Universidade Estadual de Londrina

DANIELA KAIZER TERTO

AVANÇOS NA TILAPICULTURA: SELEÇÃO GENÉTICA, PRÁTICAS DE MANEJO PRÉ-ABATE, BEM-ESTAR ANIMAL E QUALIDADE DO FILÉ

Londrina
2025

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Tese apresentada ao Programa de Pós-graduação em Ciência Animal da Universidade Estadual de Londrina - UEL, como requisito parcial para a obtenção do título de Doutora
Orientadora: Profª. Drª. Ana Maria Bridi

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“Para Deus nada é impossível.”
(Lc 1,37)

RESUMO

TERTO, Daniela Kaizer. **Avanços na tilapicultura: seleção genética, práticas de manejo pré-abate, bem-estar animal e qualidade do filé**. 2025. 87 páginas. Tese (Doutorado em Ciência Animal) – Universidade Estadual de Londrina, Londrina, 2025.

Objetivou-se investigar a relação entre seleção genética, manejo pré-abate e qualidade do filé de tilápias do Nilo (*Oreochromis niloticus*), avaliando a expressão dos genes RyR3 e m-calpaína e os efeitos de diferentes densidades de transporte em condições sazonais, com enfoque no bem-estar animal e na qualidade do filé. No primeiro artigo, foram avaliadas 78 tilápias provenientes do programa de melhoramento genético Tilamax, sendo 40 de alto valor genético e 38 de baixo valor genético para ganho de peso, em um delineamento completamente casualizado. Foram realizadas análises de expressão gênica (RT-qPCR), composição centesimal (umidade, cinzas, lipídios totais e proteína total) e qualidade do filé (cor, pH, perda de líquido por descongelamento e por cocção). A expressão gênica não diferiu entre os grupos para RyR3 ($p=0,9260$) e m-calpaína ($p=0,3808$). No entanto, o grupo de alto valor genético apresentou maior teor de cinzas ($p=0,012$), lipídios totais ($p=0,013$), além de maior luminosidade (L*) ($p<0,001$) e croma ($p<0,001$), indicando efeitos positivos da seleção genética sobre a qualidade do filé. No segundo artigo, foram avaliadas três densidades de transporte (375, 425 e 475 kg/m³) durante verão e inverno, em um delineamento completamente casualizado em esquema fatorial 3x2. Foram analisados biomarcadores de estresse (glicose e lactato), parâmetros bioquímicos (glutationa reduzida, catalase e lipoperoxidação) e atributos qualitativos do filé (pH, capacidade de retenção de água (CRA), cor e textura). O lactato aumentou no inverno sob 375 kg/m³ e no verão sob 475 kg/m³ ($p=0,0131$). O pH reduziu sob 375 kg/m³ no inverno ($p=0,0043$). A CRA foi maior no verão sob 375 kg/m³ e menor no inverno sob 425 kg/m³ ($p= 0,0112$). A coesividade da carne reduziu no verão sob 425 kg/m³ ($p=0,0051$), enquanto a gomosidade foi menor no inverno ($p=0,0056$). A resiliência dos filés aumentou sob 475 kg/m³ no inverno ($p=0,0017$). Conclui-se que ajustes sazonais na densidade de transporte são essenciais para garantir bem-estar animal e qualidade do filé, reforçando a importância de estratégias integradas de seleção genética para otimizar a produtividade e a sustentabilidade na tilapicultura.

Palavras-chave: Lactato; m-Calpaína; pH, Perfil de textura; Rianodina.

ABSTRACT

TERTO, Daniela Kaizer. **Advances in tilapia farming: genetic selection, pre-slaughter management practices, animal welfare, and fillet quality.**2025. 87 pages. Thesis (Doctorate in Animal Science) – State University of Londrina, Londrina, 2025.

The objective was to investigate the relationship between genetic selection, pre-slaughter handling, and Nile tilapia (Oreochromis niloticus) fillet quality by evaluating the expression of the RyR3 and m-calpain genes and the effects of different transport densities under seasonal conditions, focusing on animal welfare and fillet quality. In the first study, 78 tilapias from the Tilamax genetic improvement program were evaluated, with 40 fish classified as high genetic value and 38 as low genetic value for weight gain, using a completely randomized design. Gene expression analysis (RT-qPCR), proximate composition (moisture, ash, total lipids, and total protein), and fillet quality (color, pH, thawing and cooking loss) were assessed. Gene expression did not differ between groups for RyR3 ($p=0.9260$) and m-calpain ($p=0.3808$). However, the high genetic value group exhibited higher mineral content ($p=0.012$), total lipids ($p=0.013$), as well as greater lightness (L*) ($p<0.001$) and chroma ($p<0.001$), indicating positive effects of genetic selection on fillet quality.In the second study, three transport densities (375, 425, and 475 kg/m³) were evaluated during summer and winter using a completely randomized factorial 3x2 design. Stress biomarkers (glucose and lactate), biochemical parameters (reduced glutathione, catalase, and lipid peroxidation), and fillet quality attributes (pH, water-holding capacity (WHC), color, and texture) were analyzed. Lactate levels increased in winter under 375 kg/m³ and in summer under 475 kg/m³ ($p=0.0131$). pH decreased under 375 kg/m³ in winter ($p=0.0043$). WHC was higher in summer under 375 kg/m³ and lower in winter under 425 kg/m³ ($p=0.0112$). Meat cohesiveness decreased in summer under 425 kg/m³ ($p=0.0051$), while gumminess was lower in winter ($p=0.0056$). Fillet resilience increased under 475 kg/m³ in winter ($p=0.0017$).It is concluded that seasonal adjustments in transport density are essential to ensure animal welfare and fillet quality, reinforcing the importance of integrated genetic selection strategies to optimize productivity and sustainability in tilapia farming.

Key-words: Lactate; m-Calpain; pH; Texture profile; Ryanodine.

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LISTA DE ABREVIATURAS E SIGLAS

ABNT	Associação Brasileira de Normas Técnicas
CAT	Catalase
CEUA	Comissão de Ética no Uso de Animais
CONCEA	Conselho Nacional de Controle e Experimentação Animal
CRA	Capacidade de Retenção de Água
DTNB	5,5'-Ditiobis-2-Nitrobenzoico
GSH	Glutationa Reduzida
LPO	Lipoperoxidação
MAPA	Ministério da Agricultura, Pecuária e Abastecimento
MDA	Malonaldeído
PLC	Perda de Líquido por Cocção
PLD	Perda de Líquido por Descongelamento
TBARS	Substâncias Reativas ao Ácido Tiobarbitúrico
UEL	Universidade Estadual de Londrina

SUMÁRIO

1 INTRODUÇÃO

A tilapicultura é amplamente reconhecida como um segmento crucial da aquicultura global, com *Oreochromis niloticus* destacando-se por sua alta produtividade e notável capacidade de adaptação. Sua produção mundial que superou 6,65 milhões de toneladas anuais em tal ano. No Brasil, a tilápia lidera a produção aquícola, representando 65,3% do total nacional, que alcançou aproximadamente 579 mil toneladas em 2023. O país ocupa atualmente a quarta posição no ranking mundial, consolidando a tilapicultura como um setor estratégico para o desenvolvimento econômico, geração de empregos e segurança alimentar (PEIXE BR, 2024).

A preferência pela tilápia deve-se a suas características zootécnicas, como rápido crescimento, elevada eficiência alimentar, resistência a variações ambientais e qualidade superior do filé. Além disso, a espécie apresenta ampla aceitação tanto no mercado interno quanto no internacional, reforçando seu papel como um dos pilares da aquicultura moderna. Essas características posicionam a tilápia como uma espécie de grande relevância estratégica, promovendo a sustentabilidade econômica e contribuindo para a expansão dos mercados globais de pescado (SCHULTER; VIEIRA FILHO, 2017; ANIMAL BUSINESS, 2019; PEIXE BR, 2024).

Nesse contexto, o melhoramento genético desempenha um papel central na maximização da produtividade e qualidade da tilapicultura. Programas como o Genetically Improved Farmed Tilapia (GIFT) estabeleceram bases para o desenvolvimento de linhagens de alto desempenho, como a Tilamax no Brasil. Essa linhagem apresenta ganhos expressivos em peso e eficiência alimentar, atributos que contribuem para a competitividade e sustentabilidade do setor (ZARDIN, 2016). Além de promover avanços produtivos, a seleção genética permite uma compreensão mais profunda dos mecanismos moleculares que regulam o crescimento muscular em peixes, como calpaína e o receptor de rianodina tipo 3 (RyR3) que desempenham papéis cruciais na dinâmica muscular, influenciando diretamente processos como hipertrofia e hiperplasia, essenciais para a deposição de massa magra. Essas enzimas também se correlacionam com a qualidade do filé, afetando características como capacidade de retenção de água, textura e composição centesimal (GEESINK; KOOHMARAIE, 2004; BHAT et al., 2018).

Apesar dos avanços genéticos, o manejo pré-abate ainda apresenta lacunas,

especialmente no Brasil, onde a ausência de normativas específicas compromete o bem-estar animal e a qualidade do produto. O transporte, uma etapa crítica, é influenciado por fatores como densidade de estocagem e variações sazonais. Durante o verão, o aumento das temperaturas intensifica o metabolismo e a demanda por oxigênio, enquanto no inverno, a baixa temperatura desafia a homeostase térmica das tilápias. Tais condições, associadas à densidade inadequada, resultam em estresse fisiológico, alta mortalidade e comprometimento da qualidade do filé (REFAEY et al., 2017; WU et al., 2018; MAPA, 2022).

As respostas ao estresse no transporte pré-abate incluem alterações metabólicas, como elevações nos níveis plasmáticos de glicose e lactato, e biomarcadores de estresse oxidativo, como catalase (CAT), glutationa reduzida (GSH) e lipoperoxidação (LPO). Essas alterações impactam atributos de qualidade, como pH muscular, capacidade de retenção de água (CRA), cor e textura, que são determinantes para a aceitação do produto pelo mercado consumidor (HERTOG-MEISCHKE et al., 1997; CHOWDHURY et al., 2020; PONGSETKUL et al., 2022). Assim, a interação entre seleção genética e manejo pré-abate constitui uma área estratégica para otimizar a produtividade e a qualidade da tilapicultura.

Este estudo aborda as lacunas na relação entre seleção genética, bem-estar animal no transporte pré-abate e qualidade do filé de tilápias do Nilo, com foco em estratégias que atendam aos desafios da tilapicultura moderna. Portanto, foram avaliados os efeitos de linhagens genéticas de alto e baixo valor para ganho de peso sobre a qualidade da carne, bem como a influência das densidades de transporte pré-abate, considerando as interações sazonais entre verão e inverno, na modulação do bem-estar animal e nas características qualitativas do filé.

2 REFERENCIAL TEÓRICO

2.1 TILAPICULTURA

2.1.1 Histórico e Melhoramento Genético

A introdução da tilápia do Nilo (*Oreochromis niloticus*) no Brasil teve início em 1971, quando o Departamento Nacional de Obras Contra as Secas (DNOCS) trouxe os primeiros exemplares para povoamento de reservatórios públicos no Nordeste, visando fomentar a piscicultura regional. Apesar da expansão da espécie, os primeiros cultivos ainda careciam de um aprimoramento genético que garantisse maior eficiência produtiva e melhor qualidade da carne (PANORAMA DA AQUICULTURA, 2003).

Na década de 1990, a tilapicultura nacional passou por uma transformação significativa com a importação da linhagem Chitralada, vinda da Tailândia, que foi introduzida no Paraná em 1996 por meio de uma iniciativa da Associação Paranaense dos Produtores de Alevinos e da EMATER. Essa linhagem trouxe ganhos em crescimento e resistência, impulsionando a produção comercial em larga escala (EMATER, 2018). No início dos anos 2000, outro avanço ocorreu com a chegada da GenoMar Supreme Tilapia, introduzida pela Aquabel, que se tornou uma importante fornecedora de alevinos melhorados geneticamente para os piscicultores brasileiros (EMATER, 2018).

O grande salto da tilapicultura brasileira ocorreu em 2005, com a introdução da linhagem GIFT (Genetically Improved Farmed Tilapia), proveniente da Malásia, resultado de uma parceria entre a Secretaria Especial de Aquicultura e Pesca e a Universidade Estadual de Maringá (UEM), além do apoio do WorldFish Center. A partir desse programa, foi possível selecionar peixes com maior taxa de crescimento, melhor conversão alimentar e maior uniformidade nos lotes de produção, impulsionando a produtividade do setor (EMATER, 2018).

Como resultado desse avanço, a Tilamax, uma linhagem derivada da GIFT, foi desenvolvida no Brasil, consolidando-se por sua adaptação às condições de cultivo e pelo desempenho produtivo superior (ZARDIN, 2016). O programa conduzido pela

UEM busca aprimorar características como crescimento acelerado e eficiência alimentar, tornando a espécie mais competitiva no mercado. Além do bom desempenho zootécnico, a Tilamax também se destaca pela qualidade da carne, cuja textura e sabor são bem aceitos tanto no mercado interno quanto no externo, impulsionando sua comercialização (ZARDIN, 2016).

A partir de 2016, novas linhagens foram incorporadas ao mercado nacional, incluindo a SPRING-genetics, proveniente dos Estados Unidos, que trouxe inovações no aprimoramento genético para maior desempenho em diferentes sistemas de cultivo (EMATER, 2018).

2.1.2 Relevância Econômica e Expansão da Tilapicultura Brasileira

Sua eficiência produtiva e alta adaptabilidade ambiental, tornou a Tilápia do Nilo um dos principais organismos cultivados na aquicultura mundial (PEIXE BR, 2024). Atualmente, o cultivo da tilápia representa uma parcela significativa do volume global de pescado, atingindo aproximadamente 6,65 a 6,7 milhões de toneladas em 2023, com a China como maior produtora, seguida por Egito e Indonésia. Essa expansão coloca o Brasil na quarta posição no ranking mundial, consolidando sua relevância no cenário global da piscicultura e destacando a tilápia como o peixe mais cultivado no país, responsável por 65,3% da produção aquícola (ou piscícola) nacional (PEIXE BR, 2024).

Em 2023, o Brasil produziu cerca de 579.080 toneladas de tilápia, com os estados do Paraná, São Paulo e Minas Gerais liderando a produção nacional, reflexo de um ambiente favorável, de investimentos tecnológicos e de um setor que avança na profissionalização. Nesse sentido, a demanda crescente pela espécie é acompanhada por um aumento considerável no consumo doméstico: nos últimos dez anos, o consumo per capita no país passou de 1,47 kg para 2,84 kg, um aumento de 93%, refletindo uma preferência do consumidor por produtos de cultivo, devido ao sabor e acessibilidade (PEIXE BR, 2024).

Além do mercado interno, o Brasil tem aumentado sua participação no mercado internacional, exportando 6.815 toneladas de tilápia em 2023 e gerando uma receita de US$ 24,7 milhões, representando um crescimento de 4% em comparação ao ano

anterior. O mercado norte-americano surge como o principal destino, valorizando a qualidade do filé brasileiro e incentivando um potencial de crescimento no segmento de exportação para mercados *premium* (PEIXE BR, 2024).

2.2 Crescimento Muscular e Mecanismos de Regulação em Peixes

O crescimento muscular em peixes desempenha um papel central na sua produtividade e na qualidade do produto, especialmente em espécies como a tilápia do Nilo, amplamente cultivada devido à sua adaptabilidade e características zootécnicas (PEIXE BR, 2024). Este crescimento é governado por dois processos principais: hipertrofia e hiperplasia muscular (ZIMMERMAN; LOWERY, 1999).

A hipertrofia refere-se ao aumento do tamanho das fibras musculares existentes, enquanto a hiperplasia envolve a formação de novas fibras musculares. Ambos os processos ocorrem em diferentes estágios do desenvolvimento do peixe. Nos estágios iniciais, a hiperplasia é predominante, permitindo a formação de um maior número de fibras musculares pequenas. Com o avanço do crescimento, especialmente após a maturação sexual, o papel da hipertrofia torna-se mais evidente, contribuindo para o aumento do volume muscular por meio da expansão das fibras existentes (REIS NETO et al., 2014; HAMZAH et al., 2017).

Em espécies como o robalo branco (*Atractoscion nobilis*), foi demonstrado que a hiperplasia contribui para o crescimento inicial, enquanto a hipertrofia domina nas fases mais avançadas. Esse padrão também se aplica às tilápias, onde as fibras musculares brancas, responsáveis pela maior parte do crescimento somático, apresentam aumento expressivo na área transversal ao longo do tempo (ZIMMERMAN; LOWERY, 1999).

Os mecanismos moleculares que regulam o crescimento muscular em tilápias envolvem uma complexa interação de fatores genéticos e ambientais. Genes como receptores de rianodina (RyR3), calpaína e calpastatina desempenham papéis cruciais na modulação da dinâmica muscular (HAMZAH et al., 2017; ALI et al., 2020).

2.2.1 Receptores de Rianodina Tipo 3 (RyR3)

O receptor de rianodina tipo 3 (RyR3) é uma proteína transmembrana fundamental, localizada no retículo sarcoplasmático das células musculares, que atua como um canal regulador de cálcio. Ele desempenha um papel essencial na liberação de íons cálcio (Ca^{2+}) durante o processo de contração muscular, funcionando como um elemento-chave no acoplamento excitação-contração. A regulação precisa do RyR3 é crucial para o controle da função muscular, abrangendo crescimento, reparo e manutenção do tecido muscular (ZIMMERMAN; LOWERY, 1999; BHAT et al., 2018).

O RyR3 é ativado em resposta ao aumento do potencial de ação celular, que estimula os canais de cálcio dependentes de voltagem localizados na membrana do retículo sarcoplasmático. Sua ativação resulta na liberação de cálcio armazenado para o sarcoplasma, aumentando a concentração intracelular de Ca^{2+}. Esse íon é essencial para a ativação de proteínas contráteis, como actina e miosina, que são responsáveis pela contração muscular. Além disso, o cálcio desempenha um papel como modulador direto de vias de sinalização celular associadas ao crescimento muscular, incluindo a via mTOR (mammalian target of rapamycin), que regula a síntese proteica e o desenvolvimento de hipertrofia e hiperplasia musculares (TAYLOR et al., 1995; GEESINK; KOOHMARAIE, 2004).

O cálcio liberado pelo RyR3 está intimamente ligado à ativação do sistema calpaína-calpastatina, um regulador crucial da integridade estrutural do músculo. As calpaínas, proteases dependentes de cálcio, utilizam o cálcio disponibilizado pelo RyR3 para iniciar a degradação controlada de proteínas miofibrilares, como desmina e talina. Esse processo é essencial para o remodelamento muscular, permitindo adaptações estruturais e funcionais durante o crescimento e reparo do tecido (GEESINK et al., 2004; BHAT et al., 2018).

Por outro lado, a calpastatina, uma inibidora específica das calpaínas, age em equilíbrio dinâmico com o RyR3 para prevenir uma degradação proteica excessiva. Alterações na expressão ou na funcionalidade do RyR3 podem modificar diretamente a atividade do sistema calpaína-calpastatina, influenciando tanto a plasticidade muscular quanto a qualidade estrutural e sensorial do músculo. Estudos em peixes geneticamente selecionados para maior crescimento mostram que ajustes na expressão do RyR3 são frequentemente acompanhados por mudanças na regulação

da calpaína e da calpastatina, otimizando a eficiência do crescimento e garantindo a estabilidade do tecido muscular (ZIMMERMAN; LOWERY, 1999; GEESINK; KOOHMARAIE, 2004).

A função do RyR3 como modulador do cálcio intracelular e sua interação com o sistema calpaína-calpastatina são de particular relevância no contexto da aquicultura e do melhoramento genético. Linhagens que apresentam maior expressão de RyR3 demonstram eficiência no controle do cálcio, promovendo um equilíbrio entre a síntese e a degradação proteica que otimiza o crescimento muscular e preserva a qualidade do filé. Essa relação destaca o papel do RyR3 não apenas como um regulador fisiológico, mas também como um alvo estratégico para aprimorar a produtividade e a competitividade no mercado global de tilápias (TAYLOR et al., 1995; GEESINK et al., 2004).

2.2.2 Calpaína

A calpaína, um sistema de proteases intracelulares dependentes de cálcio, desempenha um papel fundamental na regulação do crescimento muscular em peixes, abrangendo os processos de hiperplasia e hipertrofia muscular. Essas enzimas exercem suas funções no remodelamento do citoesqueleto e na degradação controlada de proteínas estruturais, promovendo tanto o aumento da massa muscular quanto a qualidade da carne (BHAT et al., 2018).

Dentre as isoformas, destacam-se a μ-calpaína e a m-calpaína, que apresentam diferentes sensibilidades às concentrações de cálcio para sua ativação. A ação dessas proteases está fortemente associada à remodelação de componentes estruturais das fibras musculares, particularmente os discos Z, sendo responsáveis pela degradação de proteínas-chaves, como desmina, titina e nebulina. Essas proteínas são essenciais para a plasticidade e integridade das fibras musculares, garantindo a adaptação e reparo do tecido durante os processos de crescimento e regeneração muscular. Assim, a calpaína atua como um regulador crítico da dinâmica muscular, com implicações diretas para a produtividade (BHAT et al., 2018).

Segundo Bhat et al. (2018), O processo de ativação da calpaína é altamente dependente do cálcio, sendo que a μ-calpaína requer concentrações menores de

cálcio em comparação à m-calpaína para ser ativada. Essa ativação leva a mudanças conformacionais nas proteínas-alvo, aumentando sua suscetibilidade à proteólise. Este mecanismo é essencial para a remodelação muscular necessária durante a hiperplasia e hipertrofia. Além disso, a calpaína interage com outras vias de sinalização envolvidas na síntese proteica, como a via mTOR (mammalian target of rapamycin), que regula o crescimento e a proliferação celular. Em peixes, a calpaína desempenha um papel crítico na regulação dos níveis de proteína miofibrilar, afetando diretamente a deposição de massa magra e, consequentemente, a qualidade do filé (GEESINK; KOOHMARAIE, 2004).

De acordo com Morais et al. (2020), a avaliação de tilápias do Nilo abatidas em diferentes pesos corporais revelou que a expressão da m-calpaína foi maior nos indivíduos com maior peso ao abate. Essa expressão mostrou uma correlação positiva com atributos superiores de qualidade do filé, incluindo menor força de cisalhamento e maior capacidade de retenção de água. Os resultados também destacaram que a atividade da calpaína está intrinsecamente ligada à degradação controlada de proteínas estruturais no músculo, como desmina e talina, durante o período pós-morte. Essa degradação contribui para uma retenção hídrica aprimorada, reduzindo as perdas por gotejamento e descongelamento nos filés.

2.3 Composição Centesimal de Tilápias

A composição centesimal do filé de tilápia, revela informações essenciais sobre seu valor nutricional e funcionalidade. Os componentes majoritários incluem umidade, cinzas, proteínas, e lipídios, que variam dependendo de fatores como genética, dieta, ambiente e fase de crescimento dos peixes (SALES; OLIVEIRA, 2015).

A umidade é o principal componente do filé de tilápia, representando entre 75% e 78% de sua composição total. Esse elevado teor hídrico desempenha um papel crucial nas propriedades sensoriais do pescado, especialmente na textura e suculência, características que influenciam diretamente na aceitação do produto pelo consumidor. Contudo, esse elevado conteúdo de água também aumenta a perecibilidade do filé, exigindo estratégias rigorosas de armazenamento e transporte

para preservar sua qualidade microbiológica e sensorial ao longo da cadeia produtiva (DA SILVA et al., 2019).

A fração mineral, representada pelas cinzas, compreende cerca de 1% a 2% do peso total do filé de tilápia. Este componente é caracterizado pela presença de minerais essenciais, como cálcio, fósforo, ferro e zinco, que desempenham funções fundamentais para a saúde humana. Essas funções incluem a manutenção da estrutura óssea, a regulação de processos metabólicos e o transporte de oxigênio pelo sistema circulatório. A alta biodisponibilidade desses micronutrientes no filé de tilápia reforça sua importância em dietas nutricionalmente equilibradas, posicionando-o como uma fonte estratégica de minerais essenciais (SIMÕES et al., 2007;SOUZA et al., 2021).

As proteínas, que correspondem a aproximadamente 18% a 20% da composição do filé de tilápia, destacam-se como um dos componentes nutricionais mais relevantes desse pescado. Essa fração proteica é caracterizada por sua alta digestibilidade e pelo perfil rico em aminoácidos essenciais, como lisina e metionina, que são indispensáveis para processos fundamentais como crescimento, reparo tecidual e manutenção da saúde muscular. A qualidade biológica das proteínas presentes no filé de tilápia reforça sua relevância como fonte estratégica para a segurança alimentar global, especialmente em regiões onde o pescado é uma das principais fontes de proteína animal (DA SILVA et al., 2019; SOUZA et al., 2021).

Embora os lipídios sejam um componente menos abundante, representando entre 2% e 5% da composição, eles possuem um impacto considerável na qualidade sensorial e no valor nutricional do filé. A tilápia é uma fonte de ácidos graxos poli-insaturados, como os ômega-3, conhecidos por seus benefícios à saúde cardiovascular e neurológica (SIMÕES et al., 2007).

A seleção genética para ganho de peso em peixes impacta diretamente a composição centesimal, refletindo os objetivos dos programas de melhoramento em equilibrar rendimento do filé, eficiência metabólica e qualidade nutricional. Os teores de proteínas, variando entre 18% e 20%, são preservados ou aumentados devido à sua importância para a funcionalidade muscular, enquanto os lipídios, mais variáveis, frequentemente diminuem em linhagens otimizadas para maior eficiência alimentar, redirecionando energia para o crescimento muscular sem comprometer a qualidade nutricional (LALU et al., 2021).

Os componentes mais estáveis, como umidade (75%-78%) e cinzas (1%-2%), podem apresentar pequenas variações relacionadas às mudanças nas proporções corporais associadas ao melhoramento genético. Ainda assim, os níveis de minerais essenciais, como cálcio e fósforo, mantêm-se dentro de padrões consistentes, assegurando a densidade de nutrientes e a qualidade funcional do filé após sucessivas gerações de seleção (da SILVA et al., 2019; LALU et al., 2021).

2.4 Transporte Pré-Abate de Tilápias

O transporte pré-abate de peixes representa uma etapa crítica no manejo aquícola, influenciando diretamente o bem-estar animal e a qualidade final do produto. De acordo com o Manual de Boas Práticas no Transporte de Peixes do Ministério da Agricultura, Pecuária e Abastecimento (2022), estratégias adequadas de transporte podem mitigar os efeitos do estresse, promovendo melhores resultados produtivos. Durante este processo, os peixes são expostos a diversos estressores, como alta densidade de transporte, manuseio excessivo e variações na qualidade da água, fatores que desencadeiam respostas fisiológicas adversas, incluindo alterações hormonais, imunológicas e metabólicas, comprometendo a saúde e a sobrevivência dos animais (PARVATHY et al., 2019).

Entre os principais desafios desse manejo estão as flutuações de temperatura, concentrações de oxigênio dissolvido, níveis de amônia e pH, além das densidades inadequadas de transporte, que podem deteriorar a qualidade da água e aumentar a mortalidade (PAN et al., 2010). Essas condições exigem monitoramento constante e práticas de manuseio fundamentadas em dados científicos, visando o desenvolvimento de protocolos de transporte eficazes que atendam às demandas do setor produtivo e assegurem o bem-estar dos peixes (PARVATHY et al., 2019).

2.4.1 Densidade no Transporte de Tilápias

Embora existam diretrizes claras sobre as densidades máximas para o transporte de tilápias, como descrito no Manual de Boas Práticas no Transporte de Peixes do Ministério da Agricultura, Pecuária e Abastecimento (2022), o Brasil ainda

carece de regulamentações legais que tornem essas práticas obrigatórias. O manual fornece recomendações técnicas destinadas a reduzir os impactos do transporte sobre o bem-estar animal, incluindo parâmetros específicos para ajustar densidades de acordo com fatores como temperatura da água e tempo de transporte. Contudo, essas diretrizes têm caráter consultivo e não possuem força normativa, deixando a adoção dessas práticas dependente da iniciativa dos produtores.

A ausência de legislação específica compromete a uniformidade das práticas de manejo no transporte aquícola, aumentando a variação nos padrões de operação entre diferentes regiões e sistemas de produção. Sem regulamentação vinculativa, é comum que práticas inadequadas resultem em maior estresse, mortalidade e comprometimento da qualidade do pescado. O manual destaca que a densidade de transporte deve ser fundamentada em evidências científicas e ajustada de forma a equilibrar eficiência produtiva e saúde animal.

No entanto, sem uma legislação específica que exija a aplicação dessas recomendações, o setor permanece vulnerável a lacunas operacionais que prejudicam tanto a sustentabilidade quanto a competitividade da aquicultura brasileira. A implementação de normativas específicas para o transporte de peixes, particularmente no que tange às densidades, surge como uma necessidade estratégica para assegurar padrões elevados de bem-estar animal e qualidade final do produto (MAPA, 2022).

Altas densidades de transporte no manejo pré-abate de peixes apresentam efeitos adversos sobre o bem-estar animal, comprometendo tanto a saúde quanto a qualidade final do produto. Durante essa etapa, a aglomeração excessiva dos peixes intensifica o estresse por meio de limitações de espaço, interações sociais agressivas e deterioração progressiva da qualidade da água. Estudos indicam que densidades elevadas aumentam a competição por oxigênio dissolvido e levam ao acúmulo de resíduos nitrogenados, como amônia e nitrito, substâncias tóxicas que comprometem a sobrevivência e a homeostase dos peixes. Particularmente em tilápias, a redução no oxigênio dissolvido e o aumento da amônia não ionizada são fatores críticos para o comprometimento metabólico e diminuição da sobrevivência (WU et al., 2018).

No âmbito fisiológico, altas densidades induzem respostas adversas, incluindo aumento das enzimas hepáticas, redução dos níveis de proteínas totais e alterações na integridade muscular. Tais respostas afetam diretamente a qualidade do filé,

resultando em menor rendimento e aceitação comercial. Além disso, a abrasão física causada pela proximidade excessiva dos peixes contribui para lesões corporais que exacerbam os impactos negativos sobre o bem-estar e a saúde geral dos animais (ORINA et al., 2014).

Em contrapartida, embora associadas a menores níveis de estresse, baixas densidades durante o transporte também podem ser prejudiciais ao bem-estar dos peixes. Em espécies gregárias, como a tilápia, a interrupção do comportamento natural de formação de cardumes pode induzir estresse por isolamento e alterar o comportamento social, aumentando a atividade metabólica e a demanda por oxigênio. Consequentemente, os peixes podem apresentar maior consumo de oxigênio dissolvido e comprometimento metabólico em ambientes de baixas densidades (WU et al., 2018).

Além disso, baixas densidades prejudicam a estabilidade térmica e química do ambiente aquático, uma vez que o menor número de peixes reduz o efeito de tamponamento térmico observado em tanques mais densos. Essa instabilidade pode tornar os peixes mais suscetíveis a variações bruscas de temperatura e outros fatores estressores ambientais. No contexto econômico, baixas densidades aumentam os custos operacionais por unidade transportada sem melhorar os indicadores de qualidade do pescado. Em algumas situações, podem até levar a maiores taxas de mortalidade devido à combinação de estressores ambientais e comportamentais, evidenciando a necessidade de densidades otimizadas para garantir tanto o bem-estar animal quanto a eficiência produtiva (ORINA et al., 2014; WU et al., 2018).

2.4.2 Influência das Estações do Ano no Transporte de Tilápias

A tilápia, como espécie pecilotérmica, apresenta variações metabólicas diretamente influenciadas pela temperatura ambiente, o que torna desafiadora a determinação de densidades ideais para o transporte em diferentes condições sazonais. Durante o verão, as altas temperaturas aumentam a atividade metabólica e, consequentemente, a demanda por oxigênio dissolvido. Por outro lado, no inverno, o metabolismo reduzido permite o transporte em densidades mais elevadas, devido à menor exigência fisiológica. A ausência de normativas específicas que regulem essas

práticas, considerando as variações sazonais, compromete tanto o bem-estar dos peixes quanto a sustentabilidade da produção aquícola (REFAEY et al., 2017; BORTOLETTI et al., 2023).

No contexto do transporte pré-abate, as altas temperaturas da água observadas durante o verão apresentam desafios críticos ao bem-estar das tilápias. O aumento da temperatura reduz a solubilidade do oxigênio na água, agravando o descompasso entre a elevada demanda metabólica e a limitada disponibilidade de oxigênio dissolvido. Essa condição frequentemente resulta em hipóxia, intensificando o estresse fisiológico e, em casos extremos, aumentando a mortalidade dos peixes durante o transporte.

Além disso, as temperaturas elevadas aceleram a deterioração da qualidade da água, favorecendo a excreção de amônia pelos peixes e a proliferação de microrganismos. A amônia não ionizada, altamente tóxica, causa danos aos tecidos branquiais e compromete a osmorregulação dos animais, levando a respostas de estresse caracterizadas por elevações nos níveis de cortisol e glicose plasmáticos. Tais alterações impactam negativamente a qualidade muscular, refletindo-se na capacidade de retenção de água e em variações no pH, o que compromete tanto a textura quanto o sabor do filé (REFAEY et al., 2017; WU et al., 2018).

Por outro lado, as baixas temperaturas da água, típicas do inverno, representam outro desafio ao bem-estar das tilápias, devido à sua origem tropical e limitada capacidade fisiológica de adaptação a ambientes frios. Expostas a temperaturas abaixo de 18 °C, essas espécies apresentam uma redução acentuada na taxa metabólica, comprometendo funções vitais como respiração, digestão e resposta imunológica, aumentando assim a suscetibilidade a doenças e ao estresse oxidativo (REFAEY et al., 2017; WU et al., 2018).

Durante o transporte em baixas temperaturas, as tilápias mobilizam energia adicional para manutenção da homeostase térmica e ativação de respostas adaptativas ao frio. Esses mecanismos incluem a redução da atividade locomotora, diminuição no consumo de oxigênio e alterações no equilíbrio osmótico. Temperaturas próximas ao limite inferior de tolerância, que é de aproximadamente 12 °C, podem levar ao colapso metabólico e mortalidade. No entanto, uma faixa de temperatura considerada ideal, entre 22 °C e 28 °C, é recomendada para minimizar os impactos negativos durante o transporte pré-abate, garantindo um equilíbrio adequado entre

demandas metabólicas e estabilidade fisiológica (REFAEY et al., 2017; BORTOLETI et al., 2023).

A incapacidade de adaptação a extremos térmicos compromete ainda mais a osmorregulação e a integridade muscular, aumentando os riscos de mortalidade e a deterioração da qualidade do filé. Portanto, o controle rigoroso da temperatura da água durante o transporte é indispensável para preservar o bem-estar das tilápias e a sustentabilidade do processo produtivo (MAPA, 2022).

2.5 Estresse em Peixes

O estresse é amplamente descrito como um estímulo que promove alterações na homeostase, envolvendo a ativação do Sistema Nervoso Autônomo e respostas adaptativas do organismo (CANNON, 1935; BRETT, 1958). Em peixes como a tilápia, o transporte pré-abate representa um dos principais fatores estressores, com impacto no bem-estar animal. O estresse pode ser descrito em três estágios: primário, secundário e terciário. Na fase primária ocorre uma resposta neuroendócrina mediada pelo hipotálamo, que estimula a liberação de catecolaminas (adrenalina e noradrenalina) pelas células cromafins, resultando em alterações respiratórias e cardiovasculares, como aumento da ventilação branquial e maior captação de oxigênio. Essas mudanças visam compensar os distúrbios iniciais, mas podem agravar o estresse quando mantidas por longos períodos (MAZEAUD; MAZEAUD, 1981).

Na resposta secundária, a liberação de catecolaminas e cortisol causa alterações metabólicas, como hiperglicemia, hiperlactacemia e catabolismo proteico (MARTINS DA ROCHA et al., 2004). Se o estressor persistir, a resposta terciária manifesta-se por efeitos patológicos, incluindo imunossupressão, redução no crescimento e maior suscetibilidade a doenças, comprometendo a saúde e a qualidade dos peixes transportados (OBA et al., 2009).

2.5.1 Avaliação do Estresse no Plasma Sanguíneo de Peixes

Os parâmetros sanguíneos, como glicose e lactato, são amplamente

reconhecidos como biomarcadores confiáveis para avaliar o estado de estresse em peixes. Esses indicadores metabólicos refletem adaptações fisiológicas a estímulos estressores, sendo ferramentas essenciais no monitoramento do bem-estar animal em etapas críticas do manejo aquícola, como o transporte pré-abate (BEECHAM et al., 2006; JIANG et al., 2017).

A glicose, principal fonte de energia disponível no organismo, apresenta um aumento em situações de estresse. Esse incremento plasmático é mediado por processos de glicogenólise e gliconeogênese, estimulados pela ação de catecolaminas (epinefrina e norepinefrina) e cortisol, hormônios que desempenham papéis centrais na resposta metabólica ao estresse. Essas adaptações metabólicas são cruciais para atender às demandas energéticas intensificadas em condições adversas, como aumento da ventilação branquial e da atividade locomotora, que auxiliam na sobrevivência do peixe diante de estressores ambientais e de manejo (BARTON E IWAMA, 1991; MOMMSEN et al., 1999).

Por outro lado, o lactato surge como um subproduto do metabolismo anaeróbico, ativado quando a disponibilidade de oxigênio para os tecidos é insuficiente para suprir as demandas metabólicas. Durante o transporte pré-abate, os níveis plasmáticos de lactato elevam-se, indicando uma adaptação fisiológica a condições de hipóxia transitória. O acúmulo de lactato no plasma e nos tecidos musculares não apenas sinaliza um estado de estresse metabólico, mas também afeta diretamente a qualidade do filé, reduzindo o pH muscular e comprometendo atributos sensoriais, como textura e sabor. Assim, tanto o lactato quanto a glicose são parâmetros fundamentais para a avaliação do bem-estar animal e da qualidade final do produto em práticas de manejo na aquicultura (BEECHAM et al., 2006; PANKHURST, 2011).

De acordo com Pongsetkul et al. (2022), densidades de transporte superiores a 300 kg/m³ induzem estresse em tilápias, refletido por níveis elevados de glicose (1,76 g/L) e lactato plasmáticos (49,20 mmol/L) em densidades de 420 kg/m³. Esse aumento decorre do metabolismo anaeróbico causado pela diminuição na qualidade da água e pela limitação de oxigênio, características de altas densidades. Em contraste, densidades mais baixas, como 60 kg/m³ e 180 kg/m³, mantêm níveis menores desses biomarcadores, indicando condições menos estressantes que favorecem a homeostase metabólica.

As variações térmicas durante o transporte pré-abate de tilápias exercem influência sobre o estresse metabólico, com impactos nos níveis plasmáticos de glicose e lactato. Em condições de baixas temperaturas, abaixo da faixa ideal para a espécie (22 °C a 28 °C), observa-se uma redução acentuada na atividade metabólica, caracterizada por concentrações plasmáticas reduzidas de glicose e um acúmulo mais lento de lactato. Essa resposta metabólica atenuada reflete uma estratégia adaptativa à supressão das demandas energéticas impostas pelo ambiente frio, diminuindo temporariamente os efeitos do estresse. No entanto, essa redução na atividade metabólica compromete a capacidade dos peixes de responder a estressores, evidenciando sua vulnerabilidade fisiológica em condições subótimas (KINDLE; WHITMORE, 1986; WHITNEY, 2010).

Em contraste, temperaturas superiores a 26 °C intensificam os desafios fisiológicos enfrentados durante o transporte, aumentando os níveis plasmáticos de glicose e lactato. A elevação térmica amplifica a atividade metabólica, estimulando processos como glicogenólise e gliconeogênese. Contudo, a redução da solubilidade do oxigênio dissolvido em águas mais quentes exacerba o metabolismo anaeróbico, resultando em um acúmulo rápido de lactato e alterações no equilíbrio ácido-base. Essas condições não apenas intensificam o estresse fisiológico, mas também comprometem a qualidade muscular. Assim, o controle rigoroso da temperatura durante o transporte é indispensável para manter o bem-estar dos peixes e a integridade do filé (JUN, 2011; QIANG et al., 2012).

2.5.2 Estresse Oxidativo em Peixes

O estresse oxidativo em tilápias é exacerbado pelo desequilíbrio entre a produção de espécies reativas de oxigênio (ROS) e os sistemas antioxidantes endógenos responsáveis por neutralizá-las. Esse processo fisiológico desencadeia respostas celulares complexas, evidenciadas pela alteração nos biomarcadores antioxidantes, como glutationa redutase (GR), catalase (CAT) e lipoperoxidação (LPO). A glutationa redutase é uma enzima fundamental que atua na regeneração do glutationa reduzida (GSH), um dos principais antioxidantes celulares, responsável por neutralizar diretamente os radicais livres e preservar a homeostase redox. Sob

condições estressantes, como no transporte pré-abate, a atividade de GR é intensificada como uma resposta adaptativa à sobrecarga de ROS, refletindo o esforço celular para manter a integridade redox. Paralelamente, a catalase, uma enzima crítica na detoxificação do peróxido de hidrogênio (H_2O_2), converte-o em água e oxigênio, e sua atividade também aumenta em resposta ao estresse oxidativo induzido por hipoxia, superlotação e outras condições adversas associadas ao manejo (CHOWDHURY et al., 2020).

Já a lipoperoxidação representa um indicador direto do dano oxidativo, pois envolve a degradação de ácidos graxos poliinsaturados presentes nas membranas celulares. O aumento nos níveis de LPO durante o transporte pré-abate é um reflexo claro do comprometimento estrutural das membranas, levando à perda de integridade celular e à disfunção fisiológica. Esses efeitos fisiológicos não apenas afetam o bem-estar animal, mas também impactam negativamente atributos como a qualidade do filé (CHOWDHURY et al., 2020).

De acordo com Goes et al. (2019) e Yang et al. (2024), as densidades de transporte influenciam diretamente os parâmetros de estresse oxidativo em tilápias. Em altas densidades, como 300 kg/m³, os autores observaram um aumento na atividade das enzimas antioxidantes, como a catalase (CAT) e a glutationa redutase (GSH), como resposta ao estresse elevado causado pela deterioração da qualidade da água e pelo confinamento. Contudo, com a exposição prolongada, essas respostas compensatórias se esgotam, levando à redução da atividade enzimática e ao aumento dos níveis de lipoperoxidação (LPO), refletidos pelo acúmulo de malonaldeído (MDA), um claro indicativo de danos celulares e perda da integridade fisiológica.

Por outro lado, em baixas densidades, como 60 kg/m³, os autores relataram uma menor alteração nos parâmetros antioxidantes. Nessas condições, a atividade de GSH e CAT permaneceu próxima aos níveis basais, indicando um menor grau de estresse fisiológico. A qualidade da água foi mais bem mantida, o que limitou a produção de espécies reativas de oxigênio (ROS) e reduziu os níveis de lipoperoxidação. Dessa forma, os peixes transportados em densidades mais baixas apresentaram menor comprometimento celular e melhor capacidade de manutenção da homeostase metabólica, reforçando a importância de um manejo adequado para a preservação do bem-estar animal (GOES et al., 2019; YANG et al., 2024).

Segundo Zutshi et al. (2020), variações extremas de temperatura durante o

transporte de peixes têm um impacto determinante no estresse oxidativo e na capacidade adaptativa dos organismos. Sob altas temperaturas, o aumento acentuado do metabolismo induz uma produção excessiva de espécies reativas de oxigênio (ROS), desencadeando uma ativação compensatória das enzimas antioxidantes, como catalase (CAT) e glutationa reduzida (GSH). Essa resposta inicial visa mitigar os efeitos oxidativos, mas a exposição prolongada supera a capacidade celular de defesa, levando ao acúmulo de malonaldeído (MDA). Em contraste, baixas temperaturas, ao reduzirem o metabolismo global, limitam a geração de ROS e mantêm a atividade antioxidante, incluindo CAT e GSH, em níveis mais estáveis. Contudo, a menor demanda antioxidante também implica uma capacidade de resposta mais restrita diante de desafios adicionais, evidenciando a vulnerabilidade dos peixes em ambientes frios. Esses achados ressaltam a necessidade de um controle rigoroso das condições térmicas durante o manejo pré-abate, garantindo a mitigação do estresse oxidativo e a preservação do bem-estar animal.

2.6 Qualidade de Carne de Tilápia

A qualidade da carne de peixe é determinada por uma complexa interação de fatores que abrangem desde características genéticas até manejos específicos na fase pré-abate, tornando essencial o entendimento dessas variáveis para a otimização do produto. Becker (2002) define a qualidade da carne por duas perspectivas complementares: objetiva e subjetiva. A avaliação objetiva corresponde a parâmetros mensuráveis, amplamente utilizados na cadeia produtiva e na ciência da carne, permitindo a análise precisa de atributos físicos, químicos e tecnológicos. Por outro lado, a qualidade subjetiva está intrinsicamente relacionada às percepções do consumidor, que reconhece o produto como de qualidade com base em atributos sensoriais, nutricionais, éticos e sanitários (RAMOS; GOMIDE, 2007).

Entre os fatores que influenciam diretamente a qualidade do filé, destaca-se o estresse pré-abate, que pode desencadear uma série de respostas fisiológicas que impactam diretamente a estabilidade estrutural do tecido muscular. Entre essas respostas, destaca-se o aumento da secreção de muco cutâneo, um componente essencial na defesa imunológica e na homeostase osmótica dos peixes. No entanto,

sob condições de estresse, a composição desse muco pode ser alterada, favorecendo a proliferação microbiana e promovendo um ambiente propício para a ativação de enzimas proteolíticas e lipolíticas. Metaloproteases e serino proteases, notoriamente associadas a processos de degradação proteica, tornam-se mais ativas, promovendo a quebra das proteínas estruturais do músculo. Esse processo resulta em uma desorganização da matriz proteica, reduzindo a coesão do tecido muscular e aumentando a exsudação de líquidos. Como consequência, ocorre uma deterioração da qualidade sensorial do filé, comprometendo atributos fundamentais como textura e suculência, os quais são essenciais para a aceitabilidade do produto pelo consumidor (PÉREZ-SÁNCHEZ et al. 2017; MURPHY et al. 2020).

O pH muscular, a capacidade de retenção de água (CRA) e os parâmetros de cor são amplamente reconhecidos como indicadores críticos da qualidade de produtos cárneos. O pH muscular é um dos principais parâmetros afetados durante o *rigor mortis* em peixes, sendo influenciado pela transição metabólica para a glicólise anaeróbica. Após o abate, a interrupção do suprimento de oxigênio induz a depleção das reservas de ATP, ativando o metabolismo anaeróbico. Nessa fase, o glicogênio muscular é metabolizado em ácido lático, acumulando-se nas fibras musculares e resultando em uma redução no pH, que passa de valores iniciais de aproximadamente 7,3 em peixes vivos para intervalos entre 6,0 e 6,8, dependendo das condições de manejo pré-abate (SANTOS, 2013).

A capacidade de retenção de água é diretamente impactada pelas mudanças no pH muscular e pela estrutura proteica durante o *rigor mortis*. A redução no pH afeta as cargas elétricas das proteínas miofibrilares, particularmente em valores próximos ao ponto isoelétrico, onde a carga líquida das proteínas é mínima. Nesse estado, a interação das proteínas com as moléculas de água é prejudicada, resultando na liberação de líquidos intracelulares para o espaço extracelular, frequentemente observada como exsudação. A desnaturação proteica, intensificada por fatores de estresse pré-abate, como altas densidades de transporte e temperaturas extremas, agrava a perda de CRA, afetando negativamente atributos sensoriais como textura e suculência (HERTOG-MEISCHKE et al., 1997).

Além disso, os parâmetros de cor, representados pelos componentes L* (luminosidade), a* (intensidade do vermelho) e b* (intensidade do amarelo), estão intrinsecamente ligados ao *rigor mortis* e às alterações no pH muscular. A redução do

pH desencadeia alterações nas proteínas miofibrilares e sarcoplasmáticas, modificando a refração e a absorção da luz pelo tecido muscular. Uma acidificação acentuada eleva a luminosidade (L*), conferindo ao filé uma aparência mais pálida, frequentemente associada à exsudação de líquidos e compactação das fibras musculares. O componente a* é geralmente reduzido em condições de hipóxia severa antes do abate, devido à oxidação de hemoglobina e mioglobina em metamioglobina, resultando em uma coloração menos vibrante. Em contrapartida, o componente b* tende a aumentar como resultado da oxidação lipídica, intensificada pela degradação das membranas celulares durante o *rigor mortis* (HUSS, 1995; HALLIER et al., 2007).

Estudos conduzidos por Roth et al. (2012) e Goes et al. (2019) evidenciam que densidades elevadas de transporte, exacerbam o estresse fisiológico em tilápias, resultando em uma intensificação da glicólise anaeróbica e no consequente acúmulo de ácido lático, fatores que promovem uma redução do pH muscular. Esse cenário de estresse também compromete a capacidade de retenção de água (CRA), refletindo-se em maior exsudação de líquidos intracelulares e alterações estruturais nas proteínas miofibrilares, prejudicando atributos sensoriais como textura e suculência. Por outro lado, densidades mais baixas, mitigam os efeitos adversos do manejo, favorecendo a manutenção do equilíbrio metabólico, preservando tanto o pH quanto a CRA, e assegurando a qualidade do produto.

A temperatura durante o transporte é igualmente determinante para a qualidade do filé. De acordo com Tacchi et al. (2015), temperaturas elevadas intensificam o metabolismo anaeróbico, acelerando a acidificação muscular e reduzindo a CRA, enquanto temperaturas mais baixas retardam a degradação post mortem, permitindo a preservação da CRA e dos parâmetros de cor. No entanto, temperaturas extremamente baixas podem interferir no *rigor mortis*, afetando a uniformidade estrutural e a qualidade sensorial do filé. Esses achados corroboram as observações de Lakshmanan et al. (2007), que destacam o papel das condições de estresse e da força iônica na CRA, influenciando diretamente as características tecnológicas e sensoriais do pescado.

Quanto aos parâmetros de cor, altas densidades e temperaturas elevadas provocam alterações. A redução de L* (luminosidade) é atribuída à acidificação muscular e à perda hídrica, enquanto a diminuição de a* (vermelho) decorre da baixa oxigenação e do acúmulo de metamioglobina. Em contrapartida, o aumento de b*

(amarelo) reflete os processos de oxidação lipídica, intensificados pela degradação das membranas celulares (HALLIER et al., 2007). O transporte sob condições ideais de densidade e temperatura, por sua vez, mantém os parâmetros de cor em níveis desejáveis, garantindo uma aparência mais uniforme e fresca ao filé.

Alterações nas proteínas miofibrilares e sarcoplasmáticas, induzidas pelo *rigor mortis*, impactam diretamente as propriedades mecânicas e sensoriais do músculo, destacando a importância da análise do perfil de textura como ferramenta para monitorar e otimizar a qualidade da carne de tilápias (LAKSHMANAN et al., 2007).

A análise do perfil de textura (TPA) desempenha um papel crucial na avaliação da qualidade sensorial e tecnológica de filés de peixes, fornecendo informações detalhadas sobre as alterações bioquímicas e mecânicas que ocorrem durante o armazenamento, transporte e manuseio pré-abate. Esse método instrumental é amplamente utilizado para medir parâmetros que influenciam diretamente a percepção sensorial e funcionalidade do músculo, permitindo uma avaliação integrada da qualidade estrutural e funcional dos filés (HUSS, 1995).

Entre os parâmetros avaliados pela TPA, a dureza se destaca como uma medida da força máxima necessária para comprimir o alimento, correlacionando-se com a rigidez e a resistência do músculo. Filés com altos valores de dureza indicam textura firme e frescor, enquanto valores reduzidos podem refletir degradação estrutural. A adesividade, por sua vez, mensura a força requerida para superar a adesão entre o filé e o instrumento de medição, sendo um indicador da integridade estrutural do produto. A elasticidade, relacionada à capacidade do músculo de retornar à sua forma original após compressão, é um parâmetro que distingue filés frescos e de alta qualidade daqueles comprometidos por degradação proteica (HALLIER et al., 2007).

Parâmetros como coesividade, gomosidade, mastigabilidade e resiliência complementam essa análise, fornecendo informações adicionais sobre a integridade estrutural e funcionalidade do músculo. A coesividade reflete a resistência interna do filé à fragmentação, enquanto a gomosidade e a mastigabilidade avaliam, respectivamente, a energia necessária para fragmentar e mastigar o filé até que esteja pronto para ser engolido. Já a resiliência mede a capacidade do músculo de recuperar sua forma após a deformação inicial, sendo uma característica diretamente associada ao frescor e à qualidade mecânica do filé (LAKSHMANAN et al., 2007).

Esses parâmetros tornam-se ainda mais relevantes quando correlacionados aos fatores de manejo pré-abate, como densidade de transporte, temperatura e condições de estresse, que afetam não apenas o pH muscular, a capacidade de retenção de água (CRA) e os parâmetros de cor, mas também a textura do filé (HERTOG-MEISCHKE et al., 1997).

Zhang et al. (2022) demonstraram que densidades elevadas de estocagem pré-abate em tilápias intensificam o estresse fisiológico, resultando em filés com maior dureza e redução na elasticidade, coesividade e mastigabilidade, em comparação a densidades mais baixas. Essas alterações foram atribuídas ao aumento da glicólise anaeróbica e ao acúmulo de ácido lático, que provocaram a acidificação muscular e modificações nas propriedades das proteínas miofibrilares. O estudo destaca que o manejo adequado das densidades é essencial para preservar a qualidade sensorial e estrutural dos filés, atendendo às demandas do mercado e ao bem-estar animal.

Portanto, práticas de manejo pré-abate que assegurem o controle rigoroso da densidade de transporte e da temperatura tornam-se fundamentais para atenuar os efeitos adversos associados ao *rigor mortis*. Tais práticas contribuem para a preservação de parâmetros críticos, como o pH muscular, a capacidade de retenção de água (CRA), os atributos de cor e o perfil de textura, que são determinantes para a qualidade sensorial e tecnológica dos filés de pescado.

REFERÊNCIAS

ANIMAL BUSINESS BRASIL. Relatório técnico: Desempenho da tilapicultura brasileira. Animal Business, v. 12, n. 1, p. 34-45, 2019.

BARTON, B. A.; IWAMA, G. K. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases*, v. 1, p. 3-26, 1991.

BECKER, T. Defining meat quality. In: Meat processing: improving quality. *New York: CRC Press*, 2002. p. 3-24.

BEECHAM, R. V.; SMALL, B. C.; MINCHEW, C. D. Using portable lactate and glucose meters for catfish research: acceptable alternatives to established laboratory methods? *North American Journal of Aquaculture*, v. 68, p. 291-295, 2006.

BHAT, Z. F.; MORTON, J. D.; MASON, S. L.; BEKHIT, A. E. D. A. Role of calpain system in meat tenderness: A review. *Food Science and Human Wellness,* v. 7, n. 3, p. 196-204, 2018. DOI: https://doi.org/10.1016/j.fshw.2018.08.002.

BORTOLETTI, M.; FONSATTI, E.; LEVA, F.; MACCATROZZO, L.; BALLARIN, C.; RADAELLI, G.; CABERLOTTO, S.; BERTOTTO, D. Influence of transportation on stress response and cellular oxidative stress markers in juvenile meagre (Argyrosomus regius). *Animals*, v. 13, n. 20, 2023. DOI: https://doi.org/10.3390/ani13203288.

BRETT, J. R. Implications and assessment of environmental stress. In: LARKIN, P. A. (Ed.). The investigation of fish-power problems. Vancouver: *MacMillan Lectures in Fisheries*, 1958. p. 69-83.

CANNON, W. Stress and strains of homeostasis. American Journal of the Medical

Sciences, v. 189, p. 1-14, 1935.

CARDOSO, C. R.; SILVA, A. L.; PEREIRA, F. M.; OLIVEIRA, J. S.; SOUZA, M. R.; ANDRADE, L. C.; COSTA, D. M. Genetic improvement in tilapia: Advances and challenges. *Aquaculture International*, v. 29, n. 4, p. 1012-1025, 2021. DOI: https://doi.org/10.1007/s10499-020-00630-2.

CHOWDHURY, A.; KHAN, S. K.; SARKAR, S.; AHMED, R.; RAHMAN, T.; AKTER, F. Oxidative stress biomarkers in fish during pre-slaughter handling and transportation. *Aquaculture*, v. 532, p. 736010, 2020. DOI: https://doi.org/10.1016/j.aquaculture.2020.736010.

DA SILVA, B. C.; DA COSTA MARCHIORI, N.; SILVA, F. M.; MASSAGO, H. Genetic gain after one generation of individual selection for final weight and morphometric variables in tilapias. *Revista de Ciências Agroveterinárias*, v. 18, n. 1, p. 103-110, 2019. DOI: https://doi.org/10.5965/223811711812019103.

EMATER. Criação de tilápias. Empresa de Assistência Técnica e Extensão Rural do Distrito Federal, 2018. Disponível em: https://emater.df.gov.br/wp-content/uploads/2018/06/Cria%C3%A7%C3%A3o-de-til%C3%A1pias.pdf. Acesso em: 13 mar. 2025.

GEESINK, G. H.; KOOHMARAIE, M. Calpain/calpastatin system and meat tenderness. *Trends in Food Science & Technology*, v. 15, n. 1, p. 81-87, 2004. DOI: https://doi.org/10.1016/j.tifs.2003.09.001.

GOES, E. S. R.; SILVA, M. F.; LIMA, R. A.; OLIVEIRA, A. P.; CARVALHO, L. C. Transport density and oxidative stress in Nile tilapia: Impacts on fillet quality. *Aquaculture,* v. 514, p. 734-742, 2019. DOI: https://doi.org/10.1016/j.aquaculture.2019.734742.

HALLIER, A.; AUBRY, J.; MARTEL, P.; BRÉE, A. The effect of slaughter conditions

on colour and texture of fish muscle. *Journal of Food Science and Technology*, v. 42, p. 131-142, 2007. DOI: 10.1007/s13197-007-0023-4.

HAMZAH, A.; THOA, N. P.; NGUYEN, N. H. Genetic analysis of a red tilapia (*Oreochromis spp*.) population undergoing three generations of selection for increased body weight at harvest. *Journal of Applied Genetics*, v. 58, n. 4, p. 509-519, 2017. DOI: https://doi.org/10.1007/s13353-017-0411-8.

HERTOG-MEISCHKE, M. J. A.; VAN LAGENDONK, J.; PEARCE, M. C. J.; HOLLINGWORTH, D.; KOOLMEES, P. A. The effects of stress on meat quality of fish. *Meat Science*, v. 45, n. 1, p. 71–79, 1997.

JIANG, X.; WANG, J.; LI, S.; LIU, Y.; ZHOU, X.; ZHANG, W. Biomarkers of stress in fish exposed to pre-slaughter handling: A review. *Aquaculture*, v. 500, p. 712-726, 2017. DOI: https://doi.org/10.1016/j.aquaculture.2017.01.010.

JUN, L. Plasma biochemical response to handling and acute temperature stress in Nile tilapia (*Oreochromis niloticus*). *Aquaculture Research*, v. 42, n. 5, p. 706-710, 2011. DOI: 10.1111/j.1365-2109.2010.02664.x.

KINDLE, A.; WHITMORE, D. H. Physiological responses of tilapia to cold stress. *Aquaculture*, v. 50, p. 245-252, 1986. DOI: https://doi.org/10.1016/0044-8486(86)90210-5.

LAKSHMANAN, R.; KAPOOR, R.; GANESH, T.; SRIVASTAVA, A.; THOMAS, R. Quality of tilapia muscle during pre-slaughter handling: A biochemical and textural analysis. *Food Chemistry,* v. 107, n. 3, p. 1155-1162, 2007. DOI: https://doi.org/10.1016/j.foodchem.2007.08.040.

LALU, O.; WIDYAISWARA, M.; BALAI, A.; PERTANIAN, P.; PERKEBUNAN, D.; NTB, P. Heritabilitas dan perolehan genetik pada bobot ikan nila hasil seleksi. *E-Jurnal Binawakya*. DOI: http://ejurnal.binawakya.or.id/index.php/MBI.

751 **MAPA.** Ministério da Agricultura, Pecuária e Abastecimento. Manual de Boas
752 Práticas no Transporte de Peixes. Brasília: MAPA, 2022.

753 **MARTINS DA ROCHA, R.; CARVALHO, E. G.; URBINATI, E. C.** Physiological
754 responses associated with capture and crowding stress in matrinxã Brycon cephalus
755 (Gunther, 1869). *Aquaculture Research*, v. 35, p. 245-249, 2004.

756 **MAZEAUD, M. M.; MAZEAUD, F.** Adrenergic responses to stress in fish. In:
757 PICKERING, A. D. *Stress in fish. London: Academic Press*, 1981. p. 49-75.

758 **MOMMSEN, T. P.; VIJAYAN, M. M.; MOON, T. W.** The physiology of stress in fish:
759 Review of biochemical responses. *Fish Physiology and Biochemistry*, v. 20, n. 4, p.
760 267-276, 1999. DOI: https://doi.org/10.1023/A:1007748906954.

761 **MORAIS, C. A. R. S.; SILVA, J. G.; MARTINS, A. A.; COSTA, P. R.** Effect of
762 slaughter weight on the quality of Nile tilapia fillets. *Aquaculture,* v. 520, 2020. DOI:
763 https://doi.org/10.1016/j.aquaculture.2020.734941.

764 **MURPHY, M. J.; NEWBY, J. M.; BUTOW, P.; LOUGHNAN, S. A.; JOUBERT, A. E.;**
765 **KIRSTEN, L.; ALLISON, K.; SHAW, J.; SHEPHERD, H. L.; SMITH, J.; ANDREWS,**
766 **G.** Randomised controlled trial of internet-delivered cognitive behaviour therapy for
767 clinical depression and/or anxiety in cancer survivors (iCanADAPT Early). *Psycho-*
768 *Oncology*, v. 29, n. 1, p. 76-85, 2020. DOI: 10.1002/pon.5267.

769 **OBA, E. T.; MARIANO, V. S.; SANTOS, L. R. B.** Estresse em peixes cultivados:
770 agravantes e atenuantes para o manejo rentável. In: Manejo e sanidade de peixes
771 em cultivo. *Macapá: Embrapa Amapá*, 2009.

772 **ORINA, P. S.; MWAI, O. N.; WANJALA, R.; NJOROGE, L.; ODERO, A.** Effects of
773 transport density on fish welfare and fillet quality. *Aquaculture Research*, v. 45, n. 6,
774 p. 1090-1100, 2014. DOI: https://doi.org/10.1111/are.12394.

PAN, Y.; WANG, X.; ZHANG, L.; JIANG, H. Effects of transport density on survival and stress in grass carp (*Ctenopharyngodon idella*). *Aquaculture*, v. 24, p. 112–118, 2010. DOI: 10.1016/j.aquaculture.2009.12.015.

PANKHURST, N. W. The endocrinology of stress in fish: an environmental perspective. *General and Comparative Endocrinology*, v. 170, p. 265-275, 2011. DOI: 10.1016/j.ygcen.2010.07.017.

PANORAMA DA AQUICULTURA. A evolução da tilapicultura no Brasil. Revista Panorama da Aquicultura, 2003. Disponível em: https://panoramadaaquicultura.com.br/a-evolucao-da-tilapicultura-no-brasil/. Acesso em: 13 mar. 2025.

PARVATHY, U.; BINSI, P. K.; KUMAR, S.; MURALI, S.; RAVISHANKAR, C. N. Live fish transportation: technology assuring quality. Aqua Star, Kochi: *ICAR-Central Institute of Fisheries Technology*, mar. 2019. DOI: 10.13140/RG.2.2.12345.67890.

PEIXE BR. Associação Brasileira da Piscicultura. Anuário da Piscicultura Brasileira 2024. São Paulo: Peixe BR, 2024.

PÉREZ-SÁNCHEZ, C.; AGUIRRE, M. Á.; RUIZ-LIMÓN, P.; ÁBALOS-AGUILERA, M. C.; JIMÉNEZ-GÓMEZ, Y.; ARIAS-DE LA ROSA, I.; RODRIGUEZ-ARIZA, A.; FERNÁNDEZ-DEL RÍO, L.; GONZÁLEZ-REYES, J. A.; SEGUI, P.; COLLANTES-ESTÉVEZ, E.; BARBARROJA, N.; VELASCO, F.; SCIASCIA, S.; CECCHI, I.; CUADRADO, M. J.; VILLALBA, J. M.; LÓPEZ-PEDRERA, C. Ubiquinol effects on antiphospholipid syndrome prothrombotic profile: a randomized, placebo-controlled trial. *Arteriosclerosis, Thrombosis, and Vascular Biology*, v. 37, n. 10, p. 1923-1932, 2017. DOI: 10.1161/ATVBAHA.117.309225.

PONGSETKUL, J.; SUWAN, K.; INTHA, S.; SUWANACHAN, S.; SUKSAMRAN, S. Transport density and physiological stress in Nile tilapia: Impacts on plasma biomarkers and fillet quality. *Aquaculture,* v. 546, p. 737-749, 2022. DOI:

https://doi.org/10.1016/j.aquaculture.2021.737749.

QIANG, J.; YANG, H.; WANG, H.; KALE, A.; KUMAR, S. Responses of plasma cortisol, glucose, and lactate to acute temperature stress in genetically improved Nile tilapia (*Oreochromis niloticus*). *Fish Physiology and Biochemistry*, v. 38, n. 4, p. 1069-1076, 2012. DOI: 10.1007/s10695-011-9583-5.

RAMOS, E. M.; GOMIDE, L. A. M. Avaliação da qualidade de carnes: fundamentos e metodologias. Viçosa: Editora UFV, 2007.

REFAEY, M. M.; TIAN, X.; TANG, R.; LI, D. Changes in physiological responses, muscular composition and flesh quality of channel catfish (*Ictalurus punctatus*) suffering from transport stress. *Aquaculture*, v. 478, p. 9-15, 2017. DOI: https://doi.org/10.1016/j.aquaculture.2017.01.026.

REIS NETO, R. V.; SOUZA, E. P.; MACHADO, T. S.; NASCIMENTO, F. G. Genetic parameters and trends of morphometric traits of GIFT tilapia under selection for weight gain. *Scientia Agricola*, v. 71, n. 4, p. 259-265, 2014. DOI: https://doi.org/10.1590/0103-9016-2013-0294.

ROTH, B.; MOE, S.; MILLA, P.; GORDON, J. Pre-slaughter stress and fish quality: Physiological and sensory effects. *Journal of Aquatic Food Product Technology*, v. 21, n. 3, p. 1-10, 2012. DOI: https://doi.org/10.1080/10498850.2012.672688.

SALES, R. O.; OLIVEIRA, A. C. Evaluation of chemical composition, mineral, amino acid profile and fatty acid acid silage of Nile tilapia *Oreochromis niloticus (Linnaeus)* cultured in Indaiatuba - SP. *Revista Brasileira de Higiene e Sanidade Animal*, v. 9, n. 3, 2015. DOI: https://doi.org/10.5935/1981-2965.20150033.

SANTOS, C. A. F. Qualidade e segurança de alimentos de origem animal. *Revista de Tecnologia e Ciência Animal,* v. 8, p. 16-24, 2013.

SCHULTER, R.; VIEIRA FILHO, J. E. R. Histórico e desenvolvimento da tilapicultura no Brasil. *Revista Brasileira de Zootecnia*, v. 36, p. 48-54, 2017.

SIMÕES, M. R.; DE FÁTIMA, C.; RIBEIRO, A.; DA CONCEIÇÃO, S.; PARK, K. J.; MURR, F. E. X. Composição físico-química, microbiológica e rendimento do filé de tilápia tailandesa (*Oreochromis niloticus*). *Ciência e Tecnologia de Alimentos*, v. 27, n. 3, p. 1-7, 2007. DOI: 10.1590/S0101-20612007000300001.

TACCHI, L.; SECOMBES, C. J.; HARRISON, A. J.; KOPPANG, E. O. Environmental stress and fish health: thermal effects on muscle pH and fillet quality. *Aquaculture Research*, v. 46, p. 1234–1243, 2015. DOI: 10.1111/are.12267.

TAYLOR, R. G.; GEESINK, G. H.; THOMPSON, V. F.; KOOHMARAIE, M.; GOLL, D. E. Is Z-disk degradation responsible for postmortem tenderization? *Journal of Animal Science*, v. 73, n. 5, p. 1351–1367, 1995.

WHITNEY, J. L. The physiological and behavioral responses of rainbow trout (*Oncorhynchus mykiss*) to transportation stress under varying environmental conditions. *Aquaculture Research*, v. 41, p. 695–701, 2010. DOI: 10.1111/j.1365-2109.2009.02350.x.

WU, X.; CHEN, Z.; HU, Q.; YANG, J. Water quality and fish welfare during transport: A comprehensive review. *Aquaculture Research*, v. 49, p. 112-123, 2018. DOI: https://doi.org/10.1111/are.13435.

YANG, Z.; CHEN, H.; TANG, X.; LI, Y.; XU, F. Stress oxidative biomarkers in fish: Effects of density and temperature. *Aquaculture,* v. 560, p. 737501, 2024. DOI https://doi.org/10.1016/j.aquaculture.2023.737501.

YOSHIDA, G. M.; OLIVEIRA, C. A. L.; KUNITA, N. M.; OLIVEIRA, S. N.; ALEXANDRE FILHO, L.; RESENDE, E. K.; LOPERA-BARRERO, N. M.; RIBEIRO, R. P. Resposta à seleção de características de desempenho e morfométricas de

tilápia-do-nilo ao longo do período de cultivo. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, v. 65, n. 6, p. 1815-1822, 2013. DOI: https://doi.org/10.1590/S0102-09352013000600032.

ZARDIN, A. M. S. O. Impacto da seleção genética na morfometria e desempenho de tilápias do Nilo. Maringá: Universidade Estadual de Maringá, 2016. *Trabalho de conclusão de curso.*

ZHANG, X.; WANG, L.; LIU, F.; DONG, H.; TANG, Z. Impact of stocking density on the quality and texture of tilapia fillets. *Food Science and Technology*, v. 60, p. 1-12, 2022. DOI: https://doi.org/10.1016/j.foodres.2021.110903.

ZIMMERMAN, A. M.; LOWERY, M. S. Hyperplastic development and hypertrophic growth of muscle fibers in the white seabass (Atractoscion nobilis). J*ournal of Experimental Zoology*, v. 284, n. 3, p. 299-308, 1999. DOI: https://doi.org/10.1002/(SICI)1097-010X(19990801)284:3<299::AID-JEZ7>3.0.CO;2-.

ZUTSHI, B.; SINGH, J.; SINGH, A. K.; CHAUDHARY, D. Impact of transient temperature disturbance on oxidative stress and antioxidant responses in fish. *Environmental Science and Pollution Research*, v. 27, n. 4, p. 4261–4272, 2020. DOI: https://doi.org/10.1007/s11356-019-07012-4.

3 HIPÓTESES

3.1 HIPÓTESE ARTIGO 1

A seleção genética para ganho de peso em tilápias do Nilo promove alterações significativas na expressão dos genes RyR3 e m-calpaína, as quais contribuem diretamente para o aumento do ganho de peso e a melhoria do desempenho produtivo e da qualidade do filé.

3.2 HIPÓTESE ARTIGO 2

A densidade de transporte e as condições sazonais (verão e inverno) interagem, influenciando os biomarcadores de estresse, os parâmetros de qualidade do filé e os indicadores antioxidantes, sendo possível determinar densidades ideais que minimizem o estresse e otimizem a qualidade do produto em cada estação.

4 OBJETIVOS

4.1 OBJETIVO GERAL

Investigar a expressão dos genes RyR3 e m-calpaína em tilápias de alto e baixo valor genético e densidade de transporte no verão e inverno e suas associações com bem-estar animal e qualidade do filé.

4.2 OBJETIVOS ESPECÍFICOS

a. Comparar características morfométricas, peso corporal, peso de filé e rendimento de filé entre tilápias de alto e baixo valor genético;
b. Investigar a expressão dos genes RyR3 e m-calpaína nos grupos genéticos e sua relação com o desempenho muscular
c. Analisar a composição centesimal dos filés (umidade, cinzas, lipídios totais e proteína total) nos dois grupos genéticos;
d. Avaliar a qualidade do filé (pH, cor, perda de líquido por descongelamento e por cocção) entre os grupos;
e. Avaliar o impacto das densidades de transporte (375, 425 e 475 kg/m³) e das estações (verão e inverno) sobre os biomarcadores de estresse (glicose e lactato);
f. Investigar como densidade e estação afetam a qualidade do filé (pH, capacidade de retenção de água, cor, textura e oxidação lipídica);
g. Analisar a interação entre densidade e estação nos indicadores de estresse oxidativo (glutationa reduzida e catalase);
h. Identificar a densidade ideal para cada estação, considerando bem-estar e qualidade do filé.

5 ARTICLE 1: EXPRESSION OF RYR3 AND M-CALPAIN GENES AND THEIR ASSOCIATION WITH GROWTH PERFORMANCE AND FILLET QUALITY IN NILE TILAPIA (OREOCHROMIS NILOTICUS) WITH DIVERGENT GENETIC VALUES

**Artigo científico escrito seguindo as normas da revista Aquaculture Research*

This study evaluated growth performance and fillet quality of Nile tilapia (*Oreochromis niloticus*) strains with divergent genetic values for weight gain, with emphasis on the expression of RyR3 and m-calpain genes. A total of 78 fish from the Tilamax breeding program were assigned to two genetic groups: high genetic value (n = 40) and low genetic value (n = 38), in a completely randomized design. White muscle samples were collected for gene expression analysis by RT-qPCR, along with proximate composition and fillet quality assessments, including color, pH, thawing loss (TL), and cooking loss (CL). Data were analyzed using ANOVA followed by Tukey's test ($p < 0.05$). No significant differences were observed in the expression of RyR3 ($p = 0.9260$) and m-calpain ($p = 0.3808$) between genetic groups. However, the high genetic value group showed higher ash content ($p = 0.012$) and total lipid levels ($p = 0.013$). In terms of fillet quality, this group exhibited greater lightness (L*) and chroma ($p < 0.001$), while pH, TL, and CL did not differ between groups. These findings indicate that genetic selection for weight gain may enhance metabolic efficiency and tissue deposition without altering key structural and functional properties of muscle.

Keywords: proximate composition; muscle growth; metabolic efficiency; fillet quality; gene expression; Nile tilapia

1. Introduction

The application of genetic tools in aquaculture has significantly advanced global fish production, promoting improvements in key traits such as accelerated growth, enhanced feed conversion efficiency, and improved product quality. Among the species benefiting most from these programs, Nile tilapia (*Oreochromis niloticus*) stands out due to its phenotypic plasticity and the success of selective breeding strategies targeting weight gain, disease resistance, and fillet yield (Gjedrem et al., 2012; Food and Agriculture Organization, 2023). In this context, Brazil has consolidated its position as the fourth largest global producer of tilapia, reaching 579,080 tons in 2023, representing 65.3% of the national fish production (Peixe BR, 2024).

Among the physiological mechanisms underlying genetic improvement in aquaculture, those involved in the regulation of muscle growth are particularly relevant. These processes are modulated by key genes such as the ryanodine receptor type 3 (RyR3) and m-calpain. Understanding the functional role of these genes is essential to elucidate how genetic selection influences tissue plasticity, protein homeostasis, and the bioeconomic efficiency of growth in improved Nile tilapia strains (Geesink & Koohmaraie, 2004; Bhat et al., 2018).

RyR3 is a transmembrane protein located in the sarcoplasmic reticulum that regulates intracellular calcium homeostasis, a central process for muscle contraction, hypertrophy, and tissue regeneration. This regulation is critical for lean mass deposition and structural adaptation of muscle tissue in genetically improved fish (Taylor et al., 1995; Geesink & Koohmaraie, 2004). Complementarily, m-calpain, a calcium-dependent protease, is involved in the selective degradation of myofibrillar proteins, facilitating muscle remodeling and enabling efficient adaptation to the increased growth rates resulting from genetic selection (Geesink & Koohmaraie, 2004; Bhat et al., 2018).

In addition to their role in growth regulation, these genes may also influence fillet quality. Proximate composition, particularly moisture, protein, lipid, and ash contents, as well as physicochemical attributes such as pH, color, and water-holding capacity, are modulated by the interaction between genetic background and metabolic processes. For instance, stable pH values contribute to muscle integrity and reduce thawing and cooking losses, whereas color attributes such as lightness (L*), chroma, and hue are associated with myofibrillar organization and lipid deposition, which are critical factors for consumer acceptance (Rutten et al., 2005; Souza et al., 2021).

Therefore, this study aimed to evaluate growth performance and fillet quality in Nile tilapia strains with high and low genetic values for weight gain, based on the expression analysis of

RyR3 and m-calpain genes. We hypothesized that divergent genetic values would modulate gene expression patterns associated with muscle growth, thereby influencing metabolic efficiency and fillet quality traits.

2. Materials and Methods

All experimental procedures were approved by the Animal Ethics Committee of the Universidade Estadual de Maringá (CEUA/UEM; protocol no. 229190121).

2.1. Fish origin and selection conditions

The animals used in this study were obtained from the Nile tilapia (*Oreochromis niloticus*) breeding program (Tilamax-UEM), conducted by the Universidade Estadual de Maringá. This program aims to estimate genetic effects related to growth rate in cage culture systems and is based on a hierarchical mating design, in which one male is paired with a single female.

Fingerlings were individually identified using passive transponders after reaching approximately 10 g of body weight. Reproduction and early rearing were carried out at the Experimental Fish Farming Station of the university. Subsequently, tagged fish were transferred to the Demonstration Unit for Cage Culture Production, located in Diamante do Norte, Paraná, Brazil.

2.2. Experimental design

A completely randomized design was adopted to compare two genetic groups of Nile tilapia with divergent genetic values for body weight: a high genetic value group (Tilamax strain) and a low genetic value group. A total of 78 fish were evaluated, comprising 40 individuals in the high genetic value group and 38 in the low genetic value group.

2.3. Capture, identification, biometric evaluation, and slaughter procedures

Fish were manually captured from cage systems using a hand net. After capture, each individual was identified and allocated to its respective genetic group.

Biometric measurements included total length, width, height, and body weight. Total length was measured using a measuring tape, and both live body weight and fillet weight were recorded using a digital bench scale (Toledo Prix 3 FIT).

Prior to slaughter, fish were anesthetized by immersion in a benzocaine solution (1 g dissolved in 10 mL ethanol diluted in 10 L of water) until complete loss of consciousness. Subsequently, euthanasia was performed by spinal cord severance, following established ethical guidelines to minimize animal distress. Fish were then eviscerated and filleted, and the right fillet from each individual was used for subsequent analyses.

pH and color measurements were performed 45 min postmortem to ensure immediate assessment of these parameters. Thereafter, fillets were individually packaged and stored at −20 °C until further analyses, including proximate composition, thawing loss, and cooking loss.

2.4. Gene expression analysis

For gene expression analysis, white muscle samples were collected from all fish in both experimental groups immediately after slaughter and stored in RNAlater® solution (Invitrogen, Carlsbad, CA, USA) at −80 °C until total RNA extraction. Total RNA was extracted using TRIzol® reagent (Invitrogen), following the manufacturer's protocol, at a ratio of 1 mL of TRIzol® per 100 mg of tissue. RNA concentration was determined using a spectrophotometer at 260 nm.

RT-qPCR reactions were performed using a Roche LightCycler® Nano thermocycler under the following conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 20 s (denaturation), 60 °C for 30 s (annealing), and 72 °C for 30 s (extension). The reaction mixture consisted of 5 µL of Platinum™ SYBR™ Green qPCR SuperMix-UDG (Thermo Fisher Scientific; Cat. No. 11733046), 0.5 µL of each primer (forward and reverse), and 10 ng of total RNA.

The expression of ryanodine receptor type 3 (RyR3) and m-calpain genes (Table 2) was analyzed, using β-actin as the endogenous control gene (Table 2), as described by Morais et al. (2020).

2.5. Proximate composition analysis

2.5.1. Moisture

Moisture content was determined according to AOAC (2016). Samples were dried in an oven at 105 °C for 16–18 h until constant weight.

2.5.2. Ash

Ash content was determined following AOAC (2016). Dried samples were incinerated in a muffle furnace at 550 °C for 4 h, followed by an additional drying step at 110 °C.

2.5.3. Total lipids

Total lipid content was determined using cold extraction with petroleum ether, according to AOAC (2016) protocols.

2.5.4. Crude protein

Crude protein content was determined using the Kjeldahl method, following AOAC (2016) guidelines. Samples were subjected to digestion and distillation, and nitrogen content was quantified by titration.

2.6. Fillet quality assessment

2.6.1. pH measurement

Muscle pH was measured using a portable digital pH meter equipped with a penetration electrode (Testo, model 205), ensuring accurate determination of pH variations in the muscle tissue.

2.6.2. Color

Fillet color was evaluated on the dorsal surface using a portable colorimeter (Minolta CR-10™, Konica Minolta Inc., Osaka, Japan), configured with illuminant D65, a 10° standard observer, and an 8 mm aperture. Color parameters were recorded according to the CIE system as L* (lightness), a* (red–green), and b* (yellow–blue). Measurements were performed in triplicate. Chroma (C*) and hue angle (h*) were calculated from a* and b* values. Chroma, which represents color intensity, was calculated as: $C^* = \sqrt{(a^*)^2 + (b^*)^2}$. Hue angle (h*), representing the color tone, was calculated as: h* = atan2(b∗,a∗) according to Konica Minolta (1998).

2.6.3. Thawing loss

Thawing loss (TL) was determined according to the method described by Cason et al. (1997). Frozen fillet samples were initially weighed without packaging or labeling, and the weight was recorded. Samples were then thawed under refrigeration at approximately 4 °C for 24 h. After thawing, samples were reweighed after carefully removing excess surface moisture using paper towels.

2.6.4. Cooking loss

Cooking loss (CL) was determined according to Cason et al. (1997). A water bath was preheated to 85 °C prior to the start of the procedure. Tilapia fillet samples were individually sealed in plastic bags to prevent water infiltration and labeled to ensure traceability.

A thermometer probe was inserted into the geometric center of one representative sample to monitor internal temperature during cooking. Samples were then placed in the water bath, ensuring they remained separated and fully immersed. Fillets were cooked for 30 min, until the internal temperature reached approximately 75 °C. After cooking, samples were immediately removed, allowed to cool to room temperature, and subsequently reweighed.

2.7. Statistical analysis

All variables, except gene expression data, were tested for normality and homogeneity of variances using the Shapiro–Wilk ($p < 0.05$) and Bartlett tests ($p < 0.05$), respectively. Data were then subjected to one-way analysis of variance (ANOVA), followed by Tukey's post hoc test ($p < 0.05$) for multiple comparisons.

Statistical analyses were performed using Jamovi (version 2.6), based on R (version 4.4).

For gene expression analysis, GraphPad Prism (version 9.0) was used. Differential expression was considered significant when fold-change values were ≥ 2 or ≤ 0.5.

3. Results

3.1. B*iometric evaluation*

Biometric parameters differed between Nile tilapia groups with high and low genetic values for body weight (Table 1). Fish from the high genetic value group exhibited greater total length compared to those from the low genetic value group ($p < 0.001$). A similar pattern was observed

for width ($p < 0.001$) and height ($p < 0.001$), with mean values of 13.88 ± 0.23 cm and 1.26 ± 0.03 cm, respectively, in the high genetic value group, whereas fish from the low genetic value group presented mean values of 10.78 ± 0.27 cm and 0.95 ± 0.02 cm.

Body weight followed the same trend, being significantly higher in the high genetic value group ($p < 0.001$). Fillet weight was also greater in genetically superior fish ($p < 0.001$).

Despite the marked differences in body and fillet weights, fillet yield did not differ between groups, with mean values of 33.5 ± 0.26% for the high genetic value group and 34.0 ± 0.24% for the low genetic value group ($p = 0.188$).

3.2. Gene expression

No significant differences were observed in the expression of the RyR3 gene between genetic groups ($p = 0.9260$) (Figure 1). Similarly, m-calpain gene expression did not differ between groups ($p = 0.3808$) (Figure 2).

Fold-change values remained within the range of 1 to 2, indicating no relevant variation in the expression levels of RyR3 and m-calpain between groups (Figure 3).

3.3. Proximate composition

Moisture content did not differ between groups, with mean values of 24.7 ± 0.22% for the low genetic value group and 24.8 ± 0.36% for the high genetic value group ($p = 0.918$) (Table 3).

However, significant differences were observed for ash and total lipid contents. The high genetic value group showed higher ash content (4.23 ± 0.09%) compared to the low genetic value group (3.67 ± 0.18%) ($p = 0.012$). In addition, total lipid content was greater in the high genetic value group (17.2 ± 0.82%) than in the low genetic value group (14.2 ± 0.64%) ($p = 0.013$) (Table 3).

3.4. Fillet quality indicators

Lightness (L*) was higher in the high genetic value group (47.5 ± 0.17) compared to the low genetic value group (45.4 ± 0.23) ($p < 0.001$). The red–green component (a*) also differed between groups, with more negative values observed in the high genetic value group (−3.00 ± 0.05) compared to the low genetic value group (−2.45 ± 0.07) ($p < 0.001$).

Chroma (C*) was greater in the high genetic value group (15.33 ± 0.36) than in the low genetic value group (12.44 ± 0.28) ($p < 0.001$). Hue angle (h*) was slightly higher in the high genetic value group (20.3 ± 0.40) compared to the low genetic value group (19.6 ± 0.01) ($p < 0.001$). In contrast, no significant differences were observed for pH ($p = 0.221$), the yellow–blue component (b*) ($p = 0.876$), thawing loss (TL) ($p = 0.162$), or cooking loss (CL) ($p = 0.900$).

4. Discussion

4.1.Biometric evaluation

Fish from the high genetic value group exhibited greater growth performance and higher fillet weight, which is consistent with previous studies showing that selective breeding for increased body weight enhances skeletal and muscle development, improving feed efficiency and growth performance (Gjedrem & Baranski, 2009; Rutten et al., 2005). These results reflect the expected outcome of genetic selection programs targeting growth, where individuals with higher protein deposition rates and improved feed utilization are favored (Rescan, 2005).

Despite the greater body and fillet weights observed in the high genetic value group, fillet yield remained unchanged between groups. This finding suggests proportional growth among body components, including muscle, viscera, and skeletal structures, maintaining a stable relationship between fillet mass and total body weight. Similar patterns have been reported in selectively bred fish, where accelerated growth does not necessarily alter carcass yield proportions (de Verdal et al., 2018). Additionally, factors such as lipid distribution and bone development may contribute to limiting variation in fillet yield even in faster-growing fish (Nguyen et al., 2010).

4.2.Gene expression

Selective breeding for growth in Nile tilapia aims to enhance muscle deposition and overall production efficiency while maintaining product quality. Among the molecular mechanisms involved, the ryanodine receptor type 3 (RyR3) and m-calpain play key roles in muscle physiology. RyR3 regulates calcium release from the sarcoplasmic reticulum, a fundamental process for muscle contraction, intracellular signaling, and hypertrophic growth (Taylor et al., 1995; Geesink & Koohmaraie, 2004).

In the present study, RyR3 expression did not differ between genetic groups, indicating that selection for increased body weight did not alter the regulation of this gene. This stability may be advantageous, as disruptions in calcium homeostasis have been associated with impaired meat quality in other species. For instance, altered RyR function has been linked to pale, soft, and exudative (PSE) meat in poultry and swine, due to accelerated postmortem metabolism and rapid pH decline (Fujii et al., 1991; Oda et al., 2009). Therefore, the absence of variation in RyR3 expression suggests that calcium regulation remains preserved, which may contribute to maintaining muscle functionality and fillet quality.

The lack of differential expression also indicates that other molecular pathways may be more influential in driving growth responses in genetically improved tilapia. Selection for growth may act primarily through mechanisms related to metabolic efficiency and protein synthesis, rather than through modulation of calcium-handling genes (Zimmerman & Lowery, 1999; Bhat et al., 2018). The conservation of RyR3 expression may represent an adaptive mechanism that ensures stable muscle physiology under selective pressure.

Similarly, m-calpain expression did not differ between groups, suggesting that the proteolytic system involved in muscle remodeling remains functionally stable. m-Calpain is a calcium-dependent protease responsible for the controlled degradation of myofibrillar proteins, contributing to muscle plasticity and tissue turnover (Geesink & Koohmaraie, 2004). Its stable expression indicates that protein turnover mechanisms are maintained even in fish selected for higher growth rates.

The functional relationship between RyR3 and m-calpain is central to calcium-mediated regulation of proteolysis. Calcium release mediated by RyR3 directly influences m-calpain activation, linking calcium homeostasis to muscle protein remodeling (Taylor et al., 1995; Zimmerman & Lowery, 1999). The stability observed in both genes suggests that this regulatory axis remains conserved, ensuring adequate muscle function and structural integrity.

Overall, these findings indicate that genetic selection for weight gain does not disrupt key regulatory systems associated with calcium signaling and proteolysis. Instead, growth improvements may rely on alternative pathways while preserving essential mechanisms of muscle homeostasis, which is critical for maintaining fillet quality attributes such as texture and water-holding capacity (Bhat et al., 2018).

4.3. Proximate composition

The absence of differences in moisture content between genetic groups indicates stability in muscle water content, supporting previous findings that genetic factors have limited influence on muscle hydration in fish (Lalu et al., 2021). This stability may also reflect improved metabolic efficiency in genetically selected fish, reducing the need for compensatory adjustments in muscle water balance (da Silva et al., 2019). Such efficiency is associated with optimized feed utilization and maintenance of muscle structural integrity during growth (Sales & Oliveira, 2015).

The higher ash content observed in the high genetic value group suggests increased mineral retention, indicating metabolic effects associated with selection for growth. Enhanced mineral deposition, particularly of calcium and phosphorus, may be linked to improved skeletal development required to support accelerated growth (Souza et al., 2021). This pattern is consistent with studies showing that genetically improved fish exhibit greater efficiency in mineral utilization, contributing to structural and functional tissue development (Rutten et al., 2005).

The increase in total lipid content in the high genetic value group indicates greater lipid deposition capacity in muscle tissue. This may reflect enhanced energy allocation efficiency, where excess energy is stored as intramuscular lipids to support the physiological demands of rapid growth. Similar responses have been reported in selectively bred fish under intensive production systems (Sales & Oliveira, 2015). In addition to serving as an energy reserve, intramuscular lipids contribute to membrane integrity and overall muscle functionality (da Silva et al., 2019).

In contrast, crude protein content remained similar between groups, indicating that muscle protein levels are highly conserved despite genetic selection for growth. This suggests that increases in body weight are not primarily driven by changes in protein concentration but may instead be associated with variations in lipid and mineral deposition. Muscle protein represents a relatively stable component of skeletal muscle, regulated by tightly controlled processes of protein synthesis and degradation (Rutten et al., 2005; da Silva et al., 2019).

The maintenance of protein content may also be linked to metabolic efficiency in genetically selected fish, allowing effective utilization of dietary amino acids without altering overall protein composition. This stability is important for preserving key quality attributes such as texture, structural integrity, and nutritional value of the fillet (Kayan et al., 2015; Souza et al., 2021).

4.4. Fillet quality indicators

The higher lightness (L*) observed in fillets from the high genetic value group may be associated with structural and biochemical changes in skeletal muscle influenced by genetic selection. Myofibrillar organization is a key determinant of light reflectance, and improved fiber compactness may enhance light scattering, resulting in increased brightness (Huff-Lonergan & Lonergan, 2005). In addition, the greater intramuscular lipid content observed in this group may have contributed to increased light dispersion, further enhancing fillet lightness (Kayan et al., 2015).

Differences in the red–green component (a*) between groups may reflect variations in muscle composition and metabolic profile. The more negative a* values observed in the high genetic value group suggest lower red pigmentation, which may be associated with a higher proportion of fast-twitch (white) muscle fibers. These fibers rely primarily on anaerobic metabolism and typically exhibit lower myoglobin content compared to oxidative fibers (Damez & Clerjon, 2008; Kayan et al., 2015). Conversely, the relatively higher redness observed in the low genetic value group may be linked to a greater relative contribution of oxidative metabolism and higher pigment stability (Rutten et al., 2005).

The higher chroma (C*) values observed in the high genetic value group indicate increased color saturation, which may be related to structural organization of muscle fibers and lipid deposition. These factors influence light–tissue interactions and contribute to visual appearance (Huff-Lonergan & Lonergan, 2005; Rutten et al., 2005). Similarly, the slightly higher hue angle (h*) suggests subtle differences in color tone, potentially associated with variations in pigment state and muscle composition (Damez & Clerjon, 2008).

No differences were observed for pH, b*, thawing loss (TL), or cooking loss (CL), indicating stability of these physicochemical attributes across genetic groups. Muscle pH is closely related to postmortem glycolysis and lactic acid accumulation, and its stability suggests that genetic selection for growth did not affect postmortem metabolic processes (Damez & Clerjon, 2008). Likewise, TL and CL are associated with water-holding capacity and myofibrillar integrity, and their similarity between groups indicates preservation of muscle structural properties (Huff-Lonergan & Lonergan, 2005).

Overall, these results suggest that genetic selection for increased body weight in Nile tilapia may influence visual attributes such as lightness and color saturation without affecting key physicochemical properties, including pH and water-holding capacity. This pattern indicates that improvements in growth performance can be achieved without compromising fundamental aspects of fillet quality.

5. Conclusion

The results of this study do not support the hypothesis that genetic selection for increased body weight modulates the expression of RyR3 and m-calpain genes, as no differences were observed between genetic groups.

Growth-related selection did not affect proximate composition or key physicochemical traits, including moisture, crude protein, pH, and water losses.

These findings indicate that improved growth performance can be achieved without detectable changes in the expression of these genes or detrimental effects on fillet quality under the conditions evaluated.

Referências

AOAC. (2016). Official Methods of Analysis of AOAC International (20th ed., vol. I e II).

Bhat, Z. F., Kumar, H., & Bhat, H. F. (2018). Calpains and calpastatin in meat quality: A review. Journal of Food Science and Technology, 55(8), 2490–2501. https://doi.org/10.1007/s13197-018-3163-1.

Bhat, Z. F., Morton, J. D., Mason, S. L., & Bekhit, A. E. D. A. (2018). Role of calpain system in meat tenderness: A review. Food Science and Human Wellness, 7(3), 196–204. https://doi.org/10.1016/j.fshw.2018.08.002.

Cason, J. A., Lyon, C. E., & Papa, C. M. (1997). Effect of muscle opposition during rigor on development of broiler breast meat tenderness.

da Silva, B. C., da Costa Marchiori, N., Silva, F. M., & Massago, H. (2019). Genetic gain after one generation of individual selection for final weight and morphometric variables in tilapias. Revista de Ciências Agroveterinárias, 18(1), 103–110. https://doi.org/10.5965/223811711812019103.

Damez, J. L., & Clerjon, S. (2008). Meat quality assessment using biophysical methods related to meat structure. Meat Science, 80(1), 132–149. https://doi.org/10.1016/j.meatsci.2008.05.039.

de Verdal, Hélène, Marc Vandeputte, Walid Mekkawy, Corinne Hervet, e Bernard Chatain. "Selection for Growth in European Sea Bass (Dicentrarchus labrax) Results in Higher Growth Rate but Lower Yield Due to a Higher Viscera Proportion." Aquaculture 495 (2018): 67–74. https://doi.org/10.1016/j.aquaculture.2018.05.035.

FAO. (2023). The State of World Fisheries and Aquaculture 2023: Towards Blue Transformation. Rome: FAO. https://www.fao.org/documents/card/en/c/cb8183en.

Fujii, J., K. Otsu, F. Zorzato, S. Leon, V. K. Khanna, J. E. Weiler, P. J. O'Brien, e D. H. MacLennan. "Identification of a Mutation in Porcine Ryanodine Receptor Associated with Malignant Hyperthermia." Science 253, no. 5018 (1991): 448–451. https://doi.org/10.1126/science.1862346.

Garcia, A. L. S., de Oliveira, C. A. L., Karim, H. M., Sary, C., Todesco, H., & Ribeiro, R. P. (2017). Genetic parameters for growth performance, fillet traits, and fat percentage of male Nile tilapia (*Oreochromis niloticus*). Journal of Applied Genetics, 58(4), 527–533. https://doi.org/10.1007/s13353-017-0413-6.

Geesink, G. H., & Koohmaraie, M. (2004). Postmortem proteolysis and beef tenderization: The role of calpains and calpastatin. Meat Science, 68(3), 305–317. https://doi.org/10.1016/j.meatsci.2004.02.006.

Gjedrem, Trygve, e Maren Baranski. Selective Breeding in Aquaculture: An Introduction. Dordrecht: Springer Science & Business Media, 2009. https://doi.org/10.1007/978-90-481-2773-3.

Gjedrem, T., Rye, M., & Robinson, M. (2012). Advances by selective breeding for aquatic species: A review. Aquaculture, 350–353, 117–129. https://doi.org/10.1016/j.aquaculture.2012.04.008.

Goes, Elenice S. R., Jorge A. F. Lara, Eliane Gasparino, Ana P. Del Vesco, Marcio D. Goes, Luiz Alexandre Filho, e Ricardo P. Ribeiro. "Pre-Slaughter Stress Affects Ryanodine Receptor Protein Gene Expression and the Water-Holding Capacity in Fillets of the Nile Tilapia." PLoS ONE 10, no. 6 (2015): e0129145. https://doi.org/10.1371/journal.pone.0129145.

GraphPad Software, Inc. (2024). GraphPad Prism®, versão 9.0. San Diego, CA. https://www.graphpad.com.

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Science, 71(1), 194–204. https://doi.org/10.1016/j.meatsci.2005.04.022.

Kayan, A., Boontan, I., Jaturssitha, S., Wicke, M., & Kreuzer, M. (2015). Effect of slaughter weight on meat quality of Nile tilapia (Oreochromis niloticus). Agriculture and Agricultural Science Procedia, 5, 159–163. https://doi.org/10.1016/j.aaspro.2015.08.024.

Lalu, O., Widyaiswara, M., Madya Balai, A., Pertanian, P., Perkebunan, D., & Ntb, P. (n.d.). Heritabilitas dan perolehan genetik pada bobot ikan nila hasil seleksi. http://ejurnal.binawakya.or.id/index.php/MBI.

Minolta. (1998). Precise Color Communication: Color Control from Perception to Instrumentation. Japan: Minolta Co., Ltd.

Morais, C. A. R. S., Santana, T. P., Santos, C. A., Passetti, R. A. C., Melo, J. F. B., de Assis Fonseca de Macedo, F., Vieira, J. S., Teixeira, A. J. C., & Del Vesco, A. P. (2020). Effect of slaughter weight on the quality of Nile tilapia fillets. Aquaculture, 520, 734941. https://doi.org/10.1016/j.aquaculture.2020.734941.

National Center for Biotechnology Information (NCBI). (2024). PREDICTED: Oreochromis niloticus calpastatin (cast), transcript variant X1, mRNA. NCBI Reference Sequence: XM_013269963.3. Acessado em 18 de novembro de 2024. https://www.ncbi.nlm.nih.gov/refseq/.

Nguyen, Nhut H., R. W. Ponzoni, H. Y. Yee, K. R. Abu-Bakar, e A. Hamzah. "Can Sexual Dimorphism and Body Shape Be Altered in Nile Tilapia (Oreochromis niloticus) by Genetic Means?" Aquaculture 305, no. 1-4 (2010): 50–56. https://doi.org/10.1016/j.aquaculture.2010.04.004.

Oda, S. H. I., A. L. Nepomuceno, M. C. N. Oliveira, S. R. R. Marin, E. I. Ida, e M. C. Ledur. "Quantitative Differential Expression of Alpha and Beta Ryanodine Receptor Genes in PSE (Pale, Soft, Exudative) Meat from Two Chicken Lines: Broiler and Layer." Brazilian Archives of Biology and Technology 52, no. 6 (2009): 1519–1525. https://doi.org/10.1590/S1516-89132009000600024.

Peixe BR. (2024). Anuário Peixe BR da Piscicultura 2024. Associação Brasileira da Piscicultura. https://www.peixebr.com.br/anuario2024.

R Core Team. (2024). R: A Language and Environment for Statistical Computing. Versão 4.4. https://cran.r-project.org.

Rescan, Pierre Yves. "Muscle Growth Patterns and Regulation during Fish Ontogeny." General and Comparative Endocrinology 142, no. 1-2 (2005): 111–16. https://doi.org/10.1016/j.ygcen.2004.12.026.

Rutten, M. J. M., Bovenhuis, H., & Komen, H. (2005). Genetic parameters for fillet traits and body measurements in Nile tilapia (*Oreochromis niloticus L.*). Aquaculture, 246(1–4), 125–132. https://doi.org/10.1016/j.aquaculture.2005.01.006.

Sales, M. T., & Oliveira, A. C. (2015). Evaluation of chemical composition, mineral, amino acid profile and fatty acid silage of Nile tilapia (Oreochromis niloticus) cultured in Indaiatuba - SP. Revista Brasileira de Higiene e Sanidade Animal, 9(3), 1–8. https://doi.org/10.5935/1981-2965.20150033.

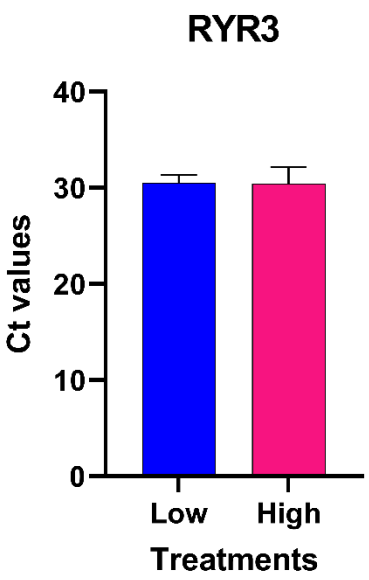
Souza, M. L. R. de, Viegas, E. M. M., Nakaghi, L. S. O., Dourado, D. M., Kronka, S. do N., & Goes, E. S. dos R. (2021). Morfologia, composição centesimal e alterações ocorridas no processo de curtimento da pele da tilápia do Nilo. Research, Society and Development, 10(8), e35810817240. https://doi.org/10.33448/rsd-v10i8.17240.

Taylor, R. G., Geesink, G. H., Thompson, V. F., Koohmaraie, M., & Goll, D. E. (1995). Is Z-disk degradation responsible for postmortem tenderization? Journal of Animal Science, 73(5), 1351–1357. https://doi.org/10.2527/1995.7351351x.

The Jamovi Project. (2024). Jamovi. Versão 2.6. https://www.jamovi.org.

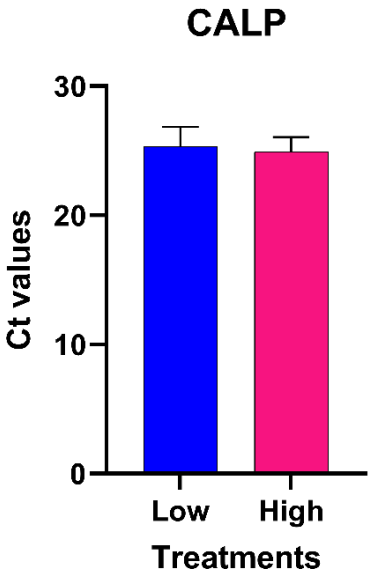
Zimmerman, A. M., & Lowery, M. S. (1999). Hyperplastic development and hypertrophic growth of muscle fibers in the white seabass (Atractoscion nobilis). Journal of Experimental Zoology, 284(3), 299–308. https://doi.org/10.1002/(SICI)1097-010X(19990801)284:3<299::AID-JEZ7>3.0.CO;2-6.

Figure 1: Comparison of RyR3 gene expression in Nile tilapia with high genetic value for weight gain. Values are presented as means, with bars indicating the standard error of the mean.



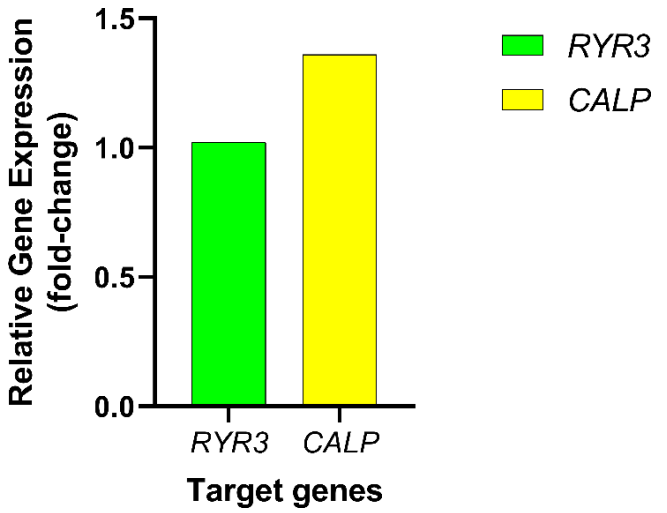
p-value: 0.9260

Figure 2: Comparison of m-calpain gene expression in Nile tilapia with low and high genetic values for weight gain. Values are presented as means, with bars indicating the standard error of the mean.



p-value: 0.3808

Figure 3: Relationship between the gene expression of RyR3 and m-calpain..



Gene expression was considered significant when fold-change values were ≥ 2 or ≤ 0.5. Values above 2 indicate upregulation, whereas values below 0.5 indicate downregulation. Values between 1 and 2 indicate no significant difference in the expression of the evaluated genes.

Table 1: Biometric parameters, body weight, and fillet yield of Nile tilapia with high and low genetic values for body weight**.**

	Low value	High value	p-value
Length (cm)	20,82±0,35	24,85±0,38	<0,001
Width (cm)	10,78±0,27	13,88±0,23	< 0,001
Height (cm)	0,95±0,02	1,26±0,03	< 0,001
Body weight (g)	856,31±40,12	1627,37±57,07	< 0,001
Fillet weight (g)	290,30±13,27	546,92±20,45	< 0,001
Fillet yield (%)	34±0,24	33,5±0,26	0,188

Tukey test at the 5% significance level.

Table 2: Primer sequences used for quantitative real-time polymerase chain reaction (RT-qPCR).

Gene	Primer sequence
Ryanodine receptor (RyR3)	5′- TGTTTCATCTGTGGGATCGG-3′
	3′-GTGTGCTCTGTCTCTCCTTG -5′
m-calpain	5′- AAGGTCAGAAGTGTGGAGAAAG -3′
	3′- TTTAGGGACATCCACAGGTTTAG - 5′
β-actin	5′- TGGTGGGTATGGGTCAGAAAG-3′
	3′-CTGTTGGCTTTGGGGTTCA -5′

Source: Morais et al. (2020)

Table 3: Proximate composition of fillets from Nile tilapia with high and low genetic values for body weight.

	Low value	High value	p-value
Moisture (%)	24,7±0,22	24,8±0,36	0,918
Ash (%)	3,67±0,18	4,23±0,09	0,012
Total lipids (%)	14,2±0,64	17,2±0,82	0,013
Total protein (%)	61,3±0,94	60,5±0,74	0,520

Tukey test at the 5% significance level.

Table 4: Fillet quality parameters (lightness (L*), red–green component (a*), yellow–blue component (b*), chroma, hue angle, thawing loss (TL), and cooking loss (CL)) of Nile tilapia with high and low genetic values for body weight.

	Low value	High value	p-value
pH	6,81±0,02	6,76±0,02	0,221
L*	45,4±0,23	47,5±0,17	<0,001
a*	-2,45±0,07	-3,00±0,05	<0,001
b*	6,15±0,11	6,17±0,10	0,876
Chroma (C*)	12,4±0,28	15,3±0,36	<0,001
Hue angle (h*)	19,6±0,01	20,3±0,01	<0,001
TL (%)	3,62±0,35	3,43±0,87	0,083
CL (%)	18,2±0,31	18,2±0,42	0,983

Tukey test at the 5% significance level.

6. ARTIGO 2 PUBLICADO:

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Pre-slaughter transport density and seasonal effects on Nile tilapia (*Oreochromis niloticus*): welfare and filet quality outcomes

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This study evaluated the effects of pre-slaughter transport density on physiological welfare indicators and fillet quality of Nile tilapia (*Oreochromis niloticus*) during summer and winter. The experiment followed a completely randomized 3 × 2 factorial design, with three transport densities (375, 425, and 475 kg/m^3) and two seasons. Stress biomarkers (glucose and lactate), oxidative stress indicators (reduced glutathione, catalase, and lipid oxidation), water quality parameters, and fillet quality traits (pH, water-holding capacity, color, and texture) were assessed. Significant density × season interactions were observed for plasma lactate ($p = 0.0472$), muscle pH ($p = 0.0091$), water-holding capacity ($p < 0.0001$), and fillet resilience ($p = 0.0043$). In summer, fish transported at 375 kg/m^3 showed lower lactate concentrations and higher water-holding capacity, whereas in winter, higher muscle pH and resilience were observed at 425 and 475 kg/m^3. Water quality variables also exhibited significant density × season interactions ($p < 0.05$). Overall, seasonal conditions were the primary drivers of physiological stress responses and postmortem fillet traits. Environmental temperature and associated water quality changes modulated metabolic demand and muscle characteristics, even under short transport duration with adequate oxygenation. Although transport density influenced specific attributes, particularly texture parameters, no consistent progressive pattern was observed across the tested range. These findings indicate that, under short-distance transport (~1.5 h), seasonal thermal management and water quality control are more critical determinants of welfare and fillet quality than moderate density adjustments within 375–475 kg/m^3.

KEYWORDS
lactate, oxidative stress, pH, texture profile, water-holding capacity

1 Introduction

Nile tilapia (*Oreochromis niloticus*) is among the most widely farmed fish species worldwide and plays a central role in global aquaculture due to its high production efficiency, broad environmental adaptability, and wide market acceptance (1). Brazil ranks among the four largest tilapia producers globally, and in 2024 the species accounted for 662,230 tons, representing 68.36% of the national farmed fish production (2). The tilapia production chain generates more than R$ 6 billion annually (2), highlighting its economic importance and reinforcing the need to improve pre-slaughter management practices. Pre-slaughter management is widely recognized as a critical stage in aquaculture, with direct implications for both fish welfare and final product quality (3, 4). Within this context, transport represents one of the most sensitive phases of the production system, as it concentrates multiple physical and environmental stressors capable of compromising animal welfare and fish quality (5, 6).

During transport, fish are exposed to several stressors, including capture, handling, and variations in stocking density, which may impair health, increase the incidence of injuries, and elevate mortality (7). Beyond the ethical implications associated with animal suffering, these practices also result in significant economic losses due to the reduced commercial value of the fish and post-transport mortality (8). Among transport-related factors, stocking density is considered one of the main determinants of fish welfare.

Excessively high densities intensify competition for oxygen and stress exposure, whereas very low densities may increase agitation and aggressive behavior, likewise compromising the physical integrity of the animals (9, 10). In Brazil, official guidelines allow maximum transport densities of up to 550 kg/m^3 for adult Nile tilapia, typically referring to fish with an average body weight of approximately 1.0 kg (10). However, these recommendations lack regional empirical validation, particularly regarding their physiological implications for welfare and their effects on meat quality (8). Although all densities evaluated in the present study fall within regulatory limits, evidence supporting their adequacy under different seasonal conditions remains limited. In this regard, seasonal thermal conditions are recognized as important modulators of Nile tilapia physiological responses to handling and transport.

The optimal thermal range for Oreochromis niloticus has been reported to lie approximately between 20.2 ˚C and 31.7 ˚C, with an optimal temperature (T_{OPT}) close to 26 ˚C, whereas temperatures outside this range are associated with reduced aerobic performance and metabolic efficiency (11). Exposure to temperatures below 20 ˚C has been associated with alterations in endocrine and metabolic stress responses, including attenuation or delays in cortisol and glucose dynamics, which may reduce physiological buffering capacity during handling and transport (12). In contrast, exposure to higher temperatures, particularly ≥30 ˚C−32 ˚C, increases metabolic demand and has been linked to oxidative stress, characterized by elevated reactive oxygen species production and mitochondrial dysfunction in tilapia tissues (13).

In Brazil, seasonality is well defined, with summer occurring between December and March and winter between June and August (14). In northern Paraná State, where the present study was conducted (Londrina region), water temperatures in aquaculture systems typically range from 26 ˚C to 30 ˚C during summer and from 18 ˚C to 22 ˚C during winter, depending on local climatic conditions and management practices (15). These seasonal temperature ranges overlap with known physiological thresholds for Nile tilapia and may influence metabolic activity, stress responsiveness, and postmortem muscle traits.

Physiological stress responses include hormonal and biochemical alterations, such as increased cortisol, glucose, and lactate concentrations, as well as osmotic and immunological disturbances that compromise homeostasis and increase fish susceptibility (16). Muscle lactate accumulation accelerates postmortem pH decline, promoting protein denaturation, reduced water-holding capacity, and texture changes, which directly affect filet quality (16, 17). Inadequate stocking densities—whether excessively high or low—tend to exacerbate these responses, linking adverse effects to both animal welfare and product quality (18).

Despite the relevance of these factors, studies that integratively assess how pre-slaughter transport density and seasonal conditions jointly affect welfare indicators and filet quality in Nile tilapia remain limited. Therefore, the present study aimed to evaluate the effects of pre-slaughter transport density on stress indicators and filet quality of Oreochromis niloticus during summer and winter. The following hypotheses were tested: (1) higher transport densities would intensify physiological stress responses, particularly during summer; (2) seasonal conditions would modulate density-dependent effects on filet quality; and (3) the relative suitability of transport density would differ between seasons due to contrasting metabolic and thermal demands.

2 Material and methods

2.1 Ethical statement

The experiment was approved by the Ethics Committee on Animal Use of the State University of Londrina (protocol no. 043.2023), and all procedures followed the guidelines of the National Council for the Control of Animal Experimentation (19).

2.2 Tilapia and transport conditions

Nile tilapia (*Oreochromis niloticus*) used in this experiment had an average body weight of 930 ± 150 g and a total length of 37 ± 3 cm. Following the guidelines established by the National Council for the Control of Animal Experimentation (19), a 24-h fasting period was applied prior to transport to ensure complete emptying of the digestive tract before slaughter. Fish originated from net cages located in the municipality of Alvorada do Sul, Paraná, Brazil.

Capture was performed manually using a dip net with an average capacity of 20 fish per operation. Subsequently, the individuals were transferred to containment bags, weighed, and allocated into transport boxes previously filled with water. Transport was carried out in fiberglass tanks with a capacity of

1,000 L, equipped with oxygen diffusers connected to pressurized cylinders. The distance traveled to the slaughterhouse was approximately 100 km, with a duration of 1 h 30 min at an average speed of 68 km h^{-1}, consistent with the operational reality of Brazilian Nile tilapia production systems, in accordance with national guidelines for the transport of live fish (20).

2.3 Experimental design

The experimental design was completely randomized in a 3 × 2 factorial arrangement, considering three different transport densities (375, 425, and 475 kg m^{-3}) and two seasons (summer and winter). The definition of densities was based on consultations with regional slaughterhouses, where the reported average was 425 kg m^{-3}. From this value, intervals of ±50 kg m^{-3} were established, avoiding the extremes recommended by MAPA, in order to represent realistic transport conditions. The choice of seasons considered regional thermal variations, in which summer and winter present water and environmental temperatures that deviate from the optimal conditions for the species.

The study comprised six independent transport trips, with three conducted during summer (March) and three during winter (August), which were considered as replications of the experimental design. Each trip involved the use of three transport tanks per density, totaling 1,275 Nile tilapia per trip. At the end of the experiment, a total of 7,650 fish were transported.

Fish were randomly allocated to transport tanks to minimize potential experimental bias. For water quality variables, the experimental unit was the transport tank. For physiological stress indicators and filet quality traits, individual fish were considered as experimental units. Transport densities were systematically rotated among tanks across three independent transport events within each season, ensuring that no tank was consistently associated with a single density. This balanced design minimized potential tank-specific effects and prevented confounding between tank and density. In addition, physiological and filet quality measurements were obtained at the individual level following transport, supporting the treatment of individual fish as independent experimental units for these analyses. Ten fish from each density were randomly selected at the end of each trip, resulting in 30 samples per trip and a total of 180 animals analyzed throughout the experiment. The number of individuals used was defined as the minimum necessary to meet the proposed objectives, in accordance with good animal welfare practices.

2.4 Environmental variables

Monitoring of environmental variables, air temperature (°C) and relative humidity (%), was performed using a data logger HOBO Ware® (Onset Computer Corporation, Bourne, MA, USA). The device was fixed to the external part of the truck, at the front of the cargo area, at the beginning of each trip and removed at the end of transport. Readings were recorded at 5-min intervals using the HOBOware® software. The data obtained are presented in Figure 1.

2.5 Water quality parameters during transport

Water quality measurements in the transport tanks were performed before the beginning of each trip. The following parameters were evaluated: water temperature and dissolved oxygen, measured using a portable YSI 550A Dissolved Oxygen Meter (YSI Inc., Yellow Springs, OH, USA), and pH and dissolved ammonia, assessed using LabconTest® colorimetric tests (Alcon, Brazil). In parallel, the same parameters were determined in the source net-cages, and water acclimation was performed when necessary.

Acclimation was carried out whenever differences >1 °C in temperature, more than 0.3 pH units, or discrepancies exceeding 1 mg L^{-1} in dissolved oxygen were detected between environments. This procedure consisted of the gradual addition of source water into the transport tanks until the physicochemical parameters were homogenized, in order to avoid osmotic and thermal shock. Upon arrival at the slaughterhouse, all parameters were reassessed in each

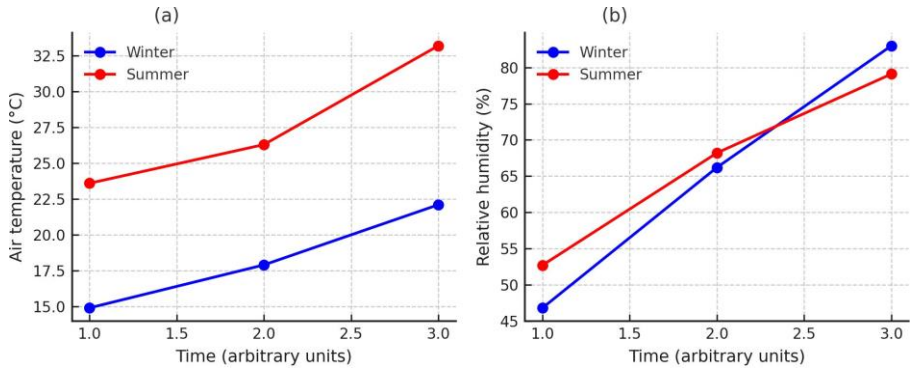

FIGURE 1
Environmental variables recorded during Nile tilapia transport in summer and winter. (a) Air temperature (°C). (b) Relative humidity (%). Lines represent mean values recorded at 5-min intervals with a HOBO Ware® data logger (Onset Computer Corporation, Bourne, MA, USA).

transport box to monitor potential changes during transport. The acclimation duration ranged from approximately 15–20 min (mean ± SD: 17.5 ± 2.5 min), which was not included in the reported transport time.

2.6 Unloading of fish

Upon arrival at the slaughter facility, Nile tilapia (*Oreochromis niloticus*) were individually removed from transport boxes using a dip net in a random manner. Each fish was handled individually to minimize stress and prevent physical injury during unloading. Ten fish from each transport-density group were randomly selected for subsequent blood and tissue analyses.

2.7 Anesthesia and blood collection

Anesthesia was performed individually by immersion in a benzocaine solution, prepared by dissolving 1 g benzocaine in 10 mL ethanol and diluting this solution in 10 L water, resulting in a final concentration of approximately 100 mg L^{-1}. Each fish was maintained individually in the anesthetic bath until complete loss of equilibrium and opercular movement, indicating deep anesthesia, in accordance with the welfare recommendations of the National Council for the Control of Animal Experimentation (19).

After confirming anesthesia (loss of reflexes and absence of opercular movement), blood samples were collected 8 ± 2 min after arrival at the slaughterhouse, by individual caudal puncture using 5 mL disposable syringes fitted with 0.8 × 25 mm needles. All fish from the same transport-density group were sampled within a maximum window of 30 min to minimize temporal variation in glucose and lactate levels. In addition, the order of blood sampling among density groups was rotated across transport events, preventing sampling from consistently starting with the same treatment and further minimizing potential temporal bias. The samples were transferred to 4 mL vacuum tubes (FirstLab®, FirstLab Indústria, Importação e Exportação de Produtos para Laboratórios Ltda., Londrina, Brazil) containing fluoride and EDTA, suitable for glucose and lactate analyses. All sampling procedures were conducted while the fish remained unconscious and were handled individually to ensure precision and minimize any residual stress response.

2.8 Euthanasia and handling

Following blood collection and before any possibility of regaining consciousness, each fish was euthanized individually by severance of the spinal cord, ensuring immediate death and preventing any recovery of consciousness.

All procedures were conducted by trained personnel, ensuring rapid, humane, and ethically compliant execution in accordance with CONCEA (19) guidelines.

2.9 Plasma evaluation

Blood samples were centrifuged immediately after collection, still at the slaughterhouse facilities, using a Centurion Scientific K3 Series centrifuge (Centurion Scientific Ltd., Chichester, UK) at 3,000 rpm (≈171 × g, considering a rotor radius of 1.7 cm) for 10 minutes at 18 °C. After centrifugation, plasma was carefully separated and transferred to 2 ml Eppendorf® (Eppendorf AG, Hamburg, Germany) tubes, then frozen and stored at −20 °C for subsequent glucose and lactate analyses.

2.10 Evaluation of filet quality indicators

After slaughter, the hydrogen potential (pH), water-holding capacity (WHC), color, and texture parameters were evaluated, with all analyses conducted on the left filet of each individual. The time elapsed between slaughter and the beginning of the analyses was 45 min, ensuring that the results reflected immediate postmortem conditions. To ensure measurement accuracy, all samples were analyzed in triplicate. All filet quality analyses were performed under controlled ambient temperature conditions (22 °C ± 1 °C), maintained throughout the evaluation period using air conditioning, to avoid potential temperature-related effects on texture and water-holding capacity measurements.

2.10.1 Hydrogen potential (pH)

pH determination was performed using a portable potentiometer equipped with a penetration electrode (Testo model 205). Measurements were taken by inserting the probe three times at different points of the filet (anterior, medial, and posterior regions). The mean pH value was calculated according to the equation:

$$pH\ mean = \frac{pH\ anterior + pH\ medial + pH\ posterior}{3}$$

2.10.2 Water-holding capacity (WHC)

WHC was determined according to the protocol described by Goes et al. (21). Raw filet samples were weighed in triplicate using an analytical balance (BEL Engineering® M214Ai, BEL Engineering srl, Monza, Italy), with each 1 g sample placed into 1.5 mL Eppendorf® tubes containing 55 mm filter paper (Whatman No. 1). Samples were centrifuged at 1,318 × g for 4 min at 4 °C using a Centurion Scientific K3 Series centrifuge. After centrifugation, the samples were reweighed and dried in an oven (DeLeo DL SE 42L) at 70 °C for 12 h.

WHC was calculated using the following formula:

$$WHC = \frac{PCSW - DSW}{ISW} \times 100$$

where PCSW = post-centrifugation sample weight, DSW = dry sample weight, and ISW = initial sample weight.

2.10.3 Color

Color evaluation was performed on the dorsal surface of the filet using a portable colorimeter Minolta CR-10^{TM} (Konica Minolta Inc., Osaka, Japan). The equipment was set with D65 illuminant, 10° standard observer, and 8 mm aperture, according to the CIE system: L^* (lightness), a^* (red-green component), and b^* (yellow-blue component). Chroma (C^*) and hue angle (h^*) values were also determined, calculated by the following formulas (22):

$$C^* = \sqrt{(a^*)^2_3 + (b^*)^2}$$

$$h^* = atan2\ a^*, b^*$$

2.10.4 Texture profile

Instrumental texture profile analysis (TPA) was performed on three cubic samples (2 cm^3) taken from each filet of all selected individuals. Analyses were performed using a Texture Analyser XT.plus® (Stable Micro Systems, Surrey, UK), as described by Bourne (23), with samples compressed to 50% of their original thickness. The experimental protocol defined pre-test, test, and post-test speeds at 2 mm/s, compression distance of 20 mm, and controlled temperature of 25 °C.

The measured texture parameters included hardness (Newton, N), adhesiveness (Newton-seconds, Ns), springiness (dimensionless), cohesiveness (dimensionless), gumminess (Newton, N), chewiness (Newton-meter, N·m), and resilience (dimensionless).

2.11 Oxidative stress biochemical parameters

Antioxidant analyses were conducted on muscle tissue samples collected from the dorsal region of the left filet. Samples were immediately frozen at −80 °C to preserve their integrity until subsequent analyses, which were performed in replicates to ensure reproducibility of the results.

2.11.1 Protein quantification

The soluble protein content was determined according to the method of Bradford (24), as the results of reduced glutathione concentration and catalase activity were expressed per mg protein.

2.11.2 Reduced glutathione concentration (GSH)

The concentration of reduced glutathione (GSH) in the tissue was determined according to the method described by Beutler et al. (25). The reaction of GSH with the substrate 5,5^r-dithiobis-2-nitrobenzoic acid (DTNB) generated thionitrobenzoate (TNB), which was quantified at 412 nm using a Victor3^{TM} spectrophotometer (PerkinElmer, USA). Results were expressed as μg GSH per mg protein.

2.11 .3 Catalase activity (CAT)

Catalase activity was quantified based on the rate of H_2O_2 decomposition in muscle tissue samples. Absorbance was monitored at 240 nm using a Victor3^{TM} spectrophotometer (PerkinElmer, USA), following the protocol described by Beutler (26). Results were expressed as μmol H_2O_2 min^{-1} per mg protein.

2.11.4 Thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation was quantified by assessing thiobarbituric acid reactive substances (TBARS), according to the protocol of Camejo et al. (27). TBARS concentration was expressed as μg MDA per mg protein.

2.12 Statistical analysis

A General Linear Model (GLM) was initially used to evaluate the effects of season (summer, winter), transport density (375, 425, and 475 kg/m^3), and their interaction on transport water quality parameters (pH, temperature, dissolved oxygen, and ammonia). These parameters were also considered as potential covariates in the subsequent analyses of physiological and filet quality traits.

For outcome variables measured at the individual level (e.g., plasma lactate, muscle pH, filet texture, water-holding capacity, and oxidative stress markers), an Analysis of Covariance (ANCOVA) was employed. The independent variables were season and transport density (both as fixed factors), while the final water quality measurements (pH, temperature, dissolved oxygen, and ammonia) were introduced as continuous covariates.

To construct the final ANCOVA models, a two-step approach was followed:

(1) Full models initially included all four water quality covariates.
(2) A stepwise backward elimination process was then applied, retaining only those covariates with statistical significance ($p < 0.10$) or demonstrated biological relevance. Dissolved oxygen and ammonia concentrations were retained in multiple models, having shown significant influence on several physiological and technological responses.

To satisfy the assumptions of ANCOVA, the homogeneity of regression slopes (i.e., parallelism between covariate effects across groups) was explicitly tested by including interaction terms between each covariate and the main factors (season and density). No significant interactions were observed ($p > 0.10$), validating the use of ANCOVA without interaction terms. Additionally, all final models were checked for residual normality (via Shapiro–Wilk test), variance homogeneity (via Levene's test), and independence of residuals, ensuring the robustness of model assumptions.

When significant main effects or interactions were detected ($p < 0.05$), means were compared using Tukey's HSD *post-hoc* test. All statistical analyses were performed using the Statistica software, version 10.0 (StatSoft Inc., Tulsa, OK, USA).

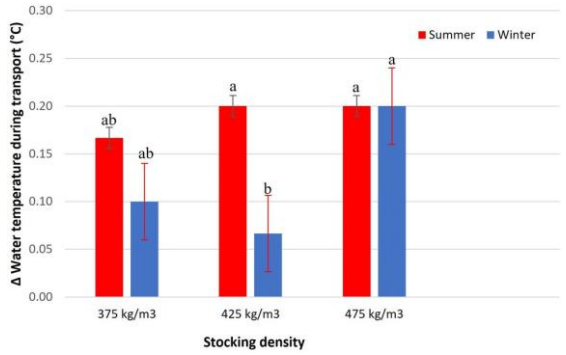

FIGURE 2
Δ Water temperature during transport (°C) of Nile tilapia at three stocking densities (375, 425, and 475 kg/m^3) in summer and winter. Bars represent mean ± standard error. Different letters indicate significant differences between treatments according to Tukey's test ($p < 0.05$).

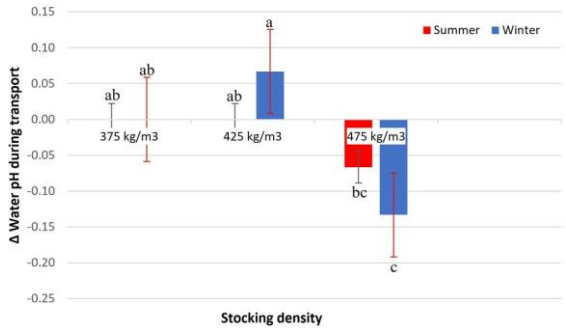

FIGURE 3
Δ Water pH during transport of Nile tilapia at three stocking densities (375, 425, and 475 kg/m^3) in summer and winter. Bars represent mean ± standard error. Different letters indicate significant differences between treatments according to Tukey's test ($p < 0.05$).

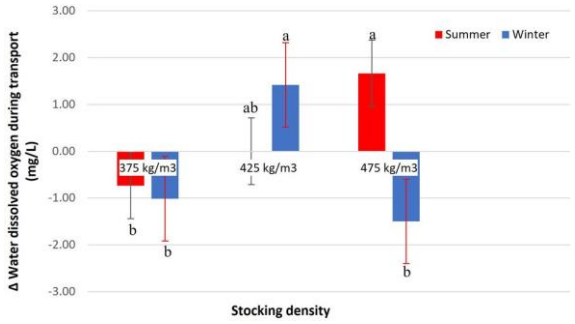

FIGURE 4
Δ Dissolved oxygen in transport water (mg/L) of Nile tilapia at three stocking densities (375, 425, and 475 kg/m^3) in summer and winter. Bars represent mean ± standard error. Different letters indicate significant differences between treatments according to Tukey's test ($p < 0.05$).

3 Results

3.1 Environmental conditions during transport

Air temperature and relative humidity recorded during transport confirmed the contrasting seasonal conditions (Figure 1). In winter, air temperature ranged from 14.9 ˚C to 22.1 ˚C, while in summer it varied between 23.6 ˚C and 33.2 ˚C. Relative humidity showed an opposite pattern, with higher values in winter (66.2%–83.0%) compared to summer (52.7%–79.1%). These environmental differences characterized the seasonal scenarios under which the transport trials were conducted.

3.2 Water quality parameters during transport

Water temperature during transport showed seasonal variations, with a significant interaction between stocking density and season ($p = 0.0472$; Figure 2). The graph presents the delta temperature (difference between the beginning and end of transport), allowing the quantification of temperature changes under different experimental conditions.

In summer, all densities exhibited a similar pattern of water temperature increase. In winter, a comparable response was observed at 375 and 475 kg/m^3. The density of 375 kg/m^3 resulted in an intermediate increase in temperature, not differing statistically from 425 kg/m^3. However, the density of 425 kg/m^3 recorded the lowest delta temperature in winter, indicating the smallest increase in water temperature during transport.

Water pH variation during transport was influenced by the interaction between stocking density and season ($p = 0.0091$; Figure 3). At 375 kg/m^3 in both seasons and at 425 kg/m^3 in summer, mean pH variations remained close to zero, with no statistical differences among them ($p > 0.05$). In contrast, 425 kg/m^3 in winter showed the greatest increase in pH, differing from the other treatments. At 475 kg/m^3, a decrease in water pH was observed in both seasons, which was more pronounced in winter compared to summer. The pH increase at 425 kg/m^3 in winter may reflect reduced metabolic CO_2 production under cold conditions, coupled with active water circulation.

The variation in dissolved oxygen in transport water was influenced by the interaction between stocking density and season ($p < 0.0001$), indicating that the effect of density differed between summer and winter (Figure 4). Stocking densities of 375 kg/m^3 in both seasons, as well as 475 kg/m^3 in winter, resulted in significant reductions in dissolved oxygen levels. In contrast, an increase in this parameter was observed at 425 kg/m^3 in winter and 475 kg/m^3 in summer. This increase in dissolved oxygen likely reflects active oxygenation rates exceeding the metabolic oxygen consumption of the fish, indicating that hypoxia was effectively prevented under these specific density and seasonal conditions. At 425 kg/m^3 in summer, dissolved oxygen remained stable, showing no substantial variation during transport. The concentration of ammonia in the transport water was influenced by the interaction between stocking density and season ($p = 0.0032$; Figure 5). In summer, all evaluated densities (375, 425, and 475 kg/m^3) resulted in a similar increase in ammonia concentration, with

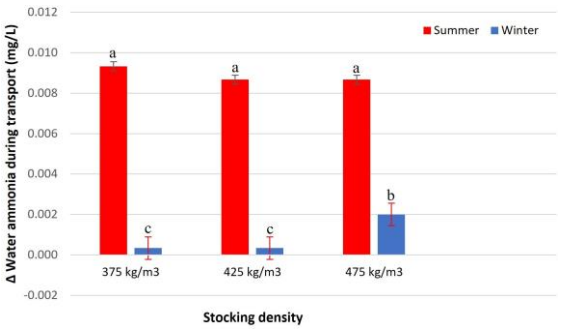

FIGURE 5
Δ Water ammonia during transport (mg/L) of Nile tilapia at three stocking densities (375, 425, and 475 kg/m^3) in summer and winter. Bars represent mean ± standard error. Different letters indicate significant differences between treatments according to Tukey's test ($p < 0.05$).

no significant differences among them. In winter, ammonia levels were consistently lower compared to summer. Among the densities evaluated in this season, 375 and 425 kg/m^3 showed the lowest accumulation of ammonia, whereas 475 kg/m^3 promoted a more pronounced increase, although still lower than the values recorded in summer. Final ammonia concentrations ranged from 0.000 to 0.014 mg/L across treatments. These concentrations were below commonly referenced operational limits of approximately 2.0 mg/L for total ammonia in freshwater fish management, as described by Boyd (28).

3.3 Plasma analysis

There was no interaction between transport density and season for glucose levels (p = 0.8443), indicating that the seasonal effect on this physiological parameter was not directly influenced by stocking density (Table 1). During winter, a significant increase in glucose levels (172.00 ± 32.49 mg dL^{-1}) was observed compared with summer (126.89 ± 40.87 mg dL^{-1}) (p = 0.0095). On the other hand, transport density did not significantly affect plasma glucose concentration (p = 0.2483).

The absence of density effects on glucose suggests that, within the tested range and with adequate oxygenation, crowding stress was not sufficient to activate the HPI axis beyond seasonal baseline differences.

In contrast, lactate levels showed a significant interaction between stocking density and season (p = 0.0131) (Figure 6). Higher concentrations were observed in winter at 375 kg/m^3 (15.0 ± 2.5 mmol L^{-1}) and in summer at 475 kg/m^3 (14.0 ± 3.0 mmol L^{-1}), which did not differ from each other. The lowest lactate level was recorded in summer at 375 kg/m^3 (4.0 ± 1.2 mmol L^{-1}). For the other conditions (425 kg/m^3 in both seasons and 475 kg/m^3 in winter), concentrations remained at intermediate levels and did not differ significantly.

3.4 Filet quality assessment

The analysis of muscle pH revealed a significant interaction between stocking density and season (p = 0.0043) (Figure 7). During summer, muscle pH values were similar across all densities (375, 425, and 475 kg/m^3), with no significant differences among them. In winter, intermediate pH values were observed at densities of 425 and 475 kg/m^3, which did not differ significantly from each other. The lowest muscle pH value was recorded at 375 kg/m^3 during winter, differing significantly from the other treatments.

There was a significant interaction between season and stocking density for filet water-holding capacity (WHC) (p = 0.0112; Figure 8). During summer, WHC values were high across all densities, with the highest value observed at 375 kg/m^3, while 425 and 475 kg/m^3 showed slightly lower but similar values (approximately 70%–72%). In winter, WHC was significantly reduced at 425 kg/m^3, which presented the lowest value (approximately 66%). In contrast, WHC values at 375 and 475 kg/m^3 during winter did not differ significantly from each other and were higher than those observed at 425 kg/m^3.

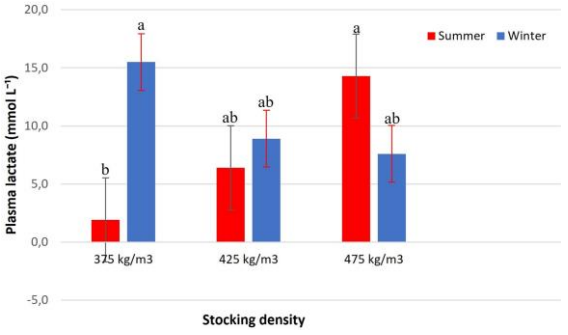

FIGURE 6
Plasma lactate (mmol L^{-1}) of Nile tilapia at three stocking densities (375, 425, and 475 kg/m^3) in summer and winter. Bars represent mean ± standard error. Different letters indicate significant differences between treatments according to Tukey's test ($p < 0.05$).

TABLE 1 Plasma glucose (mg dL^{-1}) of Nile tilapia at three stocking densities (375, 425, and 475 kg/m^3) during summer and winter transport.

Variable	Density (*D*)			Season (*S*)		*p*-value		
	375 kg/m^3	425 kg/m^3	475 kg/m^3	Summer	Winter	Density (*D*)	Season (*S*)	*D* × *S*
Glucose (mg dL^{-1})	160.00 ± 34.85	134.71 ± 47.61	162.42 ± 38.78	126.89 ± 40.87b	172.00 ± 32.49a	0.2483	0.0095	0.8443

Values are presented as mean ± standard error. The table also presents p-values for the main effects of density (*D*), season (*S*), and their interaction (*D* × *S*).
Different superscript letters indicate significant differences between treatments according to Tukey's test ($p < 0.05$).

TABLE 2 Color parameters (L^*, a^*, b^*, chroma, and hue angle) of Nile tilapia fillets at three stocking densities (375, 425, and 475 kg/m^3) during summer and winter transport.

Variable	Density (*D*)			Season (*S*)		*p*-value		
	375 kg/m^3	425 kg/m^3	475 kg/m^3	Summer	Winter	Density (*D*)	Season (*S*)	*D* × *S*
L^* surface	44.12 ± 2.81	44.89 ± 2.51	44.39 ± 2.03	42.65 ± 1.57b	45.53 ± 1.86a	0.4170	0.0010	0.9390
a^* surface	5.57 ± 1.59	5.77 ± 1.95	5.32 ± 1.53	7.06 ± 0.88a	4.62 ± 1.20b	0.5814	0.0001	0.6495
b^* surface	9.89 ± 1.41	9.75 ± 1.45	9.59 ± 0.98	10.38 ± 0.78a	9.32 ± 1.18b	0.9742	0.0290	0.5963
Chroma	11.17 ± 1.73	10.83 ± 1.66	11.47 ± 1.55	11.58 ± 1.48a	10.44 ± 1.49b	0.0630	0.0000	0.2014
Hue angle	1.06 ± 0.12	1.08 ± 0.12	1.06 ± 0.11	1.02 ± 0.10b	1.12 ± 0.11a	0.6808	0.0000	0.3026

Values are presented as mean ± standard error. The table also presents *p*-values for the main effects of density (*D*), season (*S*), and their interaction (*D* × *S*). Different superscript letters indicate significant differences between treatments according to Tukey's test ($p < 0.05$).

The evaluation of meat color parameters did not reveal a significant interaction between stocking density and season, indicating that the effects of these factors acted independently (Table 2). However, a seasonal effect was observed for lightness (L^*), with lower values recorded in summer, indicating darker filets, whereas in winter lightness was significantly higher ($p = 0.0010$), indicating lighter filets.

The red/green coordinate (a^*) varied significantly between seasons, with higher values recorded in summer, indicating redder filets, whereas in winter values were lower ($p = 0.0001$), reflecting a paler coloration. No significant effect of stocking density was observed for this parameter (Table 2).

The yellow/blue coordinate (b^*) also showed a seasonal effect, with higher values in summer, reflecting yellower filets, while in winter values were reduced ($p = 0.0290$). As with a*, stocking density did not significantly affect b^* values (Table 2).

No interaction between density and season was observed for chroma ($p = 0.2014$) (Table 2). However, a significant seasonal effect was detected ($p < 0.001$), with higher chroma in summer (11.88 ± 1.51) compared with winter (10.44 ± 1.49). Stocking density did not significantly affect chroma ($p = 0.0630$).

Similarly, no interaction between density and season was detected for hue angle ($p = 0.3026$), and no differences were found among stocking densities ($p = 0.6808$). Nevertheless, a significant seasonal effect was observed ($p < 0.001$), with higher values recorded in winter (1.12 ± 0.11) compared with summer (1.02 ± 0.10) (Table 2).

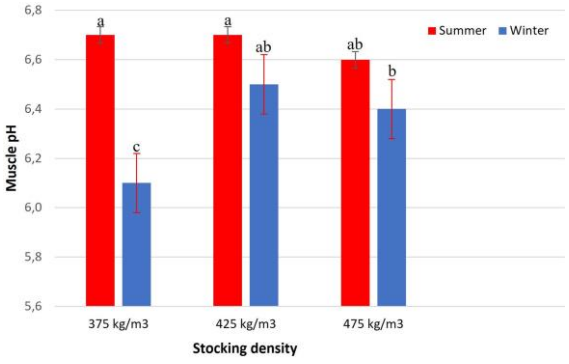

FIGURE 7
Muscle pH of Nile tilapia at three stocking densities (375, 425, and 475 kg/m^3) in summer and winter. Bars represent mean ± standard error. Different letters indicate significant differences between treatments according to Tukey's test ($p < 0.05$).

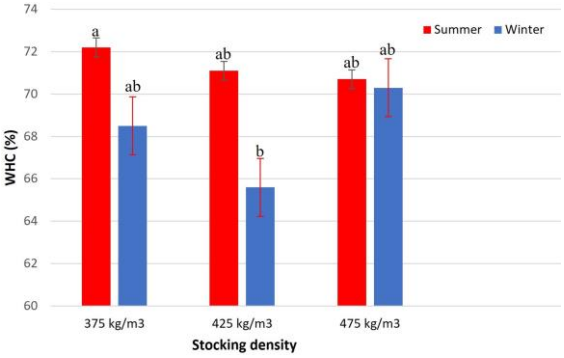

FIGURE 8
Water-holding capacity (WHC, %) of Nile tilapia filets at three stocking densities (375, 425, and 475 kg/m^3) in summer and winter. Bars represent mean ± standard error. Different letters indicate significant differences between treatments according to Tukey's test ($p < 0.05$).

Filet hardness showed no significant interaction between stocking density and season ($p = 0.9861$) (Table 3). However, a seasonal effect was observed ($p = 0.0035$), with higher values recorded in summer compared to winter. Stocking density alone did not affect hardness ($p = 0.1405$).

Adhesiveness was not affected by stocking density ($p = 0.1828$), season ($p = 0.3433$), or their interaction ($p = 0.4731$) (Table 3). Mean values ranged between −8 and −9 g·s.

Elasticity was influenced by both stocking density ($p = 0.016$) and season ($p = 0.0013$), with no interaction between the factors ($p = 0.2067$) (Table 3). Higher values were observed in summer, and among densities, the intermediate density (425 kg/m^3) showed higher elasticity compared to the others.

Chewiness was influenced by transport density ($p = 0.0025$) and season ($p < 0.0001$), with no interaction between factors ($p = 0.0913$) (Table 3). Higher values were observed in summer compared to winter, and among densities, fish transported at 425

TABLE 3 Hardness (g), adhesiveness (g · s), elasticity (dimensionless), and chewiness (g · mm) of Nile tilapia fillets at three stocking densities (375, 425, and 475 kg/m^3) during summer and winter transport.

Variable	Density (D)			Season (S)		p-value		
	375 kg/m^3	425 kg/m^3	475 kg/m^3	Summer	Winter	Density (*D*)	Season (*S*)	*D* × *S*
Hardness (g)	5613.92 ± 572	8016.05 ± 3807	4427.14 ± 2163	8609.36 ± 445a	4015.65 ± 1869b	0.1405	0.0035	0.9861
Adhesiveness (g·s)	−9.75 ± 2.07	−7.62 ± 3.07	−8.98 ± 1.65	−8.40 ± 3.17	−8.95 ± 1.60	0.1828	0.3433	0.4731
Elasticity (dimensionless)	0.37 ± 0.07b	0.47 ± 0.08a	0.40 ± 0.06b	0.47 ± 0.08a	0.39 ± 0.05b	0.0160	0.0013	0.2067
Chewiness (g·mm)	1783.23 ± 183 ab	2740.01 ± 166a	1213.87 ± 651b	3113.40 ± 148a	990.99 ± 384b	0.0025	0.0000	0.0913

Values are presented as mean ± standard error. The table also presents *p*-values for the main effects of density (*D*), season (*S*), and their interaction (*D* × *S*).
Different superscript letters indicate significant differences between treatments according to Tukey's test at $p < 0.05$. Units: Hardness (g); Adhesiveness (g·s); Elasticity (dimensionless); Chewiness (g·mm).

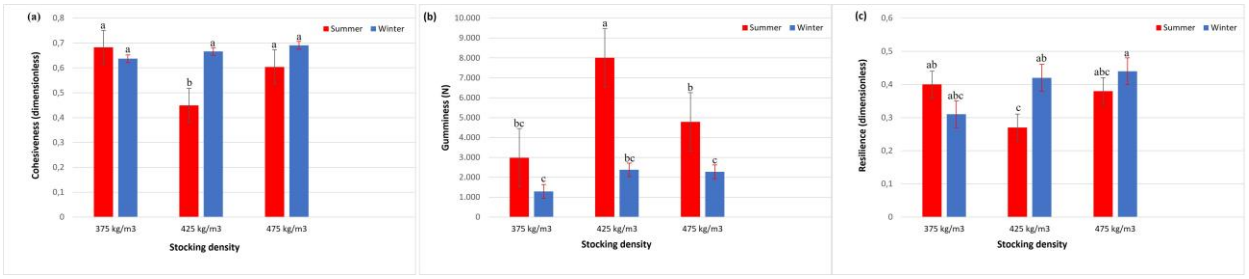

FIGURE 9
Cohesiveness (a), gumminess (b), and resilience (c) of Nile tilapia fillets at three stocking densities (375, 425, and 475 kg/m^3) in summer and winter. Bars represent mean ± standard error. Different letters indicate significant differences between treatments according to Tukey's test ($p < 0.05$).

kg/m^3 showed greater chewiness, followed by 375 kg/m^3, while the lowest values were recorded at 475 kg/m^3.

Filet cohesiveness was significantly influenced by the interaction between stocking density and season ($p = 0.0051$) (Figure 9a). In summer, the intermediate density of 425 kg/m^3 showed significantly lower cohesiveness compared to the other conditions. In winter, cohesiveness values remained high and uniform across all densities.

Gumminess was also affected by the interaction between stocking density and season ($p = 0.0056$) (Figure 9b). In summer, the highest values were observed at 425 kg/m^3, exceeding those of the other densities. In winter, gumminess values were uniform among the densities, with no significant differences.

Resilience was modulated by the interaction between stocking density and season ($p = 0.0017$) (Figure 9c). In winter, the highest resilience values were recorded at 475 kg/m^3, while in summer, the lowest values were found at 425 kg/m^3.

3.5 Biochemical parameters of oxidative stress

GSH concentration showed no interaction between stocking density and season ($p = 0.2768$) (Table 4). However, a significant effect of stocking density was observed ($p = 0.0094$), with higher values in fish transported at 475 kg/m^3 (8.06 ± 1.18 μg GSH mg^{-1} protein) compared to 375 and 425 kg/m^3. A seasonal effect was also detected ($p = 0.0261$), with higher mean concentrations in winter (7.64 ± 1.85 μg GSH mg^{-1} protein) than in summer (4.97 ± 3.76 μg GSH mg^{-1} protein). The elevated GSH concentration at 475 kg/m^3 reflects increased oxidative stress and compensatory antioxidant activation, indicating physiological challenge rather than improved welfare status.

No interaction between stocking density and season was detected for CAT activity ($p = 0.7761$) (Table 4). Similarly, no isolated effect of density ($p = 0.9113$) or season ($p = 0.0595$) was observed. Values ranged from 0.289 ± 0.15 μmol H_2O_2 min^{-1} mg^{-1} protein in summer to 0.398 ± 0.10 μmol H_2O_2 min^{-1} protein in winter, without significant differences among treatments.

TBARS concentrations were not influenced by stocking density ($p = 0.7606$), season ($p = 0.1473$), or their interaction ($p = 0.7817$) (Table 4). Mean values remained stable across treatments, at approximately 1.50 ± 0.40 nmol TBARS mg^{-1} protein, indicating that transport at different stocking densities did not significantly affect lipid oxidation in tilapia filets.

4 Discussion

4.1 Blood plasma evaluation

The physiological response of Nile tilapia (*Oreochromis niloticus*) subjected to different transport densities (375, 425, and 475 kg/m^3) during summer and winter revealed clear indicators

TABLE 4 Reduced glutathione (GSH, μg mg^{-1} protein), catalase activity (CAT, μmol H_2O_2 min^{-1} mg^{-1} protein), and thiobarbituric acid reactive substances (TBARS, μg MDA mg^{-1} protein) in Nile tilapia fillets at three stocking densities (375, 425, and 475 kg/m^3) during summer and winter transport.

Variable	Density (*D*)			Season (*S*)		*p*-value		
	375 kg/m^3	425 kg/m^3	475 kg/m^3	Summer	Winter	Density (*D*)	Season (*S*)	*D* × *S*
GSH (μg mg^{-1} protein)	6.47 ± 2.06ab	4.27 ± 4.20b	8.06 ± 1.18a	4.97 ± 3.76b	7.64 ± 1.85a	0.0094	0.0261	0.2768
CAT (μmol H_2O_2 min^{-1} mg^{-1} protein)	0.354 ± 0.13	0.344 ± 0.11	0.368 ± 0.15	0.289 ± 0.15	0.398 ± 0.10	0.9113	0.0595	0.7761
TBARS (μg MDA mg^{-1} protein)	0.507 ± 0.43	0.617 ± 0.36	0.745 ± 0.26	0.497 ± 0.21	0.754 ± 0.34	0.7606	0.1473	0.7817

Values are presented as mean ± standard error. The table also presents p-values for the main effects of density (*D*), season (*S*), and their interaction (*D* × *S*).
Different superscript letters indicate significant differences between treatments according to Tukey's test at $p < 0.05$. Units: GSH (μg mg^{-1} protein); CAT (μmol H_2O_2 min^{-1} mg^{-1} protein); TBARS (μg MDA mg^{-1} protein).

of metabolic stress, particularly in plasma glucose and lactate concentrations. The increase in plasma glucose levels observed during winter reflects an adaptive response to cold-induced stress in Nile tilapia, a warm-water species whose physiological performance is optimized for higher temperatures. Exposure to temperatures below the optimal thermal range represents a significant environmental stressor, as reduced temperature directly impairs enzymatic activity, aerobic metabolic capacity, and overall energetic efficiency in ectothermic fish (5, 29).

Under such suboptimal thermal conditions, maintaining essential physiological functions imposes a higher relative energetic cost, threatening cellular and systemic homeostasis. This imbalance triggers activation of the hypothalamic–pituitary–interrenal (HPI) axis, culminating in cortisol release as a compensatory endocrine response. Cortisol promotes metabolic adjustments, particularly through stimulation of gluconeogenesis, thereby increasing circulating glucose levels to sustain vital functions under thermally unfavorable conditions (5). Thus, the elevation of glycemia during winter reflects a hormonally mediated strategy to preserve energy availability rather than a direct effect of transport density.

This rise in glycemia, as a compensatory attempt to maintain energy homeostasis under cold stress, has been previously documented in species such as common carp (*Cyprinus carpio*) and Atlantic salmon (*Salmo salar*), suggesting that the endocrine response to low temperatures is a fundamental component of fish physiological resilience (30). However, the absence of significant density effects on glucose (Table 1, $p = 0.2483$) suggests that, under the experimental conditions with adequate oxygenation, crowding within the tested range (375–475 kg/m^3) was not sufficient to differentially activate the HPI axis. This indicates that thermal stress from season overwhelmed any potential density-related crowding stress.

The increase in plasma lactate levels observed under specific density–season combinations indicates the occurrence of acute metabolic stress associated with the energetic demands of transport. Although higher lactate concentrations were recorded during winter at 375 kg m^{-3}, similarly elevated values were also observed during summer at 475 kg m^{-3}, whereas the lowest lactate levels occurred during summer at 375 kg m^{-3}, suggesting more favorable metabolic conditions under this scenario.

In ectothermic fish, lactate production reflects the balance between aerobic metabolic capacity and the energetic demands imposed by environmental and handling stressors (5). Exposure to low temperatures reduces enzymatic efficiency and constrains aerobic metabolic rates, promoting a greater reliance on anaerobic glycolysis even under normoxic conditions. López-Jiménez et al. (31) demonstrated this effect in *Oreochromis niloticus* exposed to cold temperatures around 18 °C−20 °C, reporting increased plasma lactate levels associated with cold-induced metabolic restriction. In contrast, under warmer conditions, increased basal metabolic activity may also intensify the energetic cost of stress. Pichitkul et al. (32), evaluating *O. niloticus* exposed to 22 °C, 27 °C, and 32 °C, observed pronounced activation of the endocrine stress axis at elevated temperatures (32 °C), reflected by higher plasma cortisol concentrations and increased expression of heat shock proteins, indicating increased metabolic demand.

These findings are consistent with the synthesis presented by Barton (5), which describes how thermal deviations—both below and above the species' optimal range—amplify physiological stress responses in teleost fish through the activation of circulating corticosteroids. In the present study, lactate responses were modulated by the interaction between environmental temperature, stocking density, and transport-related energetic demand, supporting plasma lactate as a sensitive indicator of multifactorial physiological challenges during pre-slaughter transport.

4.2 Evaluation of filet quality indicators

The pH values reflected a strong seasonal influence on muscle metabolism, with higher values recorded in summer, suggesting lower lactate accumulation and more efficient aerobic metabolism at elevated temperatures, as reported by Tacchi et al. (33). Beyond the thermal influence on muscle metabolism, increased mucus secretion in response to pre-slaughter stress may have contributed to the higher pH values observed. Handling and transport stimulate mucus production as part of the fish's innate defense system, essential for osmotic and immune homeostasis. However, under chronic stress, proteolytic enzymes such as metalloproteases and serine proteases become more active, promoting the degradation of structural proteins. The resulting peptides and free amino acids

act as alkaline buffers, mitigating postmortem acidification and maintaining higher pH values (34, 35). This physiological response, while adaptive, reflects a direct consequence of stress on welfare and muscle biochemistry. In contrast, the lower pH values observed in winter were associated with intensified anaerobic glycolysis and lactate accumulation, consistent with the findings of Barton (5). The rapid consumption of glucose for energy under cold stress impairs muscular homeostasis and ultimately reduces product quality, as also described by Poli (3) and Macaraeg et al. (36).

Regarding transport density, muscle pH values observed at 425 and 475 kg/m^3 were statistically similar during winter, indicating comparable metabolic conditions between these densities. In contrast, the lowest pH values were recorded at 375 kg/m^3, suggesting greater metabolic disturbance under low-density conditions in a cold environment. These findings indicate that, during winter, reduced stocking density may increase thermal vulnerability and metabolic imbalance, whereas higher densities within the evaluated range did not intensify muscle acidification. Overall, the results reinforce that seasonal thermal conditions exert a more determinant influence on postmortem muscle metabolism than fine adjustments in transport density within the range of 375–475 kg/m^3, highlighting the importance of thermal management and adequate oxygenation as priority welfare strategies during transport (31, 37).

The higher water-holding capacity (WHC) observed in summer at 375 kg/m^3 can be attributed to the elevated pH, which moves away from the isoelectric point of muscle proteins (pH 5.0–5.5). Under these conditions, proteins carry a net negative charge, promoting electrostatic repulsion and enhanced water retention. This physiological state minimizes muscle contraction and fluid loss, preserving structural integrity and reducing the impact of transport stress. Lower postmortem acidification also reflects a more efficient aerobic metabolism, leading to greater WHC and improved meat texture (38, 39). According to Hultmann et al. (40), higher pH values are directly associated with increased WHC, contributing to the maintenance of muscle integrity and reduced exudation. During winter, although WHC was significantly reduced at 425 kg/m^3, similar WHC values were observed at 375 and 475 kg/m^3, indicating comparable water-retention responses at these two densities. Conversely, the reduction in WHC under cold conditions illustrates how decreased welfare, driven by physiological stress, translates into tangible losses in product quality (41, 42).

No interaction was detected between density and season for color parameters, although distinct seasonal patterns were evident. Lower lightness (L^*) in summer may be associated with reduced pigment oxidation, resulting in darker, more appealing filets (37). Jørpeland et al. (43) linked this phenomenon to greater myoglobin stability under higher pH, preserving the natural coloration of the meat. In contrast, increased L^* values in winter may result from dehydration and fiber compaction, enhancing light reflection on the filet surface (44). Such phenomena demonstrate the close relationship between stress physiology, pH, WHC, and color—key welfare-linked indicators of postmortem muscle quality (45).

The higher redness (a^*) in summer suggests greater retention of oxygenated myoglobin, while lower a^* values in winter indicate increased myoglobin oxidation and metmyoglobin formation, leading to paler filets (46). Similarly, elevated yellowness (b^*) during summer may be associated with heat-induced oxidative stress and pigment instability due to higher metabolic rates (43). In contrast, lower b* values in winter correspond to greater lipid oxidation and loss of pigment vibrancy, consistent with the findings of Hultmann et al. (40) and Tacchi et al. (33). Overall, these color variations provide indirect but meaningful evidence of stress-related welfare impacts on filet appearance and consumer acceptability.

For chroma, higher summer values compared to winter were attributed to increased metabolic activity and pigment oxidation driven by temperature-dependent stress. Elevated environmental temperature and handling intensity enhance oxidative metabolism, leading to stronger color saturation in summer filets (44). Conversely, the higher hue values observed in winter reflect more advanced pigment oxidation and metmyoglobin accumulation (46), reinforcing the link between environmental stress, welfare reduction, and color deterioration in fish meat.

Filet hardness was significantly greater in summer compared with winter, indicating that cold conditions promote softer meat. This outcome can be explained by the effect of temperature on enzyme activity and postmortem proteolysis. At lower temperatures, rigor mortis progression slows due to reduced ATP degradation, leading to tenderer filets (47, 48). Conversely, heat stress in summer accelerates glycogenolysis and lactate production, inducing rapid pH decline and early onset of rigor mortis, which increases toughness (16). These findings reinforce that minimizing pre-slaughter stress—through optimized handling, oxygenation, and density—is essential to preserving welfare and meat tenderness.

Adhesiveness showed low sensitivity to transport stress and seasonal variation, suggesting that this trait is more closely linked to intrinsic muscle structure than to external factors. While limited as a welfare indicator, adhesiveness highlights the relative stability of muscle cohesion under mild stress. However, when interpreted alongside pH and WHC, it complements the broader evaluation of environmental effects on product integrity (18, 49, 50).

Filet elasticity was independently influenced by stocking density and season, as no significant interaction between these factors was detected. Regardless of season, the density of 425 kg/m^3 exhibited the highest elasticity values, indicating greater muscle deformability under this condition. Overall, elasticity was also higher during summer compared to winter, independently of stocking density. These findings demonstrate additive effects of density and season on muscle elasticity, without statistical support for density recommendations specific to each season. The increase in elasticity may be associated with enhanced enzymatic activity at higher temperatures, particularly calpain-mediated proteolysis of structural proteins (47, 48).

Chewiness was independently affected by season and transport density, with no significant interaction between factors. Higher chewiness values observed during summer reflect the seasonal influence on muscle metabolism and postmortem biochemical processes, resulting in increased tissue firmness, as warmer temperatures accelerate enzymatic reactions and protein denaturation dynamics (39, 40). Regarding stocking density, chewiness was consistently highest at 425 kg/m^3 across both

seasons, followed by 375 kg/m^3, whereas the lowest values were recorded at 475 kg/m^3. This pattern indicates that intermediate densities promote greater muscle structural integrity, likely associated with a balance between muscle activity (40).

Coesiveness showed a distinct pattern, with reduced values at 425 kg/m^3 during summer compared with other treatments and seasons. This response may be attributed to elevated thermal load and intensified muscular fatigue during transport, leading to reduced structural integrity (5). Under cooler conditions, these effects were mitigated, resulting in uniform coesiveness across densities (51).

Gumminess followed a similar trend, with higher values at 425 kg/m^3 during summer, suggesting stress-induced muscle compaction and dehydration under elevated temperature. Changes in WHC and protein structure likely contributed to this phenomenon, as described by Sveinsdóttir et al. (44).

Resilience was significantly higher in winter, particularly at 475 kg/m^3, indicating better capacity of muscle to recover after deformation (48). Low temperature appears to preserve protein integrity and slow proteolytic activity, thereby maintaining structural stability and, consequently, product quality.

4.3 Oxidative stress biomarkers

The antioxidant response observed in Nile tilapia transported at different stocking densities demonstrates an important physiological mechanism of adaptation to stress, directly linked to animal welfare. The higher levels of reduced glutathione (GSH) observed in fish transported at 475 kg/m^3 are consistent with previous studies highlighting the central role of GSH in cellular defense against oxidative stress, particularly under crowding conditions (52). The increase in GSH levels under these conditions can be interpreted as a compensatory response to the accumulation of reactive oxygen species (ROS), acting as an important protective mechanism to maintain cellular homeostasis and integrity (53). However, this biochemical response reflects a physiological cost incurred by the fish to mitigate stress impacts and preserve vital functions, indicating that higher stocking densities impose a greater oxidative challenge on the organism. Therefore, stocking density emerges as a determining factor not only for productive performance but also for the magnitude of physiological challenge and welfare impairment experienced by the animals.

A clear seasonal influence was also observed, with higher GSH concentrations recorded during winter. This pattern suggests that lower temperatures stimulate a stronger antioxidant defense, as the reduced metabolic rate under cold conditions may increase ROS generation, requiring enhanced neutralization capacity to avoid redox imbalance (54). The activation of these protective pathways represents a fundamental physiological strategy for coping with environmental challenges and reflects the metabolic plasticity of tilapia in maintaining welfare under thermal stress. Even under adverse thermal conditions, elevated GSH serves as a positive marker of antioxidant resilience and adaptive capacity, characteristics associated with more sustainable and ethically managed aquaculture systems.

Beyond temperature, water quality during transport is a determining factor in modulating the antioxidant response. The reduction in dissolved oxygen, particularly at extreme densities (375 and 475 kg/m^3) during winter, may have compromised aerobic metabolism efficiency, leading to hypoxia and increased ROS production (55). Under such conditions, the enhanced antioxidant activity—including higher GSH levels—represents a compensatory mechanism aimed at cellular protection. Similarly, ammonia accumulation, more pronounced at 475 kg/m^3 in winter, may have added a cytotoxic component to physiological stress, disrupting osmotic and acid-base balance. This imbalance can trigger antioxidant defenses, as ammonia impairs mitochondrial function and amplifies ROS generation (56).

Therefore, the combination of relative hypoxia, accumulation of nitrogenous metabolites, and thermal stress acted as a physiological trigger, inducing the synthesis and maintenance of elevated GSH as a cellular protection mechanism. This response reflects the adaptive capacity of tilapia to modulate its antioxidant system in the face of multiple environmental stressors, which is essential to preserve welfare during transport. In addition to protecting cells from oxidative damage, maintaining redox balance directly contributes to preserving muscle integrity and, consequently, filet quality post-harvest. Thus, the observed antioxidant regulation reinforces the importance of management practices that minimize physiological stress, ensuring both animal welfare and the production of high-quality, value-added fish products.

Catalase (CAT), an essential enzyme responsible for decomposing hydrogen peroxide (H_2O_2) into water and oxygen, showed no interaction effect between stocking density and season, nor any isolated influence of these factors. These results suggest that CAT activity may be less sensitive to variations in oxidative stress levels under the experimental conditions tested, aligning with previous studies that reported inconsistent responses of this enzyme under different environmental contexts and stress intensities (57). The absence of significant changes in CAT activity may reflect a physiological adaptation of Nile tilapia, indicating that, even under handling and transport challenges, fish were able to maintain the efficiency of their enzymatic antioxidant defenses. This finding reinforces the importance of animal welfare in preserving cellular and metabolic integrity during pre-slaughter procedures.

Lipid oxidation, one of the main consequences of oxidative stress, was assessed indirectly through thiobarbituric acid reactive substances (TBARS), which indicate the accumulation of lipid peroxidation by-products such as malondialdehyde (MDA). However, the absence of significant variation in TBARS levels observed in this study can be attributed to two main factors: the relatively short transport duration and the efficiency of the tilapia's endogenous antioxidant defense systems—particularly those mediated by GSH and CAT (53). These mechanisms appear to have effectively neutralized reactive oxygen species (ROS), preventing the propagation of lipid peroxidation and preserving cellular membrane integrity. These findings suggest that the transport stress, while metabolically intense (as evidenced by elevated lactate), was temporally limited and did not progress to chronic oxidative damage. This underscores an important distinction: acute metabolic stress responses do not necessarily

culminate in cellular oxidative injury when transport duration is controlled.

4.4 Welfare implications of the tested density range

A critical consideration in interpreting the present results is that all tested densities (375–475 kg/m^3) substantially exceed the 300 kg/m^3 welfare threshold established by Pongsetkul et al. (58) for Nile tilapia transport. Their study, which evaluated densities from 60 to 420 kg/m^3 over 4–5 h, demonstrated that transport densities exceeding 300 kg/m^3 resulted in significantly elevated cortisol (75% increase), glucose (40% increase), and lactate (65% increase) concentrations compared to densities at or below 300 kg/m^3, alongside reduced water-holding capacity (8% reduction) and accelerated quality deterioration during subsequent refrigerated storage. In the present study, plasma lactate concentrations ranged from 4 to 15 mmol L^{-1} across treatments, with five of six density–season combinations exceeding 9 mmol L^{-1}. These values substantially exceed normal baseline levels in unstressed Nile tilapia (1–3 mmol L^{-1}) and indicate severe metabolic stress. Only summer transport at 375 kg/m^3 approached moderate stress levels (4 mmol L^{-1}), though even this value remains elevated above baseline. This physiological evidence corroborates that welfare was compromised across nearly all experimental conditions, regardless of season or density within the evaluated range. The limited differences among densities observed in our study likely reflect that we compared transport conditions within a uniformly high-stress range, where all treatments exceeded adaptive capacity to varying degrees. Under such conditions, finescale differences among densities become less apparent because the primary physiological challenge—maintaining homeostasis under thermal and crowding stress that collectively exceed compensatory capacity—is present across all treatments.

The dominant seasonal effect demonstrates that thermal stress was the primary modulator of welfare outcomes under these experimental conditions, but this does not diminish the baseline welfare concerns associated with transport densities exceeding established thresholds. Current industry practice in Brazil commonly employs transport densities between 375 and 550 kg/m^3, as reflected in national regulatory guidelines permitting up to 550 kg/m^3 for adult Nile tilapia (10). However, these regulations lack empirical welfare validation, and our results—combined with those of Pongsetkul et al. (58)—indicate that such densities impose welfare costs even when active oxygenation is provided and transport duration is limited to 1.5 h. The present findings should not be interpreted as validating the tested densities as welfareoptimal. Rather, they demonstrate that within the high-density range currently employed commercially, seasonal thermal management and water quality control exert stronger immediate effects on physiological outcomes than adjustments of 50 kg/m^3 increments within an already compromised density range.

From an animal welfare perspective, future research should investigate whether transport at densities at or below 300 kg/m^3—potentially combined with thermal control—yields welfare and quality benefits that justify increased logistical costs. Until such evidence is available, transport at densities exceeding 300 kg/m^3 should be recognized as a welfare compromise accepted for economic and operational efficiency, rather than an evidence-based best practice for fish welfare. The present study contributes to this discussion by demonstrating that, within the high-density operational context commonly used in Brazilian aquaculture, prioritizing seasonal thermal management represents a more immediately impactful strategy than finetuning density within the 375–475 kg/m^3 range, while acknowledging that achieving optimal welfare standards may ultimately require reconsideration of industry-standard transport densities.

Overall, the present findings indicate that welfare responses and filet quality outcomes during pre-slaughter transport were predominantly driven by seasonal conditions, whereas transport density within the evaluated range (375–475 kg/m^3) exerted comparatively limited and context-dependent effects. Physiological indicators, including plasma glucose, lactate, and reduced glutathione, demonstrate that the magnitude of the stress response was largely modulated by environmental temperature and associated changes in water quality during transport (e.g., dissolved oxygen and ammonia), which collectively influence HPI-axis activation and metabolic costs, even under short transport duration and active oxygenation. In parallel, postmortem muscle traits such as pH, water-holding capacity, color, and texture primarily reflected temperature-driven effects on muscle metabolism, proteolytic activity, and pigment stability. Although transport density influenced specific attributes—particularly selected texture parameters—these effects did not follow a consistent or progressive pattern across the density gradient tested. Taken together, these results refine the interpretation of density effects by demonstrating that, under short-distance transport (~1.5 h) with adequate oxygenation, seasonal thermal management and water quality control represent more critical determinants of fish welfare and filet quality than fine-scale adjustments of stocking density within the range of 375–475 kg/m^3. Importantly, this does not preclude a greater role of density under different operational scenarios, such as longer transport durations, suboptimal oxygenation, or more extreme densities, where crowding-related stress may become more pronounced.

5 Conclusion

The results of this study demonstrate that fish welfare during pre-slaughter transport is a key determinant of final filet quality in Nile tilapia, with seasonal conditions exerting a stronger and more consistent influence than transport density within the evaluated range (375–475 kg/m^3). Integrated physiological, oxidative, and technological indicators indicate that seasonal thermal stress predominantly modulated metabolic responses and postmortem muscle traits, whereas density-related effects were limited, variable, and context-dependent.

Although transport density influenced specific parameters, these effects did not support consistent or season-specific density recommendations under the tested conditions. Therefore, for short-duration transport with adequate oxygenation, management strategies prioritizing seasonal thermal control and water quality stabilization are more critical for safeguarding fish welfare and filet quality than fine-scale adjustments of transport density within this operational range. These findings reinforce the importance of seasonally informed, welfare-based transport practices for sustainable and ethically responsible tilapia production.

It is important to recognize that all transport densities evaluated in this study (375–475 kg/m^3) exceeded previously established welfare thresholds for Nile tilapia, and that elevated stress biomarkers (lactate 4–15 mmol L^{-1} vs. a normal baseline of 1–3 mmol L^{-1}) indicate compromised welfare in most treatments. Therefore, although our results demonstrate that seasonal thermal management exerts a greater influence than density optimization within the 375–475 kg/m^3 range, this conclusion applies specifically to the high-density operational context commonly employed in Brazilian commercial aquaculture, rather than to welfare-optimal conditions. The findings presented here should guide improvements in current industry practices through the prioritization of seasonal thermal management and water quality control, while recognizing that additional welfare gains may ultimately require the evaluation of transport densities below 300 kg/m^3 to determine whether commercially viable protocols can be developed that are aligned with evidence-based welfare standards.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by Ethics Committee on Animal Use (CEUA), State University of Londrina (UEL), Londrina, Paraná, Brazil. Protocol no. 043.2023. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

DT: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. AMB: Funding acquisition, Project administration, Software, Supervision, Visualization, Writing – review & editing. RC: Data curation, Software, Supervision, Visualization, Writing – review & editing. JR: Formal analysis, Methodology, Visualization, Writing – review & editing. KF: Methodology, Writing – review & editing. GF: Conceptualization, Investigation, Methodology, Writing – original draft. AGB: Investigation, Methodology, Writing – original draft. NO: Investigation, Methodology, Writing – original draft. VB: Formal analysis, Methodology, Writing – original draft. NF: Formal analysis, Methodology, Writing – original draft.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was used in the creation of this manuscript. Generative AI tools (ChatGPT, OpenAI) were used to improve English grammar and language clarity, but not to generate scientific content, data, or conclusions.

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Publisher's note

References

1. FAO. *The State of World Fisheries and Aquaculture 2022: Towards Blue Transformation*. Rome: Food and Agriculture Organization of the United Nations (2022).

2. Peixe BR. *Anuário Peixe BR da Piscicultura 2025*. São Paulo: Associação Brasileira da Piscicultura (2025).

3. Poli MB. Farmed fish welfare—suffering assessment and impact on product quality. *Ital J Anim Sci.* (2009) 8:139–60. doi: 10.4081/ijas.2009.s1.139

4. Lines JA, Spence J. Safeguarding the welfare of farmed fish at harvest. *Fish Physiol Biochem.* (2012) 38:153–62. doi: 10.1007/s10695-011-9561-5

5. Barton BA. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. *Integr Comp Biol.* (2002) 42:517–25. doi: 10.1093/icb/42.3.517

6. Ashley PJ. Fish welfare: current issues in aquaculture. *Appl Anim Behav Sci.* (2007) 104:199–235. doi: 10.1016/j.applanim.2006.09.001

7. Refaey MM, Tian X, Tang R, Li D. Changes in physiological responses, muscular composition and flesh quality of channel catfish (Ictalurus punctatus) suffering from transport stress. *Aquaculture.* (2017) 478:9–15. doi: 10.1016/j.aquaculture.2017.01.026

8. Bortoletti M, Fonsatti E, Leva F, Maccatrozzo L, Ballarin C, Radaelli G, et al. Influence of transportation on stress response and cellular oxidative stress markers in juvenile meagre (Argyrosomus regius). *Animals.* (2023) 13:3288. doi: 10.3390/ani13203288

9. Pan CH, Chien YH, Wang YJ. The antioxidant capacity response to hypoxia stress during transportation of characins (Hyphessobrycon callistus Boulenger) fed diets supplemented with carotenoids. *Aquac Res.* (2010) 41:973–81. doi: 10.1111/j.1365-2109.2009.02380.x

10. Ministério da Agricultura, Pecuária e Abastecimento (MAPA). *Manual de Boas Práticas No Transporte de Peixes*. Brasília: MAPA (2022). Available online at: https://www.gov.br/agricultura (Accessed March 15, 2024).

11. Leonard JN, Chambers LD, Halsey LG, Killen SS. Thermal performance curves and critical thermal limits of Nile tilapia (Oreochromis niloticus). *J Therm Biol.* (2022) 104:103167. doi: 10.1016/j.jtherbio.2022.103167

12. Davis KB. Temperature affects physiological stress responses to acute confinement in tilapia (Oreochromis niloticus). *Comp Biochem Physiol A Mol Integr Physiol.* (2004) 139:263–8. doi: 10.1016/j.cbpb.2004.09.012

13. Jinagool P, Sanguandee S, Jatupornchai P, Sae-ung K, Somsueb P. Effects of elevated water temperature on oxidative stress and mitochondrial function in Nile Tilapia (Oreochromis niloticus). *Aquaculture*. (2024) 579:740428. doi: 10.1016/j.aquaculture.2024.741017

14. INMET. *Normais climatológicas do Brasil (1991–2020)*. Brasília: Instituto Nacional de Meteorologia (2023).

15. IDR-Paraná. *Piscicultura no Paraná: Aspectos Climáticos e Produtivos*. Londrina: Instituto de Desenvolvimento Rural do Paraná (2022).

16. Zheng T, Jia R, Cao L, Du J, Gu Z, He Q, et al. Effects of chronic glyphosate exposure on antioxidative status, metabolism and immune response in tilapia (Oreochromis niloticus). *Comp Biochem Physiol C Toxicol Pharmacol.* (2021) 239:108878. doi: 10.1016/j.cbpc.2020.108878

17. Fiocchi E, Civettini M, Carbonara P, Zupa W, Lembo G, Manfrin A. Development of molecular and histological methods to evaluate oxidative stress biomarkers in sea bass (Dicentrarchus labrax). *Fish Physiol Biochem.* (2020) 46:1577–88. doi: 10.1007/s10695-020-00811-x

18. Zhang X, Li Y, Wang S, Chen Y. Impact of stocking density on the quality and texture of tilapia fillets. *Food Sci Technol.* (2022) 60:110903.

19. Brasil. Ministério da Ciência, Tecnologia e Inovação. Conselho Nacional de Controle de Experimentação Animal (CONCEA). *Guia brasileiro de produção, manutenção ou utilização de animais em atividades de ensino ou pesquisa científica*. 1ª ed. Brasília: MCTI (2023).

20. Brazil. *Manual de Boas Práticas para o Transporte de Peixes Vivos*. Brasília: Ministério da Agricultura, Pecuária e Abastecimento (MAPA) (2016).

21. Goes ESR, Lara JAF, Gasparino E, Del Vesco AP, Goes MD, Alexandre Filho L, et al. Pre-slaughter stress affects ryanodine receptor protein gene expression and water-holding capacity in fillets of Nile tilapia. *PLoS ONE.* (2015) 10:e0129145. doi: 10.1371/journal.pone.0129145

22. Minolta. *Precise color communication – Color control from perception to instrumentation*. Minolta Co., Ltd. (1998).

23. Bourne MC. *Food Texture and Viscosity: Concept and Measurement*. 2nd ed. New York: Academic Press (2002). doi: 10.1016/B978-012119062-0/50007-3

24. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. (1976) 72:248–54. doi: 10.1016/0003-2697(76)90527-3

25. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med*. (1963) 61:882–8.

26. Beutler E. *Red Cell Metabolism: A Manual of Biochemical Methods*. 2nd ed. New York: Grune and Stratton (1975). p. 8–18.

27. Camejo G, Wallin B, Enojärvi M. Analysis of oxidation and antioxidants using microtiter plates. *Methods Mol Biol.* (1998) 108:377–87. doi: 10.1385/0-89603-472-0:377

28. Boyd CE. *Water Quality: An Introduction*. Cham: Springer (2020). Available online at: https://link.springer.com/book/10.1007/978-3-030-23335-8 (Accessed April 2, 2024).

29. Wendelaar Bonga SE. Hormonal responses to stress in fish. *J Fish Biol.* (2011) 79:139–72.

30. Chen B, Xiao W, Li D, Zou Z, Zhu J, Yu J, et al. Characterization of glucose metabolism in high-growth performance Nile tilapia (Oreochromis niloticus). *Aquaculture.* (2024) 580:740317. doi: 10.1016/j.aquaculture.2023.740317

31. López-Jiménez D, Espinosa-Chaurand LD, Maeda-Martínez AN, Peraza-Gómez V. Combined effect of temperature, salinity and dissolved oxygen on the survival of Nile tilapia fry during transportation, at different densities and durations. *Aquaculture.* (2024) 580:740283. doi: 10.1016/j.aquaculture.2023.740283

32. Pichitkul P, Musig Y, Srisapoome P, Taparhudee W, Tunkijjanukij S, Tabthipwon P, Areechon N. Comparative assessment of plasma cortisol and Heat Shock Protein 70 expression as indicators of temperature stress in Nile tilapia (Oreochromis niloticus Linn.). *J Fish Environ*. (2015) 39:12–28.

33. Tacchi L, Lowrey L, Musharrafieh R, Crossey K, Larragoite ET, Salinas I. Effects of transportation stress and addition of salt to transport water on the skin mucosal homeostasis of rainbow trout (Oncorhynchus mykiss). *Aquaculture.* (2015) 435:120–7. doi: 10.1016/j.aquaculture.2014.09.027

34. Pérez-Sánchez J, Dío-Puertas R, Nieto JM, García de la Gándara F, Encinar JA, et al. Skin mucus of Gilthead Sea Bream (Sparus aurata L): protein mapping and regulation in chronically stressed fish. *Front Physiol.* (2017) 8:34. doi: 10.3389/fphys.2017.00034

35. Murphy AE, Elliott J, Beamish F, Scott D, et al. Exploring epidermal mucus protease activity as an indicator of stress in Atlantic sturgeon (Acipenser oxyrinchus oxyrinchus). *J Fish Biol.* (2020) 97:1322–38. doi: 10.1111/jfb.14489

36. Macaraeg NA, Vera Cruz EM, Templonuevo RMC. Influence of different temperatures and sexes on the survival and stress responses of Nile tilapia (Oreochromis niloticus L.). *Asian J Fish Aquat Res*. (2023) 23:14–23. doi: 10.9734/ajfar/2023/v23i1592

37. Dos Reis Goes ES, De Lara JAF, Gasparino E, Goes MD, Zuanazzi JSG, Lopera-Barrero NM, et al. Effects of transportation stress on quality and sensory profiles of Nile tilapia fillets. *Sci Agric*. (2018) 75:321–8. doi: 10.1590/1678-992x-2016-0387

38. Offer G, Trinick J. On the mechanism of water holding in meat: the swelling and shrinking of myofibrils. *Meat Sci.* (1983) 8:245–81. doi: 10.1016/0309-1740(83)90013-X

39. Lawrie RA. *Lawrie's Meat Science*. 6th ed. Cambridge: Woodhead Publishing (2006). doi: 10.1533/9781845691615

40. Hultmann L, Phu TM, Tobiassen T, Aas-Hansen O, Rustad T. Effects of pre-slaughter stress on proteolytic enzyme activities and muscle quality of farmed Atlantic cod (Gadus morhua). *Food Chem.* (2012) 134:1399–408. doi: 10.1016/j.foodchem.2012.03.038

41. Stien LH, Hirmas E, Bjørnevik M, Karlsen Ø, Nortvedt R, Rørå AMB, et al. The effects of stress and storage temperature on the colour and texture of pre-rigor filleted farmed cod (Gadus morhua L.). *Aquac Res*. (2005) 36:1197–206. doi: 10.1111/j.1365-2109.2005.01339.x

42. Huff-Lonergan E, Lonergan SM. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Sci.* (2005) 71:194–204. doi: 10.1016/j.meatsci.2005.04.022

43. Jørpeland G, Imsland A, Stien LH, Bleie H, Roth B. Effects of filleting method, stress, storage and season on the quality of farmed Atlantic cod (Gadus morhua L.). *Aquac Res*. (2015) 46:1597–607. doi: 10.1111/are.12312

44. Sveinsdóttir K, Martinsdóttir E, Thórsdóttir F, Schelvis R, Kole A, Thórsdóttir I. Evaluation of farmed cod products by a trained sensory panel and consumers in different test settings. *J Sens Stud.* (2010) 25:280–93. doi: 10.1111/j.1745-459X.2009.00257.x

45. den Hertog-Meischke MJA, van Laack RLJM, Smulders FJM. The water-holding capacity of fresh meat. *Vet Q.* (2011) 19:175–81. doi: 10.1080/01652176.1997.9694767

46. Roth B, Slinde E, Arildsen J. Pre or post mortem muscle activity in Atlantic salmon (Salmo salar): the effect on rigor mortis and the physical properties of flesh. *Aquaculture*. (2006) 257:504–510. doi: 10.1016/j.aquaculture.2005.10.021

47. Kiessling A, Espe M, Ruohonen K, Mørkøre T. Texture, gaping and colour of fresh and frozen Atlantic salmon flesh as affected by pre-slaughter iso-eugenol or CO_2 anaesthesia. *Aquaculture*. (2004) 236:645–57. doi: 10.1016/j.aquaculture.2004.02.030

48. Roth B, Moeller D, Veland JO, Imsland A, Slinde E. The effect of stunning methods on rigor mortis and texture properties of Atlantic salmon (Salmo salar). *J Food Sci.* (2002) 67:1462–6. doi: 10.1111/j.1365-2621.2002.tb10306.x

49. Huss HH. *Quality and Quality Changes in Fresh Fish. FAO Fisheries Technical Paper No. 348*. Rome: FAO (1995). Available online at: https://www.fao.org (Accessed May 18, 2024).

50. Lakshmanan R, Simpson BK, Badrie N. Quality of tilapia muscle during pre-slaughter handling: A biochemical and textural analysis. *Food Chem.* (2007) 107:1155–62.

51. Chowdhury S, Roy S, Rahman M. Seasonal variation in transport stress and its impact on fish fillet quality. *J Aquat Sci.* (2010) 25:215–23.

52. Martínez-Álvarez RM, Morales AE, Sanz A. Antioxidant defenses in fish: Biotic and abiotic factors. Rev Fish Biol Fish. (2005) 15:75–88. doi: 10.1007/s11160-005-7846-4

53. Almeida EA, Dias Bainy AC, Dafre AL, Gomes OF, Medeiros MHG, Di Mascio P. Oxidative stress in digestive gland and gill of the brown mussel (Perna perna) exposed to air and re-submersed. *J Exp Mar Bio Ecol.* (2005) 318:21–30. doi: 10.1016/j.jembe.2004.12.007

54. Simc˘ic˘ T, Jesenšek D, Brancelj A. Effects of increased temperature on metabolic activity and oxidative stress in the first life stages of marble trout (Salmo marmoratus). *Fish Physiol Biochem.* (2015) 41:1005–14. doi: 10.1007/s10695-015-0065-6

55. González R, Pérez E, Sánchez J. Physiological responses of Nile tilapia to hypoxia: Effects on blood parameters and oxidative stress. *Comp Biochem Physiol A Mol Integr Physiol.* (2018) 226:55–62.

56. Zhang L, Sun D, Chen Y. Impact of ammonia exposure on the physiological stress responses of Nile tilapia (Oreochromis niloticus). *Fish Physiol Biochem.* (2020) 46:973–82.

57. Bagnyukova TV, Lushchak OV, Storey KB, Lushchak VI. Oxidative stress and antioxidant defense responses by goldfish tissues to acute change of temperature from 3 to 23 ˚ C. *J Therm Biol.* (2007) 32:227–34. doi: 10.1016/j.jtherbio.2007.01.004

58. Pongsetkul J, Benjakul S, Takeungwongtrakul S, Sai-ut S. Impact of stocking density during live transportation on meat quality of Nile tilapia (Oreochromis niloticus) and their changes during storage. *J Food Process Preserv*. (2022) 46:e16523. doi: 10.1111/jfpp.16523

7. CONSIDERAÇÕES FINAIS

Os resultados desta tese demonstram que a produção de tilápias do Nilo (*Oreochromis niloticus*) pode ser otimizada por meio da integração entre melhoramento genético e manejo pré-abate, sem comprometer atributos essenciais de qualidade do filé e bem-estar animal. No contexto do melhoramento genético, a ausência de variação na expressão dos genes RyR3 e m-calpaína indica que a seleção para maior desempenho produtivo não altera esses sistemas regulatórios, sugerindo que o crescimento é promovido por outras vias metabólicas, enquanto mecanismos fundamentais relacionados à homeostase muscular permanecem preservados. Além disso, a estabilidade da composição centesimal e dos parâmetros físico-químicos reforça que o ganho de desempenho pode ser alcançado sem efeitos adversos sobre a qualidade tecnológica do filé, nas condições avaliadas.

No que se refere ao manejo pré-abate, os resultados evidenciam que o bem-estar dos peixes durante o transporte é um fator determinante para a qualidade final do filé, sendo fortemente influenciado pelas condições sazonais. O estresse térmico associado à estação do ano mostrou exercer efeitos mais consistentes sobre as respostas fisiológicas, oxidativas e sobre as características pós-morte do músculo do que as variações de densidade dentro da faixa avaliada (375–475 kg/m³). Embora a densidade de transporte tenha influenciado parâmetros específicos, esses efeitos foram limitados, variáveis e dependentes do contexto, não sustentando recomendações consistentes ou específicas por estação dentro dessa faixa operacional.

Dessa forma, estratégias de manejo que priorizem o controle térmico sazonal e a estabilidade da qualidade da água durante o transporte mostram-se mais eficazes para preservar o bem-estar animal e a qualidade do filé do que ajustes finos de densidade em sistemas de alta intensidade produtiva. É importante destacar que as densidades avaliadas refletem condições operacionais amplamente utilizadas na aquicultura comercial brasileira e podem estar acima de limites ideais de bem-estar, indicando a necessidade de estudos futuros que explorem densidades mais baixas em conjunto com viabilidade produtiva.

Em conjunto, os achados desta tese indicam que o avanço da tilapicultura depende de uma abordagem integrada, na qual a seleção genética voltada ao crescimento e a

adoção de práticas de manejo baseadas em bem-estar atuem de forma complementar. Essa integração permite aumentar a eficiência produtiva sem comprometer a qualidade final do produto, contribuindo para o desenvolvimento de sistemas aquícolas mais sustentáveis, tecnicamente robustos e alinhados às demandas éticas e de mercado.