentration of eugenol (500 ppm), then the blood (n = 9 per treatment) was collected using a syringe (useful volume 0.5 mL) with anticoagulant from the caudal peduncle and put directly into test strips to glucose (automatic meter ACCU-CHEK). Posteriorly, were collected samples of the brain, gills, liver, and muscle (n =15 per treatment). The organs were homogenized (1:5 w/w) in a buffer composed of Tris-HCl (100 mM, pH 7.75) plus EDTA (2 mM) and Mg^{2+} (5 mM). The homogenates obtained were centrifuged at 10,000 x g for 20 min at 4°C and the resultant supernatants were stored at – 80°C. The Biuret method was utilized to determine the total protein content, following the manufacturer's instructions of the Total Protein Kit Doles (Amado et al., 2011). All biochemical parameters were determined with three replicates each.

2.5. Antioxidant capacity against peroxyl radicals (ACAP)

The total antioxidant capacity against peroxyl radicals measures antioxidant competence through ROS concentration in the tissues homogenates. In a white 96-well microplate, 10 μL of samples of tissues homogenates (previously fixed to 2.0 mg protein/mL with homogenization buffer) were disposed into the wells (six wells per

sample). Each well was added by 127.5 μL of reaction buffer (30 mM HEPES, pH 7.2, 200 mM KCl, and 1 mM $MgCl_2$). Three of the six wells out of each sample were treated with or without 2,2- azobis-2-methylpropionamidine dihydrochloride (7.5 μL of ABAP at 20 μM) (a molecule that generates peroxyl radicals via thermolysis). Fluorescence was generated by adding 10 μL of a solution of 2′,7′ dichlorofluorescein diacetate (H_2DCF-DA; final concentration: 40 μM). The H_2DCF-DA (Molecular Probes) is subsequently deacetylated generating the H_2DCF, which is oxidized by ROS, producing the fluorescent compound DCF detected at wavelengths of 485 and 530 nm for excitation and emission, respectively, under 37 °C in a microplate reader (Victor 2, Perkin Elmer). During the thermal decomposition of ABAP (Sigma-Aldrich), ROS generation was monitored for 30 min, with readings at every 5 min. Total fluorescence production was calculated by integrating the fluorescence units (FU) along the time of the measurement, after adjusting the FU data to a second-order polynomial function. Results were expressed as area difference of FU× min^{-1} in the same sample, with and without ABAP addition, standardized to the ROS area without ABAP (background area). Using this methodology, a reduced relative area means higher antioxidant capacity, because low fluorescence levels obtained after the addition of ABAP, indicate high competence in neutralizing peroxyl radicals (Amado et al., 2009).

2.6. Lipid peroxidation (LPO)

The concentration of thiobarbituric acid reactive substances (TBARS) was determined following the method of Oakes and Van Der Kraak (2003). In this trial, the reaction of the malondialdehyde (MDA) – a by-product of LPO – with thiobarbituric acid (TBA, from Baker) under high temperature and acidity produces a chromogen, which can be quantitated by fluorimetry. Samples of brain (20 μL), gills (50 μL), liver (20 μL) and muscle (100μL) were aliquoted to assay tubes (in duplicate) and then added 20 μL of

butylated hydroxytoluene solution (BHT at 67 μM), 150 μL of acetic acid (at 20% and pH 3.5), 150 μL of TBA (0.8%), 20 μL of sodium dodecyl sulfate (SDS at 8.1%), and 50 μL Milli-Q water. Then after mixing, samples were heated at 95 °C for 30 min and next cooled for 10 min. An aliquot of 100 μL of Milli-Q water was added in the assay tubes with the subsequent addition of the 500 μL of n-butanol. The final solution was centrifuged (3000 ×g for 10 min at 15 °C) and the alcoholic phase was placed in a microplate reader to determine the fluorescence (excitation: 520 nm and emission: 580 nm). Results were expressed as nmol TMP mg protein^{-1}, where TMP stands for tetramethoxypropane (Acros Organics) employed as a standard.

2.7. Statistical analysis

All data were checked for normality (Shapiro-Wilk) and homogeneity of variances (Levene). When the assumptions were not satisfied, mathematical transformations were applied. Descriptive statistics were performed by calculating the mean and the standard error (SE) for each tank. Data were submitted to a linear mixed-effects model, where different inclusion LEO levels were defined as fixed effects and the experimental unit employed as a random effect (Searle, Casella and McCulloch, 2008). The Newman–Keuls post-hoc test was applied to pairwise comparisons of means among treatments. The significance level was set at $p < 0.05$ in all cases (Zar, 1984). Another statistical analysis was performed using stepwise multiple regression, where the biochemical responses (total antioxidant competence and LPO) were adjusted to a model that included both the linear and quadratic terms of transport time and açai levels in the diet as well as the interaction term between these two independent variables. Data are presented as mean ± standard error (SE).

3. Results

3.1. *Water quality*

The water quality results are shown in Table 1. Alkalinity increased over time, especially after 24 hours ($p < 0.05$). The fish fed to the control and 0.63% LEO diets were under higher temperatures compared to the other treatments after 3 hours of transport ($p < 0.05$). Fluctuations in temperature were recorded in all the feed treatments within 6 h or in subsequent transport times ($p < 0.05$). Within 3 to 12 h, the DO levels remained equivalent among all the feed groups ($p > 0.05$). However, was observed variations in the DO level over time ($p < 0.05$). Particularly, in 24 h, the DO concentration decreased significantly in the control treatment, while the fish previously fed with LEO (especially 0.63% to 2.50% LEO) presented a higher concentration ($p < 0.05$).

The CO_2 concentration increased over the transport time ($p < 0.05$), without differing among the feed treatments within each transport time ($p > 0.05$). However, a slow accumulation of CO_2 over time was observed in fish fed with 5.00% to 10.0% dietary LEO ($p < 0.05$). In the control, 0.63% and 5.00% LEO groups the pH did not change significantly over time ($p > 0.05$). However, in 1.25%, 2.50% and 10.0% LEO groups, significant fluctuations in pH were recorded over time ($p < 0.05$). TAN was accumulated over the transport time in all feed groups ($p > 0.05$). However, the increase in TAN occurred more slowly in fish fed 10.0% LEO in the diet ($p < 0.05$). Un-ionized ammonia levels showed a significant increase in all feed groups after 24h of transport ($p < 0.05$).

3.2. Glucose levels

The results of blood glucose levels are shown in Figure 1. There were no changes in blood glucose in most feed treatments over the transport period ($p > 0.05$). However, the groups fed 0.63% LEO in the diet showed a reduction in glucose levels from 6 h of

transport ($p < 0.05$), which in the subsequent times were equivalent to the levels recorded in 3 h of transport ($p > 0.05$). A similar response was observed in the fish fed with 5.00% LEO in the diet.

3.3. *Antioxidant capacity against peroxyl radicals (ACAP)*

The ACAP responses over time of exposure to transport are presented in Table 2. The time of exposure to transport impaired the brain antioxidant competence in most feed treatments ($p < 0.05$). However, no changes were found for the brain antioxidant competence of fish fed 5.00% to 10.0% LEO in the diet during 24 h of transport ($p > 0.05$). For each transport time evaluated, there were no differences in the antioxidant capacity of the brain among all the LEO groups and control group ($p > 0.05$) (supplementary material, Figure 1a). In the gills, regardless of feed treatments, there were no improvements in antioxidant status over time or within each transport time ($p > 0.05$) (supplementary material, Figure 1b).

For the control and 0.63% LEO group, the hepatic antioxidant competence has not changed over time ($p > 0.05$). However, the inclusion of 1.25% to 10.0% LEO in the diet improved the antioxidant capacity of the liver of fish transported for up to 12 h ($p < 0.05$), reducing in the fish after 24 h ($p < 0.05$). Notably, within 12h, the hepatic antioxidant capacity increased by 42% and 53% in fish treated with 1.25% to 10.0% LEO in the diet, respectively, compared to the control ($p < 0.05$). On the other hand, within 24 h of transport, the antioxidant defense system of the liver was substantially disturbed in fish fed 1.25% to 10.0% LEO in the diet than in the control group ($p < 0.05$) (supplementary material, Figure 1c).

In fish control, long transportation determined reduction in the muscle antioxidant capacity ($p < 0.05$). Although there were no changes in the 0.63% LEO group to over time ($p > 0.05$), the ACAP in the muscle was reduced after 24 h in the 1.25% LEO group ($p < 0.05$). Besides that, a punctual improvement on 6 h of transportation was found in the muscle antioxidant competence of the 5.00% and 10.0% LEO group ($p < 0.05$). Overall, no significant amelioration in the muscle antioxidant capacity was found among the diets evaluated for each transport time ($p > 0.05$) (supplementary material, Figure 1d).

3.4. Lipid peroxidation (LPO)

The results for TBARS levels in the different transport times evaluated are shown in Table 3. In the brain, the TBARS level was significantly lower in fish control only after 24 hours of transport ($p < 0.05$). The dietary administration of 0.63% and 5.00% LEO resulted in lower LPO in the brain of tambaqui transported over 6 h to 24 h compared to fish transported for 3 h in both treatments ($p < 0.05$). Variations in the MDA content of the brain were recorded for fish fed with 1.25% LEO in the diet over transport time ($p < 0.05$). A significant reduction of 42% in cerebral LPO was obtained with 5.00% LEO dietetic into 6 to 24 h of transport compared to fish transported for 3h ($p < 0.05$). However, the fish fed with 2.50% and 10.0% LEO in the diet showed no changes in the brain TBARS level until the end of the transport ($p > 0.05$). Particularly, within 12 h of transport a reduction of 84% in the cerebral LPO was found in fish fed with 5.00% to 10.0% LEO in the diet in respect to control ($p < 0.05$). All of these effects of LEO on brain ACAP are analyzed in a two-dimensional contour plotting (2D) shown in Figure 2.

The MDA content in the gills of control group was increased among 6 h and 12h of transport ($p < 0.05$). However, in all groups fed with LEO, branchial MDA content remained unchanged in respect to fish transported for 3h ($p > 0.05$). In 12 h of transport,

the gills of the fish fed with 2.50% to 10.0% LEO in the diet showed a reduction of 25% to 45% in the LPO, respectively, compared to the control ($p < 0.05$). A reduction of 23% in branchial MDA content also was observed within 24h of transport for fish treated with 5.00% to 10.0% LEO in the diet compared with fish control ($p < 0.05$) (supplementary material, Figure 2a).

It is noteworthy that LEO promote effect hepatoprotective in the tambaqui. However, the time of exposure to transport was determinant for fluctuations in the hepatic MDA content, especially in the groups fed with diets 2.50% to 10.0% ($p < 0.05$). Within 3h of transport, the administration of 0.63% LEO in the diet decreased the hepatic LPO by 48% compared to control ($p < 0.05$). After 6 h of transport, the MDA concentration in the liver was lower by 48% for fish fed with 10.0% LEO dietetic in relation of control ($p < 0.05$). The liver of fish fed with 0.63% LEO and exposed to 12 h of transport showed a reduction of 51% in the TBARS level compared to the control ($p < 0.05$). In 24 h of transport, the hepatic LPO in fish fed with LEO did not differ from the control ($p > 0.05$) (supplementary material, Figure 2b).

Among all organs, the muscle had the lowest MDA content (< 0.30 nmol of MDA /mg protein^{-1}). During the transport, punctual alterations were observed in the muscle MDA content of the control, 0.63%, 1.25% and 10.0% LEO group ($p < 0.05$), except for fish previously fed with 2.50% and 5.00% LEO ($p > 0.05$).Dietary administration of 10.0% LEO promoted a reduction of 72% in the muscle LPO of fish transported for 3h, in respect to control ($p < 0.05$). Within the other transport times, significant differences were not verified for this organ ($p > 0.05$) (supplementary material, Figure 2c).

4. Discussion

Most experimental studies focus on the physiological effects of transport on the fish, while few studies have centered on water quality (Tacchi et al., 2015; Sampaio and Freire, 2016). In this study, the increased alkalinity, especially in long transport periods, could be associated with the accumulation of ammonia in the transport water (Salbego et al., 2015; Boyd, Tucker and Somridhivej, 2016). Un-ionized ammonia excreted from gills reacts with water to generate NH_4^+ and OH^-, contributing to alkalinity (Boyd, Tucker and Somridhivej, 2016). Most fish are ectotherms, and the temperature of the water is critical to their physiological reaction rates (Baldisserotto, 2013). During simulated transport in this study, the water temperature followed the daily environmental variations, but remained within the thermal comfort ranges for the *C. macropomum* (Gomes et al., 2013).

The goal of the use of pure oxygen for the transport of live fish in a plastic bag is to reduce the deterioration of water quality - promoted by the build-up of ammonia and CO_2 over time - and maximize the survival of fish (Carneiro et al., 2009; da Silva et al., 2017). In this study, water quality showed 17.7% higher OD levels after 24 h of transport in fish previously fed with LEO compared to control group. Besides temperature, the metabolic rate can influence the consumption of oxygen (Baldisserotto, 2013), being the CO_2 a by-product of fish metabolism (Harmon, 2009). Possibly, the higher availability of DO in the water of the fish previously fed with LEO, as well as the lateness in the accumulation of CO_2 in feed treatments 5.00% to 10.0% LEO after 24 h of transport could be a result of better conditioning to manage transport stress. Similarly, the addition of essential oil of *N. grandiflora* (30 μL L^{-1}) in the water positively influenced the maintenance of the DO for tambaqui after 10 h of transport (Barbas et al., 2019).

According to Sampaio and Freire (2016), the variables CO_2 and NH_3 could be linked to water pH changes, where the increase in pH occurs due to the increased ammonia levels after long transport periods. In some açai-treatments (1.25, 2.50 and 10.0% LEO), the pH fluctuated with a punctual reduction after 6 h of transport followed by a gradual increase in the subsequent times. Ammonia is a by-product generate from fish metabolism, primarily excreted by the gills per diffusion (Harmon, 2009; Baldisserotto, 2013). Particularly, in the fish fed with 10.0% LEO, the accumulation of TAN seems to be slower. However, the build-up of N-NH_3 was similar in all food treatment over time. This result could reflect the proportion of NH_3 and NH_4^+ in water because the concentration of both are dependent on pH, temperature, and alkalinity (Baldisserotto, 2013).

The alteration in the blood glucose levels is the physiological response most commonly to evaluate the secondary stress in fish shipping (Sampaio and Freire, 2016). Under stress, the increase in glucose provides extra energy to restore homeostasis (Kumar, Subburaj and Thiagarajan, 2015). In our study, a punctual reduction in blood glucose was recorded over 6 h of transport in fish fed with 0.63% and 5.00% LEO in the diet, increasing in subsequent times. In all cases, glucose levels ranged from approximately 60 $mg.dL^{-1}$ to just over 90 $mg.dL^{-1}$, which is close to the normal levels recorded for tambaqui in aquaculture systems (Chagas et al., 2012). After 24 h of transport the fish *A. aff. bimaculatus* fed with dietary *C. longa* showed a decrease in glucose levels (Ferreira et al., 2017). On the other hand, plasma glucose remained high in pacu (*Piaractus mesopotamicus*) previously fed with up to 2.00% *Aloe vera* on diets after 4 h of transport in plastic bags (Zanuzzo et al., 2017).

Dietary LEO has not mitigated the disturbance in the antioxidant defense system induced by transport stress on the brain, gills, and muscle of tambaqui. However, was

verified an increase in the hepatic antioxidant competence of 42% and 53% in fish treated with 1.25% to 10.0% LEO, respectively, after 12 h of transportation. It is noteworthy that the fish organs can present or no antioxidant responses to antioxidant supplementation in diets as reported in previous studies (Monserrat et al., 2008; Kütter et al., 2012; Ribeiro et al., 2018; da Silva et al., 2020). In fish and mice, studies have confirmed the hepatoprotective role of *E. oleracea* associated with their functional profile of polyphenols and flavonoids improving the redox balance via direct (radical scavenging) and indirect effects (by inducing antioxidant enzyme expression) (Kim et al., 2012; Bonomo et al., 2014; de Freitas Carvalho et al., 2019). However, the dietary inclusion 1.25% to 10.0% LEO reduced hepatic antioxidant competence compared to control into 24 h of transport. According to Halliwell and Gutteridge (2015), radicals derived from flavonoids can be generated during the antioxidant activity of these phytochemicals. Nevertheless, we emphasize that lipid damage was not detected in the tambaqui liver within 24 h of transport, discarding the possibility of pro-oxidant effects from LEO.

The LEO also played a fundamental role in the protection against LPO in all organs evaluated in the *C. macropomum*. Secondary plant metabolites (e.g. phenolic compounds) stabilize and interrupt the chain reaction of reactive species via electron donation or hydrogen atoms from their OH groups (Domínguez-Avila et al., 2018). For this reason, it is considered the LEO functional action as a potential exogenous antioxidant system for fish against the initiation and propagation of chain reaction of LPO. This action preserves the structure, fluidity, and permeability of the phospholipid membrane, which prevents disturbance and inhibition in the ion transport and metabolic processes, respectively (Halliwell and Gutteridge, 2015).

The dietary use of plants for fish is widely studied from the supplementation of essential oils with positive results on growth promotion and modulation of the immune and antioxidant defense system (Sutili et al., 2018). Moreover, the properties anesthetic, antiparasitic, antibacterial and anti-stress of the essential oils has directed several types of studies about the effects of their application in water for fish transport (Saccol et al., 2016; Barbas et al., 2017b; Saccol et al., 2018; Barbas et al., 2019). Vanderzwalmen et al. (2019) reviewed the use of plant extracts as dietary supplements on stress tolerance to fish transport and attested the limitation of studies with this focus. Nevertheless, corroborating with our results, dietary *C. longa* prepares *A. aff. bimaculatus* for transport stress, increasing the activity of the antioxidant enzymes SOD and CAT in the gills and liver, respectively, followed by a reduction in the TBARS levels in both organs after transport (Ferreira et al., 2017). Additionally, Colombo et al. (2020) have reported that dietary LEO also increases the total antioxidant capacity and effectively reduces the LPO in the hepatopancreas of shrimp *L. vannamei* challenged with ammonia for 96 h.

The protection provided by LEO to tambaqui liver is crucial since this organ performs central biotransformation for the detoxification and synthesis of several essential substances, determinants for the management of oxidative stress (Abou-Seif, Hozayen and Hashem, 2019). On the other hand, the brain is highly susceptible to oxidative stress and prone to present damage (Halliwell, 2006). The gills also are the target of injury caused by pollutants, and since they are in direct contact with water, their multifunctional activity can be impaired (Wood, 2017). Finally, the muscle performed a dynamic role from a metabolic point of view, often alternating between anabolic and catabolic processes to provide a supplemental load of amino acids and energy under stress conditions (Vélez et al., 2017). In general, the administration of LEO in diets resulted in lower lipid damage in the brain, liver, and muscle of tambaqui transported for up to 12h. However, LEO still effective in

the tambaqui gills for up to 24 h under transport. Thus, considering that the LPO is the major contributor to the dysfunction cellular under oxidative stress (Storey, 1996; Halliwell and Gutteridge, 2015), the protective effect of LEO in the different organs suggests a potential capacity to promote stress resistance in fish transported.

In summary, the 30-day dietary LEO intake by tambaqui juveniles seems to have consistently to prepare this fish for transport, improving oxygen availability and consequently the water quality conditions for a long period of transport in plastic bags. Although transport time reduces the antioxidant capacity of most organs, the dietary LEO played action antioxidant in the liver up to 12 h under transport. Moreover, notable the LEO intake has demonstrated an effective protector role against LPO in the brain, gills, liver, and muscle, presumably preserving the integrity and function of the organs. Based on these results, we recommend the inclusion of 2.50% to 5.00% of LEO for the formulation of functional diets for the transport of juvenile *C. macropomum* for up to 12 h. Furthermore, studies to evaluate the effects of the açai by-product (as seeds, for example) in diets for tambaqui are also encouraged.

Acknowledgements

da Silva T.V.N. acknowledges Brazilian CAPES for the graduate scholarship (2016-2020) provided. The authors would like to thank Simião, C., Colombo, G.M., Schmitz, M.J., Ramírez, J.R.B., for their assistance with the biochemical analyses and/or technical support. We are thankful to the staff of the Tropical Species Aquaculture Laboratory - LAET/IFPA Castanhal for the support provided. Monserrat J. M. (# 308539/2016-8) and Sampaio L. A. (# 307445/2016-0) are research fellows of the Brazilian CNPq. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Conflicts of Interest

The authors have no actual or potential conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.11898033.v1, reference number 25.02.2020.

Author's Contributions

T.V.N.S, L.A.L.B, L.A.S, and J.M.M. designed the study, with intellectual contribution from all authors. M.F.T and R.M.M.G contributed with the maintenance of the fish, experimental procedures, analyses and technical support. J.M.M and T.V.N.S carried out the statistical analyses and the application of the linear mixed effects model. M.B.T contributed with the experimental diet formulations as well as technical support. T.V.N.S and J.M.M. drafted the manuscript and all authors contributed equally to the writing of the final version of the manuscript.

References

Abou-Seif, H. S., Hozayen, W. G., & Hashem, K. S. (2019). *Thymus vulgaris* extract modulates dexamethasone induced liver injury and restores the hepatic antioxidant redox system. *Beni-Suef University Journal of Basic and Applied Sciences*, *8*, 1 – 9.

Alqurashi, R. M., Commane, D. M., & Rowland, I. R. (2016). Açai fruit as a source of bioactive phytochemicals. *Journal of Life Sciences*, 10, 391 – 404.

Amado, L. L., Ramos, P. B., Freitas, R. F., Zafalon, B., Ferreira, J. L., Yunes, J. S., & Monserrat, J. M. (2009). A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: application to evaluate microcystins toxicity. *Science of the Total Environment*, 407, 2115 – 2123.

Amado, L. L., Garcia, M. L., Pereira, T. C., Yunes, J. S., Bogo, M. B., & Monserrat, J. M. (2011). Chemoprotection of lipoic acid against microcystin-induced toxicosis in common carp (*Cyprinus carpio*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 154, 146 – 153.

Aranha, L. N., Silva, M. G., Uehara, S. K., Luiz, R. R., Neto, J. F. N., Rosa, G., & de Oliveira, G. M. M. (2019). Effects of a hypoenergetic diet associated with açaí (*Euterpe oleracea* Mart.) pulp consumption on antioxidant status, oxidative stress and inflammatory biomarkers in overweight, dyslipidemic individuals. *Clinical Nutrition*, 00, 1 – 6.

Baldissera, M. D., Souza, C. F., Baldisserotto, B., Zimmer, F., Paiano, D., Petrolli, T. G., & da Silva, A. S. (2019). Vegetable choline improves growth performance, energetic metabolism, and antioxidant capacity of fingerling Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, *501*, 224 – 229.

Baldisserotto, B. (2013). Fisiologia de peixes aplicada à piscicultura. Baldisserotto, B. (ed). Santa Maria, RS. 3° ed. UFSM Ed. 352 pp.

Barbas, L. A. L., Pereira-Cardona, P. M., Maltez, L. C., Garcia, L. O., Monserrat, J. M., & Sampaio, L. A. (2017a). Anaesthesia and transport of juvenile tambaqui *Colossoma macropomum* (Cuvier, 1818) with tricaine methane-sulphonate: Implications on secondary and oxidative stress responses. *Journal of Applied Ichthyology, 33*, 720 – 730.

Barbas, L. A. L., Maltez, L. C., Stringhetta, G. R., de Oliveira Garcia, L., Monserrat, J. M., da Silva, D. T., ... & Sampaio, L. A. (2017b). Properties of two plant extractives as anaesthetics and antioxidants for juvenile tambaqui *Colossoma macropomum*. *Aquaculture*, *469*, 79 – 87.

Barbas, L. A. L., de Araújo, E. R. L., Torres, M. F., Maltez, L. C., de Oliveira Garcia, L., Heinzmann, B. M., & Sampaio, L. A. (2019). Stress relieving potential of two plant-based sedatives in the transport of juvenile tambaqui *Colossoma macropomum*. *Aquaculture*, 734681.

Barton, B. A., & Iwama, G. K. (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases*. 1: 3 – 26.

Barton, B. A. (2002). Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integrative and Comparative Biology*, 42, 517 – 525.

Berka, R., 1986. The transport of live fish: a review. EIFAC Tech. Pap. FAO Rome. 48, 52.

Bonomo, F. L., Silva, D. N., Boasquivis, P. F., Paiva, F. A., Guerra, J. F., Martins, T. A., ... & Boulanger, E. (2014). Açaí (*Euterpe oleracea* Mart.) modulates oxidative stress resistance in *Caenorhabditis elegans* by direct and indirect mechanisms. *PloS one*, *9*, e89933 – e89933.

Boyd, C. E., Tucker, C. S., & Somridhivej, B. (2016). Alkalinity and hardness: critical but elusive concepts in aquaculture. *Journal of the World Aquaculture Society*, *47*, 6 – 41.

Carneiro, P. C. F., Kaiseler, P. H. S., Swarofsky, E. A. C., & Baldisserotto, B. (2009). Transport of jundiá *Rhamdia quelen* juveniles at different loading densities: water quality and blood parameters. *Neotropical Ichthyology*, 7, 283 – 288.

Chakraborty, S. B., Horn, P., & Hancz, C. (2014). Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. *Reviews in Aquaculture*, 5: 1 – 19.

Chagas, E. C., Araújo, L. D. D., Gomes, L. D. C., Malta, J. C. D. O., & Varella, A. M. B. (2012). Efeito do cloreto de sódio sobre as respostas fisiológicas e controle de helmintos monogenóides em tambaqui (*Colossoma macropomum*). *Acta Amazonica, 42*, 439 – 444.

Colombo, G. M., Simião, C. S., Schmitz, M. J., Pedrosa, V. F., Romano, L. A., Tesser, M. B., ... & Monserrat, J. M. The role of açaí (*Euterpe oleracea* Mart. 1824) as a chemoprotective agent in the evaluation of antioxidant defence, oxidative damage and histology of juvenile shrimp *Litopenaeus vannamei* (BOONE, 1931) exposed to ammonia. *Aquaculture Research, 00, 1 – 16.*

da Silva, T. V. N., Barbas, L. A. L., Torres, M. F., Sampaio, L. A., & Monserrat, J. M. (2017). Lipid peroxidation and antioxidant capacity in *Peckoltia oligospila* (Günther, 1864) submitted to transport under different concentration of dissolved oxygen. *Aquaculture, 481*, 72 – 78.

da Silva, T. V. N., dos Santos, C. F., Torres, M. F., Barbas, L. A. L., Sampaio, L. A., Verde, P. E., Tesser, M. B., & Monserrat, J. M. (2020). Effects of dietary Amazonian açai (*Euterpe oleracea* Martius) fruit on growth performance, skin

coloration and antioxidant status of *Colossoma macropomum*. Submitted to *Aquaculture Nutrition*.

Dhanasiri, A. K. S., Kiron, V., Fernandes, J. M. O., Bergh, Ø., & Powell, M. D. (2011). Novel application of nitrifying bacterial consortia to ease ammonia toxicity in ornamental fish transport units: trials with zebrafish. *Journal of Applied Microbiology*, *111*, 278 – 292.

de Freitas Carvalho, M. M., Lage, N. N., de Souza Paulino, A. H., Pereira, R. R., de Almeida, L. T., da Silva, T. F., ... & da Costa Guerra, J. F. (2019). Effects of acai on oxidative stress, ER stress, and inflammation-related parameters in mice with high fat diet-fed induced NAFLD. *Scientific Reports*, *9*, 1 – 11.

Domínguez-Avila, J. A., Ruiz-Canizales, J., Pacheco-Ordaz, R., Villegas-Ochoa, M. A., González Aguilar, G. A. (2018). Antioxidant power. In: Nollet, L. M., Gutierrez-Uribe, J. A. (eds.). Phenolic compounds in food: Characterization and analysis. CRC Press. Taylor & Francis Group. pp. 261 – 277.

Eaton, A. D., Clesceri, L. S., Rice, E. W., & Greenberg, A. E. (2005). Standard methods for the examination of water and wastewater, (21st ed). American Public Health Association, Springfield.

Gomes, L. C., Simões, L. N., & Araújo-Lima, C. A. R. M. (2013). Tambaqui (*Colossoma macropomum*). In: Baldisserotto, B., & Gomes, L.C. (eds.), Espécies nativas para piscicultura no Brasil (2 ed.). Santa Maria: Ed. UFSM. pp. 175 – 204.

Guimarães, I. G., & Martins, G. P. (2015). Nutritional requirement of two Amazonian aquacultured fish species, *Colossoma macropomum* (Cuvier, 1816) and *Piaractus brachypomus* (Cuvier, 1818): A mini review. *Journal of Applied Ichthyology*, 31, 57 – 66.

Farsani, M. N., Hoseinifar, S. H., Rashidian, G., Farsani, H. G., Ashouri, G., & Van Doan, H. (2019). Dietary effects of *Coriandrum sativum* extract on growth performance, physiological and innate immune responses and resistance of rainbow trout (*Oncorhynchus mykiss*) against *Yersinia ruckeri*. *Fish & Shellfish Immunology*, *91*, 233 – 240.

Ferreira, P. D. M. F., Rocha, J. S., Gomes, J. R., Caldas, D. W., Martins, M. T. S., de Oliveira, J. M., ... & Zuanon, J. A. S. (2017). *Curcuma longa* supplementation in the diet of *Astyanax aff. bimaculatus* in preparation for transport. *Aquaculture Research*, *48*, 4524 – 4532.

Food and Agriculture Organization of the United Nations (FAO), 2014. The State of World Fisheries and Aquaculture, p. 243.

Halliwell, B. (2006). Oxidative stress and neurodegeneration: where are we now?. *Journal of Neurochemistry*, *97*, 1634-1658.

Halliwell, B., & Gutteridge, J. M. C. (2015). Free radicals in biology and medicine. Oxford (5th ed). Oxford University Press. EUA.

Harmon, T. S. (2009). Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. *Reviews in Aquaculture*, *1*, 58-66.

Hoseini, S. M., Yousefi, M., Hoseinifar, S. H., & Van Doan, H. (2019). Cytokines' gene expression, humoral immune and biochemical responses of common carp (*Cyprinus carpio*, Linnaeus, 1758) to transportation density and recovery in brackish water. *Aquaculture*, *504*, 13 – 21.

Hoseinifar, S. H., Dadar, M., Khalili, M., Cerezuela, R., & Esteban, M. A. (2017). Effect of dietary supplementation of palm fruit extracts on the transcriptomes of growth,

antioxidant enzyme and immune-related genes in common carp (*Cyprinus carpio*) fingerlings. *Aquaculture Research*, 48, 3684 – 3692.

Hoseinifar, S. H., Zou, H. K., Van Doan, H., Harikrishnan, R., Yousefi, M., Paknejad, H., & Ahmadifar, E. (2019). Can dietary jujube (*Ziziphus jujuba* Mill.) fruit extract alter cutaneous mucosal immunity, immune related genes expression in skin and growth performance of common carp (*Cyprinus carpio*)?. *Fish & Shellfish Immunology*, *94*, 705 – 710.

Jones, D. P. (2006). Extracellular redox state: refining the definition of oxidative stress in aging. *Rejuvenation Research*, 9, 169 – 181.

Kim, J. Y., Hong, J. H., Jung, H. K., Jeong, Y. S., & Cho, K. H. (2012). Grape skin and loquat leaf extracts and acai puree have potent anti-atherosclerotic and anti-diabetic activity in vitro and in vivo in hypercholesterolemic zebrafish. *International Journal of Molecular Medicine*, 30, 606 – 614.

Kumar, P., Thirunavukkarasu, A. R., Subburaj, R., & Thiagarajan, G. (2015). Concept of stress and its mitigation in aquaculture. In: *Advances in Marine and Brackishwater Aquaculture*. Springer, New Delhi. pp. 95 – 100.

Kütter, M. T., Monserrat, J. M., Primel, E. G., Caldas, S. S., & Tesser, M. B. (2012). Effects of dietary α-lipoic acid on growth, body composition and antioxidant status in the Plata pompano *Trachinotus marginatus* (Pisces, Carangidae). *Aquaculture*, 368, 29 – 35.

Kütter, M. T., Romano, L. A., Ventura-Lima, J., Tesser, M. B., & Monserrat, J. M. (2014). Antioxidant and toxicological effects elicited by alpha-lipoic acid in aquatic organisms. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *162*, 70 – 76.

Lushchak, V. I. (2011). Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology*, 101, 13 – 30.

Martirosyan, D., & Miller, E. (2018). Bioactive compounds: The key to functional foods. *Bioactive Compounds in Health and Disease*, *1*, 36 – 39.

Mommsen, T., Vijayan, M. & Moon, T. (1999) Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries*, 9, 211 – 268.

Monserrat, J. M., Lima, J. V., Ferreira, J. L. R., Acosta, D., Garcia, M. L., Ramos, P. B., Moraes, B. T., dos Santos, L. C., & Amado, L. L. (2008). Modulation of antioxidant and detoxification responses mediated by lipoic acid in the fish *Corydoras paleatus* (Callychthyidae). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 148, 287 – 292.

Monserrat, J. M.; da Silva, T. V. N. (2020). Effects of dietary Amazonian lyophilized fruit *Euterpe oleracea* Mart. on fish *Colossoma macropomum* submitted to transport stress. Figshare. Dataset. https://doi.org/10.6084/m9.figshare.11898033.v1

Oakes, K. D., & Der Kraak, G. J. V. (2003). Utility of the TBARS assay in detecting oxidative stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill effluent. *Aquatic Toxicology*, 63, 447 – 463.

Ostrensky, A., Marchiori, M.A., Poersch, L.H. (1992). Toxicidade aguda da amônia no processo produtivo de pós-larvas de *Penaeus paulensis*, Perez-Farfante, 1967. *Anais da Academia Brasileira de Ciências*, 64, 383 – 389.

Refaey, M. M., & Li, D. (2018). Transport stress changes blood biochemistry, antioxidant defense system, and hepatic HSPs mRNA expressions of channel catfish *Ictalurus punctatus*. *Frontiers in Physiology*, *9*, 1628.

Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., & Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives, *Aquaculture*, 433, 50 – 61.

Ribeiro, S. C., Malheiros, D. F., Guilozki, I. C., Majolo, C., Chaves, F. C. M., Chagas, E. C., ..., Yoshioka, E. T. O. (2018). Antioxidants effects and resistance against pathogens of *Colossoma macropomum* (Serassalmidae) fed *Mentha piperita* essential oil. *Aquaculture*, 490, 29 – 34.

Rosas, V. T., Monserrat, J. M., Bessonart, M., Magnone, L., Romano, L. A., & Tesser, M. B. (2019). Comparison of β-carotene and Spirulina (*Arthrospira platensis*) in mullet (*Mugil liza*) diets and effects on antioxidant performance and fillet colouration. *Journal of Applied Phycology*, *31*, 2391 – 2399.

Saccol, E. M., Toni, C., Pês, T. S., Ourique, G. M., Gressler, L. T., Silva, L. V., ... & Pavanato, M. A. (2017). Anaesthetic and antioxidant effects of *Myrcia sylvatica* (G. Mey.) DC. and *Curcuma longa* L. essential oils on tambaqui (*Colossoma macropomum*). *Aquaculture Research*, *48*, 2012 – 2031.

Saccol, E. M. H., Parrado-Sanabria, Y. A., Gagliardi, L., Jerez-Cepa, I., Mourão, R. H. V., Heinzmann, B. M., ... & Martos-Sitcha, J. A. (2018). Myrcia sylvatica essential oil in the diet of gilthead sea bream (*Sparus aurata* L.) attenuates the stress response induced by high stocking density. *Aquaculture nutrition*, *24*, 1381-1392.

Salbego, J., Becker, A. G., Parodi, T. V., Zeppenfeld, C. C., Gonçalves, J. F., Loro, V. L., ... & Baldisserotto, B. (2015). Methanolic extract of *Condalia buxifolia* added to transport water alters biochemical parameters of the silver catfish *Rhamdia quelen*. *Aquaculture*, *437*, 46 – 50.

Sampaio, F. D., & Freire, C. A. (2016). An overview of stress physiology of fish transport: changes in water quality as a function of transport duration. *Fish and Fisheries*, *17*, 1055 – 1072.

Saint-Paul, U. (2017). Native fish species boosting Brazilian's aquaculture development. *Acta of Fisheries and Aquatic Resources*, 5, 1 – 9.

Searle, S. R., Casella, G., & McCulloch, C. E. (2008). Variance Components. John Wiley & Sons, Inc. Publication.

Souza, C., Baldissera, M., Baldisserotto, B., Heinzmann, B., Martos-Sitcha, J. A., & Mancera, J. M. (2019). Essential oils as stress-reducing agents for fish aquaculture: a review. *Frontiers in Physiology*, *10*, 785.

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

Sutili, F. J., Gatlin III, D. M., Heinzmann, B. M., & Baldisserotto, B. (2018). Plant essential oils as fish diet additives: benefits on fish health and stability in feed. *Reviews in Aquaculture*, 10, 716 – 726.

Tacchi, L., Lowrey, L., Musharrafieh, R., Crossey, K., Larragoite, E. T., & Salinas, I. (2015). Effects of transportation stress and addition of salt to transport water on the skin mucosal homeostasis of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, *435*, 120 – 127.

Timmons, M.B., & Ebeling, J.M., 2010. Recirculating Aquaculture, 2nd. Ed. Cayuga Aqua Ventures, Ithaca, New York, pp. 998.

Unesco (1983). Chemical methods for use in marine environmental monitoring. Manual and Guides 12. Intergovernamental Oceanographic Commissiony, Paris, France.

Vanderzwalmen, M., Eaton, L., Mullen, C., Henriquez, F., Carey, P., Snellgrove, D., & Sloman, K. A. (2019). The use of feed and water additives for live fish transport. *Reviews in Aquaculture, 11*, 263 – 278.

Vélez, E. J., Lutfi, E., Azizi, S., Perelló, M., Salmerón, C., Riera-Codina, M., ... & Navarro, I. (2017). Understanding fish muscle growth regulation to optimize aquaculture production. *Aquaculture*, *467*, 28 – 40.

Wu, S. M., Tseng, Y. J., Lin, J. J. & Pan, B. S. (2020). Mitigação do estresse e deterioração da água com um extrato radicular de *Glycine tomentella* durante o transporte simulado de garoupa manchada de laranja (*Epinephelus coioides*). *Aquaculture* , *514* , 734485.

Wood, C. M. (2017). Toxic responses of the gill. In: Target organ toxicity in marine and freshwater teleosts. CRC Press. pp. 1 – 89.

Yousefi, M., Shabunin, S. V., Vatnikov, Y. A., Kulikov, E. V., Adineh, H., Hamidi, M. K., & Hoseini, S. M. (2020). Effects of lavender (*Lavandula angustifolia*) extract inclusion in diet on growth performance, innate immunity, immune-related gene expression, and stress response of common carp, *Cyprinus carpio*. *Aquaculture*, *515*, 734588.

Zanuzzo, F. S., Sabioni, R. E., Montoya, L. N. F., Favero, G., & Urbinati, E. C. (2017). *Aloe vera* enhances the innate immune response of pacu (*Piaractus mesopotamicus*) after transport stress and combined heat killed *Aeromonas hydrophila* infection. *Fish & Shellfish Immunology*, *65*, 198 – 205.

Zar, J.H., 1984. Biostatistical Analysis, 2nd ed. Prentice Hall, New Jersey.

Zhao, C., Xu, J., Xu, X., Wang, Q., Kong, Q., Xu, F., & Du, Y. (2019). Organ-specific responses to total ammonia nitrogen stress on juvenile grass carp

(*Ctenopharyngodon idellus*). *Environmental Science and Pollution Research*, *26*, 10826 – 10834.

Table 1. Water quality parameters of *Colossoma macropomum* previously fed for 30-days with lyophilized *Euterpe oleracea* (LEO) in diets and submitted to different times of simulated transport. Data are expressed as mean ± 1 standard error of the mean (SEM) (n =3).

Table 1		LEO (%)					
Parameters	Time (h)	Ctr	0.63	1.25	2.50	5.00	10.0
Alkalinity ($mg\ L^{-1}$)	03	18.33 ± 1.66^{Ca}	18.33 ± 1.66^{Ba}	18.33 ± 1.66^{Ca}	20.00 ± 0.00^{Ca}	18.33 ± 1.66^{Ba}	20.00 ± 0.00^{Ca}
	06	23.33 ± 1.66^{Aba}	21.66 ± 1.66^{Ba}	18.33 ± 1.66^{Ca}	15.00 ± 0.00^{Da}	18.33 ± 3.33^{Ba}	21.66 ± 1.66^{Ca}
	12	28.33 ± 1.66^{Ba}	23.33 ± 1.66^{Ba}	25.00 ± 0.00^{Ba}	27.50 ± 2.50^{Ba}	23.33 ± 1.66^{Ba}	26.66 ± 1.66^{Ba}
	24	46.66 ± 1.66^{Aa}	46.66 ± 4.40^{Aa}	43.33 ± 8.81^{Aa}	47.50 ± 2.50^{Aa}	45.00 ± 2.88^{Aa}	43.33 ± 6.66^{Aa}
pH	03	6.33 ± 0.08^{Aa}	6.35 ± 0.08^{Aa}	6.32 ± 0.03^{Aa}	6.26 ± 0.06^{Aa}	6.25 ± 0.05^{Aa}	6.49 ± 0.10^{Aa}
	06	6.34 ± 0.08^{Aa}	6.14 ± 0.07^{Aa}	6.09 ± 0.03^{Ca}	6.05 ± 0.05^{Ca}	6.05 ± 0.05^{Aa}	6.15 ± 0.04^{Ba}
	12	6.12 ± 0.04^{Aa}	6.09 ± 0.00^{Aa}	6.18 ± 0.02^{BCa}	6.00 ± 0.20^{Da}	6.11 ± 0.14^{Aa}	6.06 ± 0.02^{Ba}
	24	6.18 ± 0.00^{Aa}	6.17 ± 0.03^{Aa}	6.23 ± 0.04^{Ba}	6.13 ± 0.00^{Ba}	6.17 ± 0.02^{Aa}	6.22 ± 0.02^{Ba}
CO_2 ($mg\ L^{-1}$)	03	22.66 ± 5.23^{Ca}	22.33 ± 5.36^{Ca}	23.00 ± 2.00^{Ca}	32.00 ± 0.00^{Da}	27.33 ± 1.76^{Ba}	18.33 ± 4.66^{Ba}
	06	29.00 ± 7.23^{Ca}	44.00 ± 4.72^{Ba}	38.66 ± 4.97^{Ba}	35.00 ± 1.00^{Ca}	44.66 ± 14.24^{Ba}	40.00 ± 3.21^{Aba}
	12	56.00 ± 4.16^{Ba}	49.00 ± 3.78^{Ba}	43.33 ± 2.33^{Ba}	71.50 ± 7.50^{Ba}	50.33 ± 12.25^{Ba}	61.00 ± 4.16^{Aa}
	24	79.33 ± 3.52^{Aa}	80.66 ± 2.60^{Aa}	65.66 ± 10.20^{Aa}	91.00 ± 6.00^{Aa}	78.33 ± 2.40^{Aa}	67.66 ± 11.85^{Aa}
DO ($mg\ L^{-1}$)	03	24.01 ± 0.24^{Aa}	24.07 ± 0.08^{Aa}	24.10 ± 0.06^{Aa}	24.03 ± 0.04^{Aa}	24.47 ± 0.17^{Aa}	24.10 ± 0.20^{Aa}
	06	21.83 ± 0.37^{Ca}	22.33 ± 0.33^{Ca}	21.76 ± 0.58^{Ba}	22.45 ± 0.15^{Ca}	22.50 ± 0.05^{Ca}	22.20 ± 0.65^{Ba}
	12	23.06 ± 0.03^{Ba}	23.16 ± 0.03^{Ba}	23.13 ± 0.41^{ABa}	23.40 ± 0.00^{Ba}	23.00 ± 0.00^{Ba}	22.70 ± 0.35^{Ba}
	24	18.63 ± 1.71^{Daa}	21.80 ± 1.25^{Cc}	22.60 ± 0.55^{ABc}	23.05 ± 0.05^{Cc}	21.93 ± 0.03^{Db}	21.90 ± 0.00^{Cb}
Temperature (°C)	03	27.96 ± 0.14^{Ad}	27.15 ± 0.31^{Acd}	26.13 ± 0.02^{Dbc}	26.07 ± 0.02^{Db}	26.03 ± 0.03^{Dab}	25.97 ± 0.02^{Da}
	06	27.76 ± 0.03^{Ac}	27.63 ± 0.03^{Ac}	27.66 ± 0.05^{Ac}	27.60 ± 0.10^{Ac}	27.40 ± 0.05^{Bb}	27.20 ± 0.00^{Ba}
	12	26.83 ± 0.03^{Bb}	26.80 ± 0.00^{Ab}	26.60 ± 0.00^{Ca}	26.60 ± 0.00^{Ca}	26.93 ± 0.03^{Cc}	26.96 ± 0.06^{Cc}
	24	26.93 ± 0.03^{Ba}	26.86 ± 0.03^{Aa}	26.90 ± 0.00^{Ba}	26.90 ± 0.00^{Ba}	28.03 ± 0.06^{Ab}	28.10 ± 0.00^{Ab}
TAN ($mg\ L^{-1}$)	03	1.41 ± 0.05^{Da}	1.41 ± 0.05^{Da}	1.47 ± 0.02^{Da}	1.35 ± 0.03^{Da}	1.68 ± 0.15^{Da}	1.63 ± 0.09^{Ca}
	06	2.22 ± 0.04^{Ca}	2.19 ± 0.10^{Ca}	2.35 ± 0.13^{Ca}	2.46 ± 0.13^{Ca}	2.42 ± 0.12^{Ca}	2.19 ± 0.03^{Ca}
	12	3.95 ± 0.25^{Ba}	3.57 ± 0.22^{Ba}	3.70 ± 0.17^{Ba}	3.31 ± 0.16^{Ba}	3.55 ± 0.13^{Ba}	3.41 ± 0.28^{Ba}
	24	5.37 ± 0.07^{Aa}	5.42 ± 0.14^{Aa}	4.99 ± 0.09^{Aa}	5.81 ± 0.20^{Aa}	5.55 ± 0.31^{Aa}	5.01 ± 0.56^{Aa}
$N\text{-}NH_3$ ($\mu g\ L^{-1}$)	03	1.84 ± 0.45^{Ba}	1.75 ± 0.33^{Ba}	1.58 ± 0.13^{Ca}	1.23 ± 0.15^{Da}	1.47 ± 0.06^{Ba}	2.57 ± 0.46^{Ba}
	06	2.88 ± 0.60^{Ba}	1.73 ± 0.23^{Ba}	1.66 ± 0.21^{Ca}	1.55 ± 0.07^{Ca}	1.57 ± 0.28^{Ba}	1.70 ± 0.19^{Ba}
	12	2.75 ± 0.18^{Ba}	2.52 ± 0.20^{Ba}	2.92 ± 0.25^{Ba}	1.74 ± 0.00^{Ba}	2.82 ± 1.06^{ABa}	2.07 ± 0.06^{Ba}
	24	4.39 ± 0.10^{Aa}	4.32 ± 0.32^{Aa}	4.55 ± 0.32^{Aa}	4.20 ± 0.22^{Aa}	4.74 ± 0.34^{Aa}	4.91 ± 0.73^{Aa}

Note: Different lowercase letter superscripts indicate significant differences ($p < 0.05$) among diets within the same transport time. Different capital letter superscripts indicate significant differences ($p < 0.05$) over transport time within the same diet treatment.

Table 2. Total antioxidant capacity against peroxyl radicals (ACAP) in the brain, gills, liver and muscle of fish *Colossoma macropomum* previously fed with lyophilized *Euterpe oleracea* (LEO) in diets and submitted to simulate transport for 3, 6, 12 and 24 h in plastic bags. Data are expressed as mean ± 1 standard error of the mean (SEM) (n = 9 per treatment).

Organs	Time (h)	LEO (%)					
		Ctr.	0.63	1.25	2.50	5.00	10.0
		ACAP (relative area)					
Brain	03	0.78 ± 0.22^{Ba}	1.11 ± 0.24^{Ba}	0.84 ± 0.15^{Ba}	1.27 ± 0.20^{Ba}	1.53 ± 0.11^{Aa}	1.36 ± 0.18^{Aa}
	06	1.81 ± 0.15^{Aa}	1.30 ± 0.24^{Ba}	2.46 ± 0.19^{Aa}	1.94 ± 0.19^{Aa}	1.84 ± 0.30^{Aa}	1.92 ± 0.30^{Aa}
	12	1.39 ± 0.25^{Aa}	1.77 ± 0.34^{Ba}	1.86 ± 0.32^{Aa}	2.40 ± 0.30^{Aa}	2.37 ± 0.37^{Aa}	2.38 ± 0.52^{Aa}
	24	1.65 ± 0.16^{Aa}	2.75 ± 0.32^{Aa}	1.85 ± 0.28^{Aa}	2.11 ± 0.41^{ABa}	1.84 ± 0.05^{Aa}	1.94 ± 0.38^{Aa}
Gills	03	0.60 ± 0.19^{Aa}	0.47 ± 0.11^{Aa}	0.51 ± 0.06^{Aa}	0.38 ± 0.12^{Aa}	0.33 ± 0.13^{ABa}	0.33 ± 0.14^{Ba}
	06	0.30 ± 0.12^{Aa}	0.12 ± 0.05^{Ba}	0.22 ± 0.06^{Aa}	0.21 ± 0.05^{Aa}	0.18 ± 0.05^{Ba}	0.24 ± 0.07^{Ba}
	12	0.74 ± 0.27^{Aa}	0.94 ± 0.27^{Aa}	0.29 ± 0.08^{Aa}	0.38 ± 0.09^{Aa}	0.69 ± 0.16^{Aa}	0.57 ± 0.10^{Aa}
	24	0.39 ± 0.06^{Aa}	0.44 ± 0.08^{Aa}	0.42 ± 0.06^{Aa}	0.29 ± 0.07^{Aa}	0.37 ± 0.07^{Aba}	0.29 ± 0.06^{Ba}
Liver	03	4.70 ± 1.16^{Aa}	3.73 ± 1.12^{ABa}	6.22 ± 0.15^{Ba}	4.06 ± 1.22^{Ba}	6.83 ± 0.26^{ABa}	4.37 ± 0.67^{Ba}
	06	5.47 ± 1.05^{Aa}	3.12 ± 1.31^{Ba}	3.39 ± 1.47^{Da}	4.91 ± 1.79^{Ba}	2.56 ± 0.79^{Ca}	4.05 ± 1.54^{Ba}
	12	7.54 ± 0.48^{Aa}	5.76 ± 0.64^{ABab}	4.32 ± 0.72^{Cb}	5.18 ± 1.23^{Bab}	5.58 ± 0.43^{Bab}	3.54 ± 0.40^{Bb}
	24	5.74 ± 0.30^{Ab}	6.99 ± 0.55^{Abc}	9.28 ± 0.73^{Aac}	11.42 ± 0.62^{Aa}	10.52 ± 1.82^{Aa}	11.42 ± 1.13^{Aa}
Muscle	03	2.85 ± 0.51^{Aa}	2.42 ± 0.53^{Aa}	0.71 ± 0.10^{Ca}	1.68 ± 0.44^{Aba}	1.59 ± 0.12^{Ba}	1.60 ± 0.15^{Ba}
	06	2.01 ± 0.16^{Aa}	2.25 ± 0.21^{Aa}	2.16 ± 0.12^{Aa}	2.51 ± 0.41^{Aa}	2.61 ± 0.16^{Aa}	3.59 ± 0.42^{Aa}
	12	2.03 ± 0.27^{Aa}	2.07 ± 0.39^{Aa}	1.23 ± 0.15^{Ba}	1.92 ± 0.14^{Aba}	1.22 ± 0.14^{Ba}	1.39 ± 0.26^{Ba}
	24	1.24 ± 0.17^{Ba}	1.58 ± 0.13^{Aa}	1.96 ± 0.34^{Aa}	1.31 ± 0.34^{Ba}	1.56 ± 0.16^{Ba}	1.94 ± 0.17^{Ba}

Note: Different lowercase letter superscripts indicate significant differences ($p < 0.05$) among diets within the same transport time. Different capital letter superscripts indicate significant differences ($p < 0.05$) over transport time within the same diet treatment.

Table 3. Lipid peroxidation levels measured by concentration of thiobarbituric acid reactive substances (TBARS) in the brain, gills, liver and muscle of fish *Colossoma macropomum* previously fed with lyophilized *Euterpe oleracea* (LEO) in diets and submitted to simulate transport for 3, 6, 12 and 24 h in plastic bags. Data are expressed as mean ± 1 standard error of the mean (SEM) (n = 9 per treatment).

Organs	Time (h)	LEO (%)					
		Ctr.	0.63	1.25	2.50	5.00	10.0
		TBARS (nmol MDA mg protein^{-1})					
Brain	03	3.63 ± 1.33^{Aa}	4.35 ± 1.02^{Aa}	3.26 ± 0.95^{Aa}	1.06 ± 0.12^{Aa}	1.46 ± 0.38^{Aa}	1.07 ± 0.14^{Aa}
	06	2.07 ± 0.63^{Aa}	1.00 ± 0.21^{Ba}	0.97 ± 0.30^{Ba}	2.32 ± 0.79^{Aa}	0.84 ± 0.32^{Ba}	0.68 ± 0.15^{Aa}
	12	4.08 ± 0.10^{Aa}	2.09 ± 0.77^{Bab}	1.94 ± 0.54^{Aab}	1.46 ± 0.63^{Aab}	0.64 ± 0.13^{Bb}	0.99 ± 0.34^{Ab}
	24	1.01 ± 0.42^{Ba}	0.94 ± 0.31^{Ba}	0.60 ± 0.30^{Ba}	1.65 ± 0.53^{Aa}	0.84 ± 0.32^{Ba}	0.55 ± 0.14^{Aa}
Gills	03	2.07 ± 0.11^{Ba}	1.88 ± 0.08^{Aa}	1.79 ± 0.12^{Ba}	1.80 ± 0.13^{Aa}	1.74 ± 0.05^{ABa}	1.68 ± 0.08^{Aa}
	06	2.41 ± 0.10^{Aa}	2.25 ± 0.20^{Aa}	1.99 ± 0.10^{Aba}	2.10 ± 0.10^{Aa}	2.62 ± 0.26^{Aa}	2.20 ± 0.26^{Aa}
	12	2.71 ± 0.13^{Aa}	2.44 ± 0.19^{Aab}	2.21 ± 0.13^{Aab}	2.03 ± 0.40^{Abc}	1.50 ± 0.11^{Bc}	1.48 ± 0.14^{Ac}
	24	1.92 ± 0.09^{Ba}	1.91 ± 0.10^{Aa}	1.83 ± 0.07^{Ba}	1.75 ± 0.12^{Aa}	1.48 ± 0.07^{Bb}	1.42 ± 0.05^{Ab}
Liver	03	9.60 ± 0.92^{Aa}	5.02 ± 0.92^{Ab}	3.02 ± 0.64^{Ab}	2.12 ± 0.87^{Bb}	2.67 ± 0.25^{Bb}	1.68 ± 0.47^{Bb}
	06	7.51 ± 0.46^{Aa}	5.95 ± 1.05^{Aab}	5.35 ± 0.54^{Aab}	7.75 ± 0.81^{Aa}	6.08 ± 0.53^{Aab}	3.92 ± 0.65^{Ab}
	12	8.09 ± 0.59^{Aa}	3.95 ± 0.38^{Ac}	3.55 ± 0.33^{Ac}	6.04 ± 0.73^{ABb}	3.47 ± 0.70^{Bc}	5.54 ± 0.31^{Abc}
	24	7.29 ± 0.53^{Aa}	5.42 ± 0.70^{Aa}	4.82 ± 0.10^{Aa}	4.70 ± 0.45^{Aa}	4.14 ± 1.04^{Aba}	5.08 ± 0.44^{Aa}
Muscle	03	0.25 ± 0.04^{Aa}	0.10 ± 0.01^{Aab}	0.18 ± 0.02^{Aab}	0.13 ± 0.03^{Aab}	0.10 ± 0.02^{Aab}	0.07 ± 0.01^{Bb}
	06	0.11 ± 0.02^{Ba}	0.06 ± 0.01^{Ba}	0.05 ± 0.01^{Ba}	0.09 ± 0.02^{Aa}	0.08 ± 0.01^{Aa}	0.05 ± 0.01^{Ba}
	12	0.05 ± 0.01^{Ba}	0.09 ± 0.01^{Aa}	0.07 ± 0.01^{Ba}	0.09 ± 0.02^{Aa}	0.10 ± 0.01^{Aa}	0.04 ± 0.01^{Ba}
	24	0.07 ± 0.01^{Ba}	0.11 ± 0.01^{Aa}	0.10 ± 0.02^{Ba}	0.12 ± 0.02^{Aa}	0.06 ± 0.01^{Aa}	0.13 ± 0.02^{Aa}

Note: Different lowercase letter superscripts indicate significant differences ($p < 0.05$) among diets within the same transport time. Different capital letter superscripts indicate significant differences ($p < 0.05$) over transport time within the same diet treatment.

Figure captions

Figure 1. Blood glucose concentration (mg dL^{-1}) in fish *Colossoma macropomum* fed or not with lyophilized *Euterpe oleracea* (LEO) in the diet after simulated transport for 3, 6, 12 and 24h on plastic bags (n = 9 per treatment). Different lowercase letter superscripts in lines indicate significant differences among diet treatments within the same transport time. Different capital letter superscripts in the columns indicate significant differences over transport time within the same diet treatment. The absence of letters indicates that the results are not statistically different ($p > 0.05$) after performing the Newman–Keuls post hoc test. Values are expressed as means ± 1 standard error of the mean (SEM).

Figure 2. Two-dimensional (2D) contour plot analysis of lipid peroxidation levels measured by concentration of thiobarbituric acid reactive substances – TBARS (nmol MDA mg $protein^{-1}$) in the brain of fish *Colossoma macropomum* as a function of dietary levels of lyophilized *Euterpe oleracea* (LEO) and simulated transport for 3, 6, 12 and 24h on plastic bags (n = 9 per treatment).

Figure 1

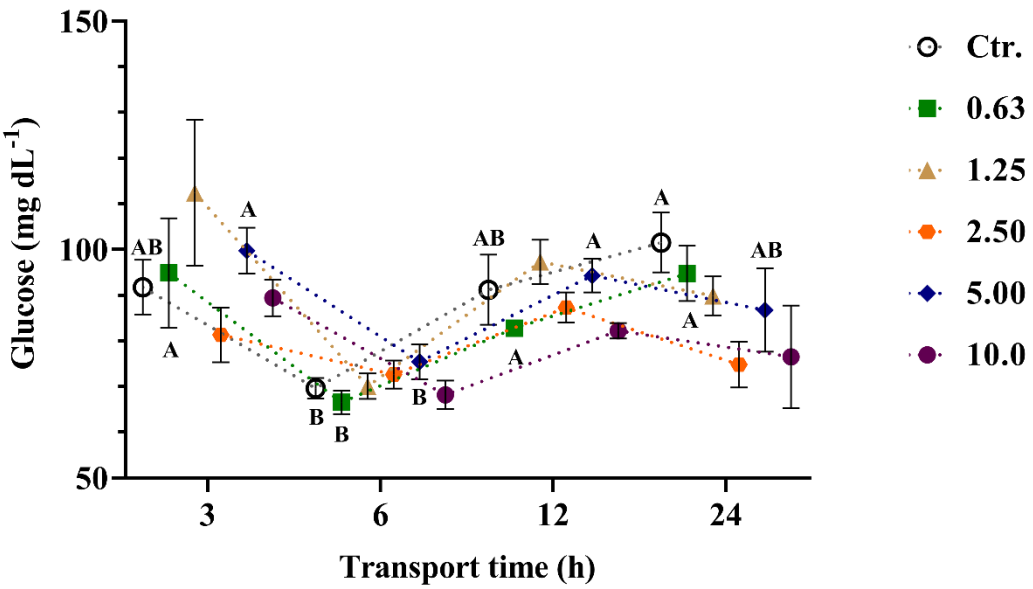
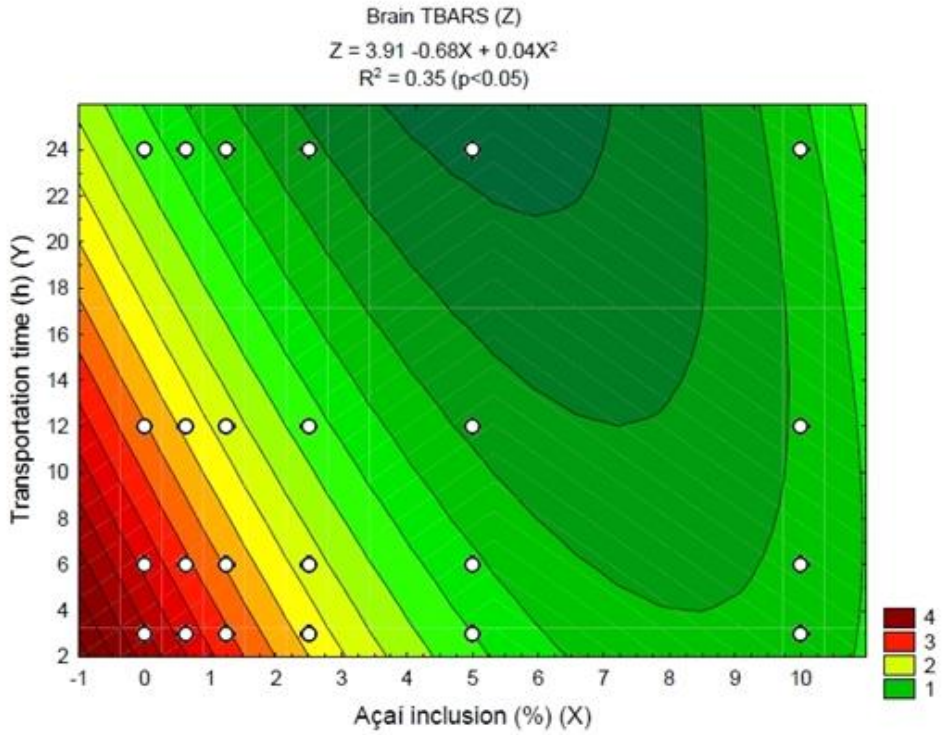

150
100
50
Glucose (mg dL-1)
3
6
12
24
Transport time (h)
AB
A
A
B
B
AB
A
A
A
AB
Ctr.
0.63
1.25
2.50
5.00
10.0

Figure 2


Brain TBARS (Z)
Z = 3.91 -0.68X + 0.04X²
R² = 0.35 (p<0.05)
Transportation time (h) (Y)
Açaí inclusion (%) (X)
24
22
20
18
16
14
12
10
8
6
4
2
-1
0
1
2
3
4
5
6
7
8
9
10
4
3
2
1

Supplementary material

Figure 1. Two-dimensional (2D) contour plot analysis of total antioxidant capacity against peroxyl radicals – ACAP (relative area) for brain (a), gills (b), liver (c), and muscle (d) of fish *Colossoma macropomum* as function of dietary levels of lyophilized *Euterpe oleracea* (LEO) and transport simulation for 3, 6, 12 and 24h on plastic bags (n = 9 per treatment).

Figure 2. Two-dimensional (2D) contour plot analysis of lipid peroxidation levels measured by concentration of thiobarbituric acid reactive substances – TBARS (nmol MDA mg protein^{-1}) for gills (a) liver (b), and muscle (c) of fish *Colossoma macropomum* as function of dietary levels of lyophilized *Euterpe oleracea* (LEO) and transport simulation for 3, 6, 12 and 24h on plastic bags (n = 9 per treatment).

Figure 1

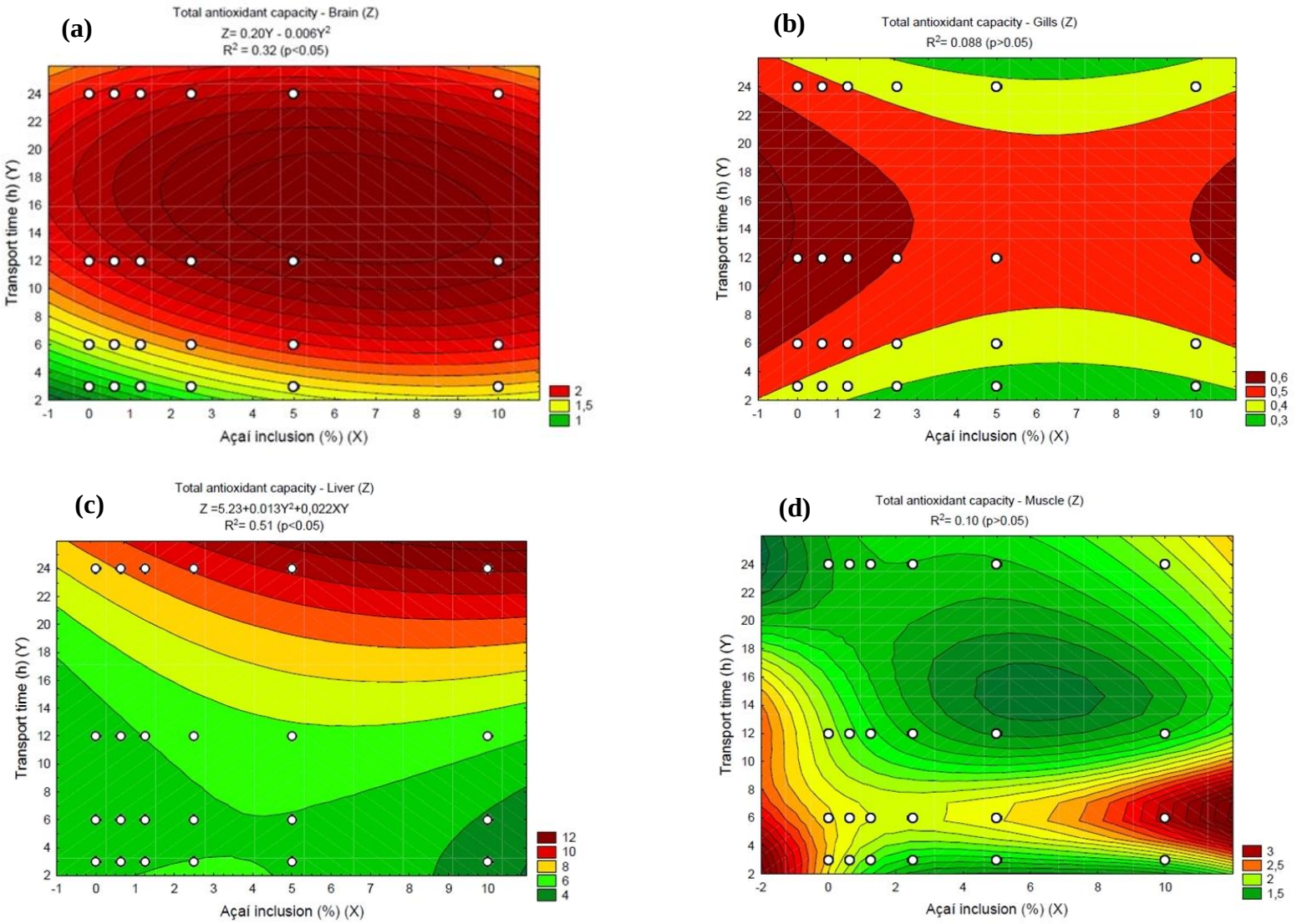
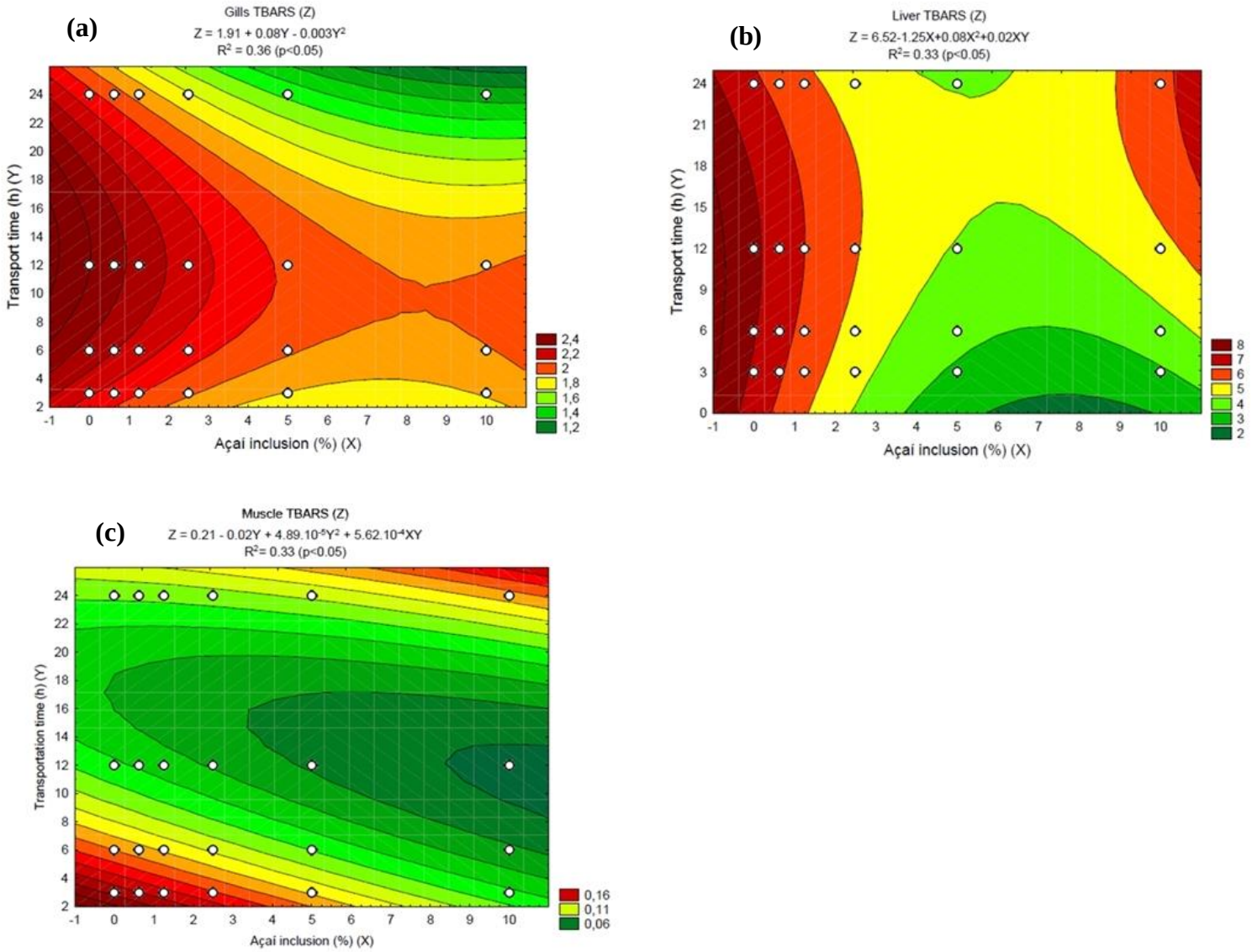

(a)
Total antioxidant capacity - Brain (Z)
Z= 0.20Y - 0.006Y²
R² = 0.32 (p<0.05)
Transport time (h) (Y)
Açaí inclusion (%) (X)
2
1,5
1
(b)
Total antioxidant capacity - Gills (Z)
R²= 0.088 (p>0.05)
Transport time (h) (Y)
Açaí inclusion (%) (X)
0,6
0,5
0,4
0,3
(c)
Total antioxidant capacity - Liver (Z)
Z =5.23+0.013Y²+0,022XY
R²= 0.51 (p<0.05)
Transport time (h) (Y)
Açaí inclusion (%) (X)
12
10
8
6
4
(d)
Total antioxidant capacity - Muscle (Z)
R²= 0.10 (p>0.05)
Transport time (h) (Y)
Açaí inclusion (%) (X)
3
2,5
2
1,5

Figure 2


(a)
Gills TBARS (Z)
Z = 1.91 + 0.08Y - 0.003Y²
R² = 0.36 (p<0.05)
Transport time (h) (Y)
Açaí inclusion (%) (X)
2,4
2,2
2
1,8
1,6
1,4
1,2
(b)
Liver TBARS (Z)
Z = 6.52-1.25X+0.08X²+0.02XY
R²= 0.33 (p<0.05)
Transport time (h) (Y)
Açaí inclusion (%) (X)
8
7
6
5
4
3
2
(c)
Muscle TBARS (Z)
Z = 0.21 - 0.02Y + 4.89.10⁻⁵Y² + 5.62.10⁻⁴XY
R²= 0.33 (p<0.05)
Transportation time (h) (Y)
Açaí inclusion (%) (X)
0,16
0,11
0,06

CAPÍTULO 4

Dietary açai (*Euterpe oleracea* Mart) attenuates seizures and lipid peroxidation in the brain of juvenile fish *Colossoma macropomum*

da Silva, T.V.N.[1], Torres, M.F.[2], Sampaio, L.A.[3], Hamoy, M.[4], Monserrat, J.M.[1,5], Barbas, L.A.L.[2*]

[1] Laboratory of Functional Biochemistry of Aquatic Organisms (BIFOA), Center for Biotechnology and Diseases of Aquatic Organisms (CBD). Federal University of Rio Grande (FURG), Institute of Oceanography (IO), Rio Grande, RS, Brazil.

[2] Tropical Species Aquaculture Laboratory (LAET), Federal Institute of Pará (IFPA), Castanhal, PA, Brazil.

[3] Estuarine and Marine Fish Laboratory (LAPEM), Federal University of Rio Grande (FURG), Institute of Oceanography (IO), Rio Grande, RS, Brazil.

[4] Pharmacology and Toxicology of Natural Products Laboratory (LFTPN), Institute of Biological Sciences (ICB), Federal University of Pará (UFPA), Belém, PA, Brazil

[5] Institute of Biological Sciences (ICB), Federal University of Rio Grande (FURG), Rio Grande, RS, Brazil.

* Corresponding author at:
Laboratório de Aquacultura de Espécies Tropicais (LAET), Instituto Federal de Educação Ciência e Tecnologia do Pará – IFPA Campus Castanhal, CP 68740 – 970, Castanhal, Pará, Brazil.
E-mail address: andre.barbas@hotmail.com (L.A.L. Barbas).

Manuscrito a ser submetido à revista *Toxicology and Applied Pharmacology*.
Formatado conforme as normas da revista.

Abstract

The study aimed to determine the effect of dietary administration of lyophilized *E. oleracea* (LEO) on pentylenetetrazole (PTZ) - induced seizures, using tambaqui juveniles (*Colossoma macropomum*) as models. Further, measurements of the lipid peroxidation (TBARS) levels in the brain were carried out. Besides the control diet (0.00% LEO), two levels of LEO inclusion were established: 5.00% and 10.0% LEO (w/w). Fish were divided into five groups (n = 5): sham control (0.9% physiological solution – i.p.), PTZ group (PTZ 150 mg / kg; i.p.), PTZ LEO 5.00%, PTZ LEO 10.0%, and BDZ-PTZ (BDZ: diazepam 10 mg / kg; i.p.). Recordings of the cerebral field potential were made by electroencephalography (EEG) and malondialdehyde (MDA) concentration was quantified in the brain, along with the characterization of behavioral responses. Fish receiving PTZ showed intense action potential bursts (APBs), which correlated with the hyperactive behavior attained. In PTZ LEO 5.00% and 10.0% groups, convulsive behavior was significantly reduced compared to the PTZ group. Fish fed 5.00% or 10.0% LEO and receiving PTZ showed less excitability and lower mean amplitude in tracings. The inclusion of 10.0% LEO in the diet prevented the increase in mean amplitude by 80%, without a significant difference to the BDZ-PTZ group. The TBARS was reduced by 60% in fish receiving dietary LEO relative to the PTZ-administered group. The results of this study demonstrated the anticonvulsant and protective role of LEO to tambaqui, where the inclusion of 5.00% LEO and above seems to be interesting for the formulation of functional fish diets.

Keywords: Amazon fish and palm fruit; pentylenetetrazole; electrophysiology; anticonvulsive activity; malondialdehyde; fish farming.

1. Introduction

Industrial effluents, sewage, agricultural and decomposition of organic matter may add to the building up of toxic compounds to the environment, such as ammonia, xenobiotics, (e.g. pesticides) and residues of drugs (e.g. tetracycline) (Nunes et al., 2015; Singh et al., 2016). These substances are generally lipophilic and easily absorbed through biological membranes, which increases their toxicity and favors the onset of deleterious effects in aquatic organisms, such as oxidative stress and neurotoxicity (Sinha et al., 2014; Bucking et al., 2017; Maltez et al., 2017).

That is because, under these conditions, reactive oxygen species (ROS) such as superoxide anion radical (O_2•-), hydrogen peroxide (H_2O_2), hydroxyl (HO•) and peroxyl (ROO•) radicals can be generated in excess, extrapolating normal physiological levels. In consequence, the defense capacity of the antioxidant system can be disrupted (Lushchak, 2014, Sies, 2015), favoring the pro-oxidants processes able of causing damage to lipids, proteins and deoxyribonucleic acid (DNA), characterizing an oxidative stress condition (Jones, 2006; Lushchak, 2011; Halliwell and Gutteridge, 2015).

To manage pro-oxidant processes, the cells developed an antioxidant defense system that consists of enzymatic defenses, such as superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase; and non-enzymatic defenses, many of them obtained from diets such as vitamins (e. g. C and E), minerals (e. g. selenium and zinc) and a variety of phytochemicals (Kütter et al., 2014; Hoseinifar et al., 2017; Sallam et al., 2017; Takahashi et al., 2017; Wu et al., 2017). In this context, the use of extract plants has shown potential in aquaculture as a replacement for synthetic substances to ensure the welfare of reared organisms as well as the final consumer (Ronquillo and Hernadez, 2016; Kuebutornye and Abarike, 2020).

It is well documented that plant dietary administration exert growth promotion; antioxidant and antimicrobial activity; enhances the immunocompetence; regulates the feed intake; possess anti-stress capabilities, and anti-pathogen potential in fish (Reverter et al., 2014; Abdel-Tawwab, 2017; da Silva et al., 2020; Fazelan et al., 2020). These biological activities are provided by the composition of phytochemicals (secondary metabolites of plants), such as alkaloid, glycosides, terpenoid, phenolic, polyphenolic, flavonoids, lectin, polypeptide compounds, and others (Piasecka et al. 2015; Shahidi et al, 2018). Literature sources also report that alkaloids and flavonoids have anxiolytic, sedative and anticovulsant activity (Jäber, Saaby & 2011; Diniz et al., 2015; Johnston, 2015). For this reason, plants have been also studied as alternative sources for the development of new anticonvulsant agents (Kamboj et al., 2009).

The açai (*Euterpe oleracea*) is an Amazonian fruit rich in anthocyanins, phenolic acids, flavonols, carotenoids and hydroxycinnamic acid (Alqurashi et al., 2016). Previous studies have reported the antioxidant and neuroprotective effects associated with the consumption of this fruit (Peixoto et al., 2016; Poulose et al., 2017; Torma et al., 2017). In mice treated with pentylenetetrazol (PTZ), a classic chemoconvulsive drug, açai completely counteracted lipid peroxidation in the cerebral cortex, which was instrumental in reducing the seizure severity (Souza-Monteiro et al., 2015). PTZ is a blocker of the gamma aminobutyric acid (GABA) A receptor, widely used in biomedical research to induce seizures in several species, including zebrafish *Danio rerio* (Wong et al., 2010).

Measurement of the brain's electrical activity can be attained using electroencephalography (EEG) (Niedermeyer and da Silva, 2005). Pioneer studies evaluating electrophysiological records were successfully conducted to evaluate the anesthetic efficacy of essential oils in Amazonian fish (Barbas et al., 2017; Fujimoto et al., 2017; de Souza et al., 2019; Vilhena et al., 2019), including EEG recordings from live fish

(Barbas et al., 2020). However, no studies are available to date on the electrophysiological responses combined with lipid peroxidation evaluation of the brain in fish previously fed with a dietetic plant, and more specifically, açai-enriched diets. Since there are several studies on the basic physiology of tambaqui fish (*Colossoma macropomum*), this species has become an interesting neotropical model species (Wood et al., 2017). Moreover, tambaqui has been used as a model in electrophysiological studies due to its handling resistance and high sensitivity to drug testing (Barbas et al., 2017a; de Souza et al., 2019; Vilhena et al., 2019), hence being chosen as a model for this study.

It is well-known that oxidative stress and seizures are intrinsically related (Souza-Monteiro et al., 2015). Therefore, considering the bioactive properties of *E. oleracea* and its anticonvulsive and antioxidant effects, already confirmed in mammals, it has been hypothesized herein that tambaqui juveniles fed açai-containing diets will experience lower lipid peroxidation and present a higher resistance to PTZ-induced seizures. Thus, the objective of this study was to investigate the electroencephalographic response, brain lipid peroxidation and characterize the behavior of *C. macropomum* juveniles fed rations containing graded levels of lyophilized açai (*E. oleracea*) and subjected to a convulsive stimulus.

2. Material and methods

This study meets the ethical standards as advised by the 'Conselho Nacional de Controle de Experimentação Animal' – CONCEA of Brazil, and complies with the Brazilian Guide for the Production, Maintenance or Use of Animals in Teaching or Scientific Research Activities ('Normativas' do CONCEA, 2016). This study was approved by the Ethics Committee on Experimental Animals of the Federal University of Pará – UFPA (CEUA nº 9966130618/2018). Experiments were conducted at the

Laboratório de Farmacologia e Toxicologia de Produtos Naturais (LFTPN), at UFPA, in Belém, PA, Brazil (1°28'27.7"S 48°27'11.4"W).

2.1. Experimental animals and feeding

Tambaqui juveniles (0.92 ± 0.01 g mean body weight) were obtained from a private commercial farm. The fish were acclimated for ten days before the begining of the experiment. During the acclimation period, fish were fed three times a day (09:00, 13:00 and 17:00 h) with the control diet (see below) at 10% body weight. Thereafter, juveniles were randomly distributed into five aquaria (200 L) at a density of 50 fish per tank to investigate the potential effect of lyophilized LEO on the growth performance and redox status of tambaqui juveniles (for more details refer to da Silva et al., 2020). For the present study, diets containing 5.00% and 10.0% inclusion of LEO, as well as a control diet were established. During the trial (30 days), fish were hand-fed four times a day (08:00, 11:30, 14:00 and 17:30 h) at a rate of 10.0% body weight. The amount of feed administered was corrected every five days, considering a feed conversion ratio of 1.2:1. The experiment was carried out in semi-static aerated water systems, with a daily partial water change of 50%.

2.2. Experimental diets

The control (0.00%), 5.00% and 10.0% LEO diets were prepared as previously described (da Silva et al., 2020). Briefly, the diets were formulated to contain 40% protein and 9% lipid on average and different levels of açai inclusion: 0 g açai Kg^{-1} (Ctr); 50 g açai Kg^{-1} (5.00%); and 100 g açai Kg^{-1} (10.0%). The ingredients were mixed in increasing order, *i,e.*, starting from those containing the least to the greatest amounts. Thereafter, warm water was added to produce a consistent and firm wet dough. The dough was pelleted and dried in an oven (65°C for 24 h). The obtained pellets were crushed and sieved (1.0 - 2.0 mm) and stored at -20 ° C. The centesimal composition analysis of diet

was performed following the Association of Official Analytical Chemists (AOAC, 1990) standard methods.

2.3. Drugs

Diazepam at 5 mg/mL (BDZ) and pentylenetetrazole at 100 mg/mL (PTZ) were obtained from Sigma-Aldrich and solubilized in sterile saline (0.9% NaCl).

2.4. Experimental design

At the end of the feeding trial, fifty fish (9.1± 1.5 g mean body weight) were kept in an environment with a regulated temperature (25 - 28 °C), a light-dark cycle of 12 hours, water temperature average of 27.29 ± 0.11 °C; oxygen 7.37 ± 0.01 mg O_2 L^{-1}; and pH 6.33 ± 0.11. The animals were assayed into five different groups (n = 6): Sham control receiving an equivalent volume of 0.9% physiological solution (i.p.); PTZ group receiving a 150 mg/kg dose of pentylenetetrazole (PTZ) i.p.; PTZ LEO 5.00%, group of animals fed a diet containing 5.00% LEO and receiving a dose of 150 mg/kg i.p. PTZ; and PTZ LEO 10.0%, group of tambaqui juveniles fed the 10.0% LEO diet submitted to PTZ at 150 mg/kg i.p. Finally, a BDZ – PTZ group was established, in which animals were administered diazepam (BDZ) via i.p., at the dose of 10 mg/kg and subsequently exposed (10 minutes later) to PTZ at 150 mg/kg via i.p.. All drug application procedures were performed using Hamilton syringes. In each treatment, five fish (n = 5) were used for the encephalographic recordings and tissue sampling. Another five fish (n = 5) were used to evaluate behavioral responses following the same experimental design. Each individual was weighed, being considered a replicate and used only once.

2.5. Assembling of electrodes and procedure of implant

The method of Pineda et al. (2011) and Barbas et al., (*data submitted*, 2020) were used for the electrophysiological recordings. The electrodes were made using two identical stainless steel rods (one for reference and another for the registration of data) measuring 3.0 mm and 1.0 mm in length and diameter (distal tip of the rod), respectively. Both stainless steel rods were coated with Teflon (Micro probe SNC – MPI – Gaithersburg, MD 20879, USA) and affixed (at a distance of 2.0 mm from each other) with a thin layer of epoxy resin. The distal part of the electrodes was responsible for the acquisition of the records, being in contact with the target brain region (recording electrode), and the proximal part (reference electrode) was connected to a high impedance amplifier.

The electrodes were implanted in the mesencephalon region concerning the median sagittal line (longitudinal) and the caudal border of the eyeball (transversal). The point of intersection between the lines corresponded to the exact site of the electrode positioning. Before the electrode implantation, fish were individually removed from the tanks and anesthetized with 60 mg L^{-1} eugenol. The animal was then restrained in a foam pad structure, receiving a continuous anesthetic solution orally administered, via gravity (60 mg L^{-1} eugenol at a 25 mL flow min^{-1}). While anesthetized, the fish were quickly equipped with the electrodes and then returned to the origin tank for recovery. The whole procedure for the recordings of the cerebral field potential was performed in a Faraday cage 24 hours after the implant and the records. Recordings took five minutes for each animal from the proposed treatments. After recordings, fish were anesthetized (high dose of eugenol) and killed with a sharp blow to the head.

2.6. Equipment and acquisition of the electroencephalographic (EEG) records

For the EEG recordings, the distal region of the electrodes was connected to a data acquisition system (Grass Technologies, Model P511) adjusted with 0.3 Hz and 300 Hz filtering and 2000X amplification; monitored with an oscilloscope (ProteK, Model 6510); and continuously scanned at a rate of 1 kHz by a computer equipped with a data acquisition board (National Instruments, Austin, TX). The data were stored on a hard disk and processed through specialized software (LabVIEW express).

2.6.1. EEG record analysis

For the analysis of acquired signals, a tool was developed using the Python programming language version 2.7. For mathematical processing, the Numpy and Scipy libraries were used and the Matplolib library was used to generate the graphs. The graphical interface was developed using the PyQt4 library (Souza-Monteiro et al., 2015). Amplitude graphs demonstrate the potential differences between the two electrodes (reference and record). The signs of the records were observed in 1,000 samples per second. Spectrograms were calculated using a Hamming window with 256 points (256/1000 seconds) each frame was generated with an overlap of 128 points per window. In each frame, the power spectral density (PSD) was calculated by the Welch average periodogram method. Frequency histograms were generated with the PSD of the signal (with 1 Hz boxes). To analyze the difference between the groups tested, a graph with the mean and standard deviation of PSD of various treatments was constructed. Each wave of the graph was generated from a set of tests, in which the PSD was generated and from this calculated the mean and standard deviation for each group. For the calculation of the PSD Hamming window was used 256 points without overlapping.

2.7. Collection of brain and lipid peroxidation (TBARS) determination

After the EEG recordings, fish were euthanized by immersion in a lethal dose of eugenol (500 mg L^{-1}) for the collection of the brain. Immediately, the tissue was frozen and stored at -80 ° C. The brain was homogenized (1:5 w/v) in a Tris–HCl (100 mM, pH 7.75) buffer with EDTA (2 mM) and Mg^{2+} (5 mM) (Amado et al., 2011). Homogenates obtained were centrifuged at 10,000 × g for 20 min at 4 °C, and the supernatants were stocked at −80 °C. Total protein content (wavelength of 550 nm) was determined in triplicate by the Biuret method (Amado et al., 2009, Amado et al., 2011) with a commercial kit (Total Protein Kit Doles) and a microplate reader (BioTek® Instruments, Inc).

The lipid peroxidation in the brain was measured using the methodology described by Oakes and Van Der Kraak (2003), which produce the reaction of the lipid peroxidation by-product (malondialdehyde - MDA) with thiobarbituric acid (TBA, from Baker) under high temperature and acidity, generating a chromogen that can be quantified by fluorimetry. Homogenized extract (30 μL) of brain was added to 20 μL butylated hydroxytoluene solution (BHT at 67 μM), 150 μL acetic acid (at 20% and pH 3.5), 150 μL TBA (0.8%), 50 μL Milli-Q water, and 20 μL sodium dodecyl sulfate (SDS at 8.1%). Samples were heated at 95 °C for 30 min. After being cooled for 10 min, 100 μL Milli-Q water and 500 μL n-butanol were added to the samples. The final solution obtained was centrifuged (3000 ×g for 10 min at 15 °C) to obtain the separation of the alcohol phase. The b-butanol phase (150 μL) was placed in a microplate reader to determine the fluorescence (excitation: 520 nm and emission: 580 nm). Results were expressed as the concentration of TBARS in nmol/mg of wet tissue, where TMP stands for tetramethoxypropane (TMP, Acros Organics) employed as a standard.

2.8. Behavioral seizure records

According to their respective treatments, juveniles of tambaqui were individually submitted to i.p. application of PTZ or BDZ-PTZ in a 1000 mL beaker. The animals were observed for 20 min and the latency to the seizure-like behavior to occur was recorded, considering the following behavioral cues: intermittent immobility (II), hyperexcitability (HY), jumping out of water (JOW), loss of posture reflex (LPR), slow tail movement (STM), visible muscular spasms (VMS), uncoordinated swimming (IS), rotational swimming (RS), tail tremor (TT), lifting of the dorsal fin (LDF), ventral swimming (VS), and return of the posture reflex (RPR).

2.9. Statistical analysis

Gaussian distribution of the data and homogeneity of variances were analyzed through Kolmogorov-Smirnov's and Levene's tests, respectively. Comparisons among mean amplitude values, as well as TBARS data were made using one-way ANOVA, in which diets with different levels of LEO and drugs were the factors. Comparisons between means of treatments were performed using Tukey post-test. A value of $p<0.05$ was considered statistically significant. The steps described above were carried out using the GraphPad Prism ® 5 software (Zar, 1996).

3. Results

3.1. Dietary LEO attenuates PTZ-induced electrical alterations

The EEG recordings obtained from sham control showed the low amplitude of tracings (Fig. 1A, left) and intensity of the signal was higher in frequencies below 10 Hz as indicated by the spectrogram (Fig. 1A, right). During this period, the amplitude recorded in the midbrain of this group was 0.0114 ± 0.0014 $mV^2/Hz \times 10^{-3}$ (Fig. 2C). The power spectral density (PSD) shows low amplitude tracings with low variability among fish in the

sham control group (Fig. 2A). When PTZ was injected, a very short latency for the initiation of central excitability was observed, which was characterized by the presence of isolated high-amplitude waves and action potential bursts (APB) with rapid evolution in the PTZ control, as it can be observed by the wave recordings (Fig. 1B, left and center) and the corresponding spectrogram of frequency (Fig. 1B, right). During the seizure-like state, the PSD of PTZ-exposed fish presented a higher amplitude, differing from the other records (Fig. 2A). The linear amplitude distribution of PTZ control was on average 0.6218 ± 0.1381 $mV^2/Hz \times 10^{-3}$, demonstrating the statistical difference to sham control (Fig. 2C).

The fish previously fed 5.00% LEO diet and treated with PTZ, showed EEG tracings with less excitability and lower amplitudes (Fig. 1C and Fig. 3B, respectively) and mean power of 0.1847 ± 0.0360 $mV^2/Hz \times 10^{-3}$, that differs significantly in respect to sham control and PTZ group (Fig. 2C). After PTZ application, fish that consumed 10.0% LEO in the diet showed a considerable reduction in wave amplitude, frequency, and APBs, suggesting a protective effect of LEO against PTZ-induced seizures (Fig. 1D and Fig. 3B, respectively). In the EEG tracings and frequency spectrogram (Hz), it is worthy of note the similar pattern s attained between PTZ LEO 10.0% and the BDZ-PTZ groups (Fig. 1D and Fig. 1E, respectively). The mean power observed in the 10.0% PTZ LEO group (0.1049 ± 0.0360 $mV^2/Hz \times 10^{-3}$) was not different from those of the sham control and BDZ-PTZ groups (Fig. 2C). However, the group treated with benzodiazepine showed similar responses compared to sham control, evidencing the antagonism of the convulsive activity elicited by pentylenetetrazole, which initially presented isolated firings (Fig. 1E). In the PSD, the benzodiazepine-treated fish did not show a statistical difference relative to the group receiving 10.0% LEO (Fig. 2B). This is observable in the linear distribution graph whereby the mean amplitude of the BDZ-PTZ group was 0.0055 ± 0.0143 $mV^2/Hz \times 10^{-3}$ (Fig. 2C).

According to the APB duration, the control animals that received PTZ only presented a mean time of 28.67 ± 3.50 seconds, demonstrating a period of intense excitability (Fig. 3A). On the other hand, the PTZ LEO 5.00% group presented a mean duration of 13.50 ± 2.66 seconds, with a statistical difference for the PTZ control in the decrease of the intensity of the seizure. The group fed 10.0% of LEO in the diet showed a greater reduction in APB duration (8.11 ± 1.16 seconds) after PTZ application, demonstrating a statistical difference to the previous groups (Fig. 3A). This can be confirmed from the mean linear amplitude observed during APB for the PTZ control group (8.381 ± 0.9532 $mV^2/Hz \times 10^{-3}$), PTZ LEO 5.00% group (5.556 ± 1.3223 $mV^2/Hz \times 10^{-3}$), and for the PTZ LEO 10.0% group (2.195 ± 0.7082 $mV^2/Hz \times 10^{-3}$), showing a clear difference in amplitude between the APB, which are significantly different (Fig. 3B). Therefore, these results indicate the açai neuroprotective action in a dose-dependent fashion.

3.2. Effectiveness of LEO in attenuating lipid peroxidation induced by convulsions

The inclusion of 5.00% LEO in the diet reduced by 60.69% the concentration of MDA in the fish brain compared to the PTZ group ($P<0.05$). Similarly, lipid peroxidation was also attenuated in the brains of fish fed the 10.0% LEO diet, differing from the PTZ group ($P<0.05$). The BDZ - PTZ treatment did not differ significantly from the PTZ group or groups fed diets containing LEO (Fig. 4).

3.3. LEO relieved behavioral alterations induced by seizures

The group of fish treated with diazepam presented delayed intermittent immobility (II) behavior concerning the other treatments, followed only by visible muscular spasms (VMS) that started in less than 2 min. PTZ group presented most of the signs related to seizures (except VMS) and required a longer time (27 min) to return the posture reflex

(RPR). The PTZ LEO 10.0% group took longer for the onset of hyperexcitability (HY), loss of posture reflex (LPR), rotational swimming (RS), tail tremor (TT), showing a faster return (in about 1.5 min) of posture reflex (RPR). The PTZ LEO 5.00% group behavior changes in intermediate time compared to the PTZ and PTZ LEO 10.0% groups, with the return of posture reflex (RPR) in just over 2 min (Table 1). Therefore, the inclusion of LEO in the diet allowed for an abbreviated and/or smoothed convulsive event, showing consistent responses in terms of APBs duration and mean power amplitude (Fig. 3A and B).

4. Discussion

The present study was set up to investigate the protective effect of *E. oleracea* in fish using the pentylenetetrazol-induced seizure model. This experimental model of seizures is effective in inducing myoclonic and generalized tonic-clonic seizures in cats, mice, rats, and primates (Jagannatha, 2015). Moreover, PTZ-induced epileptiform discharges using live animals as models represent a standard method for an effective evaluation of potential anticonvulsant compounds (Souza-Monteiro et al., 2015). PTZ is a tetrazol derivative that blocks the chloride channel coupled to the $GABA_A$ receptor complex (main inhibitory neurotransmitter in the brain) which further leads to glutamate excitation and consequent epilepsy promotion (Souza-Monteiro et al., 2015; Taiwe et al., 2016).

Açai has been the focus of many research endeavors on its ability to promote a neuroprotective effect. It has been associated with attenuation of oxidative stress in brain and seizure prevention (Spada et al., 2009; Souza-Monteiro et al., 2015; Peixoto et al., 2016; Carey et al., 2019). We highlight that the anticonvulsive potential and neuroprotective effect of this fruit has already been demonstrated *in vivo* in a mice model

(Souza-Monteiro et al., 2015), as well as a recent study which emphasized its underlying molecular mechanism *in vitro* using primary cultures of neocortical neurons and astrocytes (Arrifano et al., 2018). Previous administration of açai (10 mL/g body weight) to mice - by gavage for 4 days - partially prevented PTZ-induced seizures (to frequencies below 10 Hz and reduction of 50% of the amplitude) (Souza-Monteiro et al., 2015). EEG recordings in tambaqui showed PTZ-induced electrical alterations in the brain, as per the increased amplitudes observed. Inclusion of 10.0% LEO in the diet prevented such increases in up to 80%. Behavioral modulation was in line with this electrical pattern, since açai intake reduced the length and the intensity of the seizure-related clinical signs, as also demonstrated by the significant reductions in duration and power of the APBs.

While the effectivity of LEO in reducing seizures was verified, what could be the molecular mechanisms underlying the anticonvulsant action of açai? The central nervous system (CNS) has a high concentration of amino acids that bind to postsynaptic receptors, acting as inhibitory or excitatory neurotransmitters, dubbed GABA and glutamate, respectively. These neurotransmitters regulate several behavioral and pathological processes, such as epilepsy and neurotoxicity (Hinton and Johnston, 2018; Murray et al., 2019). These responses are generated by changes in the conductance of one or more selective ion channels. Thus, inhibitory neurotransmitters trigger a selective output current, where ion fluxes (via opening the K^+ or Cl^- channels to induce the K^+ efflux or the Cl^- influx, respectively) hyperpolarize the membrane and decrease the membrane resistance. The excitatory neurotransmitter stimulate the influx of sodium ions (via open a specific channel of cations – e.g. sodium channel) depolarize the membrane (Forman et al., 2017).

Arrifano et al. (2018) demonstrated that *E. oleracea* constituents react with $GABA_A$ receptors and modulate GABA uptake. Briefly, GABA transporters are important molecular targets for anticonvulsive drugs and, in this *in vitro* study, *E. oleracea*

significantly inhibited GABA uptake in both cortical neurons and astrocytes. Presumably, it favored the accumulation of endogenous GABA in the synaptic cleft, increasing inhibitory neurotransmission. *E. oleracea*-induced high levels of GABA may be responsible for the effects detected at the receptor since GABA can enhance benzodiazepine binding and decrease the "target at the seizure site"-binding. In this study, diazepam was used as a standard anticonvulsive drug and allowed for comparisons with animals previously fed LEO. Thus, it seems that acai compounds act on the GABAergic system, partially preventing convulsions induced by PTZ, decreasing both the frequency of the firings and mean wave amplitudes. However, we emphasize that study to determine the effects of typical agonist drugs on tambaqui are necessary to confirm this action of açai.

The PTZ-induced seizure also leads to oxidative stress in the brain, with the generation of free radicals, mainly ROS, which affect the functionality of membrane phospholipids and proteins (Diniz et al., 2015; Souza-Monteiro et al., 2015; Kovac et al. 2016). Pro-oxidants can lead to severe brain injuries as they are capable of inducing seizures via direct inactivation of glutamine synthase, favoring the atypical release of excitatory neurotransmitters (e.g., glutamic acid) (Diniz et al., 2015; Souza-Monteiro et al., 2015). Besides, ROS can inhibit the enzyme glutamate decarboxylase by lowering GABA levels in the cerebral cortex leading to seizures (Davis et al., 2001; Diniz et al., 2015). In this study, the higher concentration of MDA in the PTZ group indicates higher lipid peroxidation in the brain of these animals concerning the groups previously fed with the diets containing 5.00% and 10.0% LEO. Souza-Monteiro et al. (2015) also observed a significant reduction of TBARS in the brain of mice, ascribing this effect to the high antioxidant power of açai, which has been demonstrated in several studies, including aquatic organisms (Schauss et al., 2006; Gordon et al., 2012; Dias et al., 2013; Alqurashi et al., 2016; Colombo et al., 2020).

The LEO diets administered to tambaqui juveniles were previously evaluated for the content of flavonoids and total polyphenols, presenting a higher concentration of these antioxidants in the 5.00% and 10.0% LEO inclusion (da Silva et al., 2020). Phenolic acids (gallic acid), flavonols (quercetin), anthocyanins (mainly cyanidin 3-glucoside, and cyanidin 3-rutinoside), carotenoids (lutein, α-carotene, and β-carotene) and hydroxycinnamic acids (chlorogenic acid, caffeic acid, and ferulic acid) are the main bioactive constituents of *E. oleracea* (Alqurashi et al., 2016). Various flavonoids, including their glycosides, promote anxiolytic, sedative and anticonvulsant effects on the CNS (Jäger, Saaby, 2011; Diniz et al., 2015; Johnston, 2015) that should explain, at least partially, the observed results in our study.

Plants containing flavonoids, such as "piri-piri" (*Cyperus articulatus*), lemon balm (*Melissa officinalis*) and passion fruit (*Passiflora incarnata*) have anticonvulsive effects based on positive allosteric modulation of $GABA_A$ receptors and modulate the chloride flux (Elsas et al., 2010; Yoo et al., 2011; Herrera-Calderon et al., 2018). On the other hand, lipid (e.g., fatty acids) may also mediate anticonvulsive responses (Kumari et al., 2019). González-Trujano et al. (2018) observed that palmitic acid was partially responsible for the anticonvulsive effects of the moringa plant (*Moringa oleifera*) in mice and rats with PTZ-induced seizures. The *E. oleracea* is constituted of 105.1 ± 2.4 mg/g of gallic acid equivalent of total phenolics, 54.27 ± 0.28 mg/g of catechin equivalents of total flavonoids, as well it contains three important fatty acids: oleic acid (61.6% , 321.5 mg/g), palmitic acid (25.7%, 134.1 mg/g) and linoleic acid (12.6%, 65.9 mg/g) (Alqurashi et al., 2016). These pieces of evidence may explain the anticonvulsive and neuroprotective effect of LEO in juveniles of tambaqui during PTZ exposure, as also suggested by Arrifano et al. (2018). However, further studies on neurotransmitter or neuromodulator involvements are necessary to further the understanding of the anticonvulsant effects of *E. oleracea*.

Although *Colossoma macropomum* is a lower vertebrate, it proved to be an eligible model to be used in electroencephalographic studies, which corroborates previous reports as to the suitability of this and other fish species in this type of research (Barbas et al, 2020; Pineda et al., 2011). Finally, the findings demonstrate the anticonvulsant and neuroprotective role of LEO to tambaqui. Therefore, the inclusion of 5.00% LEO and above seems to be interesting for the formulation of functional fish diets.

Acknowledgements

da Silva T.V.N. acknowledges funding from the Brazilian CAPES for the graduate scholarship provided (2016-2020). The authors are grateful to José M. da Silva and Cecília A. F. Manoel for their assistance and technical support. We are thankful to Laboratório de Farmacologia e Toxicologia de Produtos Naturais - LFTPN ICB/UFPA Belém and Laboratório de Aquacultura de Espécies Tropicais-LAET/IFPA Castanhal and their staff for the infrastructure and support provided. Monserrat J. M. (# 308539/2016-8) and Sampaio L. A. (# 307445/2016-0) are research fellows of the Brazilian CNPq. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Conflicts of Interest

The authors have no actual or potential conflicts of interest.

Author's Contributions

T.V.N.S, J.M.M., L.A.L.B, and M.H. designed the study, with intellectual contribution from all authors. M.F.T and L.A.S. contributed with the experimental procedures and technical support. L.A.L.B, M.H., J.M.M and T.V.N.S carried out the statistical analyses. T.V.N.S, J.M.M. and L.A.L.B drafted the manuscript and all authors contributed equally to the writing of the final version of the manuscript.

References

Abdel-Tawwab, M., 2017. Turmeric powder, *Curcuma longa* L., in common carp, *Cyprinus carpio* L., diets: growth performance, innate immunity, and challenge against pathogenic *Aeromonas hydrophila* infection. *Journal of the World Aquaculture Society*. *48* (2), 303 – 312. https://doi.org/10.1111/jwas.12349

Amado, L. L., Ramos, P. B., Freitas, R. F., Zafalon, B., Ferreira, J. L., Yunes, J. S., Monserrat, J. M., 2009. A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: application to evaluate microcystins toxicity. *Science of the Total Environment*. *407* (6), 2115 – 2123. https://doi.org/10.1016/j.scitotenv.2008.11.038

Amado, L. L., Garcia, M. L., Pereira, T. C., Yunes, J. S., Bogo, M. B., Monserrat, J. M., 2011. Chemoprotection of lipoic acid against microcystin-induced toxicosis in common carp (*Cyprinus carpio*). *Comparative Biochemistry and Physiology*. Part C. 154 (3), 146 – 153. https://doi.org/10.1016/j.cbpc.2011.04.007

Alqurashi, R. M., Commane, D. M., Rowland, I. R., 2016. Açai fruit as a source of bioactive phytochemicals. *Journal of Life Sciences*. 10, 391 – 404. https://doi.org/10.17265/1934-7391/2016.08.005

AOAC (Association of Official Analytical Chemists) (1990). The official methods of analysis of the association of official analytical chemists (15th ed.). Arlington, Virginia.

Arrifano, G. P., Lichtenstein, M. P., Souza-Monteiro, J. R., Farina, M., Rogez, H., Carvalho, J. C. T., Suñol, C., Crespo-López, M. E., 2018. Clarified açaí (*Euterpe oleracea*) juice as an anticonvulsant agent: in vitro mechanistic study of GABAergic targets. *Oxidative Medicine and Cellular Longevity*. 1 – 6. https://doi.org/10.1155/2018/2678089

Barbas, L. A. L., Hamoy, M., de Mello, V. J., Barbosa, R. P. M., de Lima, H. D. S. T., Torres, M. F., do Nascimento, L. A. S., da Silva, J. K. R., Andrade, E. H. A., Gomes, M. R. F, 2017. Essential oil of citronella modulates electrophysiological responses in tambaqui *Colossoma macropomum*: A new anaesthetic for use in fish. *Aquaculture*. *479*, 60 – 68. https://doi.org/10.1016/j.aquaculture.2017.05.027

Barbas, L. A. L., Torres, M. F., da Costa, B. M. P. A., Feitosa, M. J. M., Maltez, L. C., Amado, L. L., Toda, Y. P. S., Batista, P. S., Cabral, D. A. C., Hamoy, M., 2020. Eugenol induces body immobilization yet evoking a seizure-like event in fish during short-term baths. Submitted to *Aquatic Toxicology*.

Bucking, C., 2017. A broader look at ammonia production, excretion, and transport in fish: a review of impacts of feeding and the environment. *Journal of Comparative Physiology B. 187* (1), 1 – 18. https://doi.org/10.1007/s00360-016-1026-9

Carey, A. N., Miller, M. G., Fisher, D. R., Bielinski, D. F., Gilman, C. K., Poulose, S. M., Shukitt-Hale, B., 2017. Dietary supplementation with the polyphenol-rich açaí pulps (*Euterpe oleracea* Mart. and *Euterpe precatoria* Mart.) improves cognition in aged rats and attenuates inflammatory signaling in BV-2 microglial cells. *Nutritional Neuroscience*. *20* (4), 238 – 245. https://doi.org/10.1080/102 8415X.2015.1115213

Colombo, G. M., Simião, C. S., Schmitz, M. J., Pedrosa, V. F., Romano, L. A., Tesser, M. B., Ramos, P. B., Wasielesky, Monserrat, J. M., 2020. The role of açaí (*Euterpe oleracea* Mart. 1824) as a chemoprotective agent in the evaluation of antioxidant defence, oxidative damage and histology of juvenile shrimp *Litopenaeus vannamei* (BOONE, 1931) exposed to ammonia. *Aquaculture Research*, 00, 1 – 16. https://doi.org/10.1111/are.14503

Davis, K., Foos, T., Wu, J. Y., Schloss, J. V., 2001. Oxygen-induced seizures and inhibition of human glutamate decarboxylase and porcine cysteine sulfinic acid decarboxylase by oxygen and nitric oxide. *Journal of Biomedical Science*. *8* (4), 359 – 364. https://doi.org/10.1007/BF02258378

da Silva, T. V. N., dos Santos, C. F., Torres, M. F., Barbas, L. A. L., Sampaio, L. A., ..., Monserrat, J. M., 2020. Effects of dietary Amazonian açai (*Euterpe oleracea* Martius) fruit on growth performance, skin coloration and antioxidant status of *Colossoma macropomum*. Submitted to *Aquaculture Nutrition.*

de Souza, A. da S.L., Peret, A.C., Hamoy, M., de Souza, R.A.L., Torres, M.F., Barbas, L.A.L., 2019. Propofol and essential oil of Nepeta cataria induce anaesthesia and marked myorelaxation in tambaqui Colossoma macropomum: Implications on cardiorespiratory responses. *Aquaculture*. *500*, 160 – 169. https://doi.org/10.1016/j.aquaculture.2018.10.017

Dias, A. L. S., Rozet, E., Larondelle, I., Hubert, P., Rogez, H., Quetin-Leclercq, J., 2013. Development and validation of an UHPLC-LTQ-Orbitrap MS method for non-anthocyanin flavonoids quantification in *Euterpe oleracea* juice. *Analytical and Bioanalytical Chemistry*. *405* (28), 9235 – 9249. https://doi.org/10.1007/s00216-013-7325-z

Diniz, T. C., Silva, J. C., Lima-Saraiva, S. R. G. D., Ribeiro, F. P. R. D. A., Pacheco, A. G. M., de Freitas, R. M., Quintans-Júnior, L. J., Quintans, J. S. S., Mendes, R. L., Almeida, J. R. G. D. S., 2015. The role of flavonoids on oxidative stress in epilepsy. *Oxidative Medicine and Cellular Longevity*. *171756*, 1 – 9. https://doi.org/10.1155/2015/171756

Elsas, S. M., Rossi, D. J., Raber, J., White, G., Seeley, C. A., Gregory, W. L., Mohr, C., Pfankuch, T., Soumyanath, A., 2010. *Passiflora incarnate* L. (*Passion flower*) extract selicit GABA currents in hippocampal neurons in vitro, and show anxiogenic and anticonvulsant effects in vivo, varying with extractionmethod. *Phytomedicine*. *17* (12), 940 – 949. https://doi.org/10.1016/j.phymed.2010.03.002

Fazelan, Z., Vatnikov, Y. A., Kulikov, E. V., Plushikov, V. G., Yousefi, M. (2020). Effects of dietary ginger (*Zingiber officinale*) administration on growth performance and stress, immunological, and antioxidant responses of common carp (*Cyprinus carpio*) reared under high stocking density. *Aquaculture*, *518*, 734833. https://doi.org/10.1016/j.aquaculture.2019.734833

Fujimoto, R. Y., Pereira, D. M., Silva, J. C. S., de Oliveira, L. C. A., Inoue, L. A. K. A., Hamoy, M., de Mello, V. J., Torres, M F., Barbas, L. A. L., 2017. Clove oil induces anaesthesia and blunts muscle contraction power in three Amazon fish species. *Fish Physiology and Biochemistry*. *44* (1), 245 – 256. https://doi.org/10.1007/s10695-017-0430-8

Gordon, A., Cruz, A. P. G., Cabral, L. M. C., De Freitas, S. C., Taxi, C. M. A. D., Donangelo, C. M., et al., 2012. Chemical characterization and evaluation of antioxidant properties of Açaí fruits (*Euterpe oleraceae* Mart.) during ripening. *Food Chemistry*. *133* (2), 256 – 263. https://doi.org/10.1016/j.foodchem. 2011.11.150

González-Trujano, M. E., Martínez-González, C. L., Flores-Carrillo, M., Luna-Nophal, S. I., Contreras-Murillo, G., Magdaleno-Madrigal, V. M., 2018. Behavioral and electroencephalographic evaluation of the anticonvulsive activity of *Moringa*

oleifera leaf non-polar extracts and one metabolite in PTZ-induced seizures. *Phytomedicine*. *39*, 1 – 9. https://doi.org/10.1016/j.phymed.2017.12.009

Halliwell, B., Gutteridge, J. M. C., 2015. Free radicals in biology and medicine. Oxford. 5th ed.: Oxford University Press. EUA. 944.

Herrera-Calderon, O., Santiváñez-Acosta, R., Pari-Olarte, B., Enciso-Roca, E., Montes, V. M. C., Acevedo, J. L. A., 2018. Anticonvulsant effect of ethanolic extract of *Cyperus articulatus* L. leaves on pentylenetetrazol induced seizure in mice. *Journal of Traditional and Complementary Medicine*. *8* (1), 95 – 99. https://doi.org/10.1016/j.jtcme.2017.03.001

Hinton, T., Johnston, G. A., 2018. GABA, the major inhibitory neurotransmitter in the brain. *Reference Module in Biomedical Sciences*. Hinton, T. e Johnston, GAR (2018). https://doi.org/10.1016/b978-0-12-801238-3.96594-2

Hoseinifar, S. H., Dadar, M., Khalili, M., Cerezuela, R., Esteban, M. A., 2017. Effect of dietary supplementation of palm fruit extracts on the transcriptomes of growth, antioxidant enzyme and immune-related genes in common carp (*Cyprinus carpio*) fingerlings. *Aquaculture Research*. *48* (7), 3684 – 3692. https://doi.org/10.1111/are.13192

Jagannatha, L. S., 2015. Animal models for pre-clinical antiepileptic drug research. *Science*. *34* (2), 82 – 85. https://doi.org/10.3923/std.2015.82.85

Jäger, A. K., Saaby, L., 2011. Flavonoids and the CNS. *Molecules*. *16* (2), 1471 – 14885. https://doi.org/10.3390/molecules16021471

Johnston, G. A., 2015. Flavonoid nutraceuticals and ionotropic receptors for the inhibitory neurotransmitter GABA. *Neurochemistry International*. 89, 120 – 125. https://doi.org/10.1016/j.neuint.2015.07.013

Jones, D. P., 2006. Extracellular redox state: refining the definition of oxidative stress in aging. *Rejuvenation Research*. *9* (2) 169 – 181. https://doi.org/10.1089/rej. 2006.9.169

Kamboj, P., Singh, I., Mahadevan, N., Chaudhary, G., 2009. Anticonvulsants from nature. *Pharmacognosy Reviews*. *3* (5), 108.

Kovac, S., Dinkova-Kostova, A., Abramov, A., 2016. The role of reactive oxygen species in epilepsy. *Reactive Oxygen Species*. *1* (1), 38 – 52. https://doi.org/10.20455/ros.2016.807

Kuebutornye, F. K., Abarike, E. D., 2020. The contribution of medicinal plants to tilapia aquaculture: a review. *Aquaculture International*, 1 – 19. https://doi.org/10.1007/s10499-020-00506-3

Kumari, S., Mazumder, A. G., Bhardwaj, A., Singh, D., 2019. Early α-linolenic acid exposure to embryo reduces pentylenetetrazol-induced seizures in zebrafish larva. *Prostaglandins, Leukotrienes and Essential Fatty Acids*. *143*, 15 – 20. https://doi.org/10.1016/j.plefa.2019.02.002

Kütter, M. T., Romano, L. A., Ventura-Lima, J., Tesser, M. B., Monserrat, J. M., 2014. Antioxidant and toxicological effects elicited by alpha-lipoic acid in aquatic organisms. C*omparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. *162*, 70 – 76. https://doi.org/10.1016/j.cbpc.2014.03.008

Lushchak, V. I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquatic Toxicology. *101* (1), 13 – 30. https://doi.org/10.1016/j.aquatox. 2010.10.006

Lushchak, V. I., 2014. Free radicals, reactive oxygen species, oxidative stress and its classification. *Chemical-Biological Interactions. 224*, 164 – 175. https://doi.org/10.1016/j.cbi.2014.10.016

Maltez, L. C., Stringhetta, G. R., Enamorado, A. L., Okamoto, M. H., Romano, L. A., Monserrat, J. M., Sampaio, L. A., Garcia, L., 2017. Ammonia exposure and subsequent recovery trigger oxidative stress responses in juveniles of Brazilian flounder *Paralichthys orbignyanus*. *Fish Physiology and Biochemistry*. *43* (6), 1747 – 1759. https://doi.org/10.1007/s10695-017-0406-8

Murray, T. F., 2019. Amino acid neurotransmitters - glutamate, GABA and pharmacology of benzodiazepines. In: S. L. Crowell-Davis, T. F. Murray and L. Mattos de Souza Dantas, ed., *Veterinary Psychopharmacology*, 2nd ed. Omaha, NE, USA: John Wiley & Sons, Inc, pp.11 – 19.

5039 Niedermeyer, E., da Silva, F. L. (Eds.), 2005. Electroencephalography: basic principles,
5040 clinical applications, and related fields. 2 nd ed. Philadelphia, USA: Lippincott
5041 Williams & Wilkins, pp. 1285.

5042 Nunes, B., Antunes, S. C., Gomes, R., Campos, J. C., Braga, M. R., Ramos, A. S., Correia,
5043 A. T., 2015. Acute effects of tetracycline exposure in the freshwater fish *Gambusia*
5044 *holbrooki*: antioxidant effects, neurotoxicity and histological alterations. *Archives of*
5045 *Environmental Contamination and Toxicology*. *68* (2), 371 – 381.
5046 https://doi.org/10.1007/s00244-014-0101-z

5047 Oakes, K. D., Der Kraak, G. J. V., 2003. Utility of the TBARS assay in detecting oxidative
5048 stress in white sucker (*Catostomus commersoni*) populations exposed to pulp mill
5049 effluent. *Aquatic Toxicology*. *63* (4), 447 – 463. https://doi.org/10.1016/S0166-
5050 445X(02)00204-7

5051 Peixoto, H., Roxo, M., Krstin, S., Wang, X., Wink, M., 2016. Anthocyanin-rich extract of
5052 Acai (*Euterpe precatoria* Mart.) mediates neuroprotective activities in
5053 *Caenorhabditis elegans*. *Journal of Functional Foods*. 26, 385 – 393.
5054 https://doi.org/10.1016/j.jff.2016.08.012

5055 Pineda, R., Beattie, C. E., Hall, C. W., 2011. Recording the adult zebrafish cerebral field
5056 potential during pentylenetetrazole seizures. *Journal of Neuroscience Methods*. *200*
5057 (1), 20 – 28. https://doi.org/10.1016/j.jneumeth.2011.06.001

5058 Poulose, S. M., Bielinski, D. F., Carey, A., Schauss, A. G., Shukitt-Hale, B., 2017.
5059 Modulation of oxidative stress, inflammation, autophagy and expression of Nrf2 in
5060 hippocampus and frontal cortex of rats fed with açaí-enriched diets. *Nutritional*
5061 *Neuroscience*. *20* (5), 305 – 315. https://doi.org/10.1080/1028415X.2015. 1125654

5062 Reverter, M., Bontemps, N., Lecchini, D., Banaigs, B., Sasal, P., 2014. Use of plant
5063 extracts in fish aquaculture as an alternative to chemotherapy: current status and
5064 future perspectives. *Aquaculture*, 433, 50 – 61. https://doi.org/10.1016/j.
5065 aquaculture.2014.05.048

5066 Ronquillo, M. G. & Hernandez, J. C. A. (2017). Antibiotic and synthetic growth promoters
5067 in animal diets: review of impact and analytical methods. *Food Control*, *72*, 255 –
5068 267. https://doi.org/10.1016/j.foodcont.2016.03.001

Sallam, A. E., Mansour, A. T., Srour, T. M., Goda, A. M. A., 2017. Effects of different carotenoid supplementation sources with or without sodium taurocholate on growth, feed utilization, carotenoid content and antioxidant status in fry of the European seabass, *Dicentrarchus labrax*. *Aquaculture Research*. *48* (7), 3848-3858. https://doi.org/10.1111/are.13212

Schauss, A. G., Wu, X., Prior, R. L., Ou, B., Huang, D., Owens, J., Agarwal, A., Jensen, G. S., Hart, A. N., Shanbro, E., 2006. Antioxidant capacity and other bioactivities of the freeze-dried Amazonian palm berry, Euterpe oleraceae Mart. (acai). *Journal of Agricultural and Food Chemistry*. 54 (22), 8604 – 8610. https://doi.org/10.1021/jf0609779

Shahidi, F., Chandrasekara, A., & Zhong, Y. (2018). Bioactive Phytochemicals in Vegetables. In: Handbook of vegetables and vegetable processing. Siddiq, M., & Uebersax, M. (Eds.), 2nd ed. Hoboken, NJ: John Wiley & Sons. pp. 171.

Sies, H., 2015. Oxidative stress: a concept in redox biology and medicine. *Redox Biology*. *4*, 180 – 183. https://doi.org/10.1016/j.redox.2015.01.002

Singh, S., Prakash, A., Kaur, S., Ming, L. C., Mani, V., Majeed, A. B. A., 2016. The role of multifunctional drug therapy as an antidote to combat experimental subacute neurotoxicity induced by organophosphate pesticides. *Environmental Toxicology*. *31 (8)*, 1017 – 1026. https://doi.org/10.1002/tox.22111

Sinha, A. K., AbdElgawad, H., Giblen, T., Zinta, G., De Rop, M., Asard, H., Blust, R., De Boeck, G., 2014. Anti-oxidative defences are modulated differentially in three freshwater teleosts in response to ammonia-induced oxidative stress. *Plos One*. *9* (4), e95319. https://doi.org /10.1371/journal.pone.0095319

Souza-Monteiro, J. R., Hamoy, M., Santana-Coelho, D., Arrifano, G. P., Paraense, R. S., Costa-Malaquias, A., Mendonça, J. R., da Silva, R. F., Monteiro, W. S. C., Rogez, H., Oliveira, D. L., do Nascimento, J. L. M., Crespo-Lopéz, M. E., 2015. Anticonvulsant properties of *Euterpe oleracea* in mice. *Neurochemistry International*. *90*, 20 – 27. https://doi.org/10.1016/j.neuint.2015.06.014

Spada, P. D., Dani, C., Bortolini, G. V., Funchal, C., Henriques, J. A., Salvador, M., 2009. Frozen fruit pulp of *Euterpe oleraceae* Mart. (Acai) prevents hydrogen peroxide-

induced damage in the cerebral cortex, cerebellum, and hippocampus of rats. *Journal of Medicinal Food*. *12* (5), 1084 – 1088. https://doi.org/10.1089/jmf.2008.0236

Taiwe, G. S., Tchoya, T. B., Menanga, J. R., Dabole, B., De Waard, M., 2016. Anticonvulsant activity of an active fraction extracted from *Crinum jagus* L. (Amaryllidaceae), and its possible effects on fully kindled seizures, depression-like behaviour and oxidative stress in experimental rodent models. *Journal of Ethnopharmacology*. *194*, 421 – 433. https://doi.org/10.1016/j.jep.2016.10.023

Takahashi, L. S., Biller-Takahashi, J. D., Mansano, C. F. M., Urbinati, E. C., Gimbo, R. Y., Saita, M. V., 2017. Long-term organic selenium supplementation overcomes the trade-off between immune and antioxidant systems in pacu (*Piaractus mesopotamicus*). *Fish & Shellfish Immunology*. 60, 311 – 317. https://doi.org/10.1016/j.fsi.2016.11.060

Torma, P. D. C. M. R., Brasil, A. V. S., Carvalho, A. V., Jablonski, A., Rabelo, T. K., Moreira, J. C. F., Gelain, D. P., Flôres, S. H., Augusti, P. R., de Oliveira Rios, A., 2017. Hydroethanolic extracts from different genotypes of açaí (*Euterpe oleracea*) presented antioxidant potential and protected human neuron-like cells (SH-SY5Y). *Food Chemistry*. *222*, 94 – 104. https://doi.org/10.1016/j.foodchem. 2016.12.006

Vilhena, C. S., do Nascimento, L. A. S., de Aguiar Andrade, E. H., da Silva, J. K. R., Hamoy, M., Torres, M. F., Barbas, L. A. L., 2019. Essential oil of *Piper divaricatum* induces a general anaesthesia-like state and loss of skeletal muscle tonus in juvenile tambaqui, *Colossoma macropomum*. *Aquaculture*. *510*, 169 –175. https://doi.org/10.1016/j.aquaculture.2019.05.057

Wong, K., Stewart, A., Gilder, T., Wu, N., Frank, K., Gaikwad, S., Suciu, C., DiLeo, J. Utterback, E., Chang, K., Grossman, L, Cachat, J., kalueff, A. V., 2010. Modeling seizure-related behavioral and endocrine phenotypes in adult zebrafish. *Brain Research*. 1348, 209 – 215. https://doi.org/10.1016/j.brainres.2010.06.012

Wood, C. M., de Souza Netto, J. G., Wilson, J. M., Duarte, R. M., Val, A. L., 2017. Nitrogen metabolism in tambaqui (*Colossoma macropomum*), a neotropical model teleost: hypoxia, temperature, exercise, feeding, fasting, and high environmental ammonia. *Journal of Comparative Physiology B*. *187* (1), 135 – 151. https://doi.org/10.1007/s00360-016-1027-8

Wu, F., Jiang, M., Wen, H., Liu, W., Tian, J., Yang, C. G., Huang, F., 2017. Dietary vitamin E effects on growth, fillet textural parameters, and antioxidant capacity of genetically improved farmed tilapia (GIFT), *Oreochromis niloticus*. *Aquaculture International. 25* (2), 991 – 1003. https://doi.org/10.1007/s10499-016-0089-7

Yoo, D. Y., Choi, J. H., Kim, W., Yoo, K. Y., Lee, C. H., Yoon, Y. S., Won, M., Hwang, I. K., 2011. Effects of *Melissa officinalis* L. (lemon balm) extract on neurogenesis associated with serum corticosterone and GABA in the mouse dentate gyrus. *Neurochemical Research. 36* (2). 250 – 257. https://doi.org/10.1007/s11064-010-0312-2

Zar, J. H., 1996. Biostatistical Analysis. 3 rd edition. New Jersey, Prentice - Hall, 662 pp.

Figure legends

Figure 1. Electroencephalographic recordings (EEG) from the midbrain region of juvenile tambaqui, *Colossoma macropomum* injected with petilenetetrazole (PTZ) and/or diazepam (BDZ) after a feeding trial with 5.00 and 10.0% LEO inclusion in the diet. Recordings in sham control (A), fish submitted to PTZ i.p. application (B), fish fed diet 5.00% LEO and submitted to PTZ i.p. application (C), fish fed diet 10.0% LEO and submitted to PTZ ip application (D), and fish submitted to BDZ i.p. application with subsequent PTZ i.p. injection (E). EEG over the course of 600 s (left panels), amplification of fragments in the EEG recordings (center panels), and spectrograms of frequencies (right panels).

Figure 2. Power spectral density (PSD): SHAM CONTROL (CTR), PTZ, BDZ - PTZ (A), from tambaqui fish, *Colossoma macropomum* fed diets 5.00% and 10.0% LEO compared to those receiving BDZ for the control of seizures induced by PTZ (B). Linear distribution of amplitude comparing the power in each experimental group (C). Different letters in columns indicate significant differences after one-way ANOVA and Tukey's test ($p < 0.05$), n = 5 per treatment.

Figure 3. Action potential bursts duration (APBD) in seconds (s) for PTZ, PTZ LEO 5.00%, PTZ LEO 10.0% groups (A); and linear mean power amplitude (in $mV^2/Hz \times 10^{-3}$) of the action potential bursts obtained from the PTZ, PTZ LEO 5.00%, PTZ LEO 10.0% groups (B) of tambaqui fish, *Colossoma macropomum* fed the experimental diets. Different letters in columns indicate significant differences after one-way ANOVA and Tukey test ($p < 0.05$), n = 5 per treatment.

Figure 4. Thiobarbituric reactive substances (TBARS) content (nmol TMP/mg of protein) in the brain of *Colossoma macropomum* juveniles fed or not (control) with diets containing 5.00% and 10.0% açai inclusion (*E. oleracea*) for 30 days. Data are expressed as means (red line) ± standard deviation (black whiskers). Different letters among treatments indicate significant differences after one-way ANOVA and Tukey's test ($p < 0.05$), $n = 5$ per treatment. TMP stands for tetramethoxypropane employed as a standard.

Table 1. Latencies (s) to behavioral cues in *Colossoma macropomum* fed or not with diets containing 5.00% and 10.0% inclusion of lyophilized *Euterpe oleracea* (LEO), treated with pentylenetetrazole (PTZ) and/or diazepam (BDZ).

			LEO (%)	
Behavioral cues	BDZ - PTZ	PTZ	PTZ - 5.00	PTZ - 10.0
II	45.33 ± 5.78	38.25 ± 5.51	27.25 ± 6.76	39.83 ± 4.93
HY	-	67.75 ± 7.27	108.5 ± 44.28	41.83 ± 19.46
VS	-	75.25 ± 26.38	140.5 ± 82.82	-
JOW	-	85 ± 6.81	78.25 ± 52.08	-
LPR	-	94.25 ± 5.36	85 ± 56.78	60.16 ± 20.70
IS	-	108.25 ± 62.51	-	-
LDF	-	130.5 ± 24.94	-	-
TT	-	140.75 ± 55.39	-	53.66 ± 53.66
RS	-	161.5 ± 6.03	95.5 ± 46.66	46.66 ± 30.51
STM	-	199.25 ± 72.89	-	-
VMS	101.17 ± 5.82	-	-	-
RPR	-	1619.75 ± 176.19	126.25 ± 93.26	89.16 ± 58.06

II - Intermittent immobility; HY – hyperexcitability; VS - ventral swimming; JOW - jumping out of water; LPR - loss of posture reflex; IS - incoordinated swimming; LDF - lifting the dorsal fin; TT - tail tremor; RS - rotational swimming; STM - slow tail movement; VMS - visible muscular spasms; and RPR - return of the posture reflex. Dashes denote absence of behavioral cues.

Figure 1

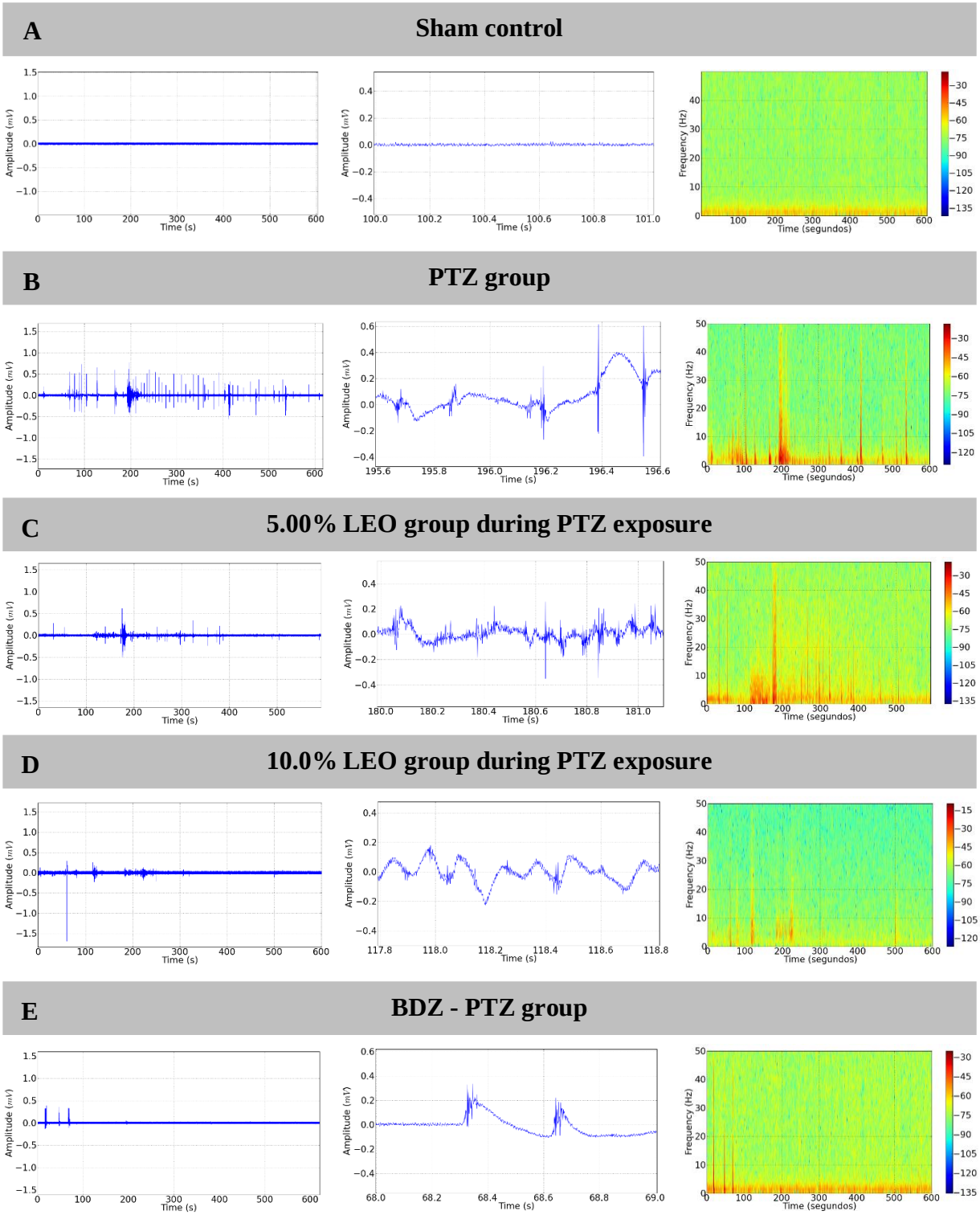
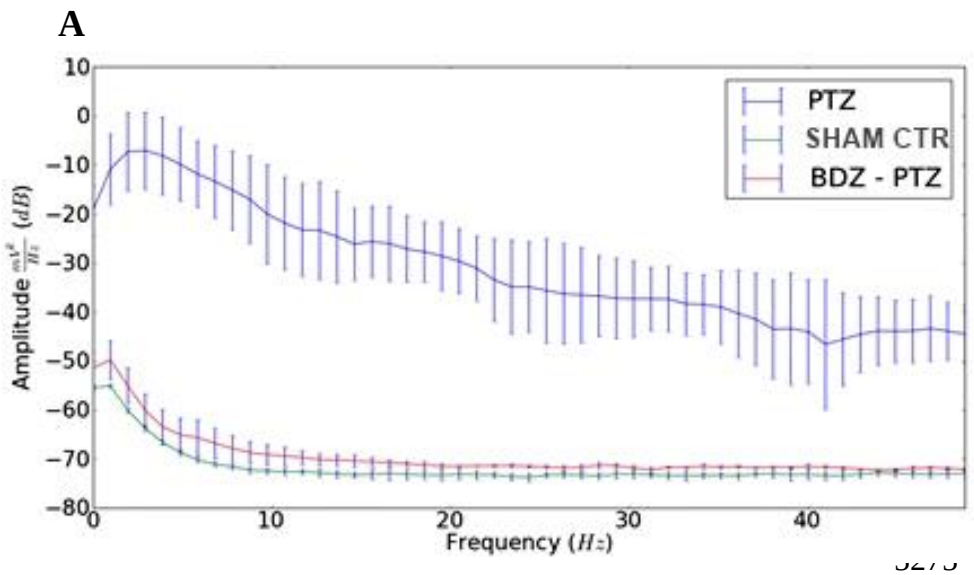
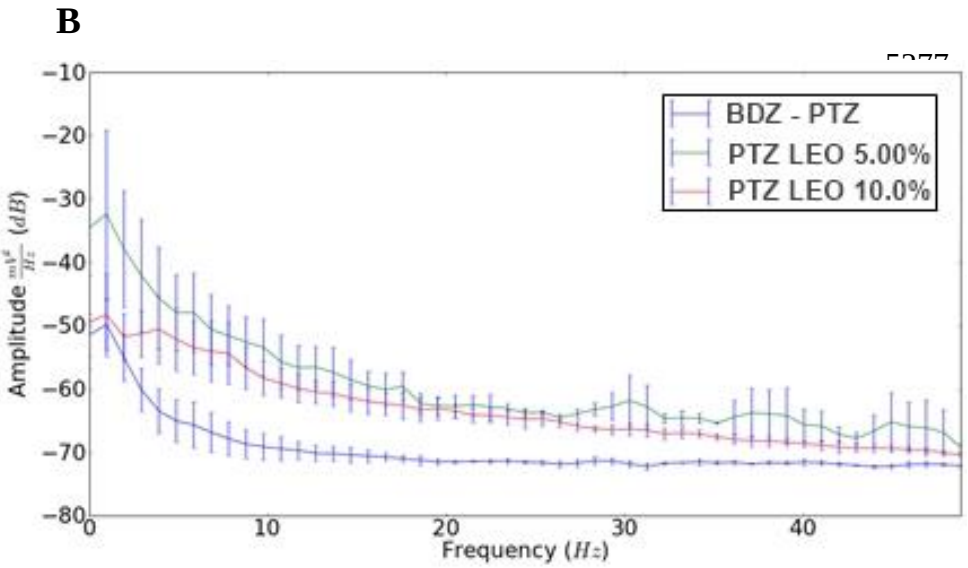

A
Sham control
B
PTZ group
C
5.00% LEO group during PTZ exposure
D
10.0% LEO group during PTZ exposure
E
BDZ - PTZ group
Amplitude (mV)
Time (s)
Frequency (Hz)
Time (segundos)

Figure 2


A
PTZ
SHAM CTR
BDZ - PTZ
Amplitude (dB)
Frequency (Hz)


B
BDZ - PTZ
PTZ LEO 5.00%
PTZ LEO 10.0%
Amplitude (dB)
Frequency (Hz)

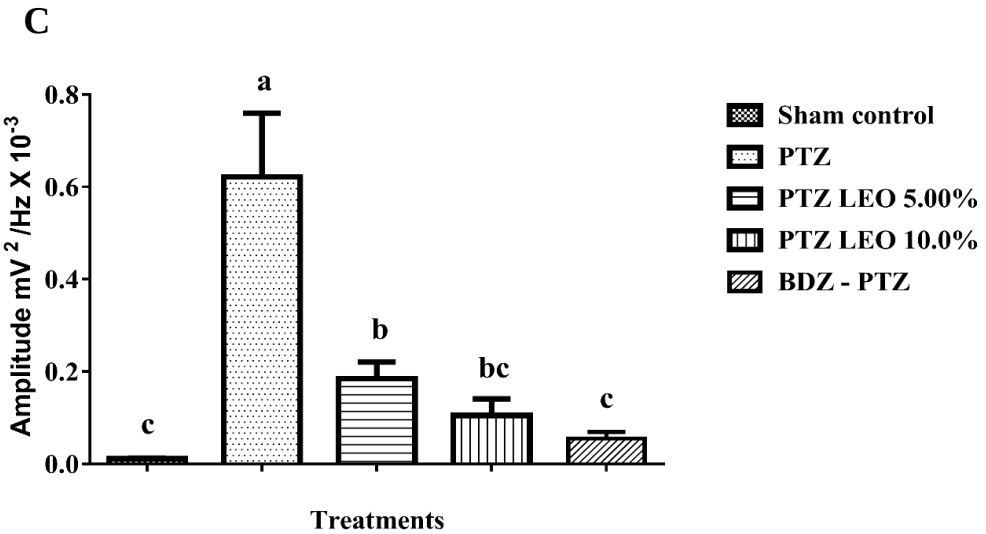

C
Amplitude mV 2 /Hz X 10-3
a
b
bc
c
c
Sham control
PTZ
PTZ LEO 5.00%
PTZ LEO 10.0%
BDZ - PTZ
Treatments

Figure 3

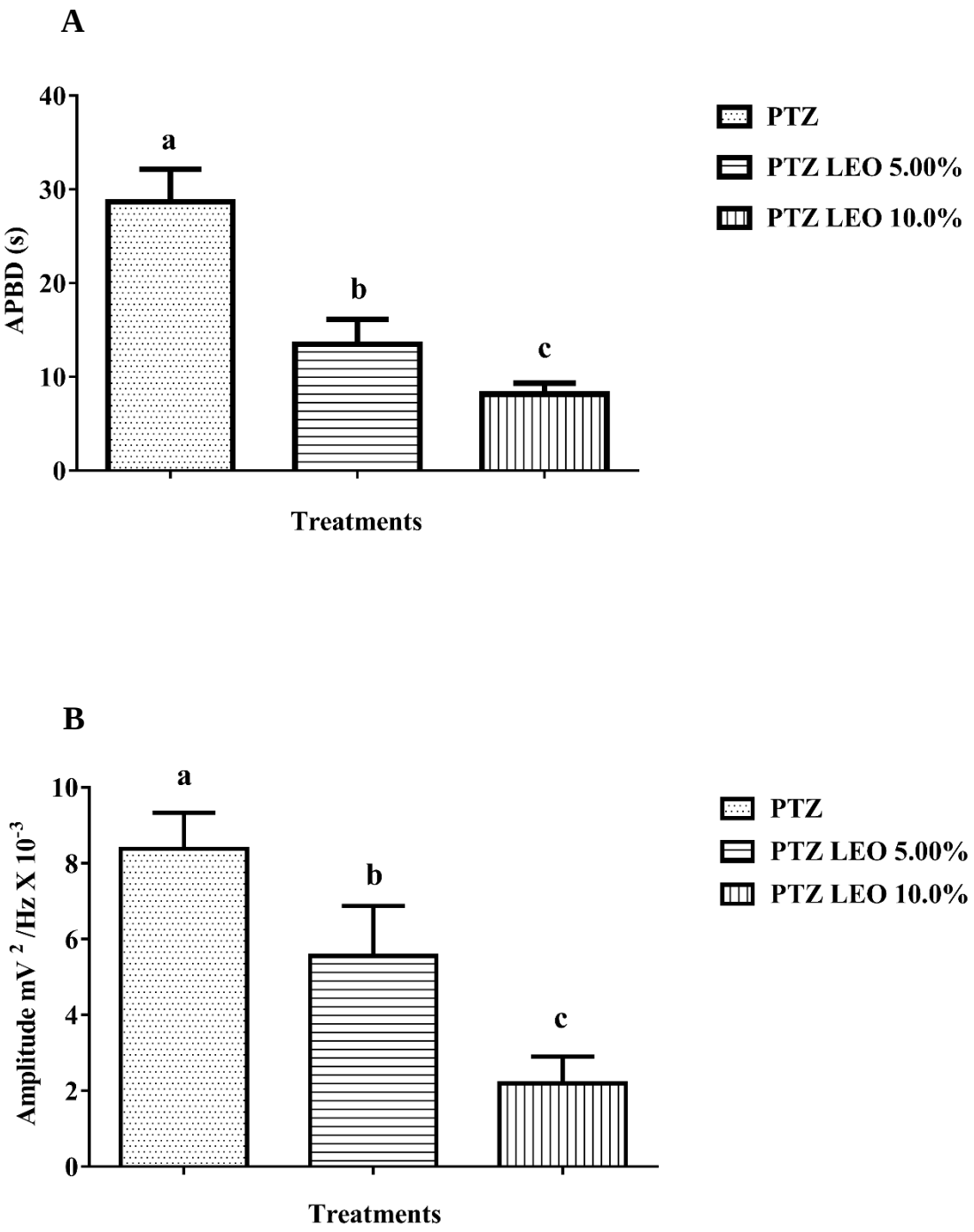
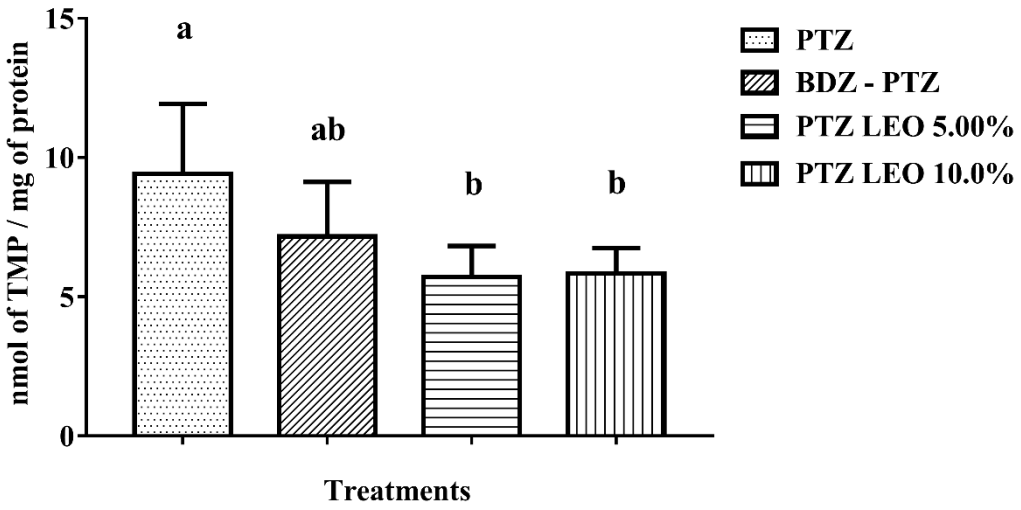

A
APBD (s)
40
30
20
10
0
a
b
c
Treatments
PTZ
PTZ LEO 5.00%
PTZ LEO 10.0%
B
Amplitude mV 2 /Hz X 10-3
10
8
6
4
2
0
a
b
c
Treatments
PTZ
PTZ LEO 5.00%
PTZ LEO 10.0%

Figure 4


15
10
5
0
nmol of TMP / mg of protein
a
ab
b
b
Treatments
PTZ
BDZ - PTZ
PTZ LEO 5.00%
PTZ LEO 10.0%

4. CONSIDERAÇÕES FINAIS

Os resultados alcançados a partir desta tese indicam que o açaí (*E. oleracea*) em dietas para tambaqui, atua como promotor do crescimento; incrementa respostas antioxidantes em órgãos específicos; modula o metabolismo energético; prepara e aumenta a resistência contra o estresse; e apresenta atividade anticonvulsiva e neuroprotetora. A partir do primeiro objetivo (Capítulo 1) deste estudo, observa-se melhora sobre a coloração (aumento da cor ciano) da pele na região dorsal. No entanto, não foram detectados aumentos no conteúdo de polifenóis, flavonoides e tampouco sobre a capacidade de eliminação de radicais (DPPH) no músculo dos peixes previamente alimentados com esta fruta. Melhor performance de crescimento foi observada com a inclusão de açaí (1,25% a 10,0% LEO), o que pode estar diretamente relacionada com a melhora nos índices de eficiência alimentar.

Porém, a otimização do conjunto de índices de eficiência alimentar (FCR, FI e PER), muito provavelmente está associado com o potencial efeito desta fruta sobre o intestino do tambaqui, o qual apresentou elevada competência antioxidante a partir da mínima inclusão de açaí na dieta (0,63% LEO). Entretanto, um maior nível de inclusão de açaí (estimado em 5,47% LEO) foi necessário para alcançar maior diminuição da peroxidação lipídica intestinal. Evidentemente, quanto maior os níveis de inclusão dietética de LEO, maior é o custo final para a produção da ração, consequentemente onerando o custo de alimentação. Por isso, a inclusão de 1,25% LEO é indicada em termos de sua viabilidade econômica, mas também pela satisfatória performance zootécnica alcançada por estes animais num período de 30 dias. Vale ressaltar que a utilização de LEO dietético promove redução da ingestão de ração a partir da inclusão de 1,25% LEO na dieta. Assim, supomos a participação de vias de sinalização molecular associadas com mecanismos de

detecção de nutrientes, além da possível atuação dos fitoquímicos do açaí na ativação da AMPK, explicando os positivos efeitos observados sobre o crescimento destes animais, hipótese que em parte pôde ser avaliada no segundo objetivo da presente tese (Capítulo 2).

No Capítulo 2, investigou-se os efeitos da administração dietética de açaí sobre o metabolismo energético muscular de tambaqui. Os resultados claramente indicaram que o açaí induz a lipólise (oxidação de triglicerídeos) e, por outro lado, eleva substancialmente o potencial metabólico (expressivo aumento sobre a atividade da ETS) muscular deste animal a partir da inclusão dietética de 1,25% LEO. Estas respostas são similares as já reportadas para animais terrestres alimentados com dietas suplementadas com outros extratos de plantas. O presente trabalho, também gerou a perspectiva de futuros estudos para investigar a hipótese acerca da semelhança estrutural entre o perfil de fitoquímicos de LEO e corticosteróides, bem como seus mecanismos sobre a modulação endócrina de fatores de crescimento. No geral, provavelmente, a melhora sobre a eficiência energética muscular promovida pelo açaí contribuiu para as melhores taxas de crescimento observadas no Capítulo 1, assim como pode ter favorecido outras funções fisiológicas, como aquelas relacionadas a resistência ao estresse. Esta última hipótese, foi então considerada no terceiro objetivo (Capítulo 3) do presente estudo.

Consistentemente, na investigação desenvolvida a partir do Capítulo 3, peixes alimentados com dietas contendo LEO por um período de 30 dias e transportados por até 24 h exibiram maior disponibilidade de oxigênio dissolvido na água de transporte. Isto nos permite considerar, que a LEO contribui para a melhora da manutenção da qualidade da água após longo período de transporte. Embora os níveis dietéticos de LEO avaliados não tenham proporcionado o aumento da capacidade antioxidante total, este fruto desempenhou fundamental ação sobre a proteção contra a peroxidação lipídica em até 12 h de transporte, com expressiva redução do conteúdo de TBARS no cérebro, brânquias e fígado. Apesar de

apresentar níveis mínimos no músculo, a LPO também foi reduzida pontualmente nos peixes tratados com LEO. Assim, o açaí dietético apresenta potencial capacidade para aumentar a resistência ao estresse de transporte, uma vez que, a promoção de proteção contra o dano lipídico se traduz na prevenção de disfunções celulares.

Finalmente, no quarto objetivo da tese (Capítulo 4) confirmou-se o potencial papel do açaí sobre a proteção contra convulsões induzidas por PTZ no cérebro de tambaqui. Particularmente, a administração dietética de 10,0% LEO por 30 dias, reduziu em 80% as amplitudes dos traçados das ondas cerebrais em tambaquis expostos ao PTZ, o que refletiu também na abreviação ou eliminação de sinais comportamentais relativos a crises convulsivas. Além disso, os níveis de TBARS foram significativamente atenuados em peixes alimentados com 5,00% a 10,0% LEO e desafiados com PTZ. Possivelmente, estes efeitos estão relacionados a capacidade do açaí para modular o sistema GABAérgico e, além disso, este fruto possui alta atividade antioxidante que efetivamente combate o acúmulo de lipídios peroxidados neste órgão (como previamente verificado no Capítulo 3). *E. oleracea*, pode, portanto, conter substancialmente os efeitos de compostos com atividade indutora de crises tônico-clônicas generalizadas em juvenis de tambaqui. Além disso, o *C. macropomum* mostrou-se elegível como um novo modelo animal para estudos eletrofisiológicos e, adicionalmente, abre caminhos para o desenvolvimento de novas pesquisas voltadas para questões de aquicultura.

Finalmente, a partir do conjunto de efeitos funcionais proporcionadas pelo açaí dietético ao *C. macropomum*, destacamos a potencial aplicação de LEO como aditivo alimentar o que, portanto, emerge como uma oportunidade para a indústria da aquicultura. Estima-se que 123,065.3 ton de ração são utilizadas alcançar a produção nacional de tambaqui (baseado numa FCR de 1,2:1). Desta forma, seriam necessários aproximadamente 1,538.3 ton de açaí liofilizado para produzir dietas funcionais contendo

1,25% de inclusão de LEO. Em termos gerais, esta demanda equivale a cerca de 16,541.5 ton de açaí fresco para produzir o montante de ração anual necessários para o setor produtivo de tambaqui. O estado do Pará é o maior produtor de açaí do Brasil, contabilizando 1.278 milhões de ton em 2018. Portanto, neste cenário, a demanda de açaí fresco para a produção de dietas funcionais para tambaqui poderia ser suprida.

5. CONCLUSÕES

Ao todo, os resultados a partir deste estudo demonstram que:

A) A inclusão de LEO a partir de 1,25% (13 g de açaí Kg^{-1}) em dietas para juvenis de tambaqui, tem efeitos positivos sobre a eficiência alimentar e performance de crescimento. Além disto, a inclusão dietética de 0,63% LEO (6 g de açaí Kg^{-1}) aumenta em 39.57% a competência antioxidante intestinal. No entanto, a intensificação da cor ciano na região dorsal do tambaqui foi possível apenas a partir da inclusão de 5,00% LEO (50 g de açaí Kg^{-1}) na dieta. Do mesmo modo, somente com a inclusão estimada em 5,47% LEO foi possível determinar o menor nível de TBARS no intestino. Considerando o custo alimentar, recomenda-se o uso de 1,25% LEO, uma vez que assim, a eficiência alimentar, crescimento e o status antioxidante intestinal são satisfatórios.

B) A inclusão de 0,63% LEO na dieta reduz em 40% o conteúdo de triglicerídeos muscular e, a partir da administração dietética de 1,25% LEO é possível aumentar em 76% o potencial metabólico deste órgão. Este resultado reflete uma forte correlação ($R^2 = 0.87$) entre a atividade do sistema de transporte de elétrons (ETS) e taxa de crescimento específico (SGR), contribuindo para explicar o significativo crescimento promovido por LEO no Capítulo 1.

C) Peixes alimentados com LEO na dieta antes do transporte experimentaram melhor condição de oxigênio dissolvido na água, com uma concentração 17,7% maior em comparação ao controle após 24 h de transporte. Todos os órgãos avaliados (brânquias, cérebro, fígado e músculo) apresentaram redução na capacidade antioxidante total, independentemente do tempo de exposição ao transporte. Contudo, é notório a efetiva redução dos níveis de TBARS no cérebro, brânquias e fígado a partir da inclusão de 2,50% a 5,00% LEO em um limiar de até 12 h de transporte. No músculo, danos lipídicos foram reduzidos significativamente em 3 h de transporte a partir da inclusão de 0,63% LEO na dieta.

D) A administração de 10,0% LEO na dieta promove redução expressiva (80%) sobre a amplitude média dos traçados registrados nos eletroencefalogramas. Além disso, a duração da salva de potencial (APB) foi substancialmente menor nesse mesmo tratamento alimentar, o que significa atenuação da excitabilidade e da intensidade convulsiva. A partir da inclusão dietética de 5,00% LEO, obteve-se redução intermediária da intensidade da convulsão induzida pelo PTZ, além de uma redução na ordem de 60,69% sobre a concentração malondialdeído - MDA (TBARS) em respeito ao controle. Essa proteção conferida através do açaí, foi refletida sobre o comportamento do tambaqui, mitigando os sinais decorrentes de crises convulsivas. Desta forma, sugere-se a inclusão de 5,00% LEO para a formulação de dietas funcionais anticonvulsivas voltadas para o setor produtivo de tambaqui.

6. PERSPECTIVAS FUTURAS

- O presente estudo confirma que o açaí (*E. oleracea*) desempenha importantes efeitos benéficos sobre o crescimento de tambaqui e sobre o uso do alimento. Contudo, a redução significativa da ingestão de ração nos peixes tratados com LEO, induz a

formulação de hipóteses acima de algumas vias de sinalização molecular envolvidas com a regulação da ingestão de alimento, bem como, pela possível atuação de LEO na modulação da AMPK. Uma diversidade de estudos para avaliar os efeitos de LEO sobre a integridade e funcionalidade intestinal; atividade prebiótica ou de enzimas digestivas; bem como para elucidar a questão da biodisponibilidade e bioatividade de compostos fenólicos no intestino são encorajados. Desta forma, se reconhece que ainda são necessários diversos estudos para expandir nossos achados nessa área de pesquisa.

- Embora as respostas induzidas pelo LEO sobre o metabolismo energético de tambaqui sejam semelhantes a já reportadas para animais de criação terrestres, uma série de lacunas foram identificadas e, portanto, futuros estudos são necessários para determinar os mecanismos endócrinos por detrás da regulação do metabolismo muscular. Adicionalmente, a evidente redução de triglicerídeos em peixes alimentados com LEO, certamente despertará interesse para o setor produtivo deste peixe, uma vez que, o teor de gordura corporal tem influência sobre o sabor do filé. Sendo assim, recomenda-se que futuros estudos avaliem os efeitos de *E. oleracea* sobre a qualidade e aspectos sensoriais do filé de tambaqui.

- Estudos com foco sobre o uso de extratos de plantas para a formulação de dietas funcionais como estratégia de preparação para o estresse de transporte são limitados. Desta forma, chama-se a atenção para esta linha de pesquisa a ser explorada. Níveis intermediários de inclusão de LEO na dieta são necessários para responder efetivamente contra o estresse de transporte e, levando em conta o custo alimentar do uso de LEO, sugere-se que estudos sejam realizados para avaliar os efeitos de subprodutos do açaí (p. ex. sementes ou o óleo derivado).

- A abordagem de estudos eletrofisiológicos é um campo pouco explorado em investigações para aquicultura e parece ser promissora ao se levar em conta, que organismos cultivados podem, em certas circunstâncias, estar propensos a sofrer com poluentes. Então, futuros estudos são encorajados para investigar os efeitos de LEO ou de seus subprodutos contra crises convulsivas parciais ou generalizadas induzidas por amônia, pesticidas ou agrotóxicos, por exemplo.