UNIVERSIDADE FEDERAL DA GRANDE DOURADOS
Faculdade de Ciências Agrárias
Programa de Pós-Graduação em Zootecnia

EFEITOS DE BIOATIVOS NUTRICIONAIS DO CERRADO BRASILEIRO SOBRE PARÂMETROS MORFOFISIOLÓGICAS E TEGUMENTARES EM PEIXES ORNAMENTAIS

Marcos Paiva Scardua

Oceanólogo

Dourados - MS

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Marcos Paiva Scardua

Orientadora: Profª Drª Cláucia Aparecida Honorato da Silva

Coorientador: Dr. Dacley Hertes Neu

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Marcos Paiva Scardua

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Drª Cláucia Aparecida Honorato da Silva
Orientadora

BIOGRAFIA DO AUTOR

Marcos Paiva Scardua, filho de João Felício Scardua e Alice Paiva Scardua, nasceu em 18 de junho de 1970 em Brasília-DF. Em janeiro de 1990, iniciou o curso Bacharelado em Oceanologia pela Universidade Federal de Rio Grande (FURG), Rio Grande-RS, onde foi bolsista da FAPERGS, graduando-se em janeiro de 1996. Em março do mesmo ano, iniciou-se o Mestrado em Aquicultura pela Universidade Federal de Santa Catarina (UFSC) em Florianópolis, onde desenvolveu estudos na área de carcinicultura marinha e foi bolsista do CNPq, submetendo-se à defesa da dissertação em 19 de outubro de 1998. De 1998 a 2000 foi Técnico em Oceanografia na Secretaria Municipal de Meio ambiente (SEMMAM) Vitória-ES. Em 1999 até outubro de 2000 foi professor na União de Escolas de Ensino Superior Capixaba, UNESC, Colatina-ES. Em 2001 ingressou no Programa de pós graduação em Bioloxia mariña e acuicultura na Universidade de Santiago de Compostela (Espanha) obtendo o diploma de estudos avançados (DEA) em 2003. Em 2005 e 2006 desenvolveu trabalhos de pesquisa na Université de Bretagne Occidentale (U.B.O) com contratos da U.B.O e do Centre National de la Recherche Scientifique (CNRS) em Brest na França. Em 2007 trabalhou no Centro de Tecnologia em Aquicultura e Meio Ambiente Ltda (CTA), Vitória-ES no Projeto Atlântida: Recifes artificiais marinhos. Uso sustenável da biodiversidade marinha no desenvolvimento da pesca, ecoturismo e maricultura no litoral do município da Serra, ES. Em 2010 foi bolsista DTI-2 na Embrapa Tabuleiros Costeiros, Aracajú-SE, dedicando-se a atividades de piscicultura familiar e ao projeto da rede Bijupirá. Em fevereiro de 2011 foi efetivado no Instituto Federal de Educação Ciência e Tecnologia do Ceará (IFCE) *campus* Aracati-CE, onde atua como professor efetivo. Entre 2016 e 2017 realizou a Especialização em Formação pedagógica para docência na educação profissional e tecnológica pelo IFCE, Fortaleza-CE. Em 2022 iniciou sua licença para realizar a capacitação no Programa de Doutorado em Zootecnia na Universidade Federal da Grande Dourados, Dourados-MS, iniciando o curso em março de 2022, procedendo à defesa de sua qualificação em 10 de março de 2025 e sua defesa de tesem dezembro de 2025.

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ABNT	Associação Brasileira de Normas Técnicas
ALT	Alanina aminotransferase
ANOVA	Análise de variância
AST	Asparatto aminotransferase
Ba	Azul do padrão RGB da nadadeira anal
Bc	Azul do padrão RGB da nadadeira caudal
BH2	Di-hidrobiopterina
BH4	Tetra-hidrobiopterina
BSA	Soro de albumina bovina
C	Controle
CA	Conversão alimentar
Ca	Cromaticidade *a* do corpo
CAT	Catalase
CATL	Catalase no fígado
CATm	Catalase no músculo
Cb	Cromaticidade *b* do corpo
CEUA	Comissão de Ética no Uso de Animais
C*I*	Luminosidade do corpo
CONCEA	Conselho Nacional de Controle de Experimentação Animal
DHI	5,6-di-idroxindol
DHICA	Ácido 5,6-c-2-carboxílico
DL_{50}	Dose necessária de um fator para matar 50% de uma população em teste
DPPH	Método utilizado para detectar a presença de compostos antioxidantes, baseado na eliminação do radical livre estável 1,1-difenil-2-picrilhidrazil (DPPH•).
EFAMD	Análise Fatorial de Dados Mistos
Ga	Verde do padrão RGB da nadadeira anal
Gc	Verde do padrão RGB da nadadeira caudal
GMP	Guanosina monofosfato
GP	Ganho em peso
GTP	Guanosina trifosfato
GTPCH	guanosina ciclohidrolase
IMP	Inosina monofosfato
K	Fator de condição
L	Comprimento total
Lab	Sistema de coordenadas Hunter: *L*: luminosidade; *a*: cromaticidade de *a*: (-100: verde,+100: vermelho); cromaticidade de *b*”(-100: azul e +100: amarelo))
L-DOPA	di-hidroxifenilalanina
MAPA	Ministério da Agricultura Pecuária e Abastecimento
MCH	Hormônio concentrador de pigmento
MSH	Hormônio dispersor de pigmento
NAS	N acetil serotonina
PB	Proteína bruta
PHE	Extrato hidroalcoólico da polpa desengordurada de pequi
PhenylH	Fenilalanina hidroxilase
PM	Farinha desengordurada da polpa de pequ

PTL	Proteína total do fígado
PTm	Proteína total músculo
PTPS	6-piruvil-tetrahidropterina sintetase
Ra	Vermelho do padrão RGB da nadadeira anal
Rc	Vermelho do padrão RGB da nadadeira caudal
RGB	Padrão que designa cores. Sendo, R: vermelho; G:verde e B: azul
SOD	Superóxido dismutase
SOD L	Superóxido dismutase no fígado
SODm	Superóxido dismutase no músculo
SPR	Sepiapterina redutase
T 1000	Dieta com1000 $mg.kg^{-1}$ de inclusão de decocto de folha guavira
T 2000	Dieta com 2000 $mg.kg^{-1}$ de inclusão de decocto de folha guavira
T 250	Dieta com 250 $mg.kg^{-1}$ de inclusão de decocto de folha guavira
T 500	Dieta com 500 $mg.kg^{-1}$ de inclusão de decocto de folha guavira
TCE	Taxa de crescimento específico
Tyr	Tirosinase
TyrH	Tirosina hidroxilase
Tyrp1	Tirosinase 1
Tyrp2	Tirosinase 2
TyrpH	Triptofano hidroxilase
Uct	Uniformidade do lote em relação comprimento total
Upf	Uniformidade do lote em relação ao peso final
UV	Radiação ultravioleta
W	Peso
XDH	Xantina desidrogenase
XO	Xantina oxidase

EFEITOS DE BIOATIVOS NUTRICIONAIS DO CERRADO BRASILEIRO SOBRE PARÂMETROS MORFOFISIOLÓGICAS E TEGUMENTARES EM PEIXES ORNAMENTAIS

RESUMO - A aquicultura é a atividade que envolve a criação de organismos aquáticos, que apresenta uma expansão na criação de espécies ornamentais. Esta modalidade vem ganhando destaque na aquicultura nacional, principalmente por apresentar-se economicamente rentável. Por ser organismos com grande potencial comercial, seu cultivo tem se tornado cada vez mais intensivo e, em ambiente natural, esses peixes tem uma alimentação baseada na combinação de ingestão de alimentos de origem animal (alto valor energético) e alimentos de origem vegetal (baixo valor energético) ricos em pigmentos. No entanto, para se obter um bom desenvolvimento desses peixes, a dieta exige uma suplementação na alimentação. Afim de otimizar ainda mais estas rações, são usados aditivos que possam incrementar a produtividade. Portanto, há crescente interesse por pesquisas que visam a utilização de fontes alternativas de compostos bioativos de alimentos e seu subprodutos (casca, sementes e bagaço), que incorporadas ao alimento podem ter efeitos com atividade antioxidante e palatabilizante, aumentando o seu valor agregado. Este trabalho tem como objetivo avaliar a inclusão de fontes de bioativos do Cerrado na alimentação de peixes ornamentais. Esta tese apresenta as considerações iniciais. **Capitulo 2**, onde investigou-se a adição de pequi (*Caryocar brasiliense*) como fonte de carotenóides e promotor de coloração, saúde e bem estar na dieta de Mato Grosso (*Hyphessobrycon eques*). Foram utilizados 150 espécimes de Mato Grosso, os quais foram distribuídos em um delineamento experimental inteiramente casualizado (três tratamentos e cinco repetições), cada unidade experimental com capacidade de 20L, ligadas a um sistema de recirculação de água durante 30 dias. Os peixes foram alimentados com dieta comercial (C), dieta comercial suplementada com 800 $mg.kg^{-1}$ de extrato hidroalcoólico da polpa desengordurada do pequi (PHE), e dieta comercial suplementada com 800 $mg.kg^{-1}$ de farinha desengordurada de pequi (PM). Foram avaliadas a sobrevivência, parâmetros de desenvolvimento, coloração, metabolismo de enzimas musculares e das enzimas hepáticas. Não houve influência das dietas nos parâmetros de desenvolvimento ($p>0,05$), porém maior consumo no grupo PM ($p < 0,05$), resultando em pior conversão alimentar. A coloração das nadadeiras foi influenciada pela dieta: na nadadeira caudal, o canal vermelho (R) foi maior no grupo controle do que no grupo PHE, enquanto o canal azul (B) foi maior no grupo PM do que no grupo PHE ($p < 0,05$). Na nadadeira anal, o grupo controle também apresentou valores de vermelho mais elevados, e os canais verde (G) e azul (B) foram significativamente afetados. A atividade da AST hepática aumentou nos peixes alimentados com PHE ($p < 0,05$), enquanto ALT, SOD e CAT foram alteradas. Em conclusão, o PM melhorou a coloração das barbatanas e reduziu a AST hepática, indicando hepatoproteção, mas diminuiu a SOD muscular, sugerindo redução da atividade antioxidante. **Capítulo 3,** avaliou-se as implicações da utilização de decocto de folha de Guavira (*Campomanesia guazumifolia*) como aditivo alimentar para *Geophagus pyrocephalus*. Foi realizado o estudado de toxicidade em *Artemia salina* e em

juvenis de *G. pyrocephalus*, além do teste de alimentação com *G. pyroccephalus*. Foram avaliadas a sobrevivência nos testes de toxicidade e histopatologia de brânquias. No teste de tolerância a alimentação a dieta foi fornecida por 30 dias. Ao final do ensaio estes foram eutanaziados e avaliados o desempenho de crescimento, enzimas metabólicas e enzimas antioxidantes. Os resultados mostraram alta toxicidade da decocção para *A. salina* e *G. pyrocephalus*, com lesões branquiais observadas em todas as concentrações. No entanto, a dieta contendo 250 $mg \cdot kg^{-1}$ do extrato proporcionou benefícios antioxidantes sem comprometer a saúde dos peixes. Doses mais elevadas induziram efeitos adversos significativos. Portanto, o uso seguro da decocção de C. guazumifolia depende da determinação da dose adequada. **Capítulo 4,** uma revisão de literatura que contextualizou a relação da fenilalanina e da tirosina com os pigmentos naturais (melaninas, pteridinas, purinas e carotenóides), o metabolismo e a pigmentação dos peixes. Apresentou-se os tipos de pigmentos, células e locais de deposição, rotas metabólicas relacionadas a cada tipo de pigmento, mecanismos e fatores que controlam que alteram a coloração, bem como as funções, reportadas na literatura, desses pigmentos e sua relação com esses aminoácidos. Demonstrando que a fenilalanina e a tirosina estão relacionadas de certa forma ao metabolismo da melanina e da pteridina e que os pigmentos podem ser metabolizados de forma distinta entre as diversas espécies de peixes, bem como durante as fases de seu desenvolvimento ontogenético, além de atuarem diretamente na coloração, fatores de crescimento, reprodução, saúde e bem estar desses animais. **Capítulo 5,** avaliou a suplementação dietética de fenilalanina no aprimoramento da coloração de peixes. Para verificar o incremento da coloração em platys (*Xiphophorus maculatus*) ao longo de um período de 21 dias, utilizamos a inclusão de 0,81%, 0,99%, 1,17%, 1,36% e 1,54% de fenilalanina em uma dieta isenta de carotenóides. A avaliação abrangeu diversos parâmetros, incluindo a coloração corporal e caudal (pedúnculo caudal), a atividade antioxidante das enzimas catalase (CAT) e superóxido dismutase (SOD) e o desempenho. Um experimento inteiramente casualizado foi conduzido, utilizando 150 espécimes de platy, dispostos em 5 tratamentos e 3 repetições. Os espécimes foram distribuídos em 15 tanques de 60 L, conectados a um sistema de recirculação de água com filtração acoplada. Observamos um aumento no peso e no comprimento padrão ($p < 0,05$), bem como baixas taxas de sobrevivência (de 49,9% até 66,7%), apesar de ($p > 0,05$). Um aumento substancial na coloração corporal (a*, b*, ΔE) e caudal (a*) foi demonstrado, conforme corroborado por correlações moderadas (r entre 0,5 e 0,75). Além disso, observou-se uma correlação entre SOD e ΔE corporal, sugerindo uma relação entre atividade antioxidante, SOD e a pteridina. A atividade enzimática avaliada permaneceu constante. Indicamos o uso de 0,93% de fenilalanina para intensificar a coloração corporal; no entanto, estudos adicionais são necessários para investigar se a suplementação nesses níveis pode prejudicar a saúde e o bem-estar dos peixes. A utilização de aditivos nutricionais nas dietas de peixes ornamentais pode ser utilizada para modular respostas antioxidantes, pigmentares e relacionadas a saúde e bem estar de peixes ornamentais.

Palavras-chaves: alimentação, efeitos na saúde, nutrição de peixes tropicais, suplementação.

EFFECTS OF NUTRITIONAL BIOACTIVES FROM THE BRAZILIAN CERRADO ON MORPHOPHYSIOLOGICAL AND INTEGUMENTARY PARAMETERS IN ORNAMENTAL FISH

ABSTRACT - Aquaculture is the activity that involves the breeding of aquatic organisms, with the breeding of ornamental species on the rise. This modality has gained importance in national aquaculture, mainly because it is economically profitable. Because they are organisms with great commercial potential, their cultivation has become increasingly intensive, and in their natural environment, these fish have a diet based on a combination of animal-based foods (high energy value) and plant-based foods (low energy value) rich in pigments. However, in order to obtain a good development of these fish, the diet needs to be supplemented in the feed. To further optimize these diets, additives are used that can optimize productivity. Therefore, there is a growing interest in research aimed at the use of alternative sources of bioactive compounds from food and its by-products (shells, seeds and bagasse), which, when incorporated in the feed, can have effects with antioxidant and palatability activity, increasing its added value. This work aims to evaluate the incorporation of sources of bioactives from the Cerrado in the diets of ornamental fish. This work presents the first considerations. **Chapter 2**, The addition of pequi (*Caryocar brasiliense*) as a source of carotenoids and promoter of color, health and well-being in the diet of Mato Grosso (*Hyphessobrycon eques*) was investigated. One hundred and fifty specimens from Mato Grosso were used, which were distributed in a completely randomized experimental design (three treatments and five replicates), each experimental unit with a capacity of 20 L, connected to a water recirculation system for 30 days. The fish were fed a commercial diet (C), a commercial diet supplemented with 800 $mg.kg^{-1}$ of hydroalcoholic extract of defatted pequi pulp (EP), and a commercial diet supplemented with 800 $mg.kg^{-1}$ of defatted pequi flour (FP). Survival, development parameters, color, metabolism of muscle enzymes and liver enzymes were evaluated. There was no influence of the diets on the development parameters ($p>0.05$). There was an increase in coloration ($p<0.05$), reduction in the activity of the hepatic enzyme AST and an increase in the activity of the antioxidant enzyme CAT in the muscle ($p<0.05$) compared to fish that consumed the EP diet. It is concluded that the use of defatted pequi flour (FP) as a dietary supplement optimizes coloration, has antioxidant activity, and promotes the well-being of *H. eques.* **CHAPTER 3:** The effects of using the decoction of Guavira leaves (*Campomanesia guazumifolia*) as a feed additive for Geophagus pyrocephalus were evaluated. The toxicity study in *Artemia salina*, in juveniles of *G. pyrocephalus* and the feeding test on *G. pyrocephalus* were conducted. Survival in the toxicity tests and histopathology of the gills were evaluated. In the feeding tolerance test, the diet was provided for 30 days. At the end of the test, the fish were euthanized and growth performance, metabolic enzymes and antioxidant enzymes were evaluated. There was no difference in weight gain, length and condition factor of the fish ($p> 0.05$) and the decoction of guavira showed high toxicity to *Artemia salina* and juveniles of *G. pyrocephalus*, gill lesions and the increase in dosage above 250 $mg.kg^{-1}$ presented deleterious effect on the liver. Concentrations of 250 $mg.kg^{-1}$

showed good antioxidant activity. **CHAPTER 4**: a literature review that contextualized the relationship of phenylalanine and tyrosine with natural pigments (melanins, pteridines, purines and carotenoids), metabolism and pigmentation of fish. The types of pigments, cells and sites of deposition, metabolic pathways associated with each type of pigment, mechanisms and factors that control and alter coloration, as well as the functions reported in the literature of these pigments and their relationship with these amino acids were presented. It has been shown that phenylalanine and tyrosine are somehow related to the metabolism of melanin and pteridine, and that the pigments can be metabolized differently among the different fish species, as well as during the phases of their ontogenetic development, in addition to acting directly on the coloration, growth factors, reproduction, health and well-being of these animals. **CHAPTER 5,** evaluated the dietary supplementation of phenylalanine in enhancing fish coloration. To verify the increase in coloration in platys (*Xiphophorus maculatus*) over a period of 21 days, we used the inclusion of 0.81%, 0.99%, 1.17%, 1.36%, and 1.54% phenylalanine in a carotenoid-free diet. The evaluation encompassed several parameters, including body and caudal (caudal peduncle) coloration, the antioxidant activity of catalase (CAT) and superoxide dismutase (SOD) enzymes, and performance. A completely randomized experiment was conducted using 150 platy specimens, arranged in 5 treatments and 3 replicates. The specimens were distributed in 15 60 L tanks connected to a water recirculation system with coupled filtration. We observed an increase in standard weight and length ($p < 0.05$), as well as low survival rates (from 49.9% to 66.7%), despite ($p > 0.05$). A substantial increase in body (a*, b*, ΔE) and caudal (a*) coloration was demonstrated, as corroborated by moderate correlations (r between 0.5 and 0.75). Furthermore, a correlation was observed between SOD and body ΔE, suggesting a relationship between antioxidant activity, SOD, and pteridine. The evaluated enzymatic activity remained constant. We recommend the use of 0.93% phenylalanine to intensify body coloration; however, further studies are needed to investigate whether supplementation at these levels may harm the health and well-being of the fish. The use of nutritional additives in ornamental fish diets can be used to modulate antioxidant, pigmentary, and health-related responses in ornamental fish.
Keywords: feeding, health effects, supplementation, tropical fish nutrition.

CAPÍTULO 1 - CONSIDERAÇÕES INICIAIS

1. INTRODUÇÃO

A aquicultura ornamental é uma atividade em crescimento (Evers et al., 2019; Biondo et al., 2024), e a necessidade por dietas que melhor se adequem ao desempenho, saúde e bem estar gera uma demanda por pesquisas para aprimorar dietas que contribuam com o crescimento, longevidade, saúde e bem estar, redução do custo de produção e com a manutenção da qualidade de água, reduzindo a poluição ambiental (Zuanon et al., 2011; Gomes et al., 2021; Yilmaz et al., 2022; Hernández-Contreras et al., 2023; Onomu e Okuthe, 2024; Reis et al., 2024 Rejes et al., 2024).

Para obter esses benefícios nos animais são utilizados como aditivos nutricionais os extrativos vegetais (Santos et al., 2020a; Santos et al., 2020b; Cruz et al. 2023; Siqueira et al. 2024), e os pigmentos, basicamente os carotenóides (Nakano e Wiegertjes, 2020; Ciji e Akhtar, 2021; Sathyaruban et al., 2021) que além de gerar benefícios relacionados com a saúde, atuam prontamente na coloração. Assiim, o uso de aditivos nutricionais nas dietas de peixes ornamentais busca o incremento e a manutenção da coloração, bem como da saúde do animal (Gomes et al., 2021), sendo que no ambiente de cultivo os peixes não possuem acesso ao alimento natural, o qual supriria suas exigências nutricionais, sendo necessário uma dieta balanceada e bem formulada (Reis et al., 2024).

A cor é um dos principais fatores que afeta o comércio destes organismos, atuando no preço final do produto (Luo et al., 2021; McLean, 2021; Vissio et al., 2021; Lau et al., 2023). Neste contexto, a dieta é um importante veículo de controle e manutenção deste parâmetro. Os principais aditivos adicionados em dietas comerciais na aquicultura relacionados a pigmentação são os carotenóides (Sanches, 2021).

Os pigmentos carotenóides atuam na cor, saúde e bem estar animal, mas não são sintetizados pelos peixes, devendo estes serem obrigatoriamente incluídos nas dietas (Sathyaruban et al., 2021). Entretanto, há outros pigmentos pouco estudados, os quais, são sintetizados naturalmente pelos peixes, que também afetam a pigmentação, e assim

podem contribuir como a valorização do produto, entre estes temos as pteridinas e a melanina (Ziegler 2003; Leclercq et al. 2009; Bilandija et al., 2013; Johnson e Fuller, 2014; Fang et al., 2022), sendo estas duas (melanina e pteridina) ligadas ao metabolismo de aminoácidos, como a fenilalanina e a tirosina (Scardua et al., 2024), as quais, em teoria, poderiam ser manipuladas através da dieta.

Em relação à saúde e ao bem estar dos peixes, os agentes estressantes (manejo, poluição, qualidade de água, adensamento, transporte) são importantes fatores que afetam o comportamento, desempenho de crescimento, reprodução, sistema imune, coloração além da saúde deixando-os mais suscetíveis a infeções e enfermidades no ambiente de cultivo (Yada e Tort, 2016; Ciji e Akhtar, 2021; Cavallino et al., 2023).

A utilização de resíduos de plantas e frutos do Cerrado na alimentação de animais de estimação, como peixes, pode ser uma alternativa viável e sustentável. Peixes ornamentais passarão a vida no aquário, sendo assim requerem cuidados específicos no envelhecimento, sendo a nutrição fundamental (Stevens et al. 2017; Segner et al. 2019; Ciji e Akhtar, 2021; Cavallino et al. 2023). Dietas para peixes senis devem atender às demandas metabólicas e incluir compostos benéficos ao metabolismo (Honorato et al., 2021). Dessa forma, a inclusão de resíduos de frutos do Cerrado na alimentação de peixes ornamentais não apenas atende às exigências nutricionais, mas também contribui para a melhoria da saúde e da estética desses animais (Porto et al., 2020; Honorato et al., 2021).

A adição de compostos bioativos na alimentação favorece o bem-estar, reduz o estresse oxidativo e intensifica a coloração, especialmente com carotenoides naturais (Honorato et al., 2021). Apesar do potencial nutricional desses coprodutos, seu uso na aquicultura ornamental ainda é limitado. Estudos indicam que resíduos de plantas e frutos do Cerrado (bacuri, bocaiúva, guavira e pequi) melhoram a coloração e o bem-estar dos peixes, agregando valor à cadeia produtiva (Honorato et al., 2021).

Existe uma enorme gama de peixes ornamentais comercializados ao redor do mundo. Aliado a isto há uma demanda por dietas específicas, o que gera a necessidade de estudos por novas fontes de pigmentos, visto que os carotenóides não são baratos e existe a preferência do mercado consumidor

por fontes naturais destes pigmentos (Sathyaruban et al., 2021).

Os peixes ornamentais sofrem constante manejo, o que pode gerar estresse e afetar a coloração, segundo Ciji e Akhtar (2021) há inúmeros tipos de indicadores/biomarcadores de estresse, tais como os não invasivos, onde são avaliados o comportamento dos peixes e os invasivos, onde são avaliados na fisiologia do animal, os marcadores endócrinos e os hormônios de estresse, os marcadores metabólicos e celulares, como os metabólitos plasmáticos (glucose e lactato) e a presença das enzimas alanina aminotransferase (ALT), aspartato aminotransferase (AST), lactato desidrogenase e creatina kinase as quais indicam dano no tecido ou nas células. Também temos os indicadores fenotípicos, índices organosomáticos, atividade cardíaca e hematologia, concentração iônica e osmolaridade, e as avaliações ômicas (Proteômica, genômica e metabolômica).

O estresse também afeta a saúde animal, e o dano oxidativo está diretamente associado à capacidade de gerar antioxidantes e à sua eficiência na defesa contra o stress oxidativo. Neste contexto as enzimas superóxido dismutase (SOD) e a catalase (CAT), são biomarcadores em peixes para avaliar a defesa antioxidante (Siqueira et al. 2024), a SOD catalisa a degradação de radicais de superóxido através da formação de H_2O_2, e a CAT possui a função de degradar o H_2O_2.

Este trabalho tem como objetivo geral avaliar a inclusão aditivos de fontes de bioativos vegetais, bem como de aminoácidos na alimentação de peixes ornamentais.

2. REVISÃO DE LITERATURA

2.1 Aquicultura ornamental

A indústria dos peixes ornamentais movimenta entre 15 e 30 bilhões de dólares anualmente (Dey, 2016; Ladisa et al., 2017; Evers et al., 2019; Biondo et al., 2024), somando a esta característica econômica, a aquariofilia é um dos principais “hobbies” do mundo, e os peixes um dos “*pets*” mais comercializados (Cardoso et al., 2021).

Neste contexto em 2018 as exportações brasileiras de peixes

ornamentais alcançaram a modesta quantia de 6,5 milhões de dólares, sendo a China (Hong Kong) e Taiwan os principais compradores (Cardoso et al., 2021). A produção pela aquicultura nacional se localiza em São Paulo e Minas Gerais (Valenti et al., 2021), e a zona da Mata de Minas Gerais a maior produtora do país. Entretanto o mercado internacional tem mais interesse nas espécies brasileiras oriundas do extrativismo, e as principais áreas do extrativismo de peixes ornamentais se localizam na Bahia, Espírito Santo e Ceará para as espécies marinhas e no ambiente de água doce se destacam as bacias hidrográficas Amazônica e a do Tocantins-Araguaia (Cardoso et al., 2021).

2.2 Espécies ornamentais comercializadas

No Brasil, as espécies de água doce dominam o mercado de produção de peixes ornamentais (Valenti et al., 2021) sendo a comercialização destinada à exportação majoritariamente para as espécies oriundas do extrativismo (Cardoso et al., 2021; Tribuzy-Neto et al., 2021). Neste contexto, o neon-cardinal (*Paracheirodon axelrodi*) é a principal espécie exportada, com mais de 90 milhões de exemplares exportadas do Amazonas, entre os anos de 2006 e 2015 (Cardoso et al., 2021; Tribuzy-Neto et al., 2021). Neste período, 375 espécies de peixes foram exportadas do estado do Amazonas sendo duas ordens, Characiformes e Siluriforrmes e Cichliformes as mais visadas com 82,6%, 16,0% e 1,24% respectivamente (Tribuzy-Neto et al., 2021). Dentre os Characiformes os Characidae representam as maiores capturas, sendo 2.307.342 espécimes do gênero *Hyphessobrycon* exportadas nesse período (Tribuzy-Neto et al., 2021).

Dentre o gênero *Hyphessobrycon* destaca-se o *Hyphessobrycon eques,* (Figura 1A e Tabela 1), um peixe popularmente conhecido como Mato Grosso (Santana et al., 2019), além de ser uma espécie de Characidae muito apreciado no aquarismo mundial (Santana et al., 2019; Gimênez Junior e Rech, 2022).

Figura 1 Imagens de Mato Grosso (*Hyphessobrycon eques*), Acará do Tapajós (*Geophagus pyrocephalus*) e Platy *(Xiphophorus maculatus*). Arquivo pessoal.

Tabela 1. Classificação dos peixes utilizados nos experimentos. Mato Grosso (*Hyphessobrycon eques*), Acará do Tapajós (*Geophagus pyrocephalus*) e do Platy (*Xyphophorus maculatus*).

Nome popular **Classificação***	**Mato Grosso**	**Acará do Tapajós**	**Platy**
Reino	Animalia	Animalia	Animalia
Filo	Chordata	Chordata	Chordata
Classe	Actinopterygii	Actinopterygii	Actinopterygii
Ordem	Characiformes	Cichliformes	Cyprinodontiformes
Família	Acestrorhamphidae	Cichlidae	Poeciliidae
Gênero	*Hyphessobrycon* Durbin, 1909	*Geophagus*	*Xyphophorus* Heckel, 1848
Espécie	*Hyphessobrycon eques* (Steindachner, 1882)	*Geophagus pyrocephalus* (Chuctaya et al., 2022)	*Xyphophorus maculatus* (Günther, 1886)

*Bases utilizadas: fishbase e Catalogue of life.

O peixe Mato Grosso (*H. eques*) atinge 40 mm de comprimento padrão (Britski et al., 2007), possuindo hábito alimentar onívoro, formando cardumes e habitando áreas de remanso, baías, nascentes e pequenas lagoas (Gimênez Junior e Rech, 2022).

Segundo Gimênez Junior e Rech (2022), a coloração de *H. eques* varia do rosa claro ao vermelho forte, com uma mancha umeral enegrecida e alongada verticalmente, olhos com margens vermelhas, nadadeiras vermelhas, exceto a dorsal que é negra com base vermelha, além da margem brancas nas nadadeiras pélvicas e anal, também há uma faixa enegrecida ao longo da margem distal da nadadeira anal.

Originalmente, o Mato Grosso é encontrado nas bacias Amazônica, Guaporé e Paraguai (Santana et al., 2019), porém devido a sua introdução, também pode ser encontrada na bacia do alto rio Paraná (Buckup et al.,

2007).

Por ser um peixe dócil, de fácil manejo e com coloração atraente, e ser uma espécie alvo de estudos do grupo de pesquisa GEPAq-UFGD, o Mato Grosso foi eleito para estudo nesta tese. Além deste, o *Geophagus pyrocephalus* (Acará do Tapajós), foi disponibilizado para pesquisa pelo Bioparque Pantanal. A última espécie utilizada, foi o *Xyphophorus. Maculatus* (platy) por possuir a descrição de presença do pigmento pteridina no seu tegmento (Henze et al., 1977).

Geophagus pyrocephalus (Acará do Tapajós ou Geophagus “red head”), (Figura 1B e Tabela 1) é uma espécie de interesse pela aquariofilia, sendo descrita recentemente no estudo de Chuctaya et al. (2022), sendo esta descrita a partir de exemplares coletados na região do baixo Tapajós através de análises genéticas baseada no gene mitocondrial cytocromo β-oxidase (Chuctaya et al., 2022).

A espécie possui uma coloração laranja avermelhada marcante na região da cabeça, sendo coletada em área lótica com alta transparência e com praia de fundo arenoso no baixo rio Tapajós. Existindo pouca informação disponibilizada sobre os aspectos de sua biologia, sabe-se que os exemplares de *G. pyrocephalus*, macho com comprimento padrão de 101,8mm e fêmea 106,7mm, estão depositados na coleção ictiológica da Universidade Federal do Rio Grande do Sul sob o código UFRGS 27440 (Chuctaya et al., 2022). Apesar de se tratar de uma espécie nova, no mercado de peixes ornamentais a espécie já era comercializada e reconhecida como *Geophagus* sp “red head” (Hallwass, 2020).

O Platy (*Xyphophorus maculatus*) (Figura 1C e Tabela 1), é um peixe de água doce que habita águas preferencialmente lentas com fundo siltoso (Yamamoto e Tagawa, 2000), embora seja encontrado em riachos. Possui hábito alimentar onívoro e é produzido mundialmente com finalidade ornamental, devido a docilidade a ao fácil manejo, também é alvo de estudos voltados a genética (Henze et al., 1977; Robins, 1991). São peixes pequenos (40 mm, macho e 60 mm a fêmea), ocorrendo desde Veracruz (México) até o norte de Belize (Keith et al., 2006), porém devido a sua introdução podem ser encontrados em mais 25 países, como no Brasil e em vários continentes, exceto a Europa (Fishbase, 2025). Devido a sua docilidade e ser uma das

poucas espécies de peixes ornamentais reportadas que possuem o pigmento pteridina.

2.3 Aditivos na alimentação de peixes ornamentais

O uso de aditivos nutricionais em dietas para peixes ornamentais tem como finalidade otimizar e manter a coloração, a saúde e o bem-estar dos animais (Gomes et al., 2021). Em sistemas de cultivo, esses peixes não têm acesso ao alimento natural que atenderia às suas necessidades nutricionais específicas, tornando imprescindível o fornecimento de dietas balanceadas capazes de suprir plenamente suas exigências fisiológicas (Reis et al., 2024).

Conforme Zuanon et al., (2011) as exigências nutricionais dos peixes ornamentais são semelhantes às dos peixes produzidos para alimentação, relevando a adição de carotenóides; entretanto, devido ao manejo constante, ficam mais expostos a situações de estresse e assim as exigências para ácidos graxos, vitaminas e minerais possam ser maiores.

O estresse pode alterar a homeostase e induzir respostas fisiológicas e comportamentais caracterizadas pela elevação de hormônios de estresse (cortisol e catecolaminas), para auxiliar os peixes a superar essa situação. Entretanto em situações crônicas esse sistema já é suficiente (Ciji e Akhtar, 2021).

As situações estressantes a que os peixes ornamentais estão expostos podem reduzir o crescimento, imunosuprimir os animais, reduzir a resistência dos peixes a doenças, afetar a pigmentação e até levar a mortalidade, sendo necessária a realização de intervenções para evitar prejuízos. Assim Intervenções nutricionais são opções recomendadas para mitigar e fortalecer os animais evitando prejuízos na produção, sendo o uso de aditivos nutricionais uma prática utilizada (Yilmaz et al., 2020; Ciji e Akhtar, 2021; Yilmaz et al., 2022).

Assim, os aditivos podem favorecer a digestibilidade, melhorar as taxas de crescimento, a resposta do sistema imune inato, melhorar a resistência dos peixes a doenças, incrementar o poder antioxidante, incrementar a pigmentação, atuar na expressão de genes relacionados ao sistema imune e a enzimas antioxidantes (superóxido dismutase, catalase e

glutationa peroxidase), possuir função hepatoprotetora, agindo desta forma na promoção da saúde e do bem estar desses animais (Yilmaz et al., 2020; Ciji e Akhtar, 2021; Gomes et al., 2021; Yilmaz et al., 2022; Cruz et al., 2023; Siqueira et al. 2024).

2.4 Aditivos nutricionais

Conforme a Instrução normativa 13/2004 do Ministério da Agricultura Pecuária e Abastecimento-MAPA (Brasil, 2004), são considerados aditivos nutricionais toda substância utilizada para manter ou melhorar as propriedades nutricionais do produto. Em 2021, foi emitida a Portaria 359/2021 (Brasil, 2021), para a emissão da lista de ingredientes e veículos autorizados pelo MAPA para uso na alimentação animal no Brasil. Já atualizada em 2024 (Brasil, 2024), constam os aditivos nutricionais permitidos, sendo estas vitaminas, provitaminas e substâncias como efeitos similares, oligoelementos ou compostos de oligoelementos, aminoácidos, seus sais e análogos, ureia e seus derivados.

Neste contexto, a função dos aminoácidos está associada com a fisiologia dos peixes em sistemas de produção, relacionando metabolismo e geração de energia, desempenhando funções estruturais indispensáveis para saúde, crescimento, reprodução e sobrevivência dos animais (Rodrigues et al., 2019). Por outro lado, a carência ou baixas quantidades de sua inclusão nas dietas, reduz crescimento e a produtividade, resultando no uso de proteínas dos tecidos para o animal se manter, prejudicando o desempenho e a produtividadae (Pezzato et al., 2004; NRC, 2011; Wu et al., 2013).

2.4.1 Suplementação com produtos naturais

Os produtos naturais, principalmente os extratos vegetais, como suplemento nutricional, são normalmente utilizados na alimentação animal, visando a promoção da saúde e bem estar é uma prática comum (Ciampi et al, 2022; Alem, 2024), principalmente devido aos benefícios associados as suas propriedades nutracêuticas (Viscardi et al., 2017; Carvalho e Conte-Junior, 2021; de Castro et al., 2023; Lescano et al., 2023) e as respostas em termos de qualidade de produto e de produção (Alem, 2024).

Os benefícios oriundos dos extrativos naturais resultam em secreção digestiva elevada, maior digestibilidade e absorção de nutrientes, estimulação do sistema imune, ação anti-bacteriana, anti-inflamatório e antioxidante (Garcia Beltrán e Esteban, 2022; Alem, 2024), são oriundos dos metabólitos secundários, compostos bioativos, também chamados de fitoquímicos (Garcia Beltrán e Esteban, 2022).

Desta forma o conhecimento da medicina tradicional, através do uso de plantas medicinais vem auxiliando na prospeção desses vegetais e seus compostos bioativos, para seu uso no aquicultura (Reverter et al., 2014; Van-Hai, 2015; Awad e Awaad, 2017; Garcia Beltrán e Esteban, 2022).

Entre esses produtos os compostos fenólicos são considerados os mais relevantes, sendo estes saponinas, glicosideos, antraquinonas, esteróides, cumarinas, sendo os terpenos, carotenóides (tetraterpeno), polifenóis (flavanóides, antocianinas, taninos eligninas) e alcalóides os mais importantes (Koldaş et al., 2015; Catelan et al., 2018; Stevanović et al., 2018; Faehnrich et al., 2021; Kuralkar e Kuralkar, 2021; Garcia Beltrán e Esteban, 2022; de Castro et al., 2023; Lescano et al., 2023).

Os flavanóides apresentam propriedades imunomoduladoras que regulam células imune, a expressão gênica e a produção de citocinas, além de apresentarem propriedades antioxidantes, pois podem induzir o aumento da expressão e atividade de enzimas antioxidantes intracelulares (superoxido dismutase, catalase e glutationa peroxidase) que reduzem os danos causados pelas espécies reativas de oxigênio (Bhuiyan et al., 2017; Yahfoufi et al., 2018; Quintans et al., 2019; Hu et al., 2025), atuando assim no bem estar e saúde dos peixes.

Dentre os flavanóides a quercetina, um polifenol lipofílico presente nas frutas, vegetais e sementes (Bigliardi e Galati, 2013) (Figura 2) é capaz de quelar íões metálicos inibindo as reações oxidativas demonstrando sua ação antioxidante (Bhuiyan et al., 2017), assim contribuindo com a homeostase dos peixes.

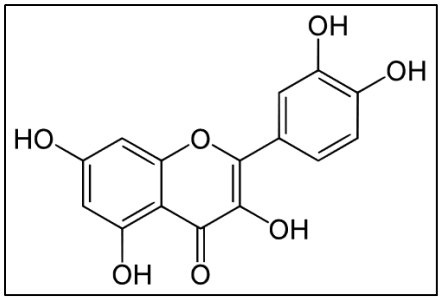

Figura 2 Estrutura química do flavanóide quercetina, fórmula geral: $C_{15}H_{10}O_7$.

Alguns estudos realizados com adição de quercetina na dieta de peixes demonstraram que esta pode promover o crescimento, a melhorar a capacidade antioxidante e imune, como nos casos com *Channa argus,* com a indicação da dose de 300 $mg.kg^{-1}$ na dieta deste peixe (Kong et al., 2022), e com *Labeo rohita*, sendo indicado a dosagem de 4,11 $g.kg^{-1}$ na dieta desta espécie (Khan et al., 2023). Já em tilápia resultou no aumentou do crescimento e da atividade das enzimas antioxidantes, catalase e superóxido dismutase (Zhai e Liu, 2014).

Foi identificada a presença de quercetina em algumas plantas do cerrado brasileiro como o pequi (*Caryocar brasiliense*) (Fracasso et al., 2023) e a guavira (*Campomanesia guazumifolia*) (Catelan et al., 2018), sendo assim, estas elegidas para a presente pesquisa.

Entre os compostos mais visados devido a suas características de bioatividade, temos os carotenóides (Hu et al., 2025), figura 3.

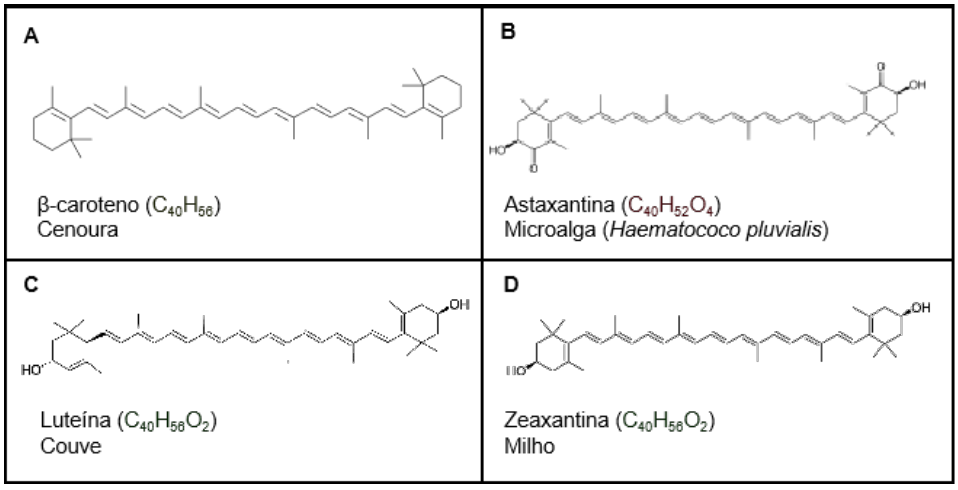

Figura 3 Carotenóides, estrutura química e fonte comum de obtenção. A: β-caroteno; B: Astaxantina; C: Luteína; D: Zeaxantina.

Segundo Britton, (2024) os carotenóides são tetraterpenos que constituem um grupo com mais de 600 pigmentos vegetais com características lipofílicas com forte poder antioxidante, sendo bastante utilizados como aditivos para dietas de peixes em cultivo, visto que estes compostos não são sintetizados por esse animais (Zuanon et al., 2011; Nakano e Wiegertjes, 2020; Sathyaruban et al., 2021; Hernández-Contreras et al., 2023; Hu et al., 2025), além de serem os pigmentos mais utilizados na aquicultura (Sanches, 2021).

Os carotenóides também são acumulados nas gônadas sendo essenciais para a reprodução, sendo reportado o aumento da fertilização, desenvolvimento do ovário, taxa de eclosão e crescimento larval para salmão e pargo europeu (Torrissen e Christiansen, 1995).

Foram relatados benefícios para a saúde e incremento de pigmentação dos peixes ornamentais através da inclusão de carotenóides (astaxantina natural e sintética) em suas dietas (Wang et al., 2006; Baron et al., 2008; Pan e Chien, 2009).

Assim observamos que os carotenóides demonstram eficácia quanto a sua adição nas dietas de peixes, resultando em peixes mais coloridos, saudáveis além de maior produtividade no cultivo.

2.4.2 Suplementação com aminoácidos

As funções básicas dos aminoácidos na fisiologia animais se relacionam à síntese de proteínas, lactação, reprodução, integridade intestinal, metabolismo de nutrientes, degradação de proteína, sinalização celular, defesa antioxidante, secreção de hormônios, balança ácido-base, crescimento, diferenciação celular (Wu et al, 2013).

Dentre os aminoácidos, a fenilalanina ($C_9H_{11}NO_2$) (Figura 4A), um aminoácido aromático apolar é responsável pela biossíntese da tirosina ($C_9H_{11}NO_3$) (Figura 4B), através da ação da enzima di-hidrobiopterina redutase (Nelson e Cox, 2014).

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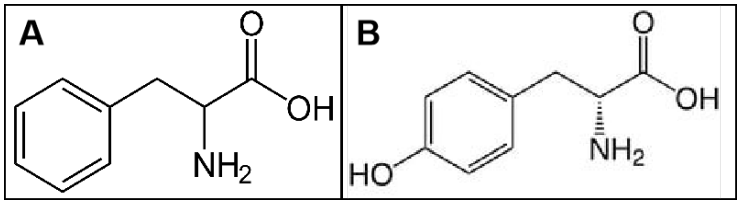

Figura 4 Aminoácidos, fenilalanina (A) e tirosina (B).

A fenilalanina atua na regulação da transcrição genética, manutenção da atividade cerebral, funções cognitivas, diferenciação celular, crescimento dos animais e produção de catecolaminas as quais atuam na destinação da gordura armazenada para à manutenção corporal e ao domínio intracelular da tirosina quinase determinado fatores de crescimento (Kim, 1993; (Liapi et al., 2008; Nelson e Cox, 2014).

A tirosina é precursora de hormônios e neurotransmissores importantes (adrenalina, noradrenalina, tiroxina, triiodotironina, melanina e dopamina) que atuam na adaptação dos animais frente as adversidades ambientais (Li et al., 2008).

Nos peixes os níveis de proteína proveniente da dieta deve conter em seu balanceamento o mínimo de aminoácidos essenciais para permitir o metabolismo de manutenção e crescimento (Pezzato et al., 2004).

O requerimento de fenilalanina varia de acordo com a espécie de peixe, sendo que uma parte dessa concentração pode ser substituída por tirosina (Rodrigues et al., 2019).

No caso da tilápia (*Oreochromis niloticus*) o requerimento é 4,84% de fenilalanina da proteína dietética (Cyrino et al., 2002) para o lambari (*Astyanax fasciatus*) 4,66% de fenilalanina e tirosnia na dieta (Furuya, et al., 2015). Conforme Furuya (2010) o requerimento de fenilalanina e tirosina para tilápia reduz a medida que o animal cresce.

A inclusão na dieta de fenilalanina beneficia a atividade das enzimas digestivas melhorando significativamente a digestão e capacidade de absorção do sistema digestivo dos peixes (Li et a., 2015), atuando também na melhora do crescimento, e a inclusão de tirosina causaria um efeito poupador da fenilalanina, restringindo a síntese (Rodrigues et al., 2019).

Os hormônios tiroideais e a dopamina, sintetizados pela tirosina-fenilalanina, aumentam a atividade das enzimas antioxidantes, desta forma

agem na eliminação de espécies reativas de oxigênio (Oommen et al. 2006; Li et al. 2015a).

Foi relatado o melhor desempenho imunitário (Li et al., 2015; Feng et al., 2015), atuação na proteção da integridade estrutural das brânquias, inibindo a ação do dano oxidativo (Feng et al., 2015) atuando como ansiolítico nas respostas ao stress durante o manejo de peixes (Li et al., 2008; Calheiros et al., 2019).

No crescimento, a fenilalanina está relacionada com a hiperplasia, desenvolvimento de novas fibras musculares (Yamashiro et al., 2016), também atua no aumento da taxa metabólica gerando ganho de peso e crescimento (Houlihan et al., 1995).

Esses dois aminoácidos também atuam no processo de pigmentação, sendo relacionados com a produção direta da eumelanina, além de algumas formas de pteridinas, além de que esses pigmentos atuam como fotoprotetores, processos de comunicação inter e intra específica e camuflagem (Baldisserotto et al., 2014; Scardua et al., 2024).

Portanto os aminoácidos fenilalanina e tirosina além e atuarem na pigmentação possuem importância fundamental no metabolismo dos peixes, possibilitando atuarem na saúde e bem estar animal.

2.4.3 Importância do estudo de metabolismo

No ambiente de cultivo e durante o processo de produção os peixes estão propensos a diversas situações que alteram o metabolismo, e podem gerar estresse, afetando a produtividade, sobrevivência, saúde e bem estar desses animais (Ciji e Akhtar, 2021).

O estresse pode alterar a homeostase dos peixes e induzir respostas fisiológicas e comportamentais obseradas através da elevação de hormônios de estresse (cortisol e catecolaminas), tais respostas podem ser adaptativas ou compensatórias, as quais irão auxiliar os peixes a superar o agente estressor. Entretanto, em situações crônicas esse sistema não é funcional, pois os animais podem perder sua adaptabilidade, gerando prejuízos no desempenho (Ciji e Akhtar, 2021).

As situações estressantes podem reduzir o crescimento, imunosuprimir os animais, reduzir a resistência dos peixes a doenças, afetar a pigmentação

e até levar a mortalidade, sendo necessária a realização de intervenções para evitar prejuízos. Assim, intervenções nutricionais são opções recomendadas para mitigar e fortalecer os animais evitando prejuízos na produção, sendo o uso de aditivos nutricionais uma prática utilizada (Yilmaz et al., 2020; Ciji e Akhtar, 2021; Yilmaz et al., 2022)..

Para acompanhar essas respostas nos peixes é necessária realização de avaliações fisiológicas, tais como avaliação do metabolismo hepático e oxidativo, os quais respondem prontamente sobre as alterações na saúde do peixe em resposta a agentes estressores (Yilmaz et al., 2020; Ciji e Akhtar, 2021; Yilmaz et al., 2022).

Avaliando essas respostas nos peixes é possível inferir sobre a saúde dos mesmos e como eles estão lidando com o que ocorre no ambiente de produção.

2.4.3.1 Metabolismo hepático

Nos peixes o fígado além de ser o maior órgão interno do corpo é responsável por biotransformar diversos produtos sendo, suas células, os hepatócitos as que atuam no metabolismo de nutrientes, armazenamento de glicose, e desintoxicação do organismo (Hinton e Lauren, 2007).

Confome Honorato et al., (2013) as funções de destaque dos hepatócitos do fígado são, a formação de proteínas plasmáticas, a desaminação de proteínas, desintoxicação pela formação de uréia para remoção de amônia e a síntese de aminoácidos.

As enzimas alanina aminotransferase (ALT) e aspartato aminotransferase (ALT) são consideradas como marcadores hepatotóxicos, estando presentes nos músculos, brânquias e no fígado, sendo importantes na interconversão de aminoácidos e outros metabólitos intermediários (Wang et al., 2006), assim, quanto maior sua presença mais aminoácidos estão sendo metabolizados em resíduos nos tecidos, ou seja, o estímulo de sua secreção está relacionado com a sobrecarga hepática.

Conforme do Carmo Ota et al., (2019), o aumento na atividade da AST e da ALT pode indicar dano aos hepatócitos, e estão associadas a lesões hepáticas agudas, e por outro lado, a sua redução sugere o efeito hepatoprotetor (Wang et al., 2006) e aumento da imunidade dos peixes (Li et

al., 2022).

2.4.3.2 Metabolismo oxidativo

O metabolismo do oxigênio é responsável por gerar radicais livres, as chamadas espécies reativas, sendo a mitocôndria a principal fonte geradora, e o sistema de defesa antioxidante inibe e reduz os danos causados por esses radicais, desta forma o estresse oxidativo é gerado pelo desequilíbrio entre a ação do sistema de defesa antioxidante e a geração dos radicais livres (Barbosa et al., 2010).

Esse sistema de defesa pode ser dividido em enzimático e não enzimático, sendo o não enzimático composto por várias substâncias antioxidantes endógenas ou dietéticas, tais como vitaminas (A, C, E), carotenóides e minerais (zinco, cobre, selênio, magnésio), já o sistema de defesa enzimático é composto por enzima como a catalase, superóxido dismutase e glutationa peroxidase (Barbosa et al., 2010).

A catalase e a glutationa peroxidase eliminam os peróxidos de hidrogênio que foram produzidos pela superóxido dismutase ao degradar os radicais de superóxido (Siqueira et al., 2024).

Desta forma, o uso desses marcadores da atividade antioxidante são importantes para caracterizar a eficiência do uso de dietas contendo substâncias que atuem neste contexto, como os carotenóides.

3. OBJETIVOS

3.1 Objetivo Geral

Este trabalho teve como objetivo avaliar se a inclusão de fonte de compostos bioativos do Cerrado na alimentação de peixes ornamentais.

3.2 Objetivos Específicos

CAPÍTULO 2 - Effects of dietary pequi (*Cariocar brasiliense*) extract and pequi meal on growth performance, coloration, and tissue-specific antioxidant responses in *Hyphessobrycon eques.*

- Avaliar a utilização de extrato de pequi e farinha de pequi (*C. brasiliense* Camb.) como suplemento alimentar do Mato Grosso (*Hyphessobrycon eques*);
- Descrever o desenvolvimento e consumo de alimento de *H. eques* alimentado com dietas suplementadas com pequi;
- Avaliação do perfil metabólico tissular e das enzimas chaves do metabolismo hepático;
- Avaliação do perfil metabólico oxidativo muscular;
- Avaliar a influência da suplementação do pequi na coloração de *H. eques.*

CAPÍTULO 3 - Toxicidade e efeitos antioxidantes do decocto da folha de Guavira (*Campomanesia guazumifolia*) como aditivo alimentar para *Geophagus pyrocephalus*

- Avaliar a utilização de decocto de folha de guavira (*Campomonesia guazumifolia*) como aditivo alimentar de G*eophagus pyrocephalus*;
- Descrever o desenvolvimento e consumo de alimento de *G. pyrocephalus* alimentado do decocto de folha de guavira;
- Avaliar a toxicidade oral e alimentar da inclusão de decocto de *Campomonesia guazumifolia* em *Geophagus pyrocephalus*;
- Avaliar o perfil metabólico tissular e das enzimas chaves do metabolismo hepático;
- Avaliar o perfil metabólico oxidativo muscular;
- Avaliar alterações branquias oriunda da inclusão de decocto de *Campomonesia guazumifolia* na dieta de *Geophagus pyrocephalus*.

CAPÍTULO 4 - O papel da fenilalanina no processo de pigmentação de peixe: uma revisão

- Descrever os tipos de pigmentares presentes nos peixes;
- Descrever as rotas metabólicas dos pigmentos presentes nos peixes;

- Descrever a relação entre as rotas metabólicas dos pigmentos presentes nos peixes e a fenilalanina.

CAPÍTULO 5 - Suplementação de fenilalanina para melhoria da qualidade cromática de platy (*Xiphophorus maculatus*): uma abordagem aplicada

- Avaliar a influência da suplementação de fenilalanina na coloração de *X. maculatus;*
- Avaliar o perfil metabólico oxidativo muscular;
- Descrever o desenvolvimento e consumo de fenilalanina em *X. maculatus*.

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CAPÍTULO 2 - EFFECTS OF DIETARY PEQUI EXTRACT AND PEQUI MEAL ON GROWTH PERFORMANCE, COLORATION, AND TISSUE-SPECIFIC ANTIOXIDANT RESPONSES IN *Hyphessobrycon eques*[1]

Marcos Paiva Scardua[1]*, Antonio Cesar Godoy[2], Leticia Maria Alburqueque Conceição[1], Jéssica Amanda Ugarte Reges[1], Daniel Ferreira Rodrigues de Oliveira[1], Dacley Hertes Neu[1], Lillian Brito Lins[3], Patricia Daniele da Silva Santos[4], Iasmim Barbosa de Souza[4], Oscar de Oliveira Santos Jr[4], Claucia Aparecida Honorato[1]

[1] Universidade Federal da Grande Dourados. Faculdade de Ciências Agrárias. Rodovia Dourados - Itahum, km 12. Cidade Universitária, Dourados/MS, 79.804-970, Brazil.
[2] Instituto Federal de Educação, Ciência e Tecnologia do Paraná. Departamento de Pesquisa em Recursos Naturais - 475 Civica Avenue, Assis Chateaubriand/PR, 85935-000, Brazil.
[3] Department of Poultry Science, Auburn University, 260 Lem Morrison Dr., Auburn, AL 36849, USA.
[4] Universidade Estadual de Maringá. Av. Colombo, 5790 - Centro de Ciências Exatas - Departamento de Química. Maringá/PR, 87020-900, Brazil
*Corresponding author: marcos.scardua@ifce.edu.br

Abstract

This study evaluated the effects of dietary supplementation with hydroalcoholic pequi extract (PHE) and pequi meal (PM) at 800 mg kg^{-1} on zootechnical performance, fin coloration, antioxidant enzymatic activity, and physiological responses in Mato Grosso (*Hyphessobrycon eques*) fish. All treatments achieved 100% survival, indicating no adverse effects. Growth parameters showed no significant differences among treatments ($p > 0.05$). Feed intake was higher in the PM group than in the control and PHE groups ($p < 0.05$), resulting in poorer feed conversion. Fin coloration was influenced by diet: in the caudal fin, the red channel (R) was higher in the control than in PHE, while the blue channel (B) was higher in PM than in PHE ($p < 0.05$). In the anal fin, the control group also showed higher red values, and both green (G) and blue (B) channels were significantly affected. Liver AST activity increased in PHE-fed fish ($p < 0.05$), whereas ALT, SOD, and CAT showed no differences. In

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conclusion, PM improved fin coloration and reduced hepatic AST, indicating hepatoprotection, but decreased muscle SOD, suggesting reduced antioxidant activity. Overall performance remained unaffected. Further studies should evaluate novel dosages to determine whether they enhance antioxidant and hepatoprotective responses in *H. eques*.

Keywords: Antioxidants, Feed supplementation, Growth performance, Metabolic enzyme function, Ornamental fish industry, Pigmentation

1. Introduction

The ornamental fish production chain differs from the food fish production chain primarily due to the high unit value of its products (Luo et al. 2021, Vissio et al. 2021, Abe et al. 2022, Lau et al. 2023). While ornamental marine fish can sell for more than USD 1,000 per kg^{-1}, marine fish intended for human consumption typically reach prices around USD 13 kg^{-1} (Leingang 2021). The ornamental fish industry generates between 15 and 30 billion dollars annually, including revenue from live organisms, equipment, and accessories (Biondo et al. 2024). This sector is characterized by a higher number of marine organisms being traded, the majority of which are sourced through extractive fishing, whereas the majority of ornamental freshwater fish sold come from aquaculture (Novák et al. 2020, Tribuzy-Neto et al. 2020, Biondo et al. 2024).

Despite being part of a multi-billion dollar industry, ornamental fish require special care to ensure good health, vibrant coloration, and mortality rate, as they are considered pets (Stevens et al. 2017). Thus, improving longevity, welfare, and health is essential (Stevens et al. 2017, Segner et al. 2019, Cavallino et al. 2023). Among the key factors influencing ornamental fish maintenance, nutrition stands out as

particularly important (Cruz et al. 2023, Svitačová et al. 2023). Within this context, carotenoids are especially relevant as functional additives (Nakano & Wiegertjes 2020) since fish are unable to synthesize these molecules *de novo*. These pigments, along with others such as melanin, purine, and pteridine, contribute significantly to coloration in ornamental species (Sathyaruban et al. 2021, Scardua et al. 2024). In addition to promoting vibrant pigmentation, carotenoids also exhibit antioxidant functions (Hu et al. 2025) and help to strengthen the immune system of fish (Lim et al. 2019, Maoka. 2020, Rana et al. 2023).

Several synthetic nutritional additives have low utilization efficiency (Sathyaruban et al. 2021, Lau et al. 2023). As a result, there is a growing market trend favoring natural sources of carotenoid pigments over synthetic alternatives (Sathyaruban et al. 2021, Lau et al. 2023). Among these natural products, carotenoid-rich additives have shown positive effects on fish health and welfare. For instance, Unver & Hamzaçebi (2020) tested red beetroot (*Beta vulgaris ruba*) and henna (*Lawsonia inermis*), alongside astaxanthin, in the cichlid *Maylandia estherae*, and found that the natural sources performed similarly to astaxanthin, which has a higher market price (Sathyaruban et al. 2021).

Wagde et al. (2018) reported enhanced growth in swordtail fish *(Xiphophorus hellerii*) with their diet was supplemented with β-carotene derived from carrots (*Daucus carota*) and spinach (*Spinacia oleracea*). Similarly, Kurnia et al. (2019) observed an improvement in the coloration of Koi carp (*Cyprinus carpio*) fed a diet supplemented with dragon fruit peel meal, compared to a commercial diet containing astaxanthin. In another study on Koi carp, Maiti et al. (2017) evaluated several carotenoid sources, including tomato peel powder, carrot peel powder, and beetroot peel powder, concluding that carrot peel powder was the most effective in enhancing

coloration and growth.

Pequi (*Caryocar brasiliense* Camb.) a fruit native to the Brazilian Cerrado, is highly valued in regional cuisine and used in medicine (Carneiro et al. 2023). It is a good source of fatty acids, vitamins (A, B1, B2) (Pinto et al. 2016, Nascimento-Silva & Naves 2019), and is also rich in phenolic compounds and carotenoid pigments (Nascimento-Silva & Naves 2019, Carneiro et al. 2023, Silva et al. 2023), which act as powerful antioxidants by removing free radicals and benefit animal health. This complexity highlights the importance of conducting experimental trials, such as the present study, to better understand how dietary components modulate both pigmentation and antioxidant responses in fish.

This study was conducted considering the carotenoid and phenolic compound composition, as well as the potential antioxidant activities of pequi (*Caryocar brasilien*se Camb.), due to the scarcity of information on its effects on aquatic species. This study aimed to evaluate the effects of pequi supplementation, including pequi meal (PM) and pequi hydroalcoholic extract (PHE), on the pigmentation, growth performance, and enzyme activity of Mato Grosso (*Hyphessobrycon eques*).

2. Materials and Methods

The experiment was conducted at the Aquatic Production Laboratory of the Federal University of Grande Dourados (UFGD), (22°11’52”S / 54°56’04”W) and was approved by the institution’s Animal Ethics Committee (CEUA) under protocol No. 23006/2023.

2.1 Materials

2.1.1 Water quality

Water temperature was recorded daily at 07:20 h and 17:20 h using a mercury thermometer placed into the recirculating water system, with values ranging between 25.5 and 30.0 °C. Other water quality parameters, including dissolved oxygen (10 to 11 mg L^{-1}), pH (6.8 to 7.5), ammonia (0.001 to 0.018 mg L^{-1}), and nitrite (0.001 to 0.250 mg L^{-1}), were measured weekly using the colorimetric method with an Alfakit ATP 100 photometer (Florianópolis, Brazil). These values were considered suitable for the rearing of most tropical fish species (Godoy et al. 2021).

2.1.2 Biological material

A total of 150 individuals of *Hyphessobrycon eques* (Steindachner 1882) were obtained from the local market, with an initial weight of 0.70 ±0.21 g, a total length of 34.97 ±3.03 mm, and a standard length of 29.77±2.43 mm. Pequi meal was also obtained from the local market (Figure 1).

Figure 1.

2.1.3 Chemicals

All chemicals used in this study were of analytical grade, including methanol, water, 2,2- diphenyl-1-picrylhydrazyl (DPPH), Trolox, and formic acid (for UPLC-MS/MS analysis).

2.2 Methods

2.2.1 Handling, treatments, and experimental design

The experiment included three dietary treatments: (1) a control group (C) fed a commercial diet without supplementation; (2) a group fed the commercial diet

supplemented with 800 mg kg^{-1} (Rahimi-Madiseh et al. 2016) of hydroalcoholic extract from defatted pequi (PHE); and (3) a group fed the commercial diet supplemented with 800 mg kg^{-1} of defatted pequi pulp meal (PM). Nutritional specifications and chemical composition are provided in Table I. Prior to the experiment, fish underwent a two week acclimation period in a 1000 L aerated tank with continuous water circulation. After acclimation, fish were randomly distributed into 15 aquariums (20 L each; 10 fish per aquarium) in a completely randomized design with three treatments and five replicates. The trial lasted 30 days, with fish fed three times daily (07:30, 11:30, and 16:30 h) to apparent satiety (Cruz et al. 2023).

Table I. Guarantee levels for the inclusion of additives in commercial extruded diets for tropical fish.

Nutritional composition specifications	
Parameter	**Value**
Moisture (Max.)	120 g kg^{-1} (12%)
Crude Protein (Min.)	360 g kg^{-1} (36%)
Crude fat (Min.)	35 g kg^{-1} (3.5%)
Crude fiber (Max.)	40 g kg^{-1} (4%)
Ash (Max.)	120 g kg^{-1} (12%)
Calcium (Max.)	40 g kg^{-1} (4%)
Phosphorus (Min.)	8000 mg kg^{-1} (0.8%)
Lysine (Min.)	1000 mg kg^{-1}
Methionine (Min.)	1000 mg kg^{-1}
Threonine (Min.)	1000 mg kg^{-1}
Tryptophan (Min.)	1000 mg kg^{-1}
Beta-Glucans (Min.)	100 mg kg^{-1}
Mannan Oligosaccharides (Min.)	50 mg kg^{-1}

Analysed parameters in the experimental diets				
Variables	**Treatments**			***p-value***
	C	**PHE (800 mg kg^{-1})**	**PM (800 mg kg^{-1})**	
Dry matter (%)	88.92±1.33	89.11±1.34	88.76±1.33	0.95

Crude protein (%)	37.04±0.56	36.98±0.55	36.64±0.55	0.65
Crude fat (%)	8.33±0.12a	7.84±0.12b	7.96±0.12b	0.01
Ash (%)	10.31±0.15	10.24±0.15	10.11±0.15	0.32
Crude Energy (kcal kg^{-1})	4350±65.25	4421±66.32	4389±65.83	0.46
Analysed Polyphenols - (mg kg^{-1})				
Gallic Acid	198.75±0.47b	199.85±0.47b	218.81±0.85a	< 0.01
Quercetin	54.01±2.15a	42.55±0.20c	47.03±1.92b	< 0.01
p-Coumaric Acid	159.60±2.41a	136.43±3.81c	145.25±2.13b	< 0.01
Ferulic Acid	162.17±0.59b	152.82±0.15c	173.52±1.36a	< 0.01
Nicotinic Acid	135.37±0.46a	136.36±1.07a	131.05±0.87b	< 0.01
Analysed antioxidant activities - (µmol kg^{-1})				
DPPH•	1217±14	1246±31	1233±26	0.41
ORAC	2940±50c	3580±42b	5325±66a	< 0.01
ABTS•+	1084±10b	1630±23a	1680±38a	< 0.01

The commercial diet was acquired from Poytara LTDA (Araraquara/SP, Brazil); Calcium pantothenate (min) 44 mg kg^{-1}; Vitamin A (min) 9. 300 IU kg^{-1}; Vitamin C (min) 500 mg kg^{-1}; Vitamin D3 (min) 240 IU kg^{-1}; Vitamin E (min) 100 IU kg^{-1}; Vitamin K3 (min) 2.6 mg kg^{-1}; Vitamin B1 (min) 8.0 mg kg^{-1}; Vitamin B2 (min) 7.0 mg kg^{-1}; Vitamin B6 (min) 12.5 mg kg^{-1}; Biotin (min) 0, 5 mg kg^{-1}; Vitamin B12 (min) 20 mcg kg^{-1}; Folic acid (min) 4.75 mg kg^{-1}; Choline (min) 300 mg kg^{-1}; Niacin (min) 180 mg kg^{-1}; Iron (min) 50 mg kg^{-1}; Iodine (min) 320 mg kg^{-1}; Manganese (min) 31.2 mg kg^{-1}; Zinc (min) 37.5 mg kg^{-1}; Inositol (min) 10 mg kg^{-1}; PHE: Hydroalcoholic extrac of pequi; PM: defatted pequi meal; C: Control diet; DPPH. (2,2-diphenyl-1-picrylhydrazyl radical scavenging activity), ORAC (Oxygen Radical Absorbance Capacity), and ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity), expressed in Trolox equivalents. Means followed by different letters indicate significant differences according to Tukey's test ($p < 0.05$). Data are expressed as mean ± standard deviation.

2.2.2 Feed proximate analysis

The proximate composition of the diets (moisture, crude protein, lipid, and ash) was determined following AOAC guidelines (AOAC 2016). Moisture: ovendried at 105 °C; crude protein: Kjeldahl method with factor 6.25; lipid: Soxtec system following Bligh & Dyer (1959); ash: muffle furnace at 550 °C for 8 h (Table I).

2.2.3 Extraction assay

To prepare extracts for antioxidant assays and phenolic quantification, 2.5 g of sample was mixed with 40 mL methanol under magnetic stirring for 4 h. Filtrates were evaporated at 37 °C under reduced pressure, and crude extracts were resuspended in 50 mL solvent. Samples were protected from light throughout.

2.2.4 Antioxidant Activity Assays

The evaluation of the antioxidant activity of the extracts was performed using three distinct methods, each providing a complementary perspective on their ability to combat free radicals. The DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical assay was conducted following the procedures established by Brand-Williams et al. (1995) and Ma et al. (2011).

The absorbance of the samples was measured at 517 nm to quantify the reduction of the DPPH radical. The results were expressed in micromoles of Trolox equivalents per gram of extract (μmol TE g^{-1} extract), allowing for a standardized comparison of the antioxidant potency. For the ORAC (Oxygen Radical Absorbance Capacity) method, a VICTOR X4 plate reader was used, according to the methodologies described by Zulueta et al. (2009) and Prior et al. (2003).

This assay measures a substance's ability to inhibit the degradation of a

fluorescent probe caused by peroxyl radicals. The antioxidant activity was expressed in micromoles of Trolox equivalents per gram of sample (μmol TE g^{-1} sample), providing a measure of the protective capacity against oxidative stress. Finally, the ABTS (2,2'-azino- bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation assay was performed based on the protocol by Thaipong et al. (2006), with some modifications. This method assesses the ability of antioxidants to neutralize the ABTS•+ radical. The results were presented in micromoles of Trolox equivalents per gram of sample (μmol TE g^{-1} sample), complementing the previous analyses and offering a comprehensive view of the total antioxidant activity (Table I).

2.2.5 Determination of Phenolic Compounds and Injection Conditions

Phenolic compounds were quantified using UPLC-MS/MS following Rodrigues et al. (2019) methodology. Standards included gallic acid, quercetin acid, p-Coumaric acid, ferulic acid and nicotinic acid. The system comprised an Acquity H-Class UPLC coupled to a Xevo TQD triple quadrupole mass spectrometer (Waters, Milford, MA, USA) operating in positive and negative modes. MassLynx and QuanLynx software (v4.1) were used for data processing. Chromatographic conditions: Mobile phase A: Water with 0.1% formic acid; Mobile phase B: Methanol; Gradient: Started at 10% B, increasing to 60% in 3 minutes, then to 80% over 1 minute, holding for 1 minute. Finally, B was increased to 100% over 5.5 minutes, held for 1.5 minutes, then returned to 10% over 4.5 minutes; Column temperature: 30 ±1° C; Flow rate: 0.150 mL min^{-1}; Injection volume: 1.5 μL; Run time: 16 minutes. Once the method conditions were established, the analytical parameters of the UHPLC-MS/MS method developed to determine the phenolic compounds (Gallic acid, quercetin acid, p-Coumaric Acid, Ferulic Acid and Nicotinic Acid) of pequi

extracts were evaluated (Table I).

2.2.6 Zootechnical performance and Color analysis

At the end of the experiment, fish were counted, weighed, and measured. Zootechnical parameters were calculated as described in Table II.

Table II. Equations for determining zootechnical performance parameters.

PARAMETER	EQUATION
Weight gain (WG)	WG = Wf − Wi
Condition factor (K)	K = (W) / (Lb)
Weight uniformity, UW (%)	UW = (X / X1) *100
Total length uniformity, Ul (%)	Ul = (X / X1) *100
Specific growth rate (SGR)	SGR = ((ln Wf − ln Wi) / t) * 100
Feed intake (FI)	Total feed consumed (g)
Feed conversion ratio (FCR)	FCR = FI / WG
Survival rate (SUR)	SUR = (Nf / Ni) *100

Wf = Final weight (g); Wi = Initial weight (g); L = Total length (cm); b = Slope coefficient of length-weight relationship; X = total number of fish with final unit; X1 = number of fish into experimental weight or lenght ± 20% inside of the final weight or lenght mean into experimental unit (Furuya et al. 1998, Abe et al. 2022); t = Experimental period (days); Nf = Final number of fish; Ni = Initial number of fish.

At both the beginning and the end of the experiment, fifteen fish per treatment (three fish per experimental unit) were selected, identified and photographed. Images were taken on a transparent plate positioned at a 20 cm height, under a natural light

source, without the use of flash (Figure 2), using an iPhone 12 Pro. Photographs of the anal and caudal fins were later analyzed using the RGB color system in Image Pro-Plus software (version 4.5, Media Cybernetics, Silver Spring, MD, USA).

Figure 2.

Body coloration was measured below the dorsal fin in the same 45 fish used for RGB analysis. Color was assessed with a Chroma Meter CR-400 portable photocolorimeter (Konica Minolta) (Figure 2 A, B and C), using the Hunter *L, a, b* coordinate system, where L* represents brightness or luminosity (0 = black, 100 = white), a* ranges from -100 (green) to +100 (red), and b* ranges from -100 (blue) to +100 (yellow) (Gouveia et al. 2003, Rezende et al. 2012, Porto et al. 2020a, Cruz et al. 2023).

2.2.7 Muscle and Hepatic Enzyme Analysis

Samples from fifteen fish per treatment (three fish per experimental unit, randomly selected) were collected for the analysis of hepatic and muscle enzymatic activity. The fish were euthanized with clove oil at a concentration of 50 mg L^{-1} for 30 seconds (Inoue et al. 2003, Honorato et al. 2017), then individually weighed and measured. Liver and muscle tissue samples were collected and immediately stored at -80 °C in an ultra-freezer until analysis.

For enzymatic analysis, 100 mg of liver and muscle tissue were homogenized in phosphate buffer (glycerol v/v in 20 mM sodium phosphate buffer and 10 mM Tris, pH 7.0) using a Potter-Elvehjem homogenizer. The homogenates were centrifuged at 4 °C for 3 minutes at 600 x g. The resulting supernatant was then centrifuged again at

6000 x g for eight minutes. The final supernatant was used to determine liver enzyme activity (ALT and AST) via spectrophotometry (Reitman & Frankel 1957) using a Bioplus S-200 semi-automatic spectrophotometer. Protein content was quantified following the method of (Kruger 2009) using Bradford reagent and a bovine serum albumin standard solution. Absorbance was read at 450 nm for both liver and muscle samples. SOD activity was determined based on pyrogallol auto-oxidation, where 1.0 U inhibits 50% of pyrogallol auto-oxidation in the presence of SOD (Beutler 1984), with absorbance measured at 420 nm. CAT activity was assessed by measuring H_2O_2 decay at 230 nm (Beutler 1984), where one CAT unit equals 1.0 μmol of H_2O_2 min^{-1} oxidation, using a molar absorptivity of (H_2O_2) $\epsilon\lambda 230$ nm = 0.071 mM cm^{-1}.

2.3 Statistical analysis

The data were grouped by treatment and assessed for normality using Bartlett's test (Bartlett 1992) and the Shapiro-Wilk test for homogeneity (Shapiro & Wilk 1965). When assumptions of normality and homogeneity were met, data were subjected to one-way analysis of variance (ANOVA). Significant differences $P<0.05$ were compared using Tukey's post hoc test (Tukey 1949). Statistical analyses were performed using the ExpDes.pt package (Ferreira et al. 2021) in R software (R Development Core Team 2025) .

For each variable, the analysis of variance was performed according to the following general model:

$$Yij = \mu + \alpha i + \varepsilon ij \quad (1)$$

in which Yij is the measured dependent variable, μ is the overall mean, αi is the effect of treatments, and εij is the random error.

2.3.1 Principal Component Analysis (PCA)

Principal Component Analysis was conducted to explore relationships among variables in the dataset. Prior to analysis, data were normalized (Equation 2). Pearson correlation coefficients were computed using (R Development Core Team 2025) with the corrplot package (Wei & Simko 2021), assessing linear relationships between variables.

$$Xi = (xi - \mu)/SD, \quad (2)$$

where Xi is the normalized value, xi the observed value, and μ and SD represent the variable's mean and standard deviation, respectively.

PCA reduces data dimensionality through linear transformation, generating principal components (PCs) that retain key information from the original variables (Wise et al. 1999, Olsen et al. 2012). We retained only PCs with eigenvalues >1 (Kaiser criterion (Kaiser 1960)), following standard practice in agricultural and aquaculture research (Olsen et al. 2012). Component loadings, indicating variable contributions to each PC, were calculated using multcomp package (Hothorn et al. 2008, Lê et al. 2008). This multivariate approach enabled comprehensive evaluation of diet effects across multiple response variables.

3 Results

3.1 Zootechnical performance

Table III presents the zootechnical performance of Mato Grosso (*Hyphessobrycon eques*) fed diets containing 800 mg kg^{-1} of hydroalcoholic pequi extract (PHE) or pequi meal (PM), compared to a control diet. Survival was 100% across all treatments, indicating no mortality or negative effects from pequi inclusion. No significant differences were found in growth indicators, including final weight,

lengths, height, weight gain, or specific growth rate. Weight and length uniformity (100%) also did not vary among groups. However, feed intake was significantly higher in the PM group compared to both control and PHE. Feed conversion ratio (FCR) was negatively affected in the PM group, showing higher values than control and PHE, indicating less efficient biomass conversion. Condition factor (0.01-0.02) remained unchanged, suggesting similar morphology despite dietary interventions. Overall, pequi meal increased feed intake but impaired feed efficiency, without promoting growth advantages during the trial.

Table III. Zootechnical performance of treatments with 800 mg kg^{-1} of hydroalcoholic extract (PHE) and defatted pequi meal (PM) compared to the control (C).

Variables	**Treatments**			***p-value***
	C	**PHE (800 mg kg^{-1})**	**PM (800 mg kg^{-1})**	
Survival (%)	100.00	100.00	100.00	-
Final weight (g)	0.90±0.16	0.95±0.17	0.92±0.17	0.7117
Weight uniformity (%)	73.33±27.89	66.67±33.34	60.00±14.91	0.6384
Total length (mm)	38.49±2.14	38.37±2.81	38.93±2.74	0.8199
Total length uniformity (%)	100.00	100.00	100.00	-
Standard length (mm)	31.87±1.48	32.13±1.45	32.08±2.17	0.9109
Height (mm)	12.96±1.28	13.45±1.13	12.90±1.41	0.4453
Weight gain (g)	0.22±0.16	0.27±0.17	0.24±0.17	0.7117
Specific growth rate	0.90±0.61	1.08±0.62	0.95±0.65	0.7305
Feed intake(g)	7,51±0,62b	7,31±0,88b	9,48±1,04a	0,0032
Condition factor	0.02±0.01	0.02±0.01	0.01±0.01	-
Feed conversion ratio	2.91±0.45b	2.88±0.45b	4.62±0.71a	0.0380

Means followed by different letters indicate significant differences according to

Tukey's test ($p < 0.05$). Data are expressed as mean ±standard deviation.

3.2 Fish Pigmentation

The effect of dietary treatments on tail and anal fin coloration in Mato Grosso is shown in Table IV. Significant differences were detected among treatments for several chromatic parameters. In the tail fin, red (R) values were higher in the control group than in the hydroalcoholic extract group, while the pequi meal treatment showed intermediate, nonsignificant values. The blue (B) channel was highest in the pequi meal group, followed by the control and hydroalcoholic extract, whereas no differences were observed for the green G) channel. For the anal fin, the control group again exhibited the highest red (R) value, significantly greater than the hydroalcoholic extract, with pequi meal showing intermediate levels. In the green (G) channel, pequi meal and control shared higher values, both differing from the hydroalcoholic extract. The blue (B) channel also varied significantly, being highest in the pequi meal group, intermediate in the control, and lowest in the hydroalcoholic extract group.

Table IV. Colorimetric parameters of tail fin, anal fin, and body of Mato Grosso (*Hyphessobrycon eques*) subjected to dietary treatments with a hydroalcoholic extract (PHE) and defatted pequi meal (PM), compared to the control group (C).

Variables	**Treatments**			***p-value***
	C	**PHE (800 mg kg^{-1})**	**PM (800 mg kg^{-1})**	
Anal fin color				
R	130.92±9.58a	119.75±8.77b	127.61±9.34ab	0.0060
G	74.75±9.84a	63.53±8.37b	74.09±9.76a	0.0027
B	25.78 ±7.33a	18.96±5.39b	29.19 ±8.30a	0.0009
Tail fin color				

R	138.85±5.37a	133.89±5.18b	137.36±5.32ab	0.0406
G	93.56±7.87	90.80±7.64	92.02±7.74	0.6226
B	40.96±10.63ab	34.67±9.00b	43.13±11.45a	0.0490
Body color				
*L**	46.74±10.18	49.87±6,29	49.46±10.73	0.6077
*a**	0.44±0.89	0.28±0.48	0.13±0.94	0.8181
*b**	-1.15±5.66	-0.57±3.70	0.79±6.08	0.5894

Means followed by different letters indicate significant differences according to Tukey's test ($p < 0.05$). Data are expressed as mean ± standard deviation. R: Red; G: Green; B: Blue; L*: 0 (black) to +100 (white); a*: -100 (green) to +100 (red); b*: -100 (blue) to +100 (yellow).

3.3 Enzymes activities

In liver tissue, ALT activity (Figure 3A) showed no significant differences, while AST (Figure 3B) increased in the PHE group, indicating enhanced amino acid metabolism. Hepatic SOD (Figure 3C) and CAT (Figure 3D) remained stable across diets. In muscle, SOD (Figure 3E) was higher in the Control group than in PM, with PHE showing intermediate values, while CAT (Figure 3F) showed no significant variation. Overall, PHE influenced liver metabolism, whereas PM mainly affected muscle antioxidant capacity, highlighting tissue-specific responses.

Figure 3.

3.4 Phenolic compounds and antioxidant activity

UPLC-MS/MS identified gallic acid, quercetin, p-coumaric acid, ferulic acid, and nicotinic acid in the diets (Table I). PHE contained more gallic and ferulic acids, while the Control diet had more quercetin and p-coumaric acid. DPPH showed no

differences among diets, but ABTS activity was lower in the Control group. ORAC results confirmed higher antioxidant capacity in pequi diets, with PHE outperforming PM. Thus, pequi supplementation enhanced dietary antioxidant potential.

3.5 Principal Component Analysis - PCA

PCA (Figure 4A) explained 22.4% (PC1) and 14.5% (PC2) of the total variance, with confidence ellipses (90%) indicating partial clustering but also wide dispersion among treatments. Variable contributions (Figure 4B) highlight Ga (RGB of the anal fin) as the main discriminator along PC1, while Bc (RGB of the caudal fin) contributed most to PC2. Together, these color parameters played a central role in group separation. Regarding biochemical traits, catalase activity in liver tissue (CAT L) showed a moderate contribution, reinforcing that antioxidant responses also influenced the multivariate distribution. These results suggest that both colorimetric parameters and tissue-specific antioxidant activities are key drivers of variability among treatments.

Figure 4.

4. Discussion

In the ornamental fish industry, increasing zootechnical performance to boost biomass production is not the primary objective. Consumers seek longlasting, beautiful, and healthy animals (Stevens et al. 2017, Sathyaruban et al. 2021, Vissio et al. 2021) leading us to consider animal welfare, pigmentation, and longevity. Therefore, a bioactive substance that positively impacts one of these factors without negatively affecting zootechnical performance would be ideal for use in this industry.

Our study showed that supplementation with pequi meal (PM) has a

comparable effect to a commercial diet, increasing pigmentation and improving liver health, but reducing antioxidant activity. However, these effects were not associated with any change in the zootechnical performance of *H. eques*.

In this context, it is common to observe significant improvements in growth performance when nutritional bioactive compounds are included in the diet (Li et al. 2022, Rana et al. 2023), though this is not always the case (Wang et al. 2006). These improvements may also result in increased growth and metabolic activity (Wang et al. 2017, Porto et al. 2020a, Xu et al. 2020), indicating species-specific or context-dependent effects.

There is growing interest in the use of natural additives derived from microalgae, plants, fruits, and plant extracts in aquafeeds, particularly for their functional benefits. These compounds can serve as antioxidants (França et al. 2022, Rana et al. 2023, Trivedi et al. 2025), support immune function (Porto et al,.2020b, Li et al. 2022), improve pigmentation (Berchielli-Morais et al. 2015, Ebeneezar et al. 2020, Cruz et al. 2023, Rana et al. 2023, Tripathy et al. 2024, Rana et al. 2024), enhance digestibility (Honorato et al. 2022), and contribute to overall fish welfare (Porto et al, 2020a). Such effects are especially relevant to ornamental species, where coloration is a key trait (Kaur & Shah 2017).

In the present study, supplementation with defatted pequi meal did not alter overall body coloration but significantly influenced fin pigmentation. This finding is particularly relevant because ornamental species, such as *H. eques*, possess distinct carotenoid metabolic pathways compared to other fish (Scardua et al. 2024). Consequently, pigmentation expression may depend not only on pigment availability but also on physiological regulation and metabolic allocation (Sköld et al. 2016, Cal et al. 2017). Furthermore, experimental evidence suggests that enhanced antioxidant

activity can suppress pigmentation by interfering with oxidative signaling mechanisms that regulate chromatophore function (Huang et al. 2023, Siqueira et al. 2024). These interactions highlight the complex balance between dietary antioxidants and visible pigmentation, reinforcing the importance of experimental trials to disentangle such effects in ornamental fish nutrition.

Antioxidant activity is an important variable influencing the effects of plants and nutraceutical compounds in fish diets (Honorato et al. 2022, Cruz et al. 2023, Siqueira et al. 2024). Carotenoids contribute to this activity by neutralizing free radicals through hydrogen atom abstraction (Sandhiya & Zipse 2022). Pequi is notably rich in carotenoids, as well as other bioactive compounds such as fatty acids, phenolic compounds, vitamin C, and tocopherols, all of which contribute to its antioxidant capacity (Leão et al. 2017, Roll et al. 2018, Silva et al. 2023). In addition, Baptista et al (2018) reported that all parts of the pequi plant exhibit strong antioxidant activity.

The phenolic compounds identified in the present study, such as gallic acid, quercetin, p-coumaric acid, ferulic acid, and nicotinic acid have previously been reported in pequi and are well recognized for their antioxidant potential (De Araujo 1995, Rocha et al. 2015, Melo et al. 2024). Importantly, Santos et al. (2022) demonstrated through *in vitro* digestion of pequi meal that the antioxidant capacity of these compounds increases after digestion, suggesting improved bioaccessibility. This pattern has also been observed in other plant derived matrices (Gullon et al. 2015, Su et al. 2017), reinforcing the idea that gastrointestinal processes can enhance the functional activity of phenolics, thereby supporting their role in modulating antioxidant responses *in vivo*.

In mammalian models, pequi supplementation has been shown to provide

oxidative protection. For example, Vale et al. (2019) demonstrated that pequi oil supplementation protected liver cells from oxidative damage in rats subjected to exhaustive swimming, as indicated by reductions in lipid peroxidation. Additionally, Torres et al. (2016) found that pequi oil enhanced endogenous antioxidant defenses by increasing superoxide dismutase (SOD) activity, a key enzyme in free radical detoxification. The activity of CAT and SOD with aflatoxin inclusion (400 μg kg^{-1}) is linked to the ability to remove free radicals and inhibit reactive compounds in metabolism, delaying damage (Godoy et al. 2024).

Beyond pigmentation, the use of natural additives enhances immunity (Dorce et al. 2025). Studies indicate that fruits not only provide nutrients but also contain significant levels of carotenoids, tocopherols, and phenolics in their pulp (Sousa et al. 2011, Siqueira et al. 2018), which alters ornamental fish coloration (França et al. 2022, Cruz et al. 2023, Dorce et al. 2025). The combined effect of antioxidant activity and coloration has been successfully reported with the use of tomato (*Solanum lycopersicum*), carrot (*Daucus carota*) (Mirzaee et al. 2012), annatto (*Bixa orellana*) (Dorce et al. 2025), jabuticaba (França et al. 2022), and hibiscus (Cruz et al. 2023).

Fish pigmentation is associated with species, the pigment's ability to color, its dietary concentration (Li et al. 2017), and especially its form of inclusion (Vanegas-Espinoza et al. 2019). This study highlights key differences between the use of plant extracts and meal. The observed responses may be related to the higher feed intake promoted by meal inclusion. Incorporating whole plant meals in diets affects palatability and feed utilization (Honorato et al. 2022), and fish metabolism, as whole plant use promotes synergy and inclusion of associated compounds, leading to more acute effects (Honorato et al. 2021). Fruit meal is generally closer to the natural product, retaining more original nutrients like fiber, vitamins, and minerals, unlike

extracts, which may undergo concentration or purification processes that remove some of these substances. Carneiro et al. (2023) report that one of the biggest challenges in extracting bioactive compounds from plants is processing, as they can easily be lost or degraded during handling.

Despite the antioxidant potential highlighted in pequi, its incorporation into the Mato Grosso diet elicited distinct responses. The hydroalcoholic extract exhibited comparable levels of SOD and CAT activity to the control diet. However, the incorporation of pequi meal (PM) resulted in a decline in SOD activity, suggesting a decrease in antioxidant activity. It is possible that the elevated levels of consumption observed in the PM treatment contributed to this response, thereby prompting a reevaluation of the dosage or its application.

Conversely, the incorporation of pequi meal (PM) into the diet resulted in a decline in AST activity, suggesting a potential role for pequi meal in supporting liver function and hepatoprotection. Increased ALT and AST activity may indicate hepatocyte damage and is associated with acute liver injury (Ota et al. 2019), and their reduction may indicate hepatoprotective effects (Siqueira et al. 2024). Reports of hepatoprotection in fish include the use of roselle (*Hibiscus sabdariffa* L.) hydroalcoholic extract in *H. eques* diets (Cruz et al. 2023), *Erythrina crista-galli* inclusion in red-eye tetra (*Moenkhausia forestii*) diets (Siqueira et al. 2024), and carotenoid inclusion in *H. eques* diets (Wang et al. 2006).

However, further studies should be conducted on pequi meal in *H. eques* diets, combining the product with vitamin sources to enhance bioavailability (Dorce et al. 2025) or amino acid inclusion (Scardua et al. 2024). In addition, the efficacy of novel dosages should be assessed to determine whether they lead to enhanced antioxidant and hepatoprotective effects in *H. eques*.

5. Conclusion

In conclusion, dietary supplementation with pequi meal (PM) at 800 mg kg^{-1} proved to be generally safe, as all groups maintained 100% survival. While zootechnical parameters were unaffected, PM increased feed intake and reduced feed efficiency but enhanced anal and caudal fin coloration, a desirable trait in ornamental fish. PM supplementation reduced hepatic AST activity, indicating potential metabolic benefits, but decreased muscle SOD activity, impairing antioxidant metabolism. However, further studies using different dosage levels are needed to determine whether muscle SOD activity in *H. eques* can be improved.

6. Author Contributions

A.C.G.: Conceptualization, Investigation, Methodology, Writing, Review editing; C.A.H.: Conceptualization, Investigation, Methodology, Writing, Review editing; D.H.N.: Conceptualization, Investigation, Methodology, Writing, Review editing; D.F.R.O.: Investigation, Methodology, Writing; I.B.S.: Investigation, Methodology, Writing; J.A.U.R.: Investigation, Methodology, Writing; L.M.A.C.: Investigation, Methodology, Writing; L.B.L.: Investigation, Methodology, Writing; M.P.S.: Conceptualization, Investigation, Methodology, Writing, Review editing; O.O.S.J.: Investigation, Methodology, Writing; P.D.S.: Investigation, Methodology, Writing;

7. Data Availability

The experimental data are available from the corresponding author on reasonable request.

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10. **Figures**

Figure 1 Pequi fruit (*Caryocar brasiliense*) (A), pequi meal (B), and Mato Grosso tetra (*Hyphessobrycon eques*) (C).

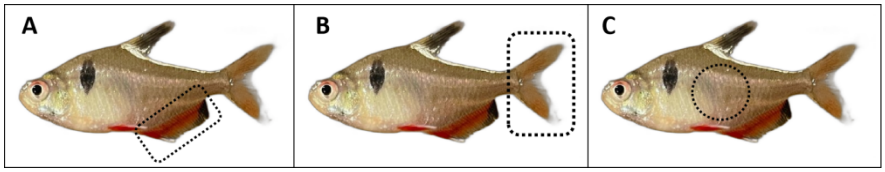

Figure 2 Color sampling location in *H. eques* for RGB: Anal fin (A), Caudal fin (B), and for L*, a*, b* coordinate system: body (C).

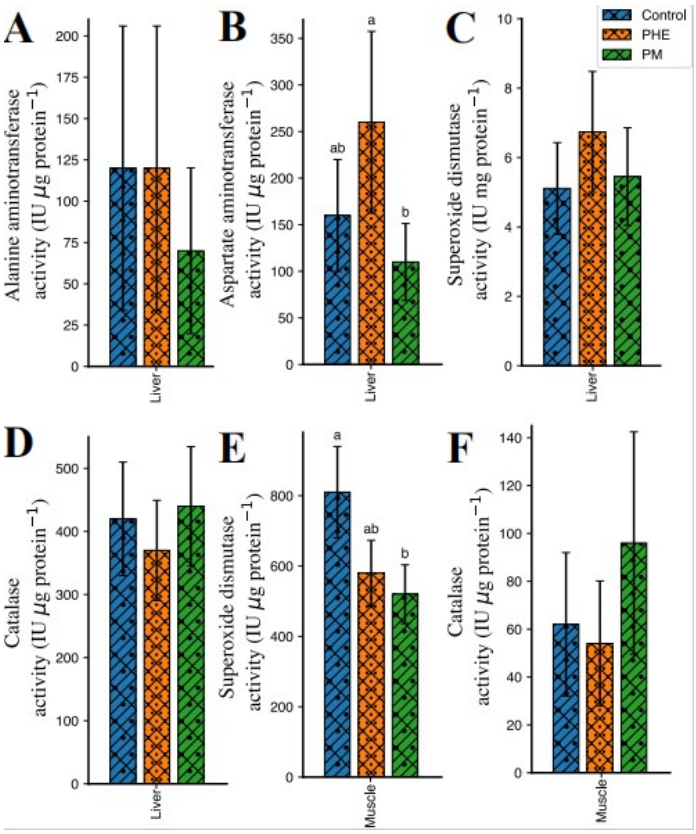

Figure 3 Enzyme activities in muscle and liver tissues of fish subjected to different dietary treatments. Different letters indicate significant differences according to Tukey's test ($p < 0.05$).

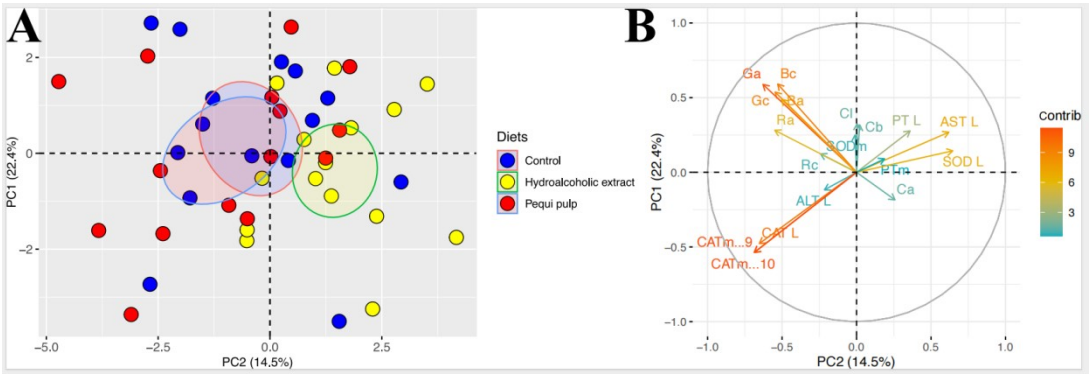

Figure 4 PCA. Biplots of PC1 and PC2, where: blue points represent the control group; red and yellow points represent the treatments with inclusion of hydroalcoholic extract and Caryocar brasiliense (pequi) meal, respectively. The rings correspond to 90% confidence ellipses estimated using the treatment means. The variance explained by each dimension is shown in parentheses. (B): Correlation of quantitative variables and their contributions within each dimension. CATm: catalase in muscle; CATl: catalase in liver; SODm: superoxide dismutase in muscle; SODl: superoxide dismutase in liver; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Cl: body lightness; Cb: body chromaticity b; Ca: body chromaticity a; Ra: red from the RGB pattern of the anal fin; Ga: green from the RGB pattern of the anal fin; Ba: blue from the RGB pattern of the anal fin; Rc, Gc, and Bc: corresponding RGB values from the caudal fins. ”Diet” refers to the experimental treatments.

CAPÍTULO 3 - TOXICITY AND ANTIOXIDANT EFFECTS OF THE DECOCTE OF THE LEAF OF Guavira (*Campomanesia guazumifolia*) AS A FOOD ADDITIVE FOR *Geophagus pyrocephalus*[2]

Abstract

The search for nutraceutical products for inclusion in animal diets is a global trend, and ornamental aquaculture follows this tendency. Guavira (*Campomanesia guazumifolia*) is a native Cerrado species recognized for its anti-inflammatory, antimicrobial, antioxidant and immunomodulatory properties. This study evaluated the potential of *C. guazumifolia* leaf decoction as a feed additive for *Geophagus pyrocephalus*. Toxicity tests were performed using *Artemia salina* at the following decoction concentrations: 0.0 (control); 1.5; 5.0; 25.0; 125.0; 250.0; 500.0 and 1000.0 $\mu g \cdot mL^{-1}$, and on G. pyrocephalus juveniles at concentrations: 0.0 (control); 0.1; 1.0; 10.0; 25.0; 50.0; 100.0; 200.0; 300.0; 400.0 and 500.0 $\mu g \cdot mL^{-1}$, in addition to a feeding trial containing the extract at different doses (0.0 (control), 250, 500, 1000 and 2000 $mg \cdot kg^{-1}$). Evaluated parameters included zootechnical performance, histopathological analysis of the gills, hepatic metabolism and muscle antioxidant activity. The results showed high toxicity of the decoction to *A. salina* and *G. pyrocephalus*, with gill lesions observed at all concentrations. However, the diet containing 250 $mg \cdot kg^{-1}$ of extract provided antioxidant benefits without compromising fish health. Higher doses induced significant adverse effects. Therefore, safe use of *C. guazumifolia* decoction depends on appropriate dose determination.

Keywords: welfare; guavira; ornamental aquaculture; health

INTRODUCTION

Medicinal plants and their derivatives are rich sources of diverse bioactive constituents with a range of biological activities (Carvalho and Conte-Junior, 2021). Plant-derived products have been widely used in traditional medicine as preventive and therapeutic alternatives (Al-Okbi and Sahar, 2014; Barnes, 2020; Manzoor et al., 2022). In this context, species from the Brazilian Cerrado have been investigated for their antiplatelet potential and are widely used in food and beverage preparation (Lescano et al., 2018; Lescano et al., 2021; Tolouei et al., 2018). Particular attention has been given to phenolic compounds such as flavonoids, which are abundant in nature and exert diverse effects on antiplatelet agents (Lamponi, 2021; Fernández-Rojas et al., 2022).

[2] Este capítulo corresponde ao artigo científico submetido à revista Fish Physiology and Biochemistry.

The Brazilian Cerrado is a biome with high biodiversity of native species, recognized for medicinal, nutraceutical and food potential (Carvalho and Conte-Junior, 2021). Among these species, *Campomanesia guazumifolia* stands out (commonly known as sete-capotes, sete-capas, capoteira, sete-casacas, arázeiro and araçá-do-mato) and is traditionally used to treat intestinal and hepatic disorders, also showing anti-inflammatory and anti-edematogenic actions (Dorigoni et al., 2001).

Recent studies have shown that leaf infusion of *C. guazumifolia* exhibits anti-inflammatory activity (Catelan et al., 2018), while the ethanolic extract presents photoprotective potential (Catelan et al., 2019). These biological activities are mainly attributed to phenolic compounds found in the plant (Catelan et al., 2018; Cialdella-Kam et al., 2017), which may be explored as feed additives to improve animal health (Viscardi et al., 2017).
In aquaculture, various plant-derived ingredients have been incorporated into diets to promote fish health (El-Barbary, 2018; Li et al., 2022). However, the effects of plant extracts on aquatic organisms remain underexplored (Siqueira et al., 2024), making it essential to understand their influence on fish health, including physiological and histological parameters, as a preliminary step for large-scale use. In ornamental fish, the inclusion of phytotherapeutic additives has shown benefits for health and welfare, positively affecting animal longevity (Reges et al., 2024).

Ornamental species, especially cichlids, are valued in aquaculture for their aesthetic appeal and market acceptance (Karsli, 2021). *Geophagus pyrocephalus* is a Brazilian cichlid known for its intense red cephalic coloration, which is a key commercial trait for aquarists (Luo et al., 2021; McLean, 2021; Vissio et al., 2021; Lau et al., 2023; Scardua et al., 2024).

Therefore, this study aimed to investigate the oral toxicity and effects of *C. guazumifolia* leaf decoction when included in the diet of *G. pyrocephalus*, in order to assess its potential as a functional feed additive for ornamental fish.

MATERIALS AND METHODS

2.1 Biological material

Leaves of *Campomanesia guazumifolia* (Figure 1A) were collected on a farm located in the municipality of Amambai, Mato Grosso do Sul. The species was identified and deposited in the Herbarium of the Federal University of Grande Dourados (UFGD), Dourados-MS, under number 4746. Leaves were dried in a circulating-air oven at 37 ± 2°C and milled in a Wiley-type mill (Marconi) with a 10 mesh sieve. The sample was then packaged, labeled and stored at room temperature.

The fish, *Geophagus pyrocephalus* (Figure 1B), were obtained from Bioparque Pantanal, located in Campo Grande, Mato Grosso do Sul, Brazil. The experiment was approved by the Animal Research Ethics Committee, University Center of Grande Dourados (UFGD) under protocol number 003/2014. SISGEN registration code: AA88DF1.

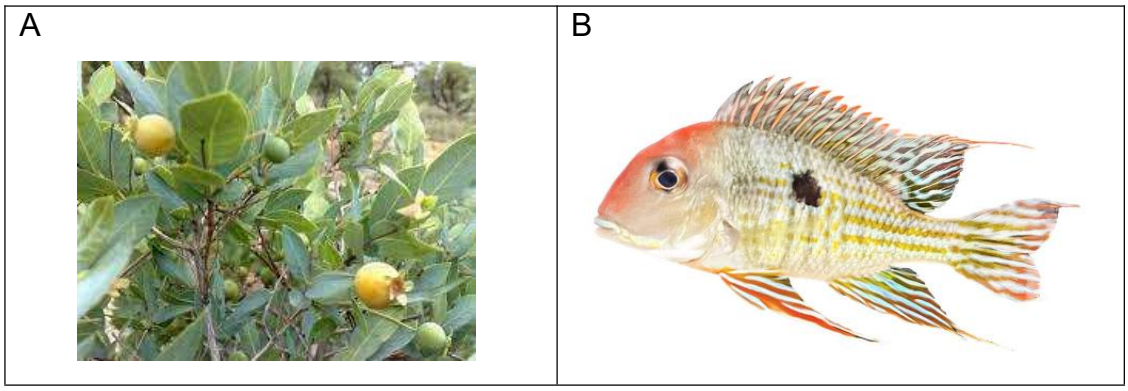

Figure 1. A) Image of *Campomonesia guazumifolia*, personal archive. B) *Geophagus pyrocephalus*, photo: Guilherme Pereira Marconato.

The decoction was prepared by boiling the leaves for 10 minutes in distilled water (VINAGRE et al., 2010). The decoction was filtered and subsequently lyophilized at -42°C under vacuum (0.045 mbar). Extraction yield was calculated based on the dry lyophilized mass, and the extract was stored at -20°C. Chemical composition was evaluated at a concentration of 1 $mg \cdot g^{-1}$ of extract.

Phenolic compound content was determined using the Folin–Ciocalteu colorimetric method (Djeridane et al., 2006). For this procedure, 0.5 mL of Folin–Ciocalteu reagent (1:10 v:v) and 1000 µL of distilled water were added to 100 µL of each sample and allowed to stand for 1 minute. Then 1.5 mL of

20% aqueous sodium carbonate solution was added and the reaction was kept in the dark for 120 minutes. Absorbance was read at 760 nm. Results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

The extract was subjected to clean-up using a C-18 SPE cartridge (50.0 mg) (Strata C18-E, Phenomenex) activated with 4.0 mL methanol (Sigma-Aldrich). The sample (3.0 mg) was solubilized in the minimum amount of mobile phase and subjected to solid-phase extraction with 5.0 mL methanol. The obtained solution was dried at room temperature, redissolved in methanol to obtain a concentration of 3.0 mg·mL-1 and filtered through a 0.45 µm filter (CHROMAFIL® Xtra). The sample was then analyzed. A direct injection was performed (FIA-ESI-IT-MS, negative mode - Mass spectrometer LTQ XL equipped with an APCI ionization source and linear ion-trap analyzer) to obtain mass spectra, and for the major peaks MSn experiments were carried out to obtain further structural information.

2.2 Toxicity assessment

The assay was approved by the Institutional Animal Use and Care Committee of the University Center of Grande Dourados/CEUA, protocol no. 003/14. The study complied with biosafety norms and the Ethical Principles in Animal Experimentation elaborated by the National Council for the Control of Animal Experimentation (CONCEA).

The toxicological analysis in *Artemia salina* followed the protocol described by Meyer et al. (1982). Artemia cysts were incubated for 48 hours in synthetic seawater (20 g·L-1) with 0.7 $g \cdot L^{-1}$ sodium bicarbonate (pH = 8), under continuous illumination (60 W) and aeration. To determine the LC50, evaluation was performed with a negative control (saline solution) and eight treatments containing the aqueous extract of *C. guazumifolia* at concentrations: 0.0 (control); 1.5; 5.0; 25.0; 125.0; 250.0; 500.0 and 1000.0 $\mu g \cdot mL^{-1}$. Triplicates of 10 individuals were used for each concentration. After 24 hours of incubation, mortality (%) of *A. salina* individuals in each concentration was recorded.

For the acute toxicity test in *G. pyrocephalus* juveniles, animals weighing 5.53 ± 1.04 g were exposed to concentrations: control (zero), 0.1; 1.0; 10.0; 25.0; 50.0; 100.0; 200.0; 300.0; 400.0 and 500.0 $\mu g \cdot mL^{-1}$ with ten

replicates for 24 hours. Fish were acclimated for seven days in laboratory conditions (60 L tanks, at a density of 10 fish per unit) with constant aeration, temperature of 27°C and a 12 h photoperiod. Fish were fed to apparent satiation twice daily with extruded commercial diet (32% crude protein).

For acute toxicity tests, feeding was suspended 24 hours before the start of the experiment and the mass-to-volume ratio was maintained near 1 $g \cdot L^{-1}$, in accordance with the recommendations of the Brazilian Association of Technical Standards - ABNT NBR 15088:2011. Water quality parameters (temperature, dissolved oxygen, turbidity and pH) were monitored during the assay.

Tolerance to different extract concentrations over 24 hours was assessed by survival. Observations were recorded hourly during the first six hours, then at six-hour intervals until 24 hours of exposure. After exposure, all fish from each aquarium had caudal vein blood collected for glucose measurement. Fish were then anesthetized (100 mg eugenol$\cdot L^{-1}$) for gill histopathology procedures. Tissue fragments were fixed in formalin for 24 hours and subsequently washed in 70% alcohol. After fixation, fragments were dehydrated, cleared and embedded in paraffin with Histosec® polymer (Merck). Sections of 2-5 µm were cut and stained with hematoxylin and eosin (HE). Microscopic analyses and documentation were performed on an Olympus BX41 photomicroscope. Gills were analyzed according to Bernet et al. (1999).

2.3 Feeding trial

The i*n vivo* feeding trial was conducted at Bioparque Pantanal, Campo Grande - MS, using 150 juveniles of *G. pyrocephalus* with an average body weight of 2.74 ± 0.82 g and total length of 5.31 ± 0.46 cm. Fish were distributed in 15 experimental units (30 L each) with ten fish per unit, totaling 30 fish per treatment, in individual recirculation systems with a 12 h photoperiod and mechanical and biological filtration.

Fish were fed diets containing different doses of *C. guazumifolia* leaf decoction (T250; T500, T1000 and T2000 $mg \cdot kg^{-1}$, in addition to a commercial diet without extract) for 30 days to apparent satiation, twice daily.

The base diet was supplied by Poytara Ltd. (40.88% crude protein and

4374.8 Kcal·kg^{-1}, 1.0 mm pellets), which was ground and mixed for 30 min at room temperature and the experimental microencapsulated pellets were thereafter reformed. Pellets were dried at 50°C for 24 h in a forced-air oven and stored in the dark at 4°C.

Zootechnical performance was evaluated at the end of the feeding trial. Variables measured included initial weight (g), final weight (g), initial length (mm), final length (mm), weight gain, length gain and condition factor (Lima-Junior et al., 2002). After biometrics, juveniles were anesthetized with eugenol (100 mg·L^{-1}), euthanized, organs removed and frozen for analyses of hepatic enzymes, antioxidant activity and total protein of muscle and liver.

For hepatic metabolic enzyme analysis, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) samples of liver (100 mg) were homogenized in sodium phosphate buffer (glycerol v/v in 20 mM sodium phosphate and 10 mM Tris buffer, pH 7.0) using a Potter–Elvehjem homogenizer. Samples were centrifuged at 4°C for three minutes at 600 × g and the supernatant subjected to a second centrifugation for eight minutes at 6000 × g. The resultant supernatant was used for ALT and AST assays. Enzyme activities were determined by a modification of the Reitman and Frankel method (1957). Readings were performed on a semi-automatic spectrophotometer (Bioplus S-200) at wavelengths appropriate for each test.

Superoxide dismutase (SOD) was assayed by the auto-oxidation of pyrogallol, which is inhibited in the presence of SOD (Beutler, 1984, modified). Absorbance readings were performed at 420 nm, where 1.0 U.I. inhibits 50% of pyrogallol auto-oxidation. Catalase (CAT) activity was assessed by monitoring the decay of H2O2 at 230 nm (Beutler, 1984). One CAT unit was defined as the amount of enzyme required to decompose 1.0 µmol of H2O2 min-1, using an extinction coefficient of ε230 = 0.071 mM·cm^{-1}. Total protein was quantified using the Bradford reagent against a BSA standard (Kruger, 2009) for both liver and muscle samples.

2.4 Statistical analysis

Toxicity assay results were expressed as mortality indices (%) and analyzed using the non-parametric Kruskal–Wallis test ($p < 0.05$), followed by the Student–Newman–Keuls test. For the feeding toxicity test, a completely

randomized design (CRD) with five treatments and three replicates was used. Normality and homogeneity of variances were checked by the Shapiro–Wilk test using Bioestat (version 5.0). Analysis of variance (ANOVA) was performed and, when significant differences ($p < 0.05$) were detected, means were compared via Tukey's test.

A multivariate analysis (Principal Component Analysis – PCA) was used to evaluate correlations between mixed qualitative variables (different decoction concentrations) and quantitative variables (enzymatic data). PCA reduces data dimensionality by forming linear combinations that discard components with low variance, highlighting the most influential variables. PCA was performed in R (R Core Team, 2022) using FactoMineR (Husson et al., 2024) and factoextra (Kassambara, 2020).

RESULTS

The decoction of *C. guazumifolia* leaves presented 728.70 ± 13.20 mg·g-1 of phenolic compounds. In the compound characterization (Table 1), five compounds were identified.

Table 1 - MS and MS^n data of the compounds identified in the decoction of *C. guazumifolia* leaves.

MS (m/z)	FIA-ESI-IT-MS MS-MS íons (m/z)	Compounds identified	Bibliographic references
301	132	quercetin pentose	Gondoin et al., 2010 Scoparo et al., 2012 Huang et al., 2015
301	146	quercetin deoxyhexoside	Simirgiotis et al., 2013 Chavez et al., 2016
316	146	myricetin deoxyhexoside	Simirgiotis et al., 2013 Chavez et al., 2016
316	163	myricetin hexoside	Kim et al., 2011 Singh et al., 2011 Fang et al., 2007
316	152	myricetin deoxyhexoside gallate	Fang et al., 2007

An increase in decoction doses resulted in increased turbidity and pH (Figure 2).

A B

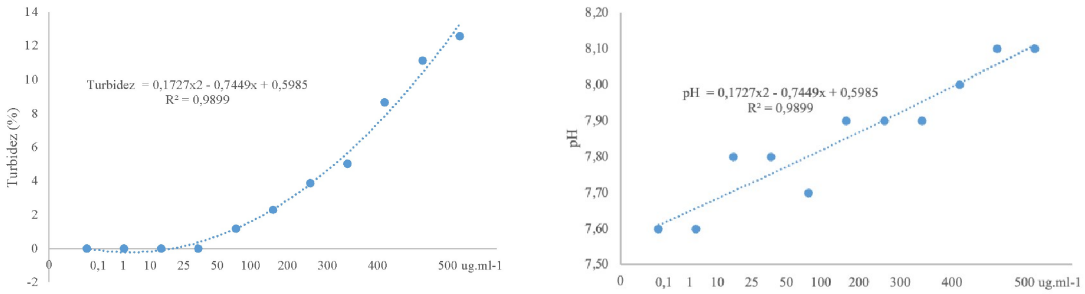

Figure 2 Turbidity (A) and pH (B) of water according to the doses of the decoction of *C. guazumifolia* leaves.

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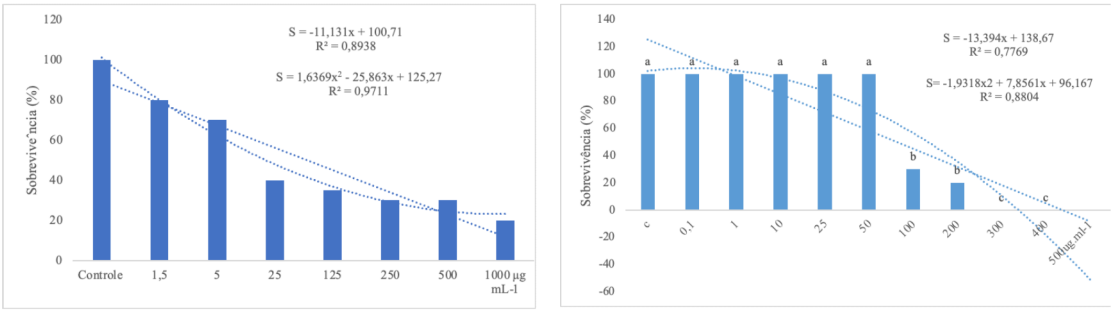
Survival (Figure 3B) of *G. pyrocephalus* juveniles was markedly affected from a dose of 100 μg·mL^{-1}. The LD50 for fish was established at 50.03 μg·mL^{-1} (Figure 3B). Survival time of juveniles dropped drastically from 100 μg·mL^{-1} inclusion (Figure 3C). Plasma glucose concentration indicated an increase in fish maintained in water containing 25 μg·mL^{-1} of *C. guazumifolia* or higher (Figure 3D).

A B



C D

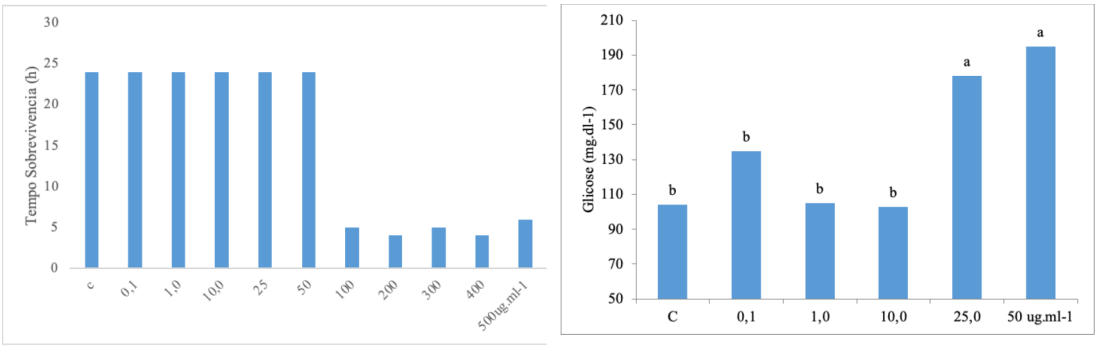


Figure 3A Shows *Artemia salina* survival in response to inclusion of *C. guazumifolia* decoction. There was a statistical difference between Artemia maintained in water without *C. guazumifolia* and those exposed to decoction doses above 25 $\mu g \cdot mL^{-1}$ (Kruskal–Wallis: H = 21.1059; p = 0.0036). The lethal dose for 50% mortality (LD50) for *A. salina* was determined as 23.29 $\mu g \cdot mL^{-1}$.

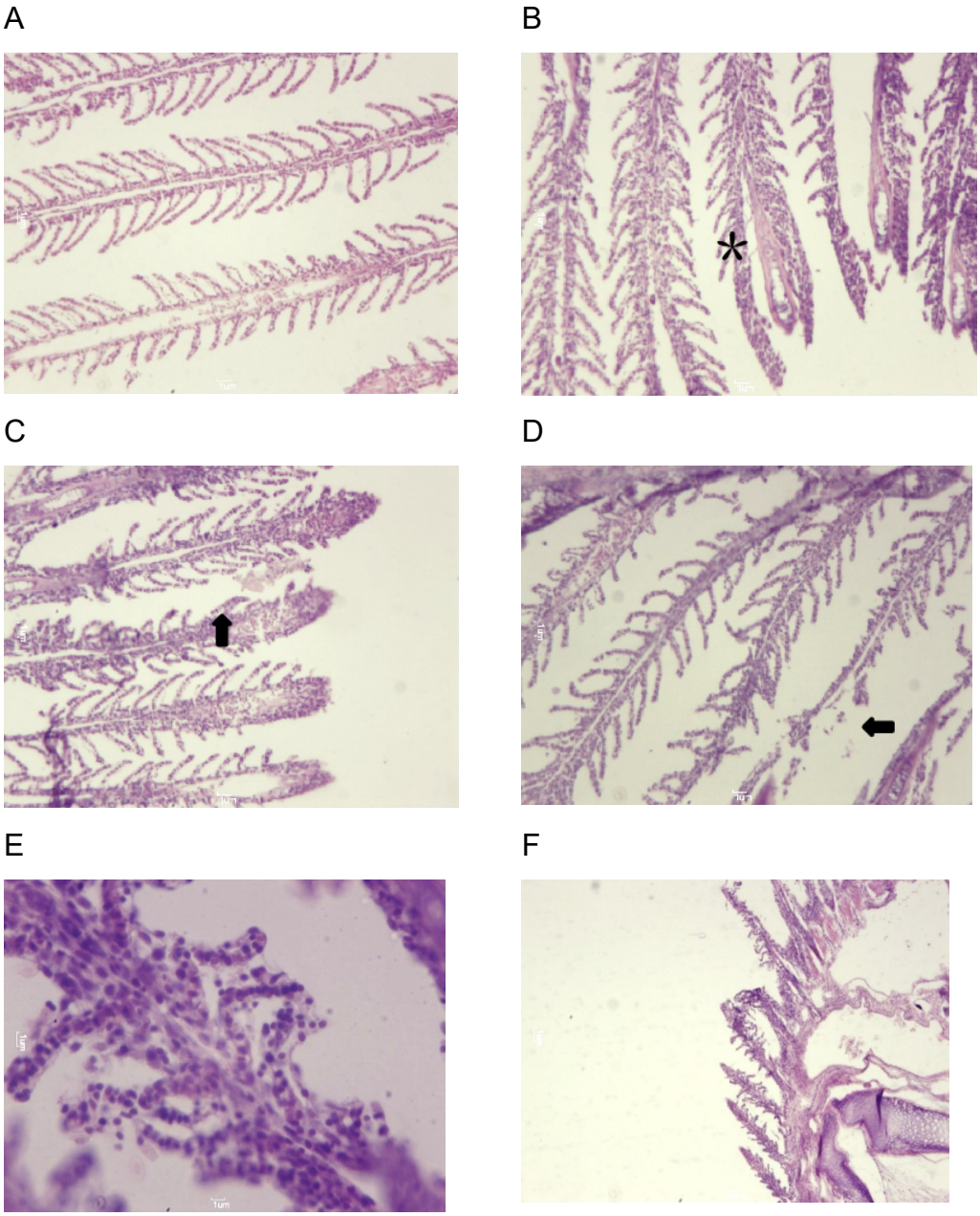


Figure 4. Photomicrograph of the gill of *Geophagus pyrocephalus* subjected to different doses of *Campomanesia guazumifolia* decoction in the toxicity experiment. (A) control treatment showing primary lamellae (PL) and secondary lamellae (SL) HE, 20x; (B) treatment containing 0.1 $\mu g.ml^{-1}$ of *C. guazumifolia* showing hyperplasia of the lining cells of the secondary lamellae

(*); (C) treatment containing 1 µg.ml^{-1} of *C. guazumifolia*. Detachment of the lamellar epithelium (arrowhead); (D) treatment containing 10 µg.ml^{-1} of *C. guazumifolia*. Rupture of cell membranes (arrowhead); (E) treatment containing 25 µg.ml^{-1} of *C. guazumifolia*. Necrosis at the base of the filamentous epithelium HE, 40x, (F) treatment containing 50 µg.ml^{-1} of *C. guazumifolia*

Gill lesions were observed at all tested concentrations of *C. guazumifolia*. At 0.1 µg·mL^{-1} there was hyperplasia of the covering cells in the interlamellar space and mucus hypersecretion (Figure 4B). At 1 to 10 µg·mL^{-1} there was hyperplasia of covering cells, development of interlamellar epithelium and apical fusion of secondary lamellae (Figures 4C and 4D). At 25 and 50 µg·mL^{-1} there was necrosis of the supporting collagen structure resulting in detachment of mucosal cells from the lamellar structure (Figures 4E and 4F).

Regarding the feeding test, no significant differences were observed in fish growth and diet consumption (Table 2). Hepatic alanine aminotransferase (ALT) activity increased with increasing decoction concentration, while other liver functions did not show significant differences ($p < 0.05$). Muscle antioxidant activity revealed increased superoxide dismutase (SOD) activity in the group fed the diet containing 250 mg of leaf decoction compared to other treatments ($p < 0.05$).

Principal component analysis showed that inclusion of 250 mg decoction in the diet (Figure 5A) promoted effects on muscle antioxidant activity (Figure 5B). Inclusion of 2000 mg showed a positive correlation with hepatic damage. The control group, T500 and T1000 were similar to each other.

Table 2: Development and hepatic and antioxidant activity of *Geophagus pyrocephalus* fed with decoction of *Campomonesia guazumifolia* leaves.

	Treatments (mg of guavira decoction.kg^{-1})					
	Control	**250**	**500**	**1000**	**2000**	***P***
Performance						
Weight gain (g)	0,352 ± 0,048	0,322 ± 0,083	0,310 ± 0,065	0,293 ± 0,050	0,280 ± 0,033	*0,210*
Length gain (mm)	3,057 ± 0,151	3,043 ± 0,162	3,129 ± 0,138	3,129 ± 0,150	3,029 ± 0,076	*0,511*

Condition factor	0,828 ± 0,056	0,762 ± 0,102	0,737 ± 0,084	0,719 ± 0,071	0,719 ± 0,031	*0,050*
Atividade hepática (UI.mg proteína^{-1})						
ALT	0,026 ± 0,013ab	0,025 ± 0,016b	0,025 ± 0,019b	0,032 ± 0,018ab	0,050 ± 0,009a	*0,021*
AST	0,034± 0,012	0,035 ± 0,008	0,035 ± 0,010	0,034 ± 0,009	0,043 ± 0,008	*0,363*
SOD	0,756 ± 0,399	1,241 ± 0,517	1,125 ± 0,696	0,830 ± 0,277	1,583 ± 0,943	*0,109*
CAT	1,814 ± 0,711	2,561 ± 1,075	2,058 ± 1,001	1,595 ± 0,834	1,332 ± 0,312	*0,089*
Atividade Antioxidante muscular (UI.mg proteín^{-1})						
SOD	1,093 ± 0,463b	2,713 ± 0,523a	1,200 ± 0,560b	1,047 ± 0,581b	1,298 ± 0,500b	*<0,001*
CAT	0,563 ± 0,253	0,592 ± 0,330	0,462 ± 0,257	0,327 ± 0,189	0,372 ± 0,094	*0,187*

Different letters on the same line indicate significant differences at the 5% level. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; SOD: Superoxide dismutase in the liver; CAT: Catalase.

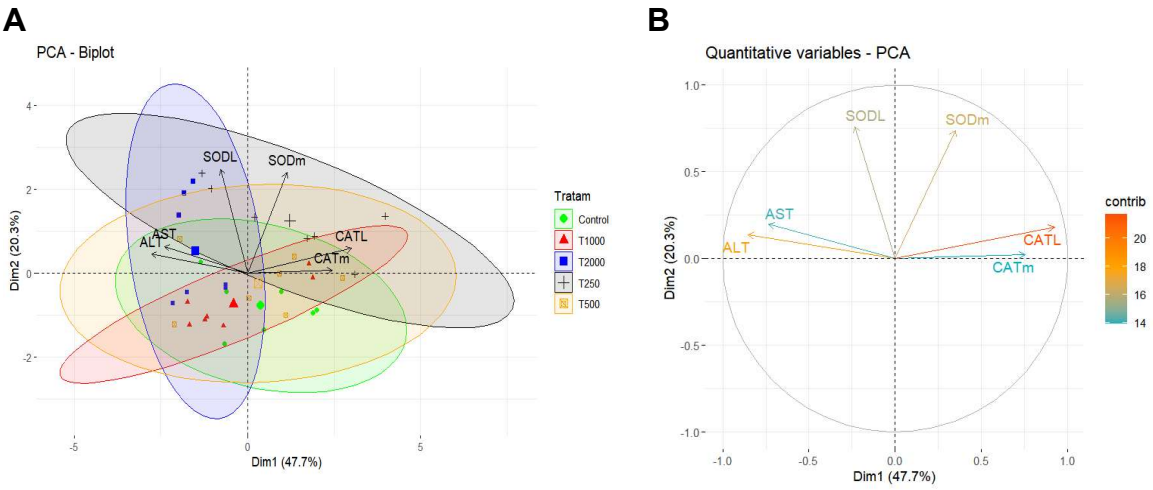


Figure 5. Visualization of the multifactorial analysis - PCA. (A) Biplots of Dimensions 1 and 2, where: the green dots represent the control group: diet without inclusion of *C. guazumifolia* leaf decoction; the black, orange, red and blue dots represent, respectively, the treatments with 250, 500, 1000 and 2000 mg.kg^{-1} of inclusion of *C. guazumifolia* leaf decoction (T250, T500, T1000 and T2000). The rings correspond to the 95% confidence ellipses, estimated using the treatment mean. The explained variance for each dimension is shown in parentheses. (B) Correlation of quantitative variables and their contributions within each dimension. Where; CATm: catalase in muscle; CATL: catalase in liver; SODm: superoxide dismutase in muscle;

SODL: superoxide dismutase in liver; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Treatments: treatments.
tratamentos.

Observing Figures 5A and 5B together, there are high contributions from CATL, ALT and SODm (reddish colors) (Figure 5B), with ALT and AST contributions increasing toward T2000, while SODm and CATL contributions increase toward T250. Corroborating this, Table 2 shows increased ALT in fish fed T2000 ($p < 0.05$). We also observed a marked increase in SODm ($p < 0.05$) in animals that consumed diet containing 250 mg leaf decoction, and a non-significant ($p > 0.05$) increase in CATL in this same experimental group.

DISCUSSION

Several studies report flavonoids in Campomanesia species (Schmeda-Hirschmann, 1995; Arruda, 2013; Ferreira et al., 2013; Michel et al., 2013; Catelan et al., 2018; de Castro et al., 2023). In the present study, five glycosylated flavonoids were identified in the decoction of *C. guazumifolia* leaves: quercetin pentoside, quercetin deoxyhexoside, myricetin deoxyhexoside, myricetin hexoside and myricetin deoxyhexoside-gallate.

Glycosylated flavonoids are one of the main classes of flavonoids and are frequently identified in plants conjugated with one or more sugar moieties (Simões et al., 2017), as reported for Campomanesia species (Arruda, 2013; Lescano et al., 2023).

Toxicity assessment is essential to ensure safety of products with therapeutic potential for consumption (Arruda, 2013; da Silva et al., 2016; Viscardi et al., 2017; Castro and Cardoso, 2023). Species of the *Campomanesia* genus have been studied for efficacy and toxicological profiles (*in vivo*), including *C. xanthocarpa* (da Silva et al., 2016) and *C. adamantium* (Viscardi et al., 2017).

The *C. guazumifolia* decoction proved highly toxic to both *A. salina* (LD50 = 23.29 $\mu g \cdot mL^{-1}$) and *G. pyrocephalus* juveniles (LD50 = 50.03 $\mu g \cdot mL^{-1}$). According to Sandoval et al. (2020), toxicity classification can be: non-toxic (LD50 > 1000 $\mu g \cdot mL^{-1}$), low toxicity (500 < LD50 $\leqslant$ 1000 $\mu g \cdot mL^{-1}$), moderate toxicity (100 < LD50 $\leqslant$ 500 $\mu g \cdot mL^{-1}$) and high toxicity (LD50 < 100 $\mu g \cdot mL^{-1}$).

Contrary to Arruda (2013) and de Castro et al. (2023), which reported absence or low toxicity of *C. guazumifolia* leaf infusions in Artemia (LD50 > 1000 $\mu g \cdot mL^{-1}$ and 917.34 $\mu g \cdot mL^{-1}$, respectively), Silva (2011) observed high toxicity for *A. salina* (LD50 = 71 $\mu g \cdot mL^{-1}$), although using different species and extract types (hexane and ethyl acetate extracts of *C. xanthocarpa* leaves).

Although *C. guazumifolia* decoction displayed toxicity at low concentrations (23.29 $\mu g \cdot mL^{-1}$ for *A. salina* and 50.03 $\mu g \cdot mL^{-1}$ for *G. pyrocephalus* juveniles), Guerrero et al. (2010) reported that hydroethanolic extract of C. pubescens at 250 and 500 $mg \cdot kg^{-1}$ was potentially free of renal and hepatic toxicity in adult male Wistar rats and suggested anti-inflammatory activity.

These results suggest that the method of preparing the extract (decoction) may be more aggressive for Artemia and fish. Other extraction methods could potentially be less toxic and possibly beneficial.

Gill tissue is directly exposed to the aquatic environment and reacts to changes in that medium. Due to its large contact area facilitating gas exchange, gills are more susceptible to exposure to pathogens and pollutants (Garcia-Santos et al., 2007; Cantanhêde et al., 2014). Gill lesions act as a defense mechanism by reducing surface area and hindering stressor action (Karlsson-Norrgren et al., 1985; Erkmen and Kolankaya, 2000; Cantanhêde et al., 2014). While protective, these responses can impair essential gas exchanges (McDonald and Wood, 1993; Cantanhêde et al., 2014).

Micronucleus alterations are associated with organism responses to aquatic pollutants (Dalzochio et al., 2016; Braga et al., 2021) and serve as indicators of clastogenic and aneugenic agents (Udroiu, 2006). Such alterations are used to assess genotoxic potential of agrochemicals in fish (Tamanho et al., 2022).

Notably, micronucleus changes and morphological gill effects do not always occur simultaneously (Dalzochio et al., 2016), suggesting that injury degree can vary with exposure to the toxicant.

Sensitivity of plant-derived products depends on composition, which is influenced by origin, management and extraction technique used, as well as extract type (Arruda, 2013; Carneiro et al., 2023; de Castro et al., 2023). The greatest benefits of bioactive use are associated with positive metabolic

effects (Honorato et al., 2021). For Nile tilapia, improvement in zootechnical indices was reported with inclusion of guavira fruit extract (Moraes et al., 2025). In ornamental aquaculture, the focus is on nutraceuticals that increase fish longevity (Reges et al., 2024).

Thus, bioactive compounds may aid hepatic function acting as hepatoprotectors (do Carmo Ota et al., 2019; Siqueira et al., 2024; Dorce et al., 2025). Increased AST and ALT activities indicate organ damage or stress (Honorato et al., 2017).

Use of plant extracts to promote fish health and metabolic adaptations has been reported in several species (França et al., 2022; Cruz et al., 2023; Siqueira et al., 2024). Elevation of oxidative enzymes has been observed in fish fed diets rich in phenolic compounds (Cruz et al., 2023; Siqueira et al., 2024). According to Viscardi et al. (2017), phenolic compounds in *C. adamantium*, particularly flavonoids, have radical-scavenging properties and inhibit lipid peroxidation. Diets were consumed normally and flavonoid stability in diets suggests these compounds are responsible for observed oxidative responses.

CONCLUSION

The study demonstrated that the evaluated extract has promising antioxidant potential, especially at lower doses. However, high doses presented significant toxic risk, with adverse effects on liver, histological alterations and negative impact on survival of aquatic organisms. These findings reinforce the importance of dosage and compositional standardization, indicating that safe use depends on adequate limitation of the administered quantity. Inclusion of 250 $mg \cdot kg^{-1}$ of *C. guazumifolia* leaf decoction shows potential for use in *G. pyrocephalus* diets.

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CAPÍTULO 4 - O PAPEL DA FENILALANINA NO PROCESSO DE PIGMENTAÇÃO DE PEIXE: UMA REVISÃO[3]

The role of phenylalanine in the fish pigmentation process: a review

Marcos Paiva Scardua1*, Shelby M. Walker2, Antonio Cesar Godoy1,3, Claucia A. Honorato1, Dacley H. Neu1†

[1]Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados, Rodovia Dourados - Itahum, km 12, Dourados, 79804-970, Mato Grosso do Sul, Brazil.

[2]School of Fisheries, Aquaculture and Aquatic Science, Auburn University, 203 Swingle Hall, Auburn, 36849, Alabama, USA

[3]Departamento de Pesquisa em Recursos Naturais, Instituto Federal de Educação do Paraná, 475 Civica Avenue, Assis Chateaubriand, 85935-000, Paraná,Brazil.

*marcos.scardua@ifce.edu.br

Abstract

In the global pet market, fish are the number one selling pets in the world. Considering a market that moves billions of dollars annually and where the body color of these animals is one of the main factors that attracts consumers to this niche, it is important to understand the pigmentation process and what can influence the metabolism of these animals, which directly affects their color. This understanding will allow for the appropriate manipulation of diets without compromising animal welfare, ultimately resulting in more attractive specimens for the market. Biological pigments, namely melanin, carotenoids, pteridine, and purines, are not only responsible for the color of fish, but also influence health, disease resistance, well-being, social interaction, growth, and reproduction. Providing these pigments in the diet is critical because some are not biosynthesized by fish. For example, pheomelanin and carotenoids are not synthesized by fish, whereas compounds such as pteridine, eumelanin, and purines are synthesized. During the synthesis phase, certain amino acids, especially phenylalanine, play a key role in the metabolic pathways. Thus, this work describes the role of phenylalanine in the metabolic pathways of these pigments, characterizes the types of pigments, highlights their importance for animals, and explains the mechanisms of action and control involved in the pigmentation process.

Keywords: Feed Supplementation; Pigments; Metabolites; Nutrition; Technological additives; Ornamental fish.

[3] Este capítulo corresponde ao artigo científico publicado na revista “International Aquatic Research, 16(2): 101-111, 2024”.

1 Introduction

Aquaculture aims to produce biomass to feed an ever-growing population (FAO 2021). However, ornamental aquaculture takes a different approach, focusing on individual traits that determine selling prices, with color being an important factor in this market (Luo et al. 2021; McLean 2021; Vissio et al. 2021; Lau et al. 2023).

Fish color is the result of a complex relationship between several biological pigments (melanin, pteridine, carotenoids, and purines) that are either ingested and metabolically converted or biosynthesized. The control mechanisms for fish skin pigmentation integrates internal factors such as: genetics, life cycle, pigment deposition ability, and body size, as well as external factors such as: feed, environmental conditions, and pigment sources (Sathyaruban et al. 2021; Vissio et al. 2021).

The color patterns and hues of a given fish species exhibit variability due to factors such as age, sex, health, stress, and reproductive stage (Vissio et al. 2021). Consequently, these colorations serve as a central visual mechanism for social communication. However, the maintenance of such coloration comes at a metabolic cost, as some of these pigments are derived from the diet, including those synthesized endogenously. This phenomenon underscores the importance of coloration as a crucial social communication mechanism, as its manifestation provides insight into the biological capabilities of each individual (Grempel and Visconti 2020).

This importance is mainly due to the multi-functionality of these pigments in fish. Beyond their role in coloration, these pigments exhibit antioxidant properties, contribute to anti-stress and anti-inflammatory responses, provide cellular photoprotection, enhance reproductive performance, influence egg and larval quality, act as provitamin A, play a role in the immune system, influence growth, and modulate lipid metabolism. Collectively, these diverse functions contribute to the overall well-being of the organism (Bell et al. 2019; Nakano and Wiegertjes 2020; Ciji and Akhtar 2021; Svitačová et al. 2023; Wassef et al. 2023). Consequently, the utilization of these pigments in the context of pigmentation becomes a resource-intensive endeavor due to the myriad metabolic pathways involved.

In the metabolic processes of these pigments, *de novo* synthesis of carotenoids is observed exclusively in plants, fungi and bacteria. The synthesis of other pigments, on the other hand, is typically found in different animal species, and certain amino acids play a crucial role in these processes. For example, in melanogenesis (the formation of melanin) and pheomelanin production, the involvement of phenylalanine, tyrosine, cysteine, and glutathione has been documented (Li et al. 2008; Bilandija et al. 2013). In the pteridine pathway, glutamine/glutamate, tryptophan, phenylalanine, and tyrosine are major contributors (Ziegler 2003; Leclercq et al. 2009). However, it is important to note that the synthesis of carotenoids, specifically via the mevalonate pathway, is not addressed in this work.

Although *de novo* synthesis of carotenoids does not occur in fish, there is compelling evidence indicating metabolic pathways through which fish can

transform assimilated carotenoids from the diet. This phenomenon was observed in goldfish, as described by Hata and Hata (1972), where astaxanthin was formed from zeaxanthin through 4-keto-zeaxanthin. Similar findings were reported in another cyprinid, *Pseudogobio esocinus*, as documented by Tsushima and Matsuno (1999). Further insights into carotenoid metabolism in fish were provided by Bijun et al. (2024), where the study reported on genes intricately involved in carotenoid metabolism in fish, focusing on key aspects such as the uptake and transport of carotenoids into tissues (SCARB and TTC39B genes), carotenoid degradation (BCO2 gene), conversion of yellow dietary carotenoids into red keto carotenoids (CYP2J19 gene), and the coding for proteins that bind to carotenoids (CRCN and GSTA2 genes). Additionally, the study presented evidence supporting a cooperative relationship between lipid metabolism and carotenoid transport and deposition, given the hydrophobic nature of carotenoids.

Regardless of whether the pigment is synthesized in the fish, aquaculture production systems typically have limited availability and access to natural diets compared to organisms developing in their natural habitat. Natural diets, which are rich in biological pigments (Sathyaruban et al. 2021), are a valuable source of these pigments. Therefore, access to pigments in aquaculture depends on supplementation of diets or live feed by producers. Of the four pigments (carotenoids, purines, melanin and pteridine) associated with fish pigmentation, three are endogenously synthesized by fish. Among these three, phenylalanine, an essential amino acid and a precursor of tyrosine, is involved in the metabolic pathways of two pigments (melanin and pteridine) (Ziegler 2003; Ito and Wakamatsu 2008; Leclercq et al. 2009; Grempel and Visconti 2020). This observation has sparked interest in exploring its potential inclusion in commercial diets for ornamental fish to assess its efficacy in enhancing the animal pigmentation process.

This review will elucidate the relationship between certain amino acids, particularly phenylalanine and tyrosine, and the metabolic pathways governing specific biological pigments responsible for fish coloration. The discussion will include the different types of biological pigments, the cells involved in pigment deposition in fish, the regulatory processes that influence pigmentation control, and the metabolic pathways that orchestrate the synthesis of biological pigments responsible for the observed coloration. Attention will also be given to the metabolic pathways associated with pigments in which phenylalanine plays a critical role.

2. Phenylalanine, pigments and regulation mechanisms

Phenylalanine, an aromatic amino acid, falls into the category of glucoketogenic amino acids, meaning it has the ability to produce both ketone bodies and glucose (Nelson et al. 2021). In addition, according to Li et al. (2008), it has the status of an essential amino acid for fish, requiring dietary intake. Phenylalanine plays a central role in several metabolic pathways, contributing to the synthesis of essential compounds, including cholesterol, steroids, carotenoids, and intermediates in the citric acid cycle (fumarate and acetoacetyl-CoA). It also contributes to the production of ketone bodies and facilitates the synthesis of tyrosine through the cofactor tetrahydrobiopterin

and the enzyme phenylalanine hydroxylase. The cooperation between phenylalanine and tyrosine extends to their involvement in the formation of several commercially important natural products, such as lignin, tannin, cinnamate, and morphine. In addition, this tandem plays a crucial role in the synthesis of essential neurotransmitters, including dopamine, epinephrine, and norepinephrine (Nelson et al. 2021).

The importance of phenylalanine in fish diets extends beyond its role in pigmentation to include vital physiological processes that actively contribute to fish growth (Khan and Abidi 2007; Rodrigues et al. 2019; Sayed and Ahmed 2023). Its importance is closely linked to various aspects of production performance, protein digestibility, immunity and overall survival. This is particularly evident because phenylalanine metabolism is closely intertwined with that of tyrosine, serving as a precursor for important hormones and neurotransmitters, including thyroxine, tri-iodo-thyronine, epinephrine, dopamine, and melanin, among others. Phenylalanine is absorbed by intestinal cells and subsequently converted to tyrosine primarily in the liver and kidney (Li et al. 2008). While phenylalanine can be converted to tyrosine, a balanced diet rich in both amino acids is critical for optimal fish development. This balance is essential due to their key roles in physiological functions, overall metabolism, and protein synthesis (Ahmed 2022). Sayed and Ahmed (2023) indicate that the best dietary inclusion ratio of phenylalanine and tyrosine (60:40) provides the best growth for catfish fry (*Heteropneustes fossilis*).

In the synthesis of biological pigments in fish, phenylalanine plays a critical role in the formation of melanin. This process involves the conversion of tyrosine to L-DOPA and its subsequent conversion to L-dopaquinone, which ultimately leads to the production of one of the melanin types (Figure 1). The synthesis of pteridine in fish is closely linked to melanogenesis, as highlighted by a metabolic pathway (Leclercq et al. 2009). This pathway involves the synthesis of tetrahydrobiopterin (4Hbiopterin), a cofactor essential for the conversion of phenylalanine to tyrosine. In addition, tetrahydrobiopterin is responsible for the synthesis of several forms of pteridines (Figure 2).

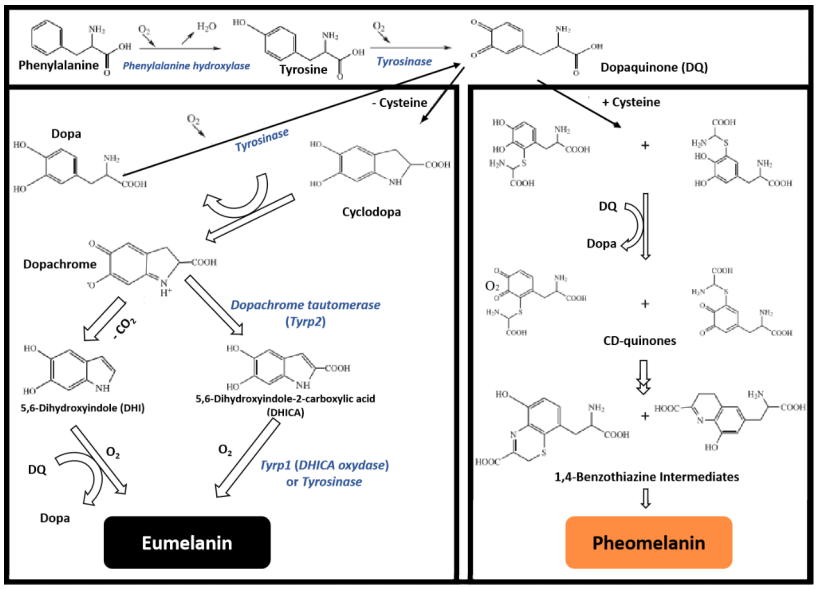

Figura 1 Melanogenesis, production of eumelanin and pheomelanin.

Source: Adapted from Ito and Wakamatsu (2008).

*Tyrp1= Tyrosinase 1 and Tyrp2 = Tyrosinase 2

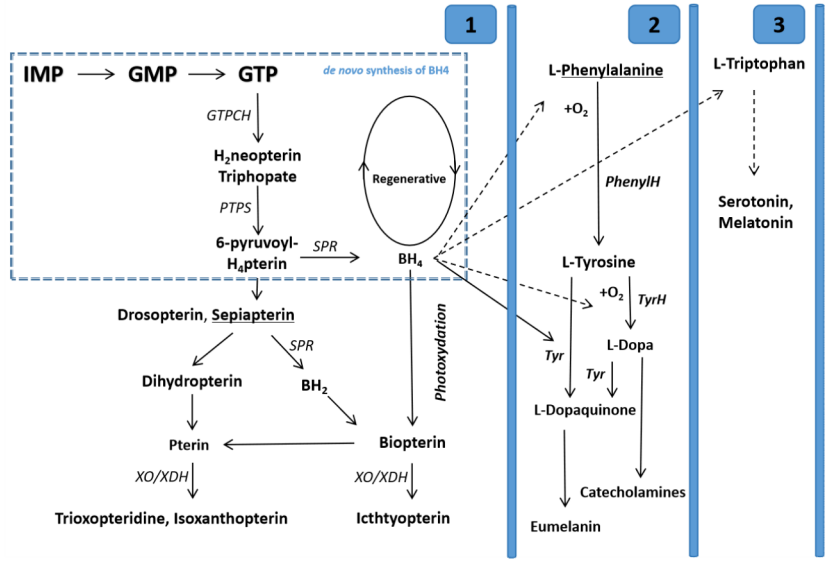

Figura 2. Synthesis of pteridine and relationships with melanogenesis and melatonin production. Adapted from Leclercq et al. (2009). IMP: Inosine monophosphate; GMP: guanosine monophosphate; GTP: guanosine triphosphate; PTPS: 6-pyruvyl tetrahydropterin synthetase; SPR: Sepiapterin reductase; BH2: Dihydrobiopterin; XO: Xanthine oxidase; XDH: Xanthine dehydrogenase; PhenylH: Phenylalanine hydroxylase; TyrH: Tyrosine hydroxylase; Tyr: Tyrosinase; TyrpH: Tryptophan hydroxylase; NAS: N acetylserotonin.

In nature, several groups of biological pigments contribute to the vibrant colors observed in fish: melanin, pteridine, carotenoids, and purines. While fish can synthesize melanin, pteridine, and purines, *de novo* synthesis of carotenoids does not occur (Johnson and Fuller 2014; Grempel and Visconti 2020; Luo et al. 2021; Yang et al. 2021; Vissio et al. 2021). Instead, some fish species exhibit the conversion of certain carotenoids into others, although these are initially ingested through their diet (Sathyaruban et al. 2021). As a result, carotenoids are naturally incorporated into fish through dietary intake in the wild or in controlled environments such as aquariums or hatcheries where they are provided in the diet.

According to Sefc et al. (2014), pigmentation in fish is a metabolically demanding and costly process that relies heavily on both biosynthesis and dietary absorption of pigments. This process serves as a reflection of an individual's ability to adapt to environmental conditions, thereby signaling the health status of each animal (Grempel and Visconti 2020). Once pigments are ingested through the fish's diet (Figure 3), they are emulsified by digestive enzymes and solubilized into micelles. These micelles are then taken up by

enterocytes in the intestine. Chylomicrons, formed from the absorbed pigments, are then transported from the intestinal mucosa to the bloodstream via lipoproteins and subsequently taken up by lymphatic vessels (Murota 2020). These chylomicrons are distributed to various tissues, including liver, muscle, skin, scales (Granneman et al. 2017; von Lintig et al. 2020; Fang et al. 2022; Yao et al. 2023), eyes (Salis et al. 2019; Svitačová et al. 2023). During the reproductive period, carotenoids may also be targeted to the gonads, ovaries and testes (Mookkan et al. 2022).

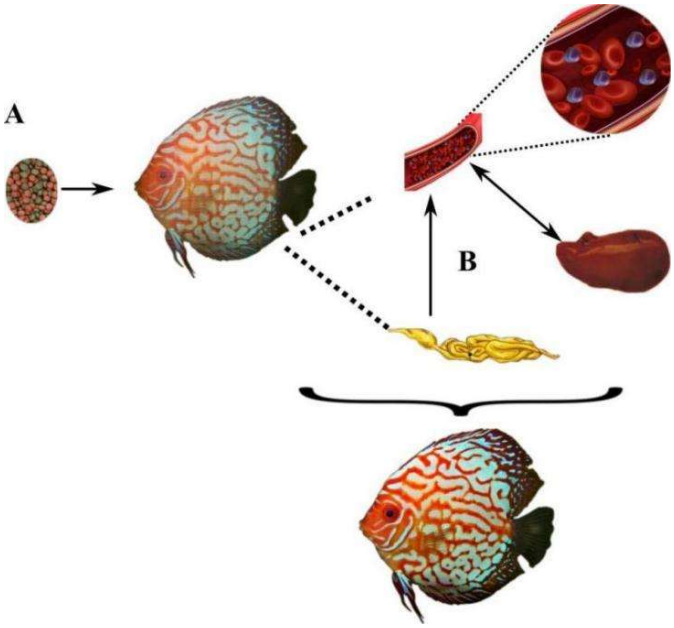

Figure 3 Scheme of absorption and destination of pigments in fish. A: Ingestion of feeds containing pigments. B: In the intestine, absorption occurs in enterocytes. Carotenoids are transported by lipoproteins through the bloodstream to lymphatic vessels where they are destined for the liver, muscles, gonads, eyes and skin.

Understanding the absorption and fate of pigments in animals is critical to understanding their presence, forms, behavior, and the colors they impart. Melanin, pteridine, carotenoids, and purines are the primary pigments found in animals. Melanin manifests in two forms: eumelanin, responsible for black and brown tones, and pheomelanin, responsible for reddish and yellowish tones (although the latter is not typically found in fish) (Irion and Nusslein Volhard 2019). Nevertheless, Zhu et al. (2016) suggest the possible occurrence of pheomelanin synthesis in red tilapia, which warrants further investigation. Extending the findings of Colanesi et al. (2012); Grempel and Visconti (2020); Sathyaruban et al. (2021); Vissio et al. (2021), it is noted that pteridines play a role in imparting reddish and yellowish colors, while carotenoids contribute to a diverse spectrum of colors. Also confirmed by Leclercq et al. (2009); Grempel and Visconti (2020), purines, when present in the form of transparent crystals, possess the ability to reflect incident light, thereby yielding a spectrum of colors including blue, silver, gold, or even white tones.

In fish, biological pigments are housed in specialized cells known as chromatophores, which are located primarily in the dermis and epidermis.

These specialized cells are generated during embryonic development by the differentiation of neural crest cells (Parichy 2006; Kottler et al. 2013; Lau et al. 2023). The cytoplasm of chromatophores contains organelles called chromatosomes, which are responsible for the accumulation of these pigments and serve as the site of their biosynthesis (Grempel and Visconti 2020). Notably, certain cells, such as erytrophores and xanthophores, have the capacity to store multiple types of pigments, as exemplified by their ability to contain both pteridine and carotenoid pigments (Ligon and McCartney 2016; Luo et al. 2021).

In contrast to birds and mammals, which have only one type of chromatophore, fish have six different types, according to Sathyaruban et al. (2021); Wu et al. (2022). These include melanophores (black and brown), erythrophores (red), xanthophores (yellow), cyanophores (blue), leucophores (white and silver), and iridophores (iridescent). Melanophores, erythrophores, xanthophores, and cyanophores are characterized by their ability to absorb light, while iridophores and leucophores reflect light. Chromatophores that reflect light contain purines (Ligon and McCartney 2016; Luo et al. 2021) and uric acid (Luo et al. 2021). Notably, leucophores are less abundant and have only been described in a few fish species (Kimura et al. 2014).

As elucidated by Yang et al. (2021), the development of fish coloration involves two main types: pigmentary coloration and structural coloration. Pigmentary coloration results from the absorption of light by intracellular pigments such as melanophores, erythrocytes, and xanthophores. On the other hand, structural coloration is formed by the reflection of light by purine crystals present in the skin or scales of fish, particularly within iridophores. Chromatophores play a key role in the dynamic display of color, responding to stimuli by either aggregating or dispersing the pigment granules within them, thereby altering the externalized color pattern. Dispersed pigments contribute to a darker skin appearance, whereas aggregated pigments create a lighter pattern (Ligon and McCartney 2016; Grempel and Visconti 2020). As classified by Grempel and Visconti (2020), the direct response of chromatophores to ambient light, independent of the nervous or hormonal systems, is considered the primary physiological response to color change. This response involves the aggregation or dispersion of pigments within fish chromatophores. In contrast, a secondary response occurs when the endocrine or nervous system is involved in the regulation of color change.

According to Bertolesi et al. (2019); Grempel and Visconti (2020); Sathyaruban et al. (2021); Vissio et al. (2021), factors that influence or are responsible for changes in fish color can be categorized as external (biotic and abiotic) or internal (genetics, life cycle, cell type, and physiological). External factors, such as the response to incident light, can rapidly induce color changes, potentially acting as a defense mechanism against predators (Iwashita et al. 2006; Grempel and Visconti 2020). Internally, genetic factors, cell type, and the nervous or endocrine system (Iwashita et al. 2006; Bertolesi et al. 2019; Grempel and Visconti 2020; Vissio et al. 2021), including hormones such as pigment concentrating hormone (MCH) and pigment dispersing hormone (MSH) (Bertolesi et al. 2019), play a role in mediating these color changes.

Fish coloration plays a crucial role in camouflage, and its effectiveness is optimized when it is matched to a specific background color. This mechanism has been conserved throughout fish evolution, with the genes responsible for pigment aggregation or dispersion and pigment synthesis remaining intact. Consequently, when fish are exposed to a light background, the pigments within the melanophores aggregate, resulting in a lighter color that enhances camouflage with the environment (PMCH and PMCHL genes). Conversely, when faced with a dark background, pigments disperse within melanophores, resulting in a darker color that reduces contrast with the environment and facilitates better camouflage (POMC gene) (Bertolesi et al. 2019). McLean (2021) also notes variation in fish color response to changes in tank bottom color, and Padhi et al. (2022) found that the black coloured tank improved body pigmentation, survival and growth of *Dawkinsia filamentosa*. Song et al. (2023) suggest that environmental adaptation of the eyes of the fish (*Plectropomus leopardus*) may be responsible for variation in skin color through several pathways, including stress protein synthesis, phenylalanine metabolism, and energy homeostasis.

Sefc et al. (2014) observed different responses depending on body region, chromatophore type, fish species, and even between populations of the same species. This highlights the challenge of elucidating the specific regulatory mechanisms at play in each fish species. Fish coloration is of great importance as a fundamental component of social and visual communication, providing insights into individual health, reproductive potential, dominance (Johnson and Fuller 2014; Sefc et al. 2014; Vissio et al. 2021), and welfare (Svitačová et al. 2024). Understanding the regulatory mechanisms of pigmentation not only contributes to the welfare of captive animals, but also helps to promote and maintain vibrant coloration.

2.1 Melanine

Melanin pigmentation serves a dual purpose in fish, providing protection against predation through the classic light ventral and dark dorsal coloration pattern (Irion and Nusslein Volhard 2019). In addition, melanin acts as a photoprotector, providing defense against harmful UV radiation (Mueller and Neuhauss, 2014; Lau et al. 2023). The synthesis of melanin, known as melanogenesis, involves the formation of pheomelanin and eumelanin (Figure 1). This complex process takes place within melanosomes, specialized organelles embedded in melanophores located in the dermis and epidermis of fish. It is generally believed that only eumelanin is present in fish (Leclercq et al. 2009; Kottler et al. 2013; Irion and Nusslein Volhard 2019).

Phenylalanine and tyrosine play fundamental roles in the melanogenic pathway. Within the cytosol of the melanophore, L-phenylalanine is converted to L-tyrosine by the enzyme phenylalanine hydroxylase, which requires oxygen as a cofactor, specifically tetrahydrobioperidine. L-tyrosine is then activated by tyrosinase and converted to dihydroxyphenyl-L-alanine (L-DOPA) within the melanosome membrane. From this point, there are two possible pathways: conjugation of dopaquinone with cysteine or glutathione, leading to the formation of pheomelanin through the action of cestenil-DOPA or glutathione-DOPA. Alternatively, it can be converted to dopachrome, which

serves as a substrate for tyrosinase 2 (Tyrp2). Tyrp2 catalyzes the formation of 5,6-dihydroxyindole-2-carboxylic acid, which is subsequently oxidized by tyrosinase 1 (Tyrp1) to eumelanin (Ito and Wakamatsu 2008; Grempel and Visconti, 2020).

Meredith and Sarna (2006) emphasize the lack of precision in determining the macromolecular structure of eumelanin, with only a general acceptance that eumelanin consists of macromolecules of 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA). The structure is considered amorphous and eumelanins are insoluble in solvents (Xiao et al. 2020). Despite recent studies, uncertainties remain regarding the exact composition of eumelanin (Cao et al. 2021). However, due to the diverse functions of these melanin compounds, including photoprotection, antioxidant, antimicrobial, radioactive absorption, anticancer properties, heavy metal absorption, thermal regulation, healing, anti-inflammatory effects, and coloration, they are biodegradable and highly valued for potential applications in medicine and the food industry. The synthesis of synthetic melanin compounds is of great interest and importance (Meredith and Sarna 2006; Xiao et al. 2020; Cao et al. 2021; Ghattavi et al. 2022).

Albinism is a genetic disorder associated with the absence of melanin pigmentation in animals. Fontes et al. (2023) found abnormal pigmentation in marine fish in the natural environment and speculate that these mutations may be associated with population decline and isolation of remote populations, but in certain environments, such as caves, where pigmentation provides no advantage and represents an unnecessary energy expenditure, some fish have evolved with partial or total loss of pigmentation, resulting in true albinos (Bilandija et al. 2013). In their study of albino fish of the species *Astyanax mexicanus*, Bilandija et al. (2013) focused on the mutation of the OCA2 gene, which is responsible for oculocutaneous albinism, specifically type 2 albinism. The researchers observed that the mutation in this gene affects the initial phase of melanin synthesis by blocking the conversion of L-tyrosine to L-DOPA. Interestingly, even with the addition of L-tyrosine, melanin synthesis does not occur; however, the addition of L-DOPA leads to successful synthesis.

The cited study by Bilandija et al. (2013) emphasized that the unused L-tyrosine serves an alternative purpose by connecting to the catecholamine pathway, which is responsible for the production of neurotransmitters such as dopamine, epinephrine, and norepinephrine. This underscores the metabolic pathway taken by L-tyrosine, which produces products that may be more useful to albino fish in their environment than the production of pigments. Although rare, the albino phenotype is highly sought after in the ornamental fish industry, primarily due to the high prices that these individuals can command (Wang et al. 2022). The generation of albino mutants can be achieved by inhibiting the central genes involved in melanogenesis, but there are few studies exploring this aspect, especially given the vast diversity of species available in the ornamental market (Bian et al. 2021; Zhang et al. 2023).

2.2 Purins

Purines are nitrogenous organic compounds that form nucleotides, of which guanine and adenine are the best known. Initially, purines are biosynthesized as nucleotides, starting from inosine monophosphate (IMP) to guanosine monophosphate (GMP) and then to guanosine triphosphate (GTP). However, the free bases adenine, guanine, xanthine, and hypoxanthine are by-products of purine nucleotide catabolism, primarily for the excretion of nitrogenous waste (Leclercq et al. 2009). The major reflective components present in teleost cells (leucophores and iridophores) are primarily compounds generated during purine metabolism, including guanine, adenine, xanthine, and hypoxanthine. Within this metabolic process, xanthine oxidase and xanthine dehydrogenase play a key role in limiting the rate of purine catabolism by catalyzing the oxidation of hypoxanthine to xanthine and xanthine to uric acid (Leclercq et al. 2009).

Similar to other pigments localized in specific chromatophores, purines accumulate in iridophores and leucophores, which are located in the skin and scales of teleosts (Yang et al. 2021). In addition, uric acid, a product of purine metabolism, is also present in these cells (Luo et al. 2021). Purines accumulate as transparent crystals that reflect incident light and produce blue, silver, gold, or white iridescence (Grempel and Visconti 2020). Notably, there is an intriguing interplay between purine, pteridine, and eumelanin through metabolic pathways. GTP serves as a common substrate in purine synthesis and is converted by guanosine cyclohydrolase (GTPCH), which through subsequent reactions leads to the formation of a pteridine, 4Hbiopteridine (BH4). BH4 is essential for the formation of various forms of pteridines and is also somewhat related to the formation of eumelanin (Figure 2).

2.3 Pteridine

As described by Leclercq et al. (2009), pteridines are a group of heterocyclic compounds with pyrimidine and pyrazine rings, classified as pterins or flavins. Many natural pteridines are readily oxidized, sensitive to light, and poorly soluble in water. Within this group, certain pteridines are colorless but fluoresce under UV light, including biopterin and xanthopterin, while others have distinct colors, such as erythropterin and drosopterin (red), leucopterin and isoxanthopterin (white), and sepiapterin and xanthopterin (yellow). Pteridine biosynthesis results in the production of yellow to reddish pigments within pterinosomes located in xanthophores and erytrophores (Braasch et al. 2008).

Pteridines have multiple functions, including immune response, antioxidant activities, neurotransmitter synthesis, and pigmentation. In particular, 7-hydroxybiopterin, extracted from the skin of the cyprinid *Devario malabaricus*, has been identified as an alarm pheromone. It is released after skin lesions and induces a fear response in conspecifics (Leclercq et al. 2009). In addition, 7-hydroxybiopterin is associated with melanogenesis through its role as a cofactor in the conversion of phenylalanine to tyrosine. This process occurs in the melanogenesis pathway, leading to the formation of dopaquinone and ultimately resulting in eumelanin synthesis.

In the study by Ziegler (2003), the regulation and biosynthesis of pteridines

during embryonic development of Danio rerio was meticulously described. As elucidated by Leclercq et al. (2009), this intricate pathway begins with the action of guanosine triphosphate hydrolase (GTP-CH) on GTP, resulting in the formation of H2neopterin triphosphate. This initiates a metabolic sequence leading to the synthesis of drosopterin, sepiapterin, and 4Hbiopteridin. Subsequently, sepiapterin and 4Hbiopteridin play key roles in the generation of a variety of pteridines, as shown in Figure 2.

2.4 Carotenoids

In aquaculture, carotenoids, in particular astaxanthin, stand out as essential pigments in commercial fish feeds. In Atlantic salmon farming, dietary astaxanthin supplementation can represent a significant portion of production costs, underscoring its importance in achieving desirable pigmentation levels (Sanches 2021). While animals do not synthesize carotenoids, certain fish species exhibit transformations of these pigments that show preferences in absorption efficiency. For example, cyprinids convert zeaxanthin to astaxanthin, while trout convert astaxanthin to zeaxanthin. Interestingly, some species such as salmon and *Pagrus major* do not convert carotenoids, but their absorption efficiency varies; salmonids absorb canthaxanthin and astaxanthin more efficiently than lutein and zeaxanthin, while channel catfish show the opposite pattern, absorbing lutein and zeaxanthin more efficiently than astaxanthin (Sathyaruban et al. 2021).

Although carotenoid synthesis is primarily dependent on plants, fungi, and bacteria, recent research, such as that by Du et al. (2021), has shed light on the genetic regulation of carotenoid transport in chromatophores of common carp (*Cyprinus carpio*). More than 40 genes have been identified as critical for fish pigmentation (Fadeev et al. 2016; Irion and Nusslein Volhard 2019; Salis et al. 2019; Du et al. 2021; Mohammed et al. 2021; Fang et al. 2022; Tang et al. 2022; Poon et al. 2023; Bijun et al. 2024), indicating the complexity of the underlying mechanisms. These findings not only contribute to our understanding of fish pigmentation, but also open avenues for potential selective breeding programs.

Ornamental fish have a high commercial value and their color directly implies this aspect (Luo et al. 2021; Vissio et al. 2021; Lau et al. 2023), as the buyer buys them for their beauty. Based on the data collected in this review, in addition to the direct practical application of promoting sales, it is possible that breeders can incorporate pigments in feed, through industrial amino acids or natural products, in appropriate doses that favor the triggering of the metabolic pathway, providing the action of pigments that promote the shine, color and health of the fish. This will contribute to the development of the feed production sector, which will be able to offer differentiated and higher quality products to the market.

Skin pigmentation is related to aspects of behavior and inter- and intraspecific social interaction of fish (Eaton et al 2016; Svitačová et al. 2024), therefore the production and maintenance of coloration can facilitate the conduct of studies that generate more appropriate management protocols for the breeding and maintenance of these animals. Furthermore, the production of more attractive

specimens in captivity helps to reduce extractivism in the natural environment when it is carried out inappropriately, thus reducing the environmental impact of this practice on both the target species and the surrounding ecosystem, thus contributing to the conservation of natural stocks, biodiversity and sustainability of the activity.

The ornamental fish market, driven by dynamic consumer and producer demands, is constantly seeking advances in pigmentation mechanisms to produce new and improved varieties. This compilation aims to provide insight into the regulatory aspects of fish pigmentation, with a particular focus on the role of phenylalanine.

3. Conclusions

Numerous amino acids play critical roles in the metabolic pathways governing fish pigmentation, with phenylalanine and tyrosine contributing prominently to coloration through the synthesis of pigments such as melanin and pteridine. While carotenoid pigments dominate commercial fish feed formulations, it is noteworthy that melanin, pteridines and purines are also of paramount importance in both inter- and intraspecific social interactions, as well as in providing protection against predation and ensuring overall animal health. Therefore, supplementing the diet not only with pigments but also with amino acids that facilitate the maintenance or synthesis of these pigments is emerging as a strategic approach in ornamental fish production.

4. Acknowledgments

4.1 Ethical Approval

This review was not an experimental trial. Only literature online was consulted.

4.2 Competing interests

The authors declare that there is no conflict of interest.

4.3 Authors' contributions

Antonio Cesar Godoy - Conceptualization; Methodology; Writing; General review

Claucia A. Honorato - Conceptualization; Methodology; General review

Dacley H. Neu - Conceptualization; Methodology; Writing; General review

Marcos Paiva Scardua - Writing; General review

Shelby Maura Walker - Writing; General review

4.4 Availability of data and materials

This review was only literature consulted.

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CAPÍTULO 5 - SUPLEMENTAÇÃO DE FENILALANINA PARA MELHORIA DA QUALIDADE CROMÁTICA DE PLATY: UMA ABORDAGEM APLICADA

Resumo

Esta é a primeira documentação sobre a utilização de fenilalanina no aprimoramento da coloração de peixes. Para verificar o incremento da coloração em platys (*Xiphophorus maculatus*) ao longo de um período de 21 dias, utilizamos a inclusão de 0,81%, 0,99%, 1,17%, 1,36% e 1,54% de fenilalanina em uma dieta isenta de carotenoides. A avaliação abrangeu diversos parâmetros, incluindo a coloração corporal e caudal (pedúnculo caudal), a atividade antioxidante das enzimas catalase (CAT) e superóxido dismutase (SOD) e desempenho. Um delineamento inteiramente casualizado foi conduzido, utilizando 150 espécimes de platy, dispostos em 5 tratamentos e 3 repetições. Os espécimes foram distribuídos em 15 tanques de 60 L, conectados a um sistema de recirculação de água com filtração acoplada. Observamos um aumento no peso e no comprimento padrão ($p < 0,05$), bem como baixas taxas de sobrevivência (49,9% até 66,7%), porém sem diferença estatística. Um aumento substancial na coloração corporal (a*, b*, ΔE) e caudal (a*) foi demonstrado, conforme corroborado por correlações moderadas (r entre 0,5 e 0,75). Além disso, observou-se uma correlação entre SOD e ΔE corporal, sugerindo uma relação entre atividade antioxidante, SOD e a pteridina. A atividade enzimática avaliada permaneceu constante. Indicamos o uso de 0,93% de fenilalanina para intensificar a coloração corporal. Porém, estudos adicionais são necessários para investigar se a suplementação nesses níveis pode afetar a saúde e o bem-estar dos peixes a longo prazo.

Palavras-chave: aditivos, fenilalanina, melanina, pigmentação, pteridina

Abstract

This report marks the inaugural documentation of the utilization of phenylalanine in the enhancement of fish coloration. To verify the coloration increase in platy (Xiphophorus maculatus) over a period of 21 days, we used 0.81%, 0.99%, 1.17%, 1.36%, and 1.54% inclusion of phenylalanine in a carotenoid-free diet. The evaluation encompassed various parameters, including body and caudal (caudal peduncle) coloration, the antioxidant activity of catalase (CAT) and superoxide dismutase (SOD) enzymes, and performance. A completely randomized experiment was conducted, using 150 platy specimens, 5 treatments, and 3 replicates. The specimens were distributed in 15 60L tanks, which were connected to a water recirculation system with coupled filtration. We observed an increase in weight and standard length ($p < 0.05$), as well as low survival rates (49.9% to 66.7%), but without statistical difference. A substantial increase in body (a*, b*, ΔE) and caudal (a*) coloration is hereby demonstrated, as substantiated by moderate correlations (r between 0.5 and 0.75). Furthermore, a correlation was observed between SOD and body ΔE, suggesting a relationship between antioxidant activity, SOD, and pteridine. The evaluated enzymatic activity remained constant. We indicate the use of 0.93% phenylalanine for body color enhancement. However, further studies are needed to investigate whether supplementation at these levels may affect the long-term health and well-being of fish.

Keywords: additives, melanin, phenylalanine, pigmentation, pteridine.

1 Introdução

A coloração da pele é um dos principais fatores que determina o valor comercial dos peixes ornamentais (Luo et al., 2021; Vissio et al., 2021; Abe et al., 2022; Lau et al., 2023), sendo fortemente influenciada pela composição da dieta, que afeta tanto a pigmentação quanto a saúde geral dos animais (Ciji & Akhtar, 2021; Lau et al., 2023). Essa coloração é produzida por cromatóforos, células especializadas em armazenar ou sintetizar pigmentos (Grempel & Visconti, 2020; Yuan et al., 2023).

Nos peixes, os cromatóforos presentes na derme e epiderme são responsáveis por acumular e sintetizar pigmentos. Nos melanóforos, encontra-se a melanina, que absorve luz e determina colorações escuras (Luo et al., 2021). Já os xantóforos e eritróforos produzem pteridinas, associadas às tonalidades amarela e laranja, e laranja a vermelho, respectivamente, podendo tanto absorver quanto refletir a luz (Zhang et al., 2017; Luo et al., 2021; Tian et al., 2022; Shi et al., 2023).

A eumelanina e as pteridinas são pigmentos sintetizados pelos próprios peixes, por meio de vias metabólicas que dependem diretamente de aminoácidos específicos envolvidos na síntese e no transporte desses compostos (Bilandžija et al., 2013; Yang et al., 2021; Bijun et al., 2024; Scardua et al., 2024). Entre esses aminoácidos destaca-se a tirosina e, de forma essencial, a fenilalanina, precursora direta da via de síntese da tirosina. A disponibilidade adequada de fenilalanina na dieta é, portanto, crucial para sustentar a produção de melanina e pteridinas, influenciando positivamente a intensidade e estabilidade da coloração dos peixes ornamentais, visto que as duas vias metabólicas estão conectadas (Ziegler, 2003; Leclercq et al., 2009; Scardua et al., 2024).

A fenilalanina é um aminoácido essencial para os peixes (Jobling, 2011), sendo precursora da tirosina, que por sua vez é precursora de uma série de hormônios e neurotransmissores (tiroxina, triiodotironina, adrenalina, noradrenalina, dopamina e melanina) relacionados com a manutenção da atividade cerebral, funções cognitivas, pigmentação e crescimento dos peixes

(Rodrigues et al., 2019).

A suplementação de aminoácidos para peixes ornamentais como o acará disco (*Symphysodon aequifasciatus*) é uma estratégia eficiente para o manejo nutricional (Santos et al., 2021). A suplementação de fenilalanina pode reduzir marcadores de estresse melhorando o bem estar em situações de produção intensiva (Salamanca et al., 2025). Em peixes este aminoácido está relacionado a diminuição de deformidade óssea em larvas, no entanto, não existem estudos sobre a suplementação de fenilalanina influenciando respostas fisiológicas de coloração e bem estar em peixes ornamentais.

O platy (*Xiphophorus maculatus*) destaca-se como um modelo promissor para estudos de coloração, pois possui dois tipos de pigmentos caracterizados, melanina (Basolo, 2006) e pteridinas (Henze et al., 1977; Rempeters et al., 1981; Basolo, 2006).

Desta forma o objetivo deste trabalho foi avaliar a suplementação de fenilalanina em dietas do platy (*Xiphophorus maculatus*) e seu papel na coloração e bem estar.

2 Material e métodos

O ensaio *in vivo* foi realizado no Laboratório de Produção Aquícola da Universidade Federal da Grande Dourados (UFGD). Foram utilizados 150 platy (*Xiphophorus maculatus*) com peso corporal médio 0,63 ± 0,19 g e comprimento total de 32,07 ± 3,33 mm, Comprimento padrão (mm) 27,46 ± 2,69; coloração corporal (*L** = 47,79 ± 3,51; *a** = 8,09 ± 3,11; b* = 31,15 ± 4,91), coloração da cauda (*L** = 43,31 ± 5,09; *a** = 3,69 ± 2,51; b* = 12,91 ± 5,57).

Os peixes foram divididos em 15 unidades experimentais (60 L de água) contendo dez peixes, totalizando 30 peixes por tratamento, em sistema de recirculação individual, com fotoperíodo de 12 horas de luz. A qualidade da água foi monitorada durante o ensaio, aferida uma vez por semana (26,7°C, 7,7 $mg.L^{-1}$ de oxigênio dissolvido, 7,5 de pH e 0,08 $mg.L^{-1}$ de NH_3.), estando de acordo com os parâmetros de qualidade para produção de peixes neotropicais (Godoy et al., 2021). E duas vezes durante a semana, era

realizada a limpeza das unidades experimentais, sifonando o excesso da matéria orgânica presente no fundo.

Os peixes foram alimentados por um período de 21 dias *ad libtum* três vezes ao dia. Para cada unidade experimental foi separado um recipiente, mantido fechado e contendo a ração referente a cada tratamento, a qual foi pesada no início e no final do experimento, permitindo calcular o consumo para cada unidade experimental. O ensaio foi aprovado pelo Comitê de Ética no Uso de Animais da Universidade Federal da Grande Dourados (Protocolo 03/2019).

2.1 Dieta experimental

Dietas isoproteicas (287 $g \cdot kg^{-1}$) de Proteína bruta e isoenergéticas (3135,6 $kcal.kg^{-1}$) com cinco níveis de fenilalanina na dieta dos peixes (0,81 - 0,99 - 1,17 - 1,36 -1,54%,), foram testadas (Tabela 1). As dietas foram umedecidas e micro peletizadas e confeccionadas com granulometria de 500µm. As dietas foram secas em estufa a 65 °C e armazenadas em sacos escuros sob refrigeração.

Tabela 1: Composição das dietas experimentais.

Ingredientes (g)	Fenilalanina (%)				
	0,81	0,99	1,17	1,36	1,54
Farelo de trigo	55,00	55,00	55,00	55,00	55,00
Farinha de carne e ossos (45%)	22,42	22,42	22,42	22,42	22,42
Óleo de soja	7,50	7,38	7,25	7,13	7,00
Ác glutâmico	5,00	5,06	5,12	5,19	5,25
L-alanina	5,00	4,87	4,75	4,63	4,50
Farinha de peixe (55%)	2,00	2,00	2,00	2,00	2,00
L-fenilalanina	0,00	0,19	0,38	0,56	0,75
L-lisina	0,75	0,75	0,75	0,75	0,75
L-treonina	0,55	0,55	0,55	0,55	0,55
DL-metionina	0,24	0,24	0,24	0,24	0,24
L-triptofano	0,12	0,12	0,12	0,12	0,12
Antifúngico	0,10	0,10	0,10	0,10	0,10
BHT	0,02	0,02	0,02	0,02	0,02
Sal	0,30	0,30	0,30	0,30	0,30
Premix	1,00	1,00	1,00	1,00	1,00

Composição analisada (mg kg^{-1})					
Matéria seca (%)	90,71±0,22b	93,01± 0,40a	90,45 ± 0,07b	89,34±0,30c	92,54±0,09a
Cinzas	12,54±0,40ab	12,42±0,11ab	12,668±0,04a	12,00±0,21b	12,62±0,13a
Lipid	10,49± 0,11c	11,36±0,10a	10,99±0,09ab	10,13±0,22c	10,93±0,21b
Prot	28,65±0,57	28,71±0,67	28,05 ± 0,22	28,55±0,52	28,74±0,66
Composição estimada (mg kg^{-1})					
Amido	17,24	17,24	17,24	17,24	17,24
Fenilalanina total	0,81	0,99	1,17	1,36	1,54
Fenilalanina + tirosina	1,16	1,34	1,52	1,71	1,89
Fósforo disponível)	1,40	1,40	1,40	1,40	1,40
Lisina total	1,62	1,62	1,62	1,62	1,62
Metionina + cistina	0,69	0,69	0,69	0,69	0,69
Energia Digestível (kcal kg^{-1})	3138,80	3137,20	3135,60	3134,00	3132,40

2.2 Desenvolvimento, bem estar e Coloração do peixes

No 21º dia os peixes foram eutanasiados com óleo de cravo a uma concentração de 50 mg L^{-1} durante 30 segundos (Honorato et al. 2017), sendo assim mensurados o peso e comprimento, e coloração individual para os cálculos de desempenho zootécnico: PF: peso final (g); S: sobrevivência (%); GP: ganho em peso (g); GC: ganho em comprimento (mm); K: fator de condição; CT: comprimento total (mm); CP: comprimento padrão (mm); C: consumo (g). Conforme as fórmulas abaixo:

Ganho em peso = peso final – peso inicial;

Taxa de crescimento específico:
(((*ln* do peso final – *ln* do peso inicial)/dias de experimento) × 100)

Consumo = peso da ração final - peso da ração inicial

Conversão alimentar aparente: CAA = consumo de ração/ganho em peso

Sobrevivência: S = (número de peixes final/número inicial de peixes) × 100

Fator de condição: $K= W/L^b$, onde W = peso total, L= comprimento e b = coeficiente angular da relação peso/comprimento.

No início e ao final do experimento, nove animais por tratamento foram

identificados, (três peixes por unidade experimental), para obtenção da coloração da nadadeira caudal (pedúnculo caudal) e corporal (Figura 1), com o auxílio de um fotocolorímetro portátil Chroma Meter CR-400 (Konica Minolta®), por meio do sistema de coordenadas de Hunter L, a, b. Sendo L*, brilho ou luminosidade variando entre 0 (preto) e, 100 (branco), a cromaticidade de a* variando entre -100 (verde) e +100 (vermelho), e a cromaticidade de b* variando entre -100 (azul) e +100 (amarelo), (Cruz et al. 2023), também foi obtido o valor de ganho de coloração (ΔE), referente a diferença de cor final e inicial para cada tratamento, utilizando o software LAB Color Chart v2 (Teixeira et al., 2024).

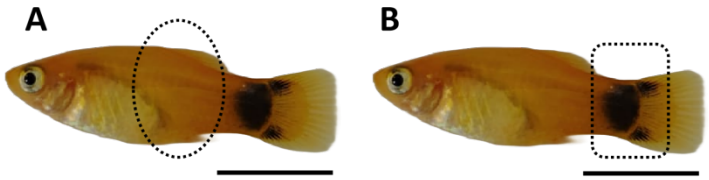

Figura 1. Locais de amostragem da coloração de platy (*X. maculatus*) demarcado com linha pontilhada. A: cor corporal, B: cor caudal. Barra de escala: 1 cm.

Após o procedimento de biometria, nove peixes por tratamento foram anestesiadas com eugenol (100 mg.L^{-1} de água) (Honorato e Nascimento, 2016), eutanasiados e congelados para a realização das análises e atividade antioxidante e de proteína total.

Para análise das enzimas metabólicas amostras de músculo (100 mg) foram homogeneizadas com tampão fosfato de sódio (glicerol v/v em tampão fosfato de sódio 20 mM e Tris 10 mM - pH 7,0) em homogenizador tipo Potter-Elvehjem. Posteriormente, esta amostra foi centrifugada a 4°C por três minutos a 600 x g e o sobrenadante submetido a uma nova centrifugação por oito minutos a 6000 x g.

O sobrenadante foi utilizado para os ensaios enzimáticos. A SOD foi avaliada pelo auto oxidação do pirogalol, que é inibida na presença de SOD (Beutler, 1984, modificado). As leituras de absorbância foram realizadas a 420 nm, considerando que 1,0 UI inibe 50 % da auto oxidação do pirogalol. A atividade da CAT foi avaliada pela leitura do decaimento do H_2O_2 a 230 nm (Beutler, 1984). Uma unidade de CAT foi definida como a quantidade de

enzima necessária em 1,0 µmol de oxidação de H_2O_2 min^{-1}, e a absortividade molar usada foi (H_2O_2) $\varepsilon\lambda 230 = 0{,}071$ $mM.cm^{-1}$. A proteína foi determinada de acordo com a metodologia de Kruger (2009), utilizando-se reagente de Bradford contra uma solução padrão de soro de albumina bovina, e procedendo com leitura a 595 nm.

2.3 Análise estatística

A normalidade dos dados e sua variância foram verificadas (Shapiro e Wilk 1965; Bartlett 1992), e quando atendidas, aplicamos a análise de variância (ANOVA) seguida pelo teste de separação de médias de Tukey (1949), com nível de 5% de significância. Realizamos a análise de regressão cúbica, onde foram apresentados os modelos ajustados ($P<0{,}05$). Utilizamos a Correlação de Pearson para analisar a relação entre as dietas e todos os dados, sendo a força de seu coeficiente, r, indicada conforme Gu et al., (2022): 0 a 0,25 sem correlação, 0,25 a 0,50 correlação fraca, 0,50 a 0,75 correlação moderada e de 0,75 a 1,00 correlação forte. Os dados de sobrevivência sofreram transformação do arco-seno para posterior análise (Zar, 1984). As análises foram realizadas utilizando o software RStudio, (R Core Team, 2025) com auxílio dos pacotes ExpDes.pt, dplyr, ggplot2 e corrplot.

3 Resultados

A suplementação dietética com diferentes níveis de fenilalanina proporcionou efeitos significativos sobre o comprimento padrão final e o ganho em peso. O fator de condição foi superior nos peixes alimentados com 1,17% de fenilalanina, indicando melhor estado corporal. Não foram observadas diferenças significativas para os demais parâmetros de desenvolvimento e a atividade antioxidante (Tabela 2).

O comprimento padrão apresentou efeito quadrático ($P = 0{,}0022$), com o melhor desempenho observado em peixes alimentados com 1,17% de

fenilalanina, que exibiram o maior valor médio (28,81 ± 0,23 mm). O ganho em peso respondeu de forma quadrática (P = 0,0069), sendo também maximizado no nível de 1,19% de fenilalanina.

A coloração corporal apresentou variação nos parâmetros a*, b* e ΔE, em função dos níveis dietéticos de fenilalanina (Tabela 3). Os melhores resultados de coloração corporal foram observados nos níveis dietéticos de 1,17% e 1,54% de fenilalanina, que apresentaram os maiores valores de a* e b*, indicando maior intensidade das tonalidades vermelha e amarela. O tratamento com 1,17% destacou-se também por apresentar o maior ΔE, representando a diferença total de cor mais perceptível e expressiva. A cromaticidade b* corporal apresentou tendência cúbica (P = 0,0079), sendo assim a tonalidade amarela maximizada no nível de 0,93% de fenilalanina.

Para a coloração caudal, o nível de 0,81% de fenilalanina proporcionou os valores mais elevados de a*, indicando maior intensidade da coloração avermelhada e também um baixo valor de luminosidade (L*) na cauda, sugerindo coloração mais escura. Os parâmetros L* e b* não apresentaram diferenças estatísticas, enquanto o ΔE variou entre os tratamentos, embora sem tendência clara associada à concentração de fenilalanina. O a* caudal também apresentou tendência cúbica, apresentando maior intensidade do vermelho para 0,81% de fenilalanina.

A análise do espaço cromático CIELAB revelou que a suplementação dietética com fenilalanina promoveu variações consistentes nos componentes cromáticos da coloração corporal e caudal dos platys. As elipses de agrupamento destacaram padrões distintos entre os tratamentos (Figura 2), evidenciando que níveis intermediários de fenilalanina tendem a promover maior intensificação da coloração, enquanto níveis extremos (menores ou maiores) resultaram em respostas mais heterogêneas ou menos saturadas. A distribuição espacial dos pontos reforça que a fenilalanina influenciou diretamente a intensidade e o padrão de coloração dos peixes, com efeito mais pronunciado na cauda do que no corpo.

Tabela 2 – Desenvolvimento e atividade antioxidante de platy suplementados com fenilalanina.

	Suplementação com fenilalanina (%)						
	0,81	**0,99**	**1,17**	**1,36**	**1,54**		
Desempenho						***P***	**Efeito**
Peso final (mg)	0,61 ± 0,11	0,73 ± 0,14	0,81 ± 0,04	0,65 ± 0,07	0,64 ± 0,16	0,2553	
Comprimento total final (mm)	32,90 ± 2,10	33,60 ± 0,40	34,6 ± 1,30	34,80 ± 1,30	32,0 ± 2,40	0,2606	
Comprimento padrão final (mm)	25,84 ± 0,54b	27,83 ± 1,11ab	28,81 ± 0,23a	28,16 ± 0,78ab	26,67 ± 1,61ab	*0,0245*	quadrática
Ganho em peso (mg/Peixe)	-0,23 ± 0,27b	0,20 ± 0,03ab	0,32 ± 0,20a	0,07 ± 0,11ab	-0,07 ± 0,19ab	*0,0266*	quadrática
Consumo	0,096 ± 0,021	0,098 ± 0,007	0,117 ± 0,026	0,136 ± 0,004	0,126 ± 0,017	*0,066*	
Sobrevivência (%)	66,67 ± 16,67	66,67 ± 16,67	55,56 ± 9,62	49,89 ± 16,84	49,89 ± 16,84	*0,4909*	
Fator de condição	0,41 ± 0,07	0,49 ± 0,09	0,54 ± 0,02	0,44 ± 0,04	0,44 ± 0,10	-	
Metabolismo antioxidante de músculo e pele (U.mg de proteina^{-1})							
Superperoxido desmutase	1,20 ± 0,75	1,04 ± 0,39	1,41 ± 0,63	1,50 ± 1,02	0,80 ± 0,38	*0,2749*	
Catalase	0,65 ± 0,52	0,52 ± 0,47	0,16 ± 0,11	0,73 ± 0,69	0,68 ± 0,17	*0,1211*	
Equações							
Comprimento padrão	$Y = 1,786 + 44,734X - 18,572X^2$, $R^2 = 0.6398$, Phe: 1,20%					*0,0022*	
Ganho em peso	$Y = -4,321 + 7,701X - 3,232X^2$, $R^2 = 0.5636$, Phe: 1,19%					*0,0069*	

Tabela 3 – Avaliação da coloração do dorso e cauda de platy suplementados com fenilalanina.

	Suplementação com fenilalanina (%)						
	0,81	**0,99**	**1,17**	**1,36**	**1,54**	*P*	*Efeito*
Coloração corporal							
*L**	51,48 ± 0,74	51,63 ± 1,25	50,12 ± 2,16	53, 92 ± 2,88	53,03 ± 2,85	*0,3011*	
*a**	11,75 ± 0,84a	11,95 ± 0,69a	13,81 ± 0,23a	9,12 ± 1,41b	11,60 ± 0,76a	*0,0011*	*NS*
*b**	30,81 ± 1,30ab	31,72 ± 1,16a	29,74 ± 1,36ab	27,16 ± 1,25b	31,90 ± 2,24a	*0,0188*	*cúbica*
ΔE	9,50 ± 1,17ab	5,69 ± 0,33b	12,78 ± 2,55a	10,75 ± 3,31ab	10,57 ± 2,81ab	*0,0391*	*NS*
Coloração caudal							
*L**	57,30 ± 2,50	58,40 ± 1,66	58,12 ± 2,05	55,35 ± 2,80	61,35 ± 0,41	*0,0553*	
*a**	10,05 ± 3,01a	4,83 ± 1,33c	7,10 ± 1,38abc	8,72 ± 2,52ab	5,36 ± 0,38bc	*0,0393*	*cúbica*
*b**	15,84 ± 3,67	13,87 ± 3,56	13,86 ± 1,16	15,63 ± 4,19	12,74 ± 3,10	*0,7538*	
ΔE	20,72 ± 6,14	16,41 ± 3,15	13,45 ± 3,99	13,38 ± 3,72	21,84 ± 3,53	*0,0974*	
Equações							
Coloração corporal							
*b**	$Y = -156,179 + 525,922X - 477,761X^2 + 139,953X^3$, $R^2 = 0.6454$, Phe: 0,93%					*0,0079*	
Coloração caudal							
a*	$Y = 273,308 - 705,752X + 608,307X^2 - 170,803X^3$, $R^2 = 0.5951$, Phe: 0,81%					*0,0158*	

L* = Luminosidade (0 preto, 100 branco); a* = coordenada vermelho/verde (+a indica vermelho e –a indica verde); b* = coordenada amarelo / azul (+b indica amarelo e –b indica azul); ΔE = diferença de cor inicial e final; NS: não significativo (p>0,05).. Dados: média e desvio padrão. Letras distintas reportam diferença significativa pelo teste de Tukey, 5%).

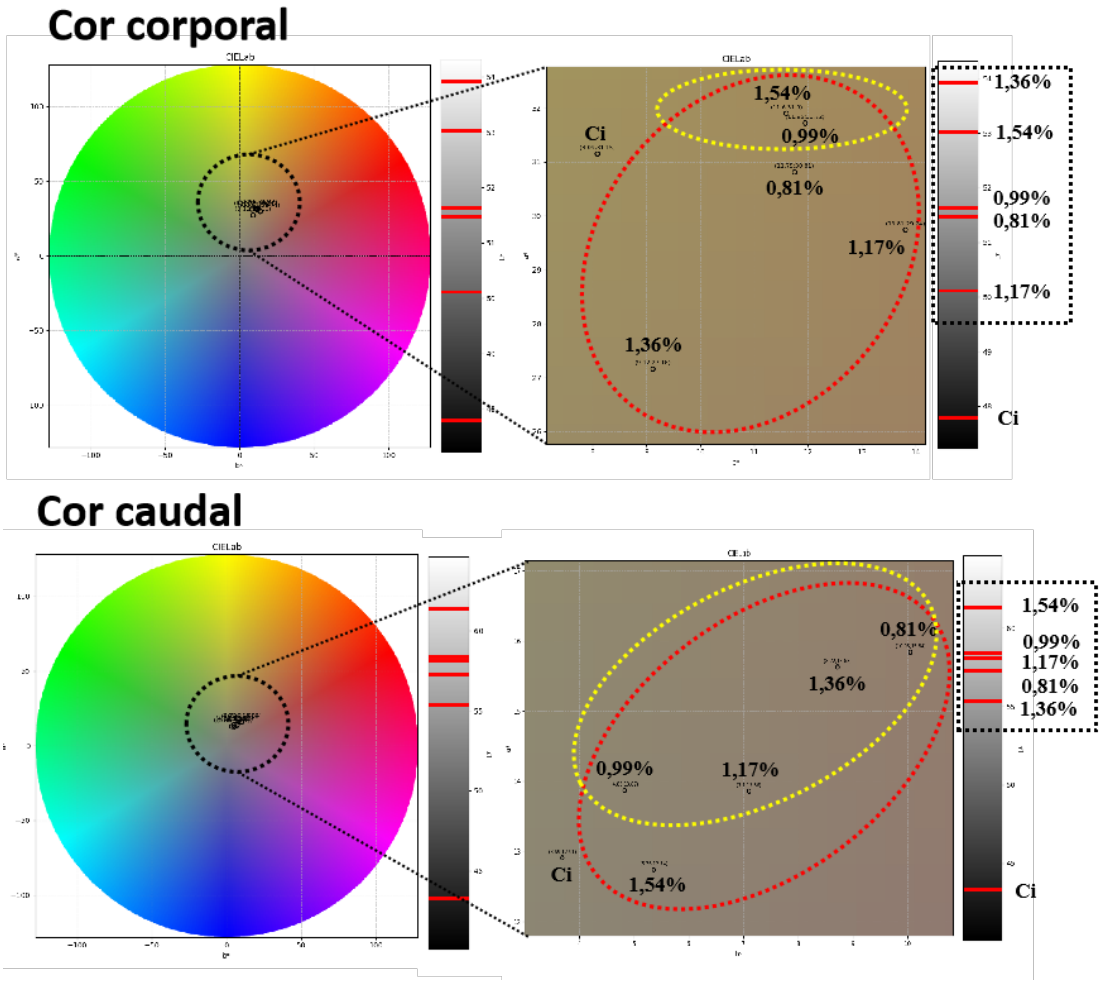

Figura 2. Gráfico da coloração corporal e caudal de platy (*X. maculatus*) alimentado com dietas suplementadas com fenilalanina. Elipse amarela: ganho na cromaticidade b* em relação a cor inicial. Elipse vermelha: ganho na cromaticidade a* em relação a cor inicial. Linha negra pontilhada: ganho na luminosidade em relação a cor inicial dos peixes (Ci).

A distribuição das coordenadas cromáticas (*a* e b*) nos tratamentos revela que a suplementação com fenilalanina modulou a expressão dos pigmentos responsáveis pela coloração dos platys. No corpo dos peixes, o deslocamento dos pontos médios em direção aos quadrantes positivos de *a* (vermelho) e *b* (amarelo), especialmente nos níveis intermediários (1,17% e 1,36%) e no nível elevado (1,54%) (Figura 2).

Na região caudal, a distribuição das coordenadas apresentou maior amplitude e formação de agrupamentos mais definidos entre os tratamentos. Os níveis 0,81% e 1,36% mostraram deslocamento para valores mais elevados de *a* e *b*, enquanto 0,99% e 1,54% se concentraram em regiões de menor intensidade

cromática, refletindo diferenças na expressão pigmentária em função da dieta.

Foram observadas correlações moderadas e altas, positivas, entre os indicadores de desempenho (fator de condição, ganho em peso, comprimento total, comprimento padrão e peso final) e os parâmetros de coloração caudal (ΔEcd e L*), além da atividade de SOD com o ganho de cor corporal (ΔEcp) (Figura 3). Houve correlação moderada positiva entre o consumo alimentar e o ganho de cor corporal (ΔEcp) (r = 0,56). O fator de condição apresentou correlação moderada e forte com o ganho de peso, peso final, comprimento padrão e comprimento total.

Na coloração caudal, houve correlação moderada positiva entre os parâmetros cromáticos a* e b* (r = 0,74) e entre L* e ΔEcd (r = 0,61). Também foram observadas correlações moderadas negativas entre a* e L* (r = –0,65) e entre b* e L* (r = –0,53). Na coloração corporal, observou-se correlação moderada negativa entre a* e L* (r = –0,68). Além disso, verificou-se correlação moderada positiva entre a atividade de SOD e o ganho de cor corporal (ΔEcp) (r = 0,72) (Figura 4).

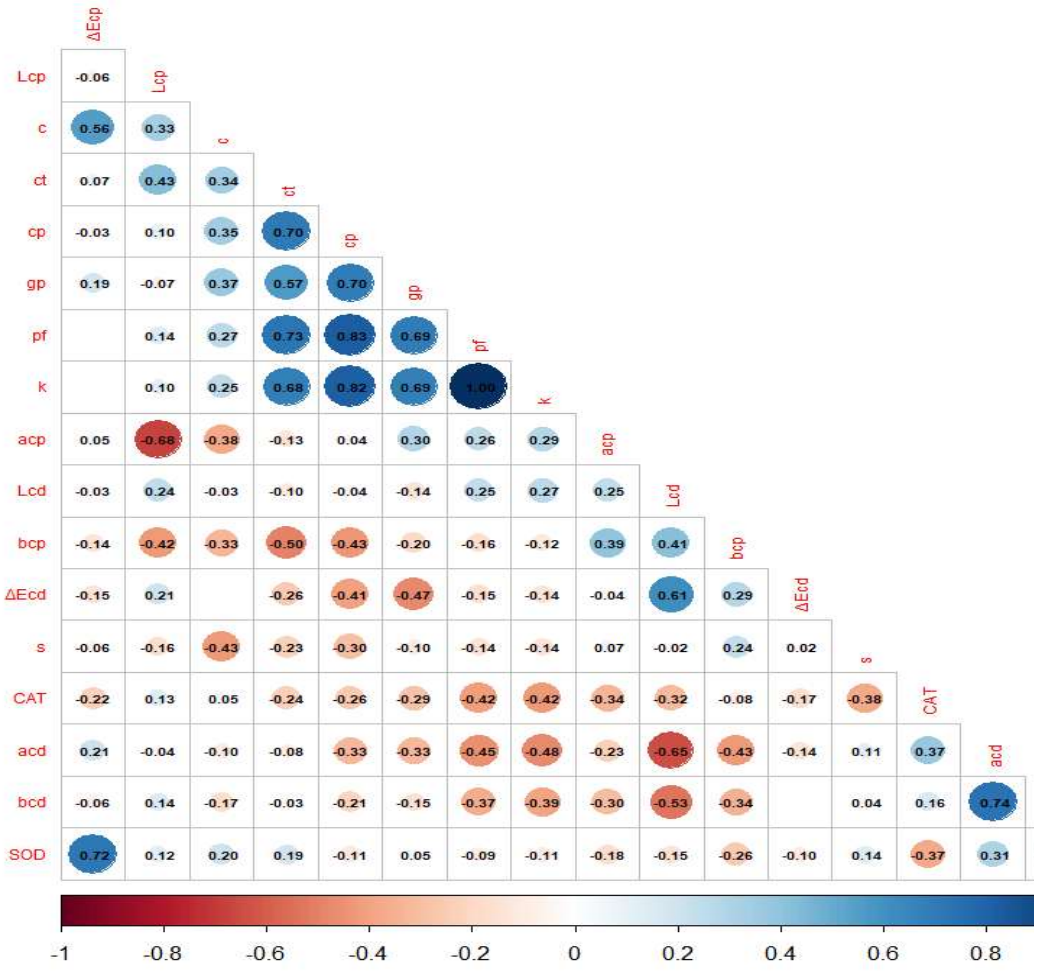

Figura 3. Correlação de Pearson. c: consumo; pf: peso final; ct: comprimento total;

cp: comprimento padrão; gp: ganho de peso; k: fator de condição; s: sobrevivência; Lcp: luminosidade corporal; acp: a* corporal: bcp: b* corporal; ΔEcp: diferença corporal de cor inicial e final; Lcd: luminosidade caudal; acd: a* caudal; bcd: b* caudal; ΔEcd: diferença caudal de cor inicial e final; CAT: catalase; SOD: superóxido dismutase.

4 Discussão

Os resultados indicaram que a suplementação dietética com fenilalanina influenciou a coloração coporal e caudal dos peixes e, em menor escala, o desempenho zootécnico. O comprimento padrão e o ganho em peso responderam de forma significativa aos níveis dietéticos, sugerindo que a fenilalanina contribui para o desenvolvimento estrutural, possivelmente por seu papel na síntese proteica e nos processos metabólicos associados. A suplementação de aminoácidos em peixes ornamentais ainda é incipiente, sendo mais pesquisada em peixes para consumo (Rodrigues et al., 2019; Sharf e Khan, 2023; Yi et al., 2023). A inclusão de aminoácidos na dieta pode otimizar as funções digestivas e metabólicas resultando em indices de desenvolvimento e homogeneidade do lote (Yamashiro et al 2016; Rodrigues et al., 2019; Yi et al., 2023) e atenuar estresse, atuando no bem estar dos peixes (Salamanca et al., 2021).

O maior fator de condição observado no nível de 1,17% reforça essa interpretação, indicando melhor estado corporal e maior eficiência no uso dos nutrientes. Conforme Sultana et al., (2023) o fator de condição pode ser utilizado para indicar o bem estar, sendo influenciado por diversos fatores, entre eles as condições ambientais, a dieta e as condições fisiológicas dos animais.

Embora os demais parâmetros de desempenho não tenham apresentado diferenças significativas, as alterações observadas na coloração corporal e caudal demonstram que a fenilalanina exerce efeito direto sobre a pigmentação. Como precursor da tirosina e da via de síntese de melanina, a fenilalanina provavelmente aumentou a disponibilidade de substrato para pigmentação, especialmente nos níveis de 1,17% que maximizaram a coloração corporal, e posteriormente com valores ajustados pela regressão cúbica para 0,93% e 0,81% de inclusão, para maximização da cor caudal e corporal respectivamente. As respostas observadas em a*, b* e ΔE sustentam a hipótese de que a suplementação modulou a atividade de cromatóforos e a deposição de pigmentos.

A fenilalanina, provavelmente possa modular indiretamente a biossíntese de pigmentos ao participar do ciclo de regeneração da tetraidrobiopteridina (BH4), um cofator indispensável para reações hidroxilativas e processos de oxidorredução envolvidos na formação de pteridinas. Durante a exposição à luz, a BH4 sofre foto-oxidação gerando biopteridina, a qual pode ser reconvertida ou desviada para a formação de derivados pteridínicos de maior estabilidade (Leclercq et al., 2009). O aumento do fluxo por essa via sugere que a disponibilidade elevada de fenilalanina possa sustentar a manutenção do estado redox da BH4 e ampliar a síntese de pteridinas cromógenas nos xantóforos e eritróforos, favorecendo assim a intensificação de tonalidades alaranjadas encontradas em nossos platys.

Em teleósteos, existe plasticidade entre os cromatóforos, permitindo que melanóforos se diferenciem em xantóforos e o inverso, o que reforça a proximidade metabólica entre as rotas de síntese de melanina e de pteridinas (Leclercq et al., 2009). Também é descrito que a melanogênese depende da disponibilidade de tirosina e do estado funcional da tetraidrobiopteridina (BH4), cofator essencial para a atividade da fenilalanina hidroxilase (Leclercq et al., 2009). Dessa forma, talvez seja possíivel que o fornecimento de fenilalanina, que eleva a oferta de tirosina, e a manutenção da BH4 possam modular conjuntamente a produção de melanina e pteridinas (Leclercq et al., 2009). Esses mecanismos ajudam a explicar a associação entre a coloração cutânea, os níveis de biopteridinas e a atividade da fenilalanina hidroxilase descrita para peixes, indicando a integração dessas vias na determinação da pigmentação (Scardua et al., 2024).

A ausência de diferenças significativas na atividade antioxidante sugere que os níveis de fenilalanina utilizados não foram suficientes para alterar a resposta oxidativa sistêmica. No entanto, a correlação positiva entre SOD e a variação de cor corporal (ΔEcp) indica que o metabolismo antioxidante pode estar associado à síntese de pigmentos, reforçando a ideia de que a pigmentação é um processo metabolicamente ativo (Huang et al., 2023). A melanina e pteridina possuem atividade antioxidante nos animais (McGraw, 2005), sendo algumas responsáveis em modular a resposta antioxidante (Martínez e Barbosa, 2010). Por exemplo, o observado em trutas selvagens, onde a coloração a base de melanina sinaliza peixes com melhor capacidade antioxidante (Parolini et al., 2018).

A suplementação de fenilalanina modulou de forma significativa a coloração de *Xiphophorus maculatus*, revelando maiores intensidades de coloração corporal com 0,93% de fenilalanina, que resultou em maior intensificação da componente

cromática *b* e na coloração caudal o nível de 0,81% revelou a maior intensidade da cromaticidade “a”. Esses achados indicam que a fenilalanina atua sobre vias pigmentares associadas à síntese de pteridinas e melaninas, contribuindo para maior saturação e qualidade visual. Embora os efeitos sobre o desempenho tenham sido limitados, o fator de condição e as correlações fisiológicas observadas sugerem manutenção do bem-estar. Dessa forma, a fenilalanina apresenta-se como um aditivo nutricional promissor para otimizar a coloração e potencialmente o valor comercial de platys na aquicultura ornamental, reforçando sua aplicabilidade prática e seu papel na melhoria da qualidade visual dos animais.

5 Conclusão

A fenilalanina configura-se como um aditivo nutricional viável para aprimorar a coloração do platy, especialmente nos níveis baixos (0,81% para coloração caudal e 0,93% para coloração corporal), que resultaram em maior intensificação das componentes cromáticas.

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CAPÍTULO 6 - CONSIDERAÇÕES FINAIS

Através da revisão de aminoácidos observou-se que as rotas metabólicas existentes no peixes permitem a produção destes pigmentos (melanina e pteridina). O uso da farinha desengordurada de pequi na dieta se mostrou promissor e favoreceu a pigmentação e o bem estar do Mato Grosso, denotando a possibilidade de seu uso nas dietas e demais espécies de peixes ornamentais, entretanto, requer mais pesquisas, visando reverter seu impacto sobre o metabolismo antioxidante

O decocto de guavira como aditivo na dieta de *Geophagus pyrocephalus* apontou resultados positivos para a atividade antioxidante com a dieta T250, porém sua toxidez aumentou de acordo com a dosagem, o que ficou evidenciado através dos testes de toxicidade com artemia e com os juvenis da própria espécie. Demonstrando assim que talvez outra forma de inserção na dieta seja menos agressivo, já que o uso deste produto em outros animais demonstrou ação positiva, porém com outros métodos de inserção.

A utilização de fenilalanina na dieta de platy incrementou a coloração apontando que há rota metabólica que interage a fenilalanina com a pteridina e a melanina o que permitirá novas tendências na manufaturação de dietas para peixes ornamentais.

O uso combinado de dietas contendo níveis seguros de fenilalanina e aditivos naturais ricos em carotenóides poderá favorecer uma maior expressão da pigmentação nos peixes ornamentais, além de trazer os benefícios atribuídos aos aditivos naturais relacionados a saúde e bem estar dos peixes.

Desta forma um pequeno avanço no setor de produção de rações no setor de piscicultura ornamental pode ser alavancado, pois poderão ser produzidas dietas com maior qualidade que favoreçam a produção e manutenção da pigmentação dos peixes, favorecendo seu desenvolvimento, sua saúde e bem-estar.