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FERNANDA PICOLI

USO DO FARELO DE SOJA FERMENTADO (FSFM) E DA BIOMASSA DE *SPIRULINA* (*Arthrospira platensis*) EM SUBSTITUIÇÃO À FARINHA DE PEIXE PARA JUVENIS DE TILÁPIA-DO-NILO (*Oreochromis niloticus*) CULTIVADOS EM SISTEMA DE BIOFLOCOS

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Tese apresentada ao Programa de Pós-Graduação em Ciência Animal, da Universidade do Estado de Santa Catarina, como requisito para obtenção do título de Doutor (a) em Ciência Animal, Área de concentração: Produção Animal.
Orientador: Prof. Dr. Thiago El Hadi Perez Fabregat
Coorientador: Prof. Dr. Diogo Luiz de Alcantara Lopes

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Tese apresentada ao Curso de Pós-Graduação em Ciência Animal, na Universidade do Estado de Santa Catarina, como requisito parcial para obtenção do título de Doutor (a) em Ciência Animal, área de Concentração em Produção Animal.

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“Que ninguém se engane, só se consegue a simplicidade através de muito trabalho”.

(Clarice Lispector)

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RESUMO

O constante e expressivo crescimento da atividade aquícola, faz necessária a intensificação dos sistemas de cultivo e o desenvolvimento de dietas mais eficientes, baratas e biodisponíveis. Neste sentido, o uso de ingredientes alternativos à farinha de peixe, como o farelo de soja fermentado (FSFM) e a biomassa de *Spirulina* (*Arthrospira platensis*), devem ser considerados. Para tal, foram realizados dois experimentos. No experimento 1, objetivou-se avaliar o efeito da inclusão do FSFM em dietas vegetais sobre o desempenho zootécnico e a saúde intestinal de juvenis de tilápia-do-Nilo (*Oreochromis niloticus*) cultivados em sistema de bioflocos (BFT). O FSFM foi avaliado em quatro níveis de inclusão em dietas vegetais (7, 14, 21 e 28%) em comparação com uma dieta controle positivo com farinha de peixe e uma dieta controle negativo sem proteína animal e sem FSFM. O delineamento foi o inteiramente ao acaso com seis tratamentos e quatro repetições. O experimento teve duração de 54 dias. Todas as dietas eram isoproteicas (33% de PB) e isoenergéticas (4.000 kcal de EB/kg). Os juvenis (1,635 ± 0,198) foram distribuídos em 24 tanques (70 L) na densidade de sete animais por unidade experimental. No final do experimento, foram analisados os parâmetros zootécnicos e a histomorfometria intestinal. A inclusão de 7% de FSFM melhorou o crescimento de juvenis de tilápia-do-Nilo alimentados com dietas vegetais, os resultados obtidos para esta dieta não diferiram dos obtidos com a dieta contendo farinha de peixe. O aproveitamento das dietas contendo FSFM não diferiu do controle positivo. Nos níveis acima de 21% de inclusão de FSFM houve evidências de melhora na saúde intestinal dos juvenis de tilápia-do-Nilo. No experimento 2, objetivou-se avaliar o efeito do sistema de cultivo (águas claras x BFT) e da inclusão de biomassa de *Spirulina* em substituição da farinha de peixe (0%, 33, 66 e 100%) sobre o desempenho, histomorfometria intestinal, bioquímico sérico e estresse oxidativo de juvenis de tilápia-do-Nilo recebendo níveis subótimos de proteína. O delineamento foi inteiramente casualizado em esquema fatorial 4x2 com quatro repetições e o experimento teve duração de 48 dias. Foram formuladas quatro dietas isoproteicas (28% PB) e isoenergéticas (4.000 kcal EB/kg). Os juvenis (0,23±0,01g) foram distribuídos em 16 tanques circulares (70 L) na densidade de 7 peixes por tanque. Nenhuma interação foi observada entre os sistemas e os tratamentos avaliados. O cultivo em sistema BFT evitou os efeitos negativos de dietas contendo níveis subótimos de proteína sobre o desempenho zootécnico, histomorfometria intestinal e metabolismo proteico de juvenis

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de tilápia-do-Nilo. Nos peixes cultivados neste sistema, houve evidências de melhorias na resposta antioxidante e menor estresse oxidativo fisiológico. A biomassa de *Spirulina* pode substituir totalmente a farinha de peixe nas dietas de juvenis de tilápia-do-Nilo. A substituição de 66% da farinha de peixe por biomassa de *Spirulina* melhorou o desempenho dos animais, independente do sistema de cultivo.

Palavras-chave: Desempenho zootécnico. Histomorfometria intestinal. Nutrição animal. Saúde animal. Sistema de bioflocos.

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ABSTRACT

The constant and expressive growth of the aquaculture activity makes it necessary to intensify the rearing systems and the development of more efficient, cheap and bioavailable diets. To this end, the use of alternative ingredients to fish meal, such as fermented soybean meal (FSFM) and *Spirulina* biomass (*Arthrospira platensis*), should be considered. For this porpose, two experiments were carried out. In experiment 1, the objective was to evaluate the effect of including FSFM in plant-based diets on the zootechnical performance and intestinal health of juvenile Nile tilapia (*Oreochromis niloticus*) grown in a biofloc system (BFT). The FSFM was evaluated at four levels of inclusion in plant-based diets (7, 14, 21 and 28%) compared to a positive control diet with fishmeal and a negative control diet with no animal protein and no FSFM. The design was completely randomized with six treatments and four replications. The experiment lasted 54 days. All diets were isoprotein (33% CP) and isoenergetic (4,000 kcal EB/kg). Juveniles (1.635 ± 0.198) were distributed in 24 tanks (70 L) at the density of seven animals per experimental unit. At the end of the experiment, zootechnical parameters and intestinal histomorphometry were analyzed. The inclusion of 7% FSFM improved the growth of Nile tilapia juveniles fed plant-based diets, the results obtained for this diet did not differ from those obtained with the diet containing fish meal. The use of diets containing FSFM did not differ from the positive control. At levels above 21% of inclusion of FSFM there was evidence of improvement in the intestinal health of Nile tilapia juveniles. In experiment 2, the objective was to evaluate the effect of the culture system (clear water x BFT) and the inclusion of *Spirulina* biomass in replacement of fish meal (0%, 33, 66 and 100%) on performance, intestinal histomorphometry, serum biochemistry and oxidative stress of Nile tilapia juveniles receiving suboptimal levels of protein. The design was completely randomized in a 4x2 factorial scheme with four replications and the experiment lasted 48 days. Four isoproteic (28% CP) and isoenergetic (4,000 kcal EB/kg) diets were formulated. Juveniles (0.23±0.01g) were distributed in 16 circular tanks (70 L) at a density of 7 fish per tank. No interaction was observed between the evaluated systems and treatments. rearing in BFT system avoided the negative effects of diets containing suboptimal levels of protein on zootechnical performance, intestinal histomorphometry and protein metabolism of Nile tilapia juveniles. In fish grown in this system, there was evidence of improvements in antioxidant response and lower physiological oxidative stress.

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Spirulina biomass can completely replace fish meal in diets of Nile tilapia juveniles. The replacement of 66% of fish meal by *Spirulina* biomass improved animal performance, regardless of the rearing system.

Keywords: Zootechnical performance. Intestinal histomorphometry. Animal nutrition. Animal health. Biofloc system.

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APRESENTAÇÃO

A tese em questão é composta por dois capítulos, que seguem:

- **CAPÍTULO I – REVISÃO BIBLIOGRÁFICA;**

- **CAPÍTULO II – MANUSCRITOS:**

    - **Manuscrito I –** Mixed culture microorganisms fermented soybean meal improves productive performance and intestinal health of Nile tilapia (*Oreochromis niloticus*) juveniles fed plant-based diets in a biofloc system. Este manuscrito foi redigido de acordo com as normas da Revista *Aquaculture Research.*

    - **Manuscrito II –** Biofloc system avoid the negative effects of diets with suboptimal protein levels on zootechnical performance, intestinal histomorphometry and protein metabolism of Nile tilapia fed *Spirulina* biomass as alternative protein source. O manuscrito foi adequado nas normas da Revista *Aquaculture International.*

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CAPÍTULO 1 – REVISÃO BIBLIOGRÁFICA

1. INTRODUÇÃO

Nos sistemas intensivos de produção de peixes, os custos com alimentação podem chegar a até 60% de todas as despesas (BARBOSA *et al.*, 2020). A farinha de peixe é um ingrediente importante nas dietas aquícolas, trata-se de uma proteína de elevado valor biológico e sua exclusão nas formulações pode levar a piora nos índices zootécnicos (GLENCROSS *et al.*, 2011; TURCHINI; TRUSHENSKI; GLENCROSS, 2019). Está cada vez menos disponível comercialmente e seu custo é elevado e permanece em constante oscilação (WORLD BANK, 2020). Esse incremento no seu custo resulta em maiores valores para produzir animais aquáticos (EL BASUINI *et al.*, 2016; 2017; HOSSAIN *et al.*, 2016). Neste contexto, a busca por ingredientes alternativos é uma estratégia para desenvolver dietas que reduzam os custos e otimizem o desempenho produtivo (BULBUL *et al.*, 2015; ZHANG *et al.*, 2016; DANIEL, 2018; ZHANG *et al.*, 2021). Ingredientes alternativos são subprodutos ou produtos mais acessíveis, de origem vegetal ou animal, provenientes de atividades agrícolas e/ou indústrias de processamento (ABOWEI; EKUBO, 2011).

O farelo de soja é uma das fontes proteicas vegetais mais utilizadas, por sua disponibilidade no mercado e perfil de aminoácidos (SØRENSEN *et al.*, 2009; DANIEL, 2018). A substituição total da farinha de peixe por esse ingrediente nem sempre é possível, devido à baixa palatabilidade, elevado teor de fibra e a deficiência de alguns aminoácidos essenciais, que tendem a limitar a utilização deste farelo (EL-DAHHAR; EL-SHAZLY, 1993; LIMA *et al.*, 2014). Estratégias como a fermentação microbiana, podem melhorar o perfil aminoacídico e a disponibilidade de nutrientes, além de reduzir os componentes fibrosos e impulsionar o desempenho e a saúde dos animais (HONG; LEE; KIM, 2004; SOTOUDEH *et al.*, 2016; VAN NGUYEN *et al.*, 2018). A fermentação é uma técnica de bioconversão ou decomposição metabólica, realizada por microrganismos (bactérias e/ou fungos), que transformam substratos complexos em compostos simples (BALAKRISHNAN; PANDEY, 1996). Trata-se de uma técnica de conservação que também melhora as propriedades nutricionais e funcionais dos ingredientes (ROSS; MORGAN; HILL, 2002; FRIAS *et al.*, 2008). Durante o processo fermentativo, ocorre a produção de metabólitos secundários com ação prebiótica (antibióticos, peptídeos bioativos de baixo peso molecular, enzimas e fatores de

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crescimento) e o incremento da população de microrganismos benéficos com função probiótica (MUKHERJEE; DUTTA; CHAKRABORTY, 2015; DAWOOD; KOSHIO, 2019; WANG *et al.*, 2019).

Já foi demonstrado que o farelo de soja fermentado (FSFM) pode substituir parcialmente a farinha de peixe das dietas sem comprometer o desempenho dos peixes (CHOI *et al.*, 2019; WANG *et al.*, 2019; HE et al, 2020a; 2020b). Também já foram observados inúmeros efeitos do FSFM sobre a saúde dos peixes, como: melhorias nos parâmetros bioquímicos (NOVRIADI *et al.*, 2018), no metabolismo lipídico, na capacidade antioxidante total (JIAN *et al.*, 2018) e na saúde intestinal (WANG *et al.*, 2016; LI *et al.*, 2019; WANG *et al.*, 2019). Além disso, incremento das respostas inflamatórias e da atividade de enzimas digestivas (SHIU *et al.*, 2015; SOTOUDEH *et al.*, 2016; LI *et al.*, 2020) também foram observadas. A utilização do farelo de soja em substituição à farinha de peixe foi avaliada em dietas para tilápias e a substituição pode ser feita de maneira parcial (AL-KENAWY; EL-NAGGAR; ABOU ZEAD, 2008; LIN; LUO, 2011; SHARMA; SAINI, 2017) ou total (EL-SAYD; GABER, 2002; AJANI *et al.*, 2016).

Outro ingrediente alternativo é a microalga filamentosa *Spirulina* (*Artrospira platensis)*, um dos principais organismos fitoplanctônicos estudados para nutrição aquática (AL-ZAYAT, 2019; PLAZA *et al.*, 2019; LEITE *et al.*, 2019; SALAH EL-DIN; ABDULLAH; SAYED, 2021). É considerada uma rica fonte de proteína e aminoácidos essenciais de alta digestibilidade, também possui minerais, vitaminas, ácidos graxos e pigmentos (DE LARA ANDRADE *et al.*, 2005). A *Spirulina é* utilizada na produção animal como fonte de proteínas e corantes (HABIB *et al.* 2008). Na piscicultura tem sido avaliada como suplemento alimentar (MANSOUR *et al.*, 2021; SALAH EL-DIN; ABDULLAH; SAYED, 2021; SOLIMAN; HAMED; SAYED, 2021) ou como substituta parcial ou total da farinha de peixe (CAO *et al.*, 2018; ERDOGAN, 2019; ROOHANI *et al.*, 2019; RAJI *et al.*, 2020), sem prejuízos à saúde ou ao desempenho de tilápias e diversos outros peixes.

Nesse contexto, o uso de alimentos fermentados, como o farelo de soja, em dietas exclusivamente vegetais, com o intuito de promover o crescimento e a saúde de tilápias-do-Nilo cultivadas em sistema de bioflocos, ainda precisa ser avaliado. Além disso, nenhum trabalho foi conduzido com o uso de biomassa de *Spirulina* na alimentação tilápias-do-Nilo, cultivadas em dois sistemas de recirculação de água (águas claras e bioflocos). Fundamentado nisso e na importância da tilápia-do-Nilo (*Oreochromis*

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niloticus) para a piscicultura nacional, foram realizados dois experimentos. No primeiro, foi avaliado o efeito da inclusão do farelo de soja fermentado (FSFM) em dietas vegetais sobre o desempenho zootécnico e a saúde intestinal de juvenis de tilápia-do-Nilo cultivados em sistema de bioflocos (BFT). No segundo, avaliou-se o efeito da inclusão de biomassa de *Spirulina* (*A. platensis)* em substituição à farinha de peixe no desempenho zootécnico, histomorfometria intestinal, bioquímica sérica e estresse oxidativo de juvenis de tilápia-do-Nilo (*O. niloticus*) cultivados em diferentes sistemas de recirculação de água (águas claras e bioflocos).

2. PISCICULTURA NACIONAL E A DEMANDA POR FARINHA DE PEIXE

De acordo com dados da Associação Brasileira de Piscicultura no ano de 2021 a produção piscícola brasileira teve um aumento de 4,7% em relação ao ano anterior (802.930 toneladas) totalizando 841.005 toneladas (PEIXE BR, 2022). O crescimento acumulado é de 45% desde o ano de 2014. Ainda de acordo com a Associação, a tilápia-do-Nilo é a espécie mais produzida, são 543.005 toneladas correspondendo a 65,3% do total da produção de peixes. Houve um incremento de 9,8% em relação ao ano de 2020 (486.155 t). O ranking nacional dos maiores produtores de tilápia começa pelo estado do Paraná, maior produtor, com 182.000 t; seguido de São Paulo, com 76.140 t, Santa Catarina, com 41.700 t e, finalmente, o Mato Grosso do Sul, com 34,450 t.

Até o ano de 2050, estima-se que a população mundial atinja os 9,7 bilhões de habitantes (UN, 2019). Com esse incremento populacional, cresce também a demanda por proteína animal e atividades mais sustentáveis. De maneira a suprir esses gargalos, a intensificação dos sistemas produtivos aliada à incessante busca por ingredientes mais sustentáveis é inevitável e necessária. A piscicultura é uma atividade em constante expansão (FAO, 2022; PEIXE BR, 2022) e a procura por sustentabilidade nesse setor depende de muitos fatores, incluindo alimentação com menores custos. Nos sistemas intensivos de produção de peixes, os custos com alimentação podem chegar a até 60% de todas as despesas (BARBOSA *et al.*, 2020).

A farinha de peixe é um ingrediente importante e comumente utilizado em dietas aquícolas. Trata-se de uma proteína de elevado valor biológico (acima de 55% proteína bruta – NRC, 2011) e sua exclusão nas formulações pode levar a piora nos índices zootécnicos (GLENCROSS *et al.*, 2011; TURCHINI; TRUSHENSKI; GLENCROSS, 2019). Além do excelente perfil de aminoácidos, destaca-se também o conteúdo de

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vitaminas lipossolúveis e hidrossolúveis, altas quantidades de ácidos graxos poliinsaturados de cadeia longa e minerais (MASUMOTO *et al.*, 1996). Uma vez que, esse ingrediente está cada vez menos disponível comercialmente, seu custo, além de elevado, permanece em constante oscilação (WORLD BANK, 2020). Isso não é somente devido ao aumento da demanda pela indústria de rações, mas também pela forte pressão de pesca sobre as espécies forrageiras, ocasionando depleção de alguns desses estoques (NAYLOR *et al.*, 2009). Com incremento no seu custo, maiores valores para produzir animais aquáticos serão alcançados (EL BASUINI *et al.*, 2016; 2017; HOSSAIN *et al.*, 2016). Nos últimos anos, constantes são as pesquisas que visam a substituição da farinha de peixe por ingredientes alternativos, que reduzam os custos com alimentação e otimizem o desempenho produtivo (BULBUL *et al.*, 2015; ZHANG *et al.*, 2016; DANIEL, 2018).

3. INGREDIENTES ALTERNATIVOS À FARINHA DE PEIXE

3.1 FARELO DE SOJA FERMENTADO

O farelo de soja é uma das fontes proteicas vegetais mais utilizadas na nutrição animal, por possuir alto teor proteico, perfil aminoacídico balanceado e disponibilidade no mercado (SØRENSEN *et al.*, 2009; 2018; MUKHERJEE; CHAKRABORTY; DUTTA, 2016; SHARAWY; GODA; HASSAAN, 2016; WANG *et al.*, 2016; DANIEL, 2018; JANNATHULLA *et al.*, 2018). É um produto originado da extração de óleo dos grãos de soja e possui, aproximadamente, 48% de proteína bruta (MUKHERJEE; CHAKRABORTY; DUTTA, 2016).

Resultados recomendam a substituição da farinha de peixe pelo farelo de soja de maneira parcial, entre 25% e 75% para tilápias (AL-KENAWY; EL-NAGGAR; ABOU ZEAD, 2008; LIN; LUO, 2011; AJANI *et al.*, 2016; SHARMA; SAINI, 2017). Esses resultados possibilitam o incremento de fontes de proteína vegetal às dietas sem prejudicar o desempenho (EL-SAIDY; GABER, 2002; AL-KENAWY; EL-NAGGAR; ABOU ZEAD, 2008; LIN; LUO, 2011; AJANI *et al.*, 2016; SHARMA; SAINI, 2017). Também foram observadas melhorias na utilização de nutrientes e na saúde dos animais (AJANI *et al.*, 2016), bem como, na produção total e no retorno econômico líquido (AL-KENAWY; EL-NAGGAR; ABOU ZEAD, 2008).

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A substituição total da farinha de peixe nem sempre é possível (HANSEN *et al.*, 2011; BONALDO *et al.*, 2011; FERRARA *et al.*, 2015). Apesar das inúmeras qualidades, o farelo de soja apresenta alguns fatores antinutricionais (ANF`s) (LIMA *et al.*, 2014). Dentre eles, inibidores de proteases, lecitinas, ácido fítico, saponinas e fibras, além de ser um ingrediente pouco palatável e escasso em alguns aminoácidos essenciais (metionina e lisina) (EL-DAHHAR; EL-SHAZLY, 1993; LIMA *et al.*, 2014). Fatores que tendem a limitar o aproveitamento das proteínas e de outros nutrientes pelo animal. A suplementação com aminoácidos pode possibilitar uma suplementação total (AJANI *et al.*, 2016; EL-SAYD; GABER, 2002), mas outros autores relatam diminuição no consumo (MAHMOUD; KILANY; DESSOUKI, 2014). Todos esses gargalos, justificam a aplicação da técnica de fermentação para este ingrediente (JAKOBSEN *et al.*, 2015; MONIRUZZAMAN *et al.*, 2018; JANNATHULLA *et al.*, 2019).

A fermentação é uma técnica de preservação e processamento dos alimentos que também melhora suas propriedades nutricionais e funcionais originais (FRIAS *et al.*, 2008). O processo consiste na inoculação e fornecimento de condições ideais para o crescimento de microrganismos benéficos (fungos e/ou bactérias) em ingredientes de origem animal ou vegetal. O objetivo é realizar a conversão biológica de substratos complexos em compostos simples, formando produtos habituais da fermentação (dióxido de carbono e álcool) (BALAKRISHNAN; PANDEY, 1996). O produto final também será rico em populações de microrganismos probióticos e metabólitos secundários como antibióticos, peptídeos bioativos de baixo peso molecular, enzimas e fatores de crescimento, todos com ação prebiótica (BALAKRISHNAN; PANDEY, 1996; KADER *et al.*, 2011; MUKHERJEE; DUTTA; CHAKRABORTY, 2015; DAWOOD; KOSHIO, 2019; WANG *et al.*, 2019).

Para a nutrição animal, a principal técnica de fermentação utilizada é a fermentação em estado sólido (FES) (YANG; ZENG; QIAO, 2021). Essa técnica baseia-se na inoculação de microrganismos em grãos ou farelos e na utilização de teores de umidade entre 40 e 80% (ORIOL *et al.*, 1988a, 1988b; BORZANI *et al.*, 1999; YANG; ZENG; QIAO, 2021) visando a produção de ingredientes secos fermentados na forma de grão inteiro ou pó (SUBRAMANIYAM; VIMALA, 2012). *Aspergillus* spp., *Rhizopus* spp. e *Lactobacillus* spp. são os principais microrganismos utilizados neste método (SUBRAMANIYAM; VIMALA, 2012) visando a produção de enzimas, ácidos orgânicos e de biopesticidas (LU; MADDOX; BROOKS, 1998; BATTAN; SHARMA; KUHAD, 2006). Também pode ser utilizada para o desenvolvimento de ingredientes para

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alimentação animal (MUKHERJEE; DUTTA; CHAKRABORTY, 2015; DAWOOD; KOSHIO, 2019; WANG *et al.*, 2019).

Os principais microrganismos utilizados na técnica de fermentação são do gênero *Bacillus* (WONGPUTTISIN *et al.* 2007; TENG *et al.*, 2012), *Aspergillus* (ILYAS *et al.*, 1995; HIRABAYASHI *et al.*, 1998; HONG; LEE; KIM, 2004; KISHIDA *et al.*, 2000; FENG *et al.*, 2007a; 2007b), *Lactobacillus* (AMADOU *et al.*, 2010a; 2010b; CHI; CHO, 2016; WANG *et al.*, 2016) e *Saccharomyces* (CHI, CHO, 2016; HASSAAN *et al.*, 2018). Cada um deles é responsável pela produção de diferentes produtos (ácido lático, etanol, ácido acético, entre outros), já que respondem a cada substrato de forma distinta (NIBA *et al.*, 2009). Por exemplo, *Lactobacillus* tendem a produzir ácido lático e cítrico, já as leveduras, etanol e CO_2 (SUBRAMANIYAM; VIMALA, 2012).

Estudos mostram que a fermentação aumenta a biodisponibilidade e a digestibilidade de nutrientes, além de diminuir os antinutrientes das proteínas de origem vegetal (WANG *et al.*, 2016; HASSAAN *et al.*, 2018). Nesse processo fermentativo, há a degradação de macromoléculas proteicas em compostos solúveis de baixo peso molecular pelos microrganismos atuantes dessa bioconversão. Esses compostos, formados por pequenas cadeias aminoacídicas e por peptídeos bioativos, são mais bem assimilados, melhorando a digestão e absorção de nutrientes e a saúde dos animais (AZARM; LEE, 2012; SANJUKTA; RAI, 2016). Ainda neste sentido, outras vantagens e benefícios da fermentação para o FSFM já foram observados, que seguem:

• Melhoria da qualidade nutricional e da atividade antioxidante do farelo de soja (DA SILVA *et al.*, 2018);

• Fonte de enzimas, ácidos orgânicos e fatores de crescimento (KADER *et al.*, 2011; MUKHERJEE; DUTTA; CHAKRABORTY, 2015; DAWOOD; KOSHIO, 2019; HA *et al.*, 2019; WANG *et al.* 2019);

• Aumento da atividade da enzima fibrinolítica, da digestibilidade in vitro da tripsina e da solubilidade do nitrogênio (MUKHERJEE; CHAKRABORTY; DUTTA, 2016; SHARAWY; GODA; HASSAAN, 2016; WANG *et al.*, 2016; JANNATHULLA *et al.*, 2018);

• Incremento dos teores de proteína bruta em aproximadamente 10%, quando comparado ao ingrediente original, sem alterar o perfil de aminoácidos essenciais (HONG; LEE; KIM, 2004);

• Diminuição dos teores de fibra, ANFs e teores tóxicos dos ingredientes, facilitando a absorção, o aproveitamento dos nutrientes e a palatabilidade. Dessa forma,

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melhorias na eficiência alimentar e no desempenho dos animais podem ser alcançados (MAKKAR, 1993; YAMAMOTO *et al.*, 2010; JAKOBSEN *et al.*, 2015);

• Ação imunoestimulante e tolerância a estressores ambientais, melhorando a saúde dos animais cultivados (MONIRUZZAMAN *et al.*, 2018; JANNATHULLA *et al.*, 2019).

O FSFM é um ingrediente rico em peptídeos bioativos, pode apresentar teor proteico e aminoacídico elevado, além de menores ANF's e alérgenos, em comparação ao farelo de soja (SHIU *et al.*, 2015; WANG *et al.*, 2016; CHI; CHO, 2016; LI *et al.*, 2019). Também é rico em compostos bioativos com função reguladora e funcional, podendo agir como anti-hipertensivo, antimicrobiano, antioxidante e antidiabético (SINGH; VIJ; HATI, 2014; SANJUKTA; RAI, 2016).

O FSFM vem sendo amplamente testado como substituto para a farinha de peixe e alimento funcional em dietas aquícolas. O FSFM pode substituir entre 25 e 60% da farinha de peixe das dietas piscícolas, sem prejudicar o desempenho e ainda, trazer inúmeros benefícios para a saúde dos animais, conforme pode ser observado na Tabela 1. Efeitos benéficos para a saúde dos peixes com a inclusão de FSFM nas dietas também já foram relatados: melhoria das respostas imunes inespecíficas, redução de inflamações, modulação da microbiota e aumento da atividade de enzimas digestivas (YAMAMOTO *et al.*, 2010; SHIU *et al.*, 2015; LEE; AZARM; CHANG, 2016; ILHAM; FOTEDAR, 2016; SOTOUDEH *et al.*, 2016; NOVRIADI *et al.*, 2018; CHOI *et al.*, 2019; RAHIMNEJAD *et al.*, 2019; LI *et al.*, 2020). Além disso, alterações nos parâmetros bioquímicos e hematológicos, no metabolismo lipídico e na saúde hepato-intestinal e aumento da capacidade antioxidante total também foram observados (YAMAMOTO *et al.*, 2010; AZARM; LEE, 2012; BARNES; BROWN; NEIGER, 2015; WANG *et al.*, 2016; JIANG *et al.*, 2018; NOVRIADI *et al.*, 2018; WANG *et al.*, 2019).

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Tabela 1 - Estudos com o uso do farelo de soja fermentado (FSFM) para diversas espécies de peixes.

Parâmetros testados	**Espécie/ Peso (g)**	**Níveis testados**	**Principais resultados/Conclusão**	**Referência**
Desempenho	Tilápia híbrida *(Oreochromis niloticus× O. aureus)* / 0,36	Controle: Sem FP; Dieta I: 0-20% de FP ou 57-31,6% de DSBM; Dieta II: 0-20% de FP ou 51-28,3% de FSFM com metionina encapsulada	Menor desempenho em dietas sem FP. A adição de metionina encapsulada não melhorou o crescimento do grupo sem FP.	Wu *et al.*, 2003
Desempenho, eficiência alimentar, digestibilidade, hematologia, status biliar e morfologia hepato-intestinal	Truta arco-íris (*Oncorhynchus mykiss)* / 12,0	Dieta FP; Dieta FS; Dieta FSFM I: adição inicial de água de 30% (fermentado por 7 h); Dieta FSFM II: 5% (10 h).	Melhor desempenho e eficiência alimentar nos grupos alimentados com dieta FP e FSFM II; Melhor saúde biliar e hepato-intestinal em dietas com o fermentado	Yamamoto *et al.*, 2010
Desempenho, parâmetros bioquímicos e digestibilidade	Pargo negro *(Acanthopagrus schlegeli)* / 1,17	FSFM (0, 80, 160, 240 e 320 g/kg) suplementado com metionina, lisina e taurina em substituição a FP	Substituição de FP por FSFM suplementado em até 40% sem prejuízos ao desempenho	Azarm; Lee, 2012
Desempenho, atividade digestiva enzimática e alterações no fígado e intestino distal	Garoupa *orange-spotted (Epinephelus coioides*) / 4,46	Controle com FP, substituição de FM por FS em 10%, 20 e 30% e em 10, 20, 30 e 40% por FSFM	Substituição de FP por FSFM em até 30% sem prejuízos ao desempenho e eficiência alimentar. Melhora hepática, intestinal e digestiva ao substituir FP por FS e FSFM	Shiu *et al.*, 2015
Desempenho, índices organossomáticos e histologia intestinal	Truta linhagens Shasta x McConaughy (*Oncorhynchus mykiss)* / 23,4	Dietas com 0, 35 ou 50% de FSFM e 40, 15 ou 0% de FP	Dietas contendo até 35% de FSFM e 15% de FP são aceitáveis para ambas as linhagens, sem prejuízos ao desempenho e histologia intestinal	Barnes; Brown; Neiger, 2015
Desempenho, atividade enzimática e fisiologia sanguínea	Barramundi (*Lates calcarifer)* / 5,04	FS ou FSFM suplementado ou não com selênio orgânico em substituição a FP	Melhoria no desempenho com o uso de selênio orgânico tanto para FS quanto para FSFM, mas não diferiu da dieta com FP.	Ilham; Fotedar, 2016

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Parâmetros testados	Espécie/ Peso (g)	Níveis testados	Principais resultados/Conclusão	Referência
Desempenho, composição corporal e atividade digestiva enzimática	Truta marrom Caspian (*Salmo truta caspius)* / 2,1	FP substituída por FS e FSFM não tratada ou irradiada por raios gama aos 15 e 30 kGy	O nível de radiação gama utilizado não foi eficiente, mas a fermentação do FS melhorou o peso final, atividade enzimas proteolíticas e composição corporal	Sotoudeh *et al.*, 2016
Digestibilidade da matéria seca e da proteína do FSFM em dietas e comparação com FS e FM	Garoupa *orange-spotted (Epinephelus coioides*) / 22,01	Controle com FP; Dietas teste com 300 g/kg de FS ou FSFM em substituição a FP	Melhoria na digestibilidade aparente das dietas com FSFM	Zhuo; Liu; Lin, 2016
Desempenho, resposta imune e morfologia intestinal	Psetta máxima - pregado *(Scophthalmus maximus)* / 5,11	Controle com FP; Dietas teste com substituição da FP por FS ou FSFM *Lactobacillus plantarum* em 15, 30, 45 e 60%	Substituição da proteína do FP pelo FSFM com *L. plantarum* em até 45% sem prejuízos as variáveis estudadas.	Wang *et al.*, 2016
Desempenho, composição corporal e atividade antioxidante enzimática	Rockfish *(Sebastes schlegeli)* / 1,2	FSFM (0, 8, 16, 24, 32%) substituindo a proteína da FP em 0, 10, 20, 30 e 40%, aproximadamente	Substituição da proteína do FP pelo FSFM em até 40% sem efeito negativo ao desempenho dos animais	Lee; Azarm; Chang, 2016
Microbiota intestinal	Salmão-do-Atlântico (*Salmo salar*) / 50	Controle com 30% FP, FS com 30% ou FSFM com 30%	O uso de FSFM promoveu saúde e proteção intestinal no salmão	Catalán *et al.*, 2017
Desempenho, perfil de ácidos graxos e composição corporal	Robalo japonês *(Lateolabrax japonicus)* / 13,3	FSFM (0, 25, 50 e 75%) em substituição a FP	Substituição de FP por FSFM em até 25% sem prejuízos ao desempenho, qualidade da carne e utilização da ração	Liang *et al.*, 2017
Desempenho e viabilidade econômica	Anabas (*Anabas testudineus*) / 1,03	Controle; FSFML substituindo o FP em 0, 25, 50, 75 ou 100% da PB do FP	Desempenho similares ao controle foram observados até o nível de 50% de FSFML. Economicamente o FSFML pode ser incluído em até 50% da proteína da FP	Kader *et al.*, 2018

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Parâmetros testados	Espécie/ Peso (g)	Níveis testados	Principais resultados/Conclusão	Referência
Desempenho, condição morfológica e bioquímica sérica do fígado e intestino distal	Pampo-verdadeiro *(Trachinotus carolinus)* / 17,01	FSFM (0, 25, 50, 75 e 100%) em substituição a FS	Melhoria nos parâmetros bioquímicos conforme a inclusão de FSFM. Melhoria na saúde intestinal nas duas maiores inclusões do fermentado	Novriadi *et al.*, 2018
Desempenho, composição corporal e parâmetros bioquímicos plasmáticos	Achigã *(Micropterus salmoides)* / 17,1	FSFMR (0, 20, 40 60g/kg da proteína) em substituição ao FS	O melhor desempenho foi observado na substituição de 40g/kg. Melhoria do metabolismo lipídico e da capacidade antioxidante	Jiang *et al.*, 2018
Desempenho, morfologia e microbiota intestinal	Corvina amarela *(Larimichthys crocea)* / 10,0	FSFM (0, 15, 30, 45, 60 e 75%) em substituição a FP	Substituição pelo FSFM em até 45% da FP sem efeitos negativos no desempenho e integridade intestinal dos animais	Wang *et al.*, 2019
Desempenho e utilização da ração	Truta arco-íris (*Oncorhynchus mykiss*)	Controle com FM; FSFM 1 e FSFM 2 (técnicas de fermentação), ambos com 20, 40 e 60% em substituição a FP	Melhoria do estresse oxidativo com o uso de FSFM. Substituição da FP em até 40% pelos fermentados, sem efeitos no crescimento e utilização da ração	Choi *et al.*, 2019
ANF’s, desempenho, digestibilidade e saúde intestinal	Psetta máxima - pregado *(Scophthalmus maximus)* / 8,53	Controle com FP (0%), 30%, 45% e 60% com FS e 30%, 45% e 60% com FSFM	Redução dos ANF’s com a fermentação, melhoria no desempenho e digestibilidade até 45% de FSFM; melhoria da saúde intestinal nos dois maiores níveis	Li *et al.*, 2019
Desempenho, status antioxidante, microbiota intestinal, morfologia e respostas inflamatórias	Psetta máxima - pregado *(Scophthalmus maximus)* / 7,57	Controle com FP; 45% de FS ou de FSFM com *Enterococcus faecium*	A fermentação neutralizou os efeitos negativos do FS, melhorou a capacidade antioxidante, suprimiu as respostas inflamatórias e modulou a microbiota intestinal	Li *et al.*, 2020

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Parâmetros testados	Espécie/ Peso (g)	Níveis testados	Principais resultados/Conclusão	Referência
Desempenho, composição corporal e aminoacídica, parâmetros bioquímicos e histologia intestinal	Achigã *(Micropterus salmoides)* / 4,43	Controle 35g/kg de FP; Substituição de FP por FS e por FSFM em 15%, 30%, 45% e 60%	Substituição pelo FSFM em até 30% da FP sem prejuízos aso desempenho, a digestibilidade e a saúde intestinal	He *et al.*, 2020a
Desempenho, histologia e microbiota intestinal	Achigã *(Micropterus salmoides)* / 4,43	Controle 35g/kg de FP; Substituição de FP por FS e por FSFM em 30% e 60%	Substituição pelo FSFM em até 30% da FP nas dietas sem impactos negativos no desempenho e a saúde intestinal	He *et al.*, 2020b
Desempenho, histologia intestinal, atividade enzimática, expressão de genes pró-inflamatórios	Garoupa genciana (*Epinephelus fuscoguttatus* × *E. lanceolatus*) / 9,0	Controle FP; Substituição de FP por FSFM em 20% e em 40%	Substituição pelo FSFM em 20-40% da FP afetou o desempenho, a morfologia intestinal e a expressão de genes relacionados ao sistema imunológico	Zhang *et al.*, 2021
Desempenho, composição da carcaça e hepática	Robalo asiático (*Lates calcarifer*) / 1,27	Controle – FP sem atrativos; Controle + FP com atrativos; Substituição da FP em 20, 40, 60, 80 e 100% por mistura de proteínas de 60% PBM e 40% FSFM	Substituição pelo FSFM em até 60% da FP sem prejuízos ao desempenho	Hong *et al.*, 2021
Desempenho, microbiologia, atividade enzimática e histologia intestinal	Bagre sul-africano – jundiá (*Rhamdia quelen)* /	Controle com FP; Substituição de FP por FSFM *Lactobacillus acidophilus* em 7, 14, 21 e 28%	Substituição pelo FSFM até 21% sem prejuízos ao desempenho. Menor contagem de *Vibrionaceae* em 7-21%. O FSFM não afetou a contagem de bactérias heterotróficas, atividade enzimática e histologia intestinal.	De Oliveira *et al.*, 2022

FP: Farinha de Peixe; FS: Farelo de Soja; FSFM: Farelo de Soja Fermentado; DSBM: Farelo de Soja Descascado; PBM: Subproduto de aves; Aa's: aminoácidos; kGy: quilo Gray; kDa: quilo Dalton; FSFMR: Resíduo de Farelo de Soja Fermentado; FSFML: Fermentado com mistura de farinha de soja e subproduto de lula; ANF's: Fatores Antinutricionais.

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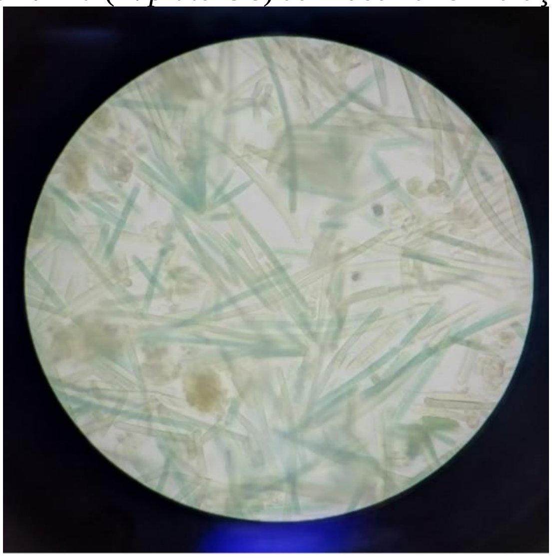
3.2 BIOMASSA DE *SPIRULINA* (*Arthrospira platensis*)

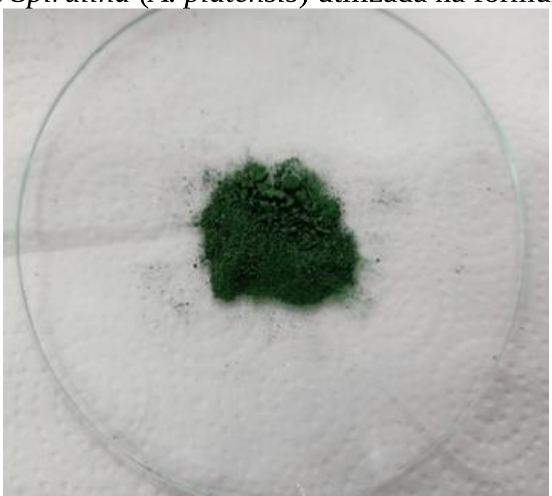
As proteínas de origem unicelular, incluindo microalgas, leveduras e bactérias, são fontes alternativas promissoras para substituir a farinha de peixe das dietas piscícolas (HARDY, 2010). São utilizadas frequentemente como ingredientes para organismos aquáticos em diferentes fases de desenvolvimento (ERDOGAN, 2019; RAJI *et al.*, 2020; MANSOUR *et al.*, 2021). Dentre elas, a *Spirulina* é uma das candidatas potenciais para essa substituição. É uma microalga filamentosa de superfície lisa que possui a ficocianina e a clorofila como pigmentos fotossintéticos principais e responsáveis por sua cor azul-esverdeada (CAPELLI *et al.*, 2010; ALI; SALEH, 2012) (Figuras 1 e 2.).

Figura 1 – Biomassa de *Spirulina* (*A. platensis*) utilizada na formulação das dietas (40x).



Fonte: Elaborada pelo autor, 2022.

Figura 2 - Biomassa de *Spirulina* (*A. platensis*) utilizada na formulação das dietas.



Fonte: Elaborada pelo autor, 2022.

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A *Spirulina* é composta por quantidades de ácidos graxos poliinsaturados ômega-6 expressivas, especialmente, ácido gama-linolênico e ácido linoleico (HENRIKSON, 1995; COLLA *et al.*, 2004; BABADZHANOV *et al.*, 2004). Além disso, em sua composição podem ser encontrados bons níveis de ferro, cálcio, fósforo, vitaminas (vitamina E e B12) e pigmentos (ficocianina, carotenoides e clorofila-a) (CAPELLI *et al.*, 2010; ALI; SALEH, 2012). Por não possuir celulose em sua parede celular e a ausência de fitatos e oxalatos, facilita a assimilação de nutrientes a nível intestinal (GUTIÉRREZ-SALMEÁN *et al.*, 2015). É uma rica fonte de proteínas digestíveis (até 70% do seu teor proteico), e possui um perfil de aminoácidos bem equilibrado (AL-DHABI; ARASU; 2016; TOKU¸SOGLU; ÜNAL, 2003). Atua também no tratamento de várias condições patológicas humanas, como hepatotoxicidade, doenças cardiovasculares, câncer, hiperlipidemia, entre outras (HABIB *et al.*, 2008; NAGAOKA *et al.*, 2005). Além de possibilitar maiores inclusões na substituição de diversos componentes na formulação de rações para os animais (AHMADZADENIA *et al.*, 2011; SIRAKOV *et al.*, 2012).

Nos últimos anos, pesquisas relataram melhorias no desempenho com o uso da *Spirulina* como suplemento ou como substituta à farinha de peixe para tilápias (Tabela 2 e 3) (PLAZA *et al.*, 2019; AL-ZAYAT, 2019; ELABD; WANG; SHAHEEN; 2020; MANSOUR *et al.*, 2021) e outras espécies de peixes, como o bagre africano (*Clarias gariepinus*) (RAJI *et al.*, 2018; 2020), a carpa comum (*Cyprinus carp*) (ABDULRAHMAN; AMEEN 2014) e a truta marrom (*Salmo truta caspius*) (ROOHANI *et al.*, 2019). Além disso, benefícios ligados à saúde dos peixes como: melhoria de parâmetros bioquímicos (ABDEL-DAIM *et al.*, 2020; MANSOUR *et al.*, 2021; SOLIMAN; HAMED; SAYED, 2021), histológicos (ABU-ELALA *et al.*, 2016), capacidade antioxidante (AWED *et al.*, 2020; ELABD; WANG; SHAHEEN; 2020; SALAH EL-DIN; ABDULLAH; SAYED, 2021) também merecem destaque.

De acordo com a Tabela 2 e 3, a *Spirulina* pode ser utilizada na dieta de tilápias como suplemento em até 20%. Como substituta da farinha de peixe, em até 75% para a mesma espécie e para o bagre africano (*Clarias gariepinus*) (RAJI *et al.*, 2018; ROSSENAU *et al.*, 2021), a carpa comum (*Cyprinus carp*) (ABDULRAHMAN; AMEEN, 2014) e a truta marrom (*Salmo truta caspius*) (ROOHANI *et al.*, 2019). A total substituição da farinha de peixe pela biomassa de *Spirulina* (100%) também já foi relatada para a carpa prussiana (*Carassis auratus* gibelio var. CAS III) (CAO *et al.*, 2018) e para o bagre africano (RAJI *et al.*, 2020), sem prejuízos à saúde ou ao desempenho desses animais.

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Tabela 2- Estudos com o uso da *Spirulina* (*Arthrospira platensis*) como suplemento para a tilápia-do-Nilo (*Oreochromis niloticus*).

Parâmetros testados	**Peso (g)**	**Níveis testados como suplemento**	**Principais resultados/Conclusão**	**Referência**
Desempenho, utilização de ração, composição corporal centesimal e parâmetros hemato-bioquímicos	0,83	Controle: Dieta vegetal sem SP; Dietas vegetais com inclusão de 0.125, 0.25, 0.5, 0.75 e 1% SP	A suplementação de 0,5-0,65% de SP em dieta vegetal melhorou o desempenho, a utilização de ração e parâmetros hemato-bioquímicos	Mansour *et al.*, 2021
Desempenho, utilização de ração e composição da carcaça	6,0	Controle: DB sem SP; Dietas com inclusão de 2,5, 0.5, 5 e 7,5% de SP	A suplementação de 7,5% de SP melhorou o desempenho, utilização proteica e parâmetros sanguíneos	Al-Zayat, 2019
Desempenho, parâmetros hematológicos, microbiota intestinal e qualidade do filé	109,0	Controle: DB sem SP; Dieta com inclusão de 3% de SP	A suplementação de 3% de SP melhorou o desempenho e a cor do filé, sem modificar a microbiota intestinal. Aumento do cortisol e dos triglicerídeos	Plaza *et al.*, 2019
Desempenho e composição do filé	1,0	Controle: DB sem SP; Dieta com inclusão de 20% de SP	A suplementação de 20% de SP aumentou o nível proteico dos filés e manteve o desempenho (exceto para S e CAA) igual ao controle.	Leite *et al.*, 2019
Parâmetros reprodutivos	55,0	Controle: DB sem SP; Dietas com inclusão de 5, 7,5, 10 e 20% de SP	A suplementação de 0,5-2% de SP melhorou parâmetros reprodutivos	Wahb; Sangak, 2017
Capacidade antioxidante, fragmentação do DNA e fração proteica (eletroforese em gel - SDS-PAGE)	9,5	Controle (T1): Sem exposição EtBr e sem SP; T2: EtBr 10 $\mu g\ L^{-1}$; T3: EtBr 10 $\mu g\ L^{-1}$ EtBr + 200 $mg\ L^{-1}$ SP; T4: EtBr 100 $\mu g\ L^{-1}$; T5: EtBr 100 $\mu g\ L - 1$ EtBr + 200 $mg\ L^{-1}$ SP	A suplementação com 200 $mg\ L^{-1}$ de SP protegeu as contra impactos negativos ao DNA e à capacidade antioxidante de tilápias expostas ao brometo de etídio	Salah El-Din; Abdullah; Sayed, 2021
Perfil bioquímico, histopatológico (cérebro, fígado e rins), estresse oxidativo e índices hematológicos	9,5	Controle (T1): DB sem exposição; T2: DB expostos 15 mg/L $CuSO_4$; T3: DN + 0,25% de SP expostos 15 mg/L $CuSO_4$; T4: DB expostos 15 mg/L CuO-NPs; T5: DB + 0,25% SP expostos 15 mg/L CuO-NPs	A suplementação com 0,25% de SP à peixes expostos ao $CuSO_4$ e CuO-NPs apresentaram proteção contra alterações histopatológicas, bioquímicas, hematológicas e estresse oxidativo	Soliman; Hamed; Sayed, 2021

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Parâmetros testados	Peso (g)	Níveis testados como suplemento	Principais resultados/Conclusão	Referência
Desempenho, capacidade antioxidante tecidual (índices antioxidantes e expressão transcriptômica de genes antioxidantes)	40,0	Controle: DB; SS: intoxicado 5,8 mg/L de SS; SP: dieta 1% de SP; SP/SS: dieta 1% de *Spirulina* e intoxicado com 5,8 mg/L de SS	A suplementação de 1% de SP não alterou o desempenho dos animais, mas suprimiu o estresse oxidativo tecidual induzido pela exposição ao SS	Awed *et al.*, 2020
Desempenho, enzimas digestivas e bioquímicas, imunológicas, status antioxidante, resistência a *Aeromonas veronii* e desafios estressores físicos	26,0	Controle: DB sem SP; Dietas com inclusão de 0,25% e 0,5% de nanopartículas de SP	A suplementação com 0,25-0,5% de nanopartículas de SP melhorou o desempenho, resposta imune, antioxidantes, enzimas digestivas, expressão gênica e controlou infecção	Elabd; Wang; Shaheen; 2020
Biomarcadores antioxidantes enzimáticos e bioquímicos e peroxidação lipídica	60,0	Controle: sem SP; Dieta 1% de SP e sem exposição CPF (15 g/L); Dieta exposição CPF sem SP; Dietas exposição CPF 0,5%-1% SP	A suplementação com 1% de SP melhorou os biomarcadores antioxidantes enzimáticos e bioquímicos e a peroxidação lipídica	Abdel-Daim *et al.*, 2020
Desempenho e índices viscerais, composição e textura dos filés	26,8	Controle: DB; Dietas 0,25% e 0,5% de SP; Dietas 0,25% e 0,5% de *S. cerevisiae;* Dietas 0,25% e 0,5% de *Rubrivivax gelatinosus*	A suplementação com 0,25-0,5% de SP não afetou o desempenho, aumentou a qualidade nutricional e preservou as características de textura dos filés.	Grassi *et al.*, 2020
Peso médio final, histopatológico, anomalias cromossômicas, danos no DNA e parâmetros bioquímicos-hematológicos	100,0	Controle: sem SP e sem exposição a FC; FC: DB + exposição a FC; FC+SP: DB + 0,5% de SP + exposição a FC; SP: DB + 0,5% SP	A suplementação com 0,5% de SP reduziu o aparecimento de anomalias cromossômicas, os danos no DNA e melhorou o eritrograma	Mahmoud *et al.*, 2019
Marcadores de dano hepático e renal, bioquímico sérico e *status* antioxidante	60,0	Controle: DB; SP1%: DB + 1% SP; DZN: DB + diazinon (0,28 mg/L); DZN-SP0,5: DB + DZN + 0,5% SP; DZN-SP1: DB + DZN + 1% SP	A suplementação com 1% de SP melhorou os marcadores de dano hepático e renal, o bioquímico sérico e incrementou o *status* antioxidante	Abdelkhalek *et al.*, 2017
Desempenho, composição corporal e parâmetros bioquímicos	7,0	Controle: DB sem SP; Dieta com 1% de *A. fusiforms;* Dieta com 1% de *A. platensis*	A suplementação de 1% SP (*A. fusiforms* e *A. platensis)* melhorou desempenho, composição corporal e parâmetros bioquímicos	Belal, Khalafalla e El-Hais, 2012

SP: *Spirulina*; FP: Farinha de Peixe; DB: Dieta basal; S: Sobrevivência; CAA: Conversão alimentar aparentes; $CuSO_4$: sulfato de cobre; CuO-NPs: óxido de cobre; EtBr: brometo de etídio; FC: produtos químicos; CPF: clorpirifós; SS: sulfato de sódio

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Tabela 3 - Estudos com o uso da *Spirulina* (*Arthrospira platensis*) como substituto da farinha de peixe para tilápias e outras espécies de peixes.

Parâmetros testados	Espécie/ Peso (g)	Níveis testados como substituto	Principais resultados/Conclusão	Referência
Desempenho, composição da carcaça e hematológico	Tilápia Híbrida Vermelha (*Oreochromis niloticus* x *O. mossambicus*) / 0,206	Controle: DB com FP; Dietas com substituição da FP pela *Spirulina* em 50, 75, 100%	A substituição de 75% da FP pela *Spirulina* não diferiu do controle com FP no desempenho e na composição da carcaça dos animais. Menor custo-benefício em dietas com uso da *Spirulina* em relação à FP	El-Sheekh; El-Shourbagy; Shalaby; Hosny; 2014
Desempenho, índices organossomáticos, utilização da ração, composição da carcaça e parâmetros bioquímico sanguíneos	Tilápia-do-Nilo *(Oreochromis niloticus)* / 0,89	Controle: DB sem *Spirulina*; Dietas com inclusão de 30, 45, 60 e75% (0% FP) de *Spirulina*	A substituição de 30% da FP pela *Spirulina* melhorou o desempenho e os índices organossomáticos, a utilização da ração, a composição da carcaça e reduziu os triglicerídeos em relação ao controle	Velasquez *et al.*, 2015
Desempenho, composição corporal e mineral e histologia hepato-intestinal	Tilápia-do-Nilo *(Oreochromis niloticus)* / 0,02	Controle: Dieta vegetal com glúten de milho como fonte proteica; Dietas com algas 0, 25, 50, 75, 100% de substituição ao glúten; Dieta com 100% de *Spirulina* em substituição ao glúten	A substituição de 100% do glúten de milho das dietas vegetais pela *Spirulina* com melhora do desempenho	Hussein *et al.*, 2013
Desempenho, digestibilidade aparente de nutrientes	Bagre africano - *catfish* (*Clarias gariepinus*) / 58,05	Controle: DB com FP; Dieta com substituição da FP pela SP em 100%; Dieta com substituição da FP pela *Chlorella vulgaris* em 100%	A substituição de 100% da FP pela SP melhorou o desempenho. Maior coeficiente de digestibilidade aparente de macronutrientes	Raji *et al.*, 2020
Desempenho, composição corporal e análise sensorial	Carpa comum (*Cyprinus carp*) / 32,7	Controle: DB com FP; Dietas com substituição da FP pela SP em 5, 10, 15 e 20%	A substituição de 5-20% da FP pela SP + vitamina C melhoraram o ganho de peso e a sobrevivência. O tratamento com 10% *Spirulina* apresentou melhor qualidade sensorial	Abdulrahman; Ameen 2014

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Parâmetros testados	Espécie/ Peso (g)	Níveis testados como substituto	Principais resultados/Conclusão	Referência
Desempenho, índices organossomáticos, composição corporal e pigmentação da pele	Golfinho do Malawi (*Cyrtocara moorii*) / 3,15	Controle: DB com FP; Dietas com substituição da FP pela SP em 5, 10, 15 e 20%	A substituição entre 5-15% da FP pela SP melhorou o desempenho e a eficiência alimentar. A pele dos animais alimentados com 15% de SP em substituição a FP apresentou maior pigmentação (carotenoides)	Erdogan, 2019
Desempenho, resposta hemato-bioquímica e antioxidante	Bagre africano - *catfish* (*Clarias gariepinus*) / 42,07	Controle: DB com FP; Dieta com substituição da FP pela SP em 50 e 75%; Dieta com substituição da FP pela *Chlorella vulgaris* em 50 e 75%	A substituição de 75% da FP pela SP melhorou o desempenho e a resposta hemato-bioquímica e antioxidante	Raji *et al.*, 2018
Desempenho, qualidade da carne e perfil ácidos graxos	Bagre africano - *catfish* (*Clarias gariepinus*) / 50,0	Controle: DB com FP; Dieta com substituição da FP pela SP em 100%	A substituição de 100% da FP pela SP: prejudicou o GDP, mas a CAA não foi afetada; alterou características visuais e da qualidade da carne/pele; maiores valores/relação de AG ômega-3 e 6	Rosenau *et al.*, 2021
Desempenho, composição corporal, perfil de ácidos graxos e aminoácidos e pigmentação	Truta marrom Caspian (*Salmo truta caspius*) / 11,0	Controle: DB com FP; Dietas com substituição da FP pela SP em 2, 4, 6 e 8%	A substituição de 8% da FP pela SP melhorou o desempenho, a composição de carcaça e a pigmentação.	Roohani *et al.*, 2019
Desempenho, utilização da ração e parâmetros digestivos	Carpa prussiana (*Carassis auratus gibelio var. CAS III*) / 5,0	Controle: DB com FP; Dietas com substituição da FP pela SP em 50, 75 e 100%. Dietas com substituição da FP pela SP em 100% + 2% fosfato dicálcico e 100% + 0.28% lisina cristalina	A substituição parcial ou total (50, 75 ou 100%) da FP pela SP não prejudicou o desempenho. **Substituição total de SP** com ou sem suplementação melhorou a digestibilidade das dietas e a capacidade antioxidante em relação ao controle	Cao *et al.*, 2018

SP: *Spirulina*; FP: Farinha de Peixe; DB: Dieta basal; GDP: Ganho de peso; CAA: Conversão alimentar aparente.

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4. SISTEMA DE BIOFLOCOS (BFT)

O cultivo em bioflocos ou “BFT” (sigla em inglês para *Biofloc Technology*) é um sistema de cultivo intensivo de baixa renovação de água através formação de uma rica comunidade microbiana que realiza a ciclagem dos nutrientes (EMERENCIANO; GAXIOLA; CUZON, 2013). É um sistema com grande potencial para a atividade aquícola já que possibilita a intensificação da atividade atrelado à menores riscos de doenças, além do mínimo de prejuízo ao meio ambiente (MARISCAL-LAGARDA *et al.*, 2012; BUHMANN *et al.*, 2015; HU *et al.*, 2015).

No sistema BFT, o crescimento de comunidades microbianas específicas é estimulado através da aeração intensa e da manutenção correta da relação carbono:nitrogênio (C:N) do meio (AVNIMELECH, 2007; EMERENCIANO *et al.*, 2012). São diversos os microrganismos presentes nesses flocos, em sua maioria, bactérias (autotróficas e heterotróficas), mas também leveduras, microalgas, ciliados, rotíferos e nematódeos (MONROY-DOSTA *et al.*, 2013). Esses microrganismos desempenham papel essencial na qualidade da água, fazendo a reciclagem da matéria orgânica, além de servirem de alimento para os organismos aquáticos cultivados e competirem diretamente com outros microrganismos patogênicos no meio (AZIM; LITTLE, 2008; EMERENCIANO; GAXIOLA; CUZON, 2013; EMERENCIANO *et al.*, 2017).

As principais bactérias benéficas encontradas no sistema BFT são: *Sphingomonas paucimobilis*, *Pseudomonas luteola*, *Pseudomonas mendocina*, *Micrococcus*, *Bacillus sp.*, *Nitrospira sp. e Nitrobacter sp*. (MONROY-DOSTA *et al.*, 2013). São gêneros dominantes e estão relacionados a um efetivo biocontrole dos microrganismos patogênicos do meio (HARGREAVES, 2006; AVNIMELECH, 1999, 2009; CRAB; DEFOIRDT; VERSTRAETE, 2012). Além disso, constituem uma rica biomassa microbiana (THOMPSON; ABREU; WASIELESKY, 2002; MONROY-DOSTA *et al.*, 2013), que poderá ser utilizada como alimento para as espécies cultivadas (MONROY-DOSTA *et al.*, 2013; MARTÍNEZ-CÓRDOVA *et al.*, 2015). Bactérias patogênicas do gênero *Aeromonas e Vibrio* tendem a serem encontradas nas primeiras semanas de cultivo em BFT e, posteriormente, cessam sua multiplicação (MONROY-DOSTA *et al.*, 2013). Isso ocorre por exclusão competitiva, conforme a proliferação de bactérias benéficas aumenta (WU *et al.*, 2012; MONROY-DOSTA *et al.*, 2013).

Estudos que permitem uso de níveis de proteína subótimos para peixes é uma prática essencial para viabilizar a atividade e avaliar o potencial dos animais e dos sistemas (FURUYA *ET AL.*, 2005; COSTA; MELO; CORREIA, 2009; AZIM; LITTLE, 2008; DA SILVA *et al.*, 2018; HISANO *et al.*, 2019). A biomassa microbiana produzida neste sistema pode ser utilizada como fonte natural de alimento, suprindo até 50% da exigência de proteína da tilápia (AVNIMELECH, 2007). Em decorrência disso, é possível reduzir mais de 30% da proteína bruta das dietas desses animais sem

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prejudicar o desempenho (AZIM; LITTLE, 2008). Adicionalmente, menores taxas de conversão alimentar em comparação ao cultivo em água clara, também foram alcançadas (LUO *et al.*, 2014).

4.1 CULTIVO DE TILÁPIA EM BFT

As tilápias são adaptadas a esse sistema, principalmente por serem animais filtradores, rústicos e de crescimento rápido (AVNIMELECH, 2011; 2015). Elas podem alimentar-se dos flocos presentes no meio de cultivo, independentemente do tamanho dos agregados (EKASARI, 2014). Esses flocos, por sua vez, são ricos em proteína, podendo variar entre 24 e 32% (AZIM; LITTLE, 2008; ELÍAS *et al.*, 2015; LIMA *et al.*, 2018). A tecnologia BFT possibilita ainda, o cultivo de tilápias em altas densidades e elevadas produtividades (10 a 40 kg/m³) (AVNIMELECH, 2005), até mesmo em relação a um cultivo em água clara (EKASARI, 2014).

Um aumento da densidade de 15 para 45 tilápias/m³ na fase de engorda em BFT, resultou em maior produtividade (LIMA *et al.*, 2015), similar ao observado por Widanarni, Ekasari e Maryam (2012). Acredita-se que o cultivo em BFT pode melhorar também a saúde das tilápias, uma vez que, os flocos contêm diversos compostos bioativos, incluindo ácidos graxos essenciais, carotenoides, aminoácidos livres e clorofilas (JU *et al.*, 2008), minerais (TACON *et al.*, 2002) e vitamina C (CRAB; DEFOIRDT; VERSTRAETE, 2012). Todos esses compostos são conhecidos por seus efeitos positivos em organismos aquáticos, como incremento do *status* antioxidante, crescimento, reprodução e resposta imune dos animais (TACON *et al.*, 2002; JU *et al.*, 2008; CRAB; DEFOIRDT; VERSTRAETE, 2012).

Os animais cultivados neste sistema podem ainda, se beneficiar da quantidade reduzida de patógenos exógenos, já que é um sistema fechado. Alguns estudos demonstraram que a presença de bactérias potencialmente patogênicas, como o *Vibrio harveyi* é reduzida no BFT em condições de cultivo adequadas (CRAB *et al.*, 2010; CRAB; DEFOIRDT; VERSTRAETE, 2012; ZHAO *et al.*, 2012).

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CAPÍTULO II – MANUSCRITOS

Os resultados desta tese estão apresentados na forma de manuscritos (artigos científicos). Eles estão redigidos de acordo com as normas das revistas onde serão/foram submetidos/publicados – *Aquaculture Research* e *Aquaculture International*.

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ARTIGO I

Mixed culture microorganisms fermented soybean meal improves productive performance and intestinal health of Nile tilapia (*Oreochromis niloticus)* juveniles fed plant-based diets in a biofloc system

Fernanda Picoli, Suelyn de Oliveira Marques, Alana Duarte de Oliveira, Cristina Gradin Nunes, Suélen Serafini, Bruna Klein, Nandara Soares de Oliveira, Nataly Neves Oliveira dos Santos, Aline Zampar, Diogo Luiz de Alcantara Lopes, Thiago El Hadi Perez Fabregat

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Mixed culture microorganisms fermented soybean meal improves productive performance and intestinal health of Nile tilapia (*Oreochromis niloticus)* juveniles fed plant-based diets in a biofloc system

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Abstract

This study aimed to evaluate the effect of plant-based diets containing different inclusion levels (7%, 14%, 21% and 28%) of mixed culture microorganisms fermented soybean meal (FSBM) on the zootechnical performance and intestinal health of juvenile Nile tilapia (*Oreochromis niloticus*) reared in a biofloc system. The FSBM diets were compared to a positive control diet with fish meal and a negative control diet with no animal protein. All diets were isoprotein (33% CP) and isoenergetic (4300 kcal GE/kg). The design was completely randomized with four replications and the experiment lasted 54 days. Juveniles (1.635 ± 0.198 g) were distributed in 24 tanks (70 L) at a density of seven animals per tank. Fermentation increased probiotic microorganisms count (lactic acid bacteria 4.75 ± 0.21 log CFU g^{-1} and yeast 3.30 ± 0.54 log CFU g^{-1}) and improved nutritional characteristics of FSBM. In fish fed the diet containing 7% FSBM, growth performance did not differ from fish fed the fish meal diet. FSBM inclusion improved food efficiency of plant-based diets. The inclusions above 21% of FSBM increased the intestinal villi height. In the inclusion of 28% of FSBM, the goblet cell was higher compared to the other plant-based diets. The inclusion of 7% FSBM allows a total replacement of fish meal without compromising the growth performance of the animals.

Keywords: Goblet cell, Lactic acid bacteria, Probiotic microorganism, Villi height, Yeast.

1. INTRODUCTION

Feed costs can reach up to 60% of operational expenses in intensive fish production systems (Barbosa *et al.* 2020). Fish meal is important in aquaculture diets as a protein source with high biological value. Its exclusion from the formulations can lead to poor performance and health (Gomes, Rema & Kaushik, 1995; Glencross, Rutherford, & Jones, 2011; Turchini, Trushenski, & Glencross, 2019). Commercial availability of fish meal is decreasing (World Bank, 2020). The increase in the price of fish meal impacts the production costs of aquatic animals (El Basuini *et al.*, 2016, 2017; Hossain, Koshio, Ishikawa, Yokoyama & Sony, 2016). In this context, alternative biotechnological techniques should be considered for the development of plant-based diets that reduce costs and optimize production performance (Bulbul *et al.*, 2015; Zhang *et al.*, 2016; Daniel, 2018).

Soybean meal is widely used as a vegetable protein source due to its availability in the market, amino acid profile and affordable price (Sørensen, Stjepanovic, Romarheim, Krekling & Storebakken, 2009; Daniel, 2018). However, the complete replacement of fish meal by soybean meal in fish diets is not always possible (Goda, El-Haroun & Chowdhury, 2007; Hansen, Hemre, Karlsen, Koppe & Rosenlund, 2011; Bonaldo *et al.*, 2011; Ferrara *et al.*, 2015). Several factors can make its replacement unfeasible, such as poor palatability, high fiber content and deficiency of essential amino acids (Dunsford, Knabe & Hacnsly, 1989; El-Dahhar & El-Shazly, 1993). Strategies such as microbial fermentation of soybean meal can address

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these problems, boosting the performance and immune system of animals (Hong, Lee & Kim, 2004; Refstie, Sahlström, Bråthen, Baeverfjord & Krogedal, 2005; Sotoudeh, Amiri Moghaddam, Shahhosseini & Aramli, 2016; Van Nguyen, Hoang, Van Khanh, Duy Hai & Hung, 2018).

Fermentation can result in useful bioconversions or metabolic decomposition. Complex substrates are transformed into simple compounds by the action of microorganisms (Balakrishnan & Pandey, 1996). It can be a preservation method that also improves the nutritional and functional properties of ingredients (Ross, Morgan & Hill, 2002; Frias, Song, Martínez-Villaluenga, De Mejia, & Vidal-Valverde, 2008). During the fermentation process, there may be an increase in populations of probiotic microorganisms and secondary metabolites with prebiotic activitiy (Balakrishnan & Pandey, 1996; Mukherjee, Chakraborty & Dutta, 2015; Dawood & Koshio, 2019; Wang *et al.*, 2019). Fermentation can be done with a single microorganism or with a mixed culture of microorganisms composed of bacteria and yeast. In mixed fermentation, there is a better use of the substrate, since these combined cultures have a wider range of enzymes and are capable of attacking a wide variety of compounds in the substrate (Macfadyen, 1903; Fossi, Tavea, Fontem, Ndjouenkeu & Wanji, 2014; Dawood & Koshio, 2019).

It has been demonstrated that fermented soybean meal (FSBM) can partially replace fish meal in diets without compromising fish performance (Wu, Chung, Lin, Chen & Huang, 2003; Yamamoto *et al.*, 2010; Azarm & Lee, 2014; Barnes, Brown & Neiger, 2015; Shiu *et al.*, 2015; Lee, Azarm & Chang, 2016; Ilham & Fotedar, 2016; Sotoudeh *et al.*, 2016; Wang *et al.*, 2016; Liang *et al.*, 2017; Jiang *et al.*, 2018; Choi *et al.*, 2019; Wang *et al.*, 2019; He *et al.*, 2020a, 2020b, De Oliveira *et al.*, 2022). In addition, FSBM can have beneficial effects on fish health, such as improvements in biochemical parameters (Novriadi, Rhodes, Powell, Hanson & Davis, 2017), lipid metabolism, antioxidant response (Jiang *et al.*, 2018; Li *et al.*, 2020) and intestinal health (Yamamoto *et al.*, 2010; Shiu *et al.*, 2015; Sotoudeh *et al.*, 2016; Wang *et al.*, 2016; Li *et al.*, 2019; Wang *et al.*, 2019).

Nile tilapia production has increased in recent years (GOAL, 2019; FAO, 2020). Production in a biofloc technology (BFT) system is already being evaluated as an alternative for traditional semi-intensive production system in ponds (Azim & Little, 2008; Luo *et al.*, 2014; Lima, Souza, Wambach, Silva & Correia, 2015; Lima, Souza, Girao, Braga & Correia, 2018).). It is an intensive cultivation system, with reduced water use, less effluent emissions and increased productivity (Avnimelech, 2009). However, intensive systems pose challenges to animals, as they may suffer higher levels of stress than in traditional systems (Gabriel &

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Akinrotimi, 2011; Mohapatra, Chakraborty, Kumar, DeBoeck & Mohanta, 2012). BFT production is done in small spaces and high population densities. When these factors are coupled with the constant fluctuations in water quality parameters and changes in management practices, they can easily affect the animal's growth and overall health (Fischer *et al.,* 2020; Romano *et al.,* 2020).

The use of soybean meal as a replacement for fish meal has been evaluated in diets for tilapia and total replacement is not always possible (El-Sayd & Gaber, 2002; Al-Kenawy, El Naggar & Abou Zead, 2008; Lin & Luo, 2011; Ajani, Orisasona, Omitoyin & Osho, 2016; Sharda Sharma & Saini, 2017). *Saccharomyces cerevisiae* fermented soybean meal allowed only a partial replacement of fish meal in plant-based diets for Nile tilapia (Hassaan, Soltan & Abdel-Moez, 2015). Fermentation using a mixed microbial cultures can provide better results and is a strategy that still needs to be evaluated as a tool for promoting the growth and health of Nile tilapia fed plant-based diets. Furthermore, the use of fermented ingredients in a BFT system still needs to be evaluated. Therefore, the objective of this study was to evaluate the effect of plant-based diets containing different inclusion levels of a fermented soybean meal (FSBM) prepared with a mixed microbial culture on the zootechnical indices and intestinal health of juvenile Nile tilapia (*Oreochromis niloticus*) reared in a BFT system.

2. MATERIALS AND METHODS

2.1 Experimental design

The experiment was carried out at the Aquaculture Laboratory of the State University of Santa Catarina – UDESC, Campus Chapecó (Santa Catarina, Brazil) and was approved by the Ethics Committee on the use of Animals (CEUA), under protocol number 3851120320. FSBM was evaluated at four inclusion levels in plant-based diets (7, 14, 21 and 28%) and compared to a positive control diet with fish meal and a negative control diet with no animal protein nor FSBM. The design was completely randomized (CRD) with six treatments and four replications, and it lasted for 54 days.

2.2 FSBM production and characterization

The production of the fermented soybean was done according to an adaptation of the solid-state fermentation technique (Azarm & Lee, 2014). For this purpose, the soybean meal

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was autoclaved at a temperature of 100°C for 20 minutes and inoculated with Aquate Fertilizer® (Alltech™) in the proportion of 15 g kg^{-1}. The composition of the Aquate Fertilizer® is show in Table 1. Distilled water was added in the proportion of 50% of the weight of the soybean meal and the mixture was placed in a polypropylene tray and kept in a forced air incubator at 36°C. Every 12 hours, the material was weighed, and distilled water was added to keep the moisture level in 50%. After the fermentation process was completed (48 hours), the fermented soybean meal (FSBM) was placed again in a forced air incubator at 36°C until it reached a constant weight. Subsequently, it was stored in a freezer at a temperature between −14°C and −25°C.

Proximal composition of soybean meal and FSBM (Table 2) was determined according to methods described in the Association of Official Analytical Chemists (AOAC, 2000). Lactic acid bacteria and yeast concentrations were determined on raw soybean meal and FSBM (Table 2). For this purpose, samples of one gram were serially diluted (1:10) in sterile saline solution at 0.65%, and then seeded on MRS (Man Rogosa & Sharpe) agar, to count lactic acid bacteria, and in Sabouraud + chloramphenicol medium (0.5 g L^{-1}) to count yeasts. Inoculated Petri plates were incubated at a constant temperature of 37°C for 48 hours and at 25°C for 72 hours, respectively (Silva *et al.*, 2013; Abdel-Razek, 2019), for total colony forming unit (CFU) counts. Soluble protein concentration was determined by the Bradford Method (Bradford, 1976) with bovine serum albumin (Sigma-Aldrich, St. Louis, MO) as a standard. All samples were analyzed in triplicate (Table 2).

The pH analysis was also performed according to the potentiometric method (AOAC, 2000). 10 g of FSBM and 100 mL of distilled water at 25°C were mixed (electronic shaker, brand DiagTech, model DT3110H) in an Erlenmeyer (250 mL) for 30 minutes. This mixture was transferred to a 500 mL beaker, where it remained at rest for 10 minutes and, subsequently, measurements were carried out with the aid of a bench-top microprocessed pH meter (Alfakit™, AT-355) (Table 2). Finally, the FSBM amino acid composition was determined according to White, Harty and Fry (1986), and for soybean meal, data from Rostagno *et al.* (2017) were used (Table 3).

2.3 Experimental diets

The experimental diets were isoprotein (33% crude protein) and isoenergetic (4,300 kcal of gross energy kg^{-1}) and were formulated (Table 4) in order to meet the nutritional requirements of protein (El-Sayd & Gaber, 2002) and amino acids for tilapia (Santiago &

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Lovell, 1988). The amino acid compositions of the diets are shown in Table 5. Inclusion levels of fermented soybean meal were determined based on similar studies (Hassaan, Soltan & Abdel-Moez, 2015; Wang *et al.*, 2016; De Oliveira *et al.*, 2022). In addition to FSBM, the formulated diets contained fish meal, soybean meal as protein sources, as well as wheat flour and soy oil as energy sources. The fish meal was purchased from Agroforte™ (Laguna, Santa Catarina, Brazil), and the other ingredients were purchased from local suppliers. All ingredients were ground in an industrial processor and sieved in 0.71 mm mesh. Subsequently, they were mixed with the addition of water (50%), pelletized in a 1 mm "spaghetti" matrix (Malta®, model 1036, no. 8 mouthpiece) and placed in an incubator (Cienlab®, model CE-220/150) at 55°C for 48 h. The diets were stored in plastic containers and placed in refrigerators (4°C) until use.

The counting of yeasts and lactic acid bacteria was also performed in the experimental diets (Table 4). For this purpose, one-gram samples were serially diluted (1:10) in sterile saline solution at 0.65%, and then seeded on MRS (Man Rogosa & Sharpe) agar for the counting of lactic acid bacteria and on Sabouraud + chloramphenicol agar (0.5 g L^{-1}) to count yeasts (*Saccharomyces cerevisiae*). After sowing in Petri dishes, the plates with MRS were incubated at a constant temperature of 35°C for 48 hours (Silva *et al.*, 2013), and the plates with Sabouraud + chloramphenicol were incubated at 25°C for 72 hours (Abdel-Razek, 2019). At the end of the respective periods, the total counts of colony forming units (CFU) were performed, as shown in Table 4.

2.4 Animals and facilities

The fish were acquired from Acqua Sul Piscicultura, located in the city of Ilhota, Santa Catarina State, and previously acclimated to the experimental conditions. A total of 168 Nile tilapia juveniles with an average initial weight of 1.635 ± 0.198 g were distributed in 24 circular polyethylene tanks (useful volume of 70 L) connected to a recirculating aquaculture system (RAS). The tanks were equipped with an individual aeration system using a porous stone (1 x 2 cm) coupled through a radial air compressor and distributed over two benches. These benches were arranged and connected to two circular tanks (useful volume: 850 L) called "macrocosms" that contained a peripheral water pump (Intech®, model BP500) responsible for the distribution and recirculation of water in the system. Each macrocosm was equipped with a heater resistor (Nobre Brasil®, 3,000 W), connected to a digital temperature controller with sensor (XH-W3001®, 12 V, China).

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Each experimental unit received an initial biofloc inoculum (10 L) from previous Nile tilapia cultures in BFT system and non-chlorine water until completing the useful volume of 70 L. For the formation and maintenance of the microbial community as well as suitable conditions of the culture medium, a C:N ratio of 10–15:1 was maintained (Pérez-Fuentes, Hernández-Vergara, Pérez-Rostro & Fogel, 2016) with sugarcane molasses being used as an external source of carbon. Thus, a biomass of 2.25 kg m^{-3} of tilapia was also maintained in each macrocosm, fed with commercial feed (32% CP supplied one time per day at 12 p.m.).

The animals were fed once a day (12 p.m.) with an initial feeding rate of 10% of the biomass/day until they reached twice their initial weight (Lopez-Betancur *et al.*, 2020). Subsequently, a rate of 5% of biomass/day was maintained until the end of the experimental period (Lopez-Betancur *et al.*, 2020). The monitoring of the animals' biomass was carried out every 18 days through biometrics of all experimental units.

Water temperature, dissolved oxygen (Alfakit™ oximeter, model AT170) and pH (Alfakit™ pH meter, model AT315) were measured daily as indicators of water quality. Analyses of nitrogen compounds (ammonia, nitrite, nitrate), orthophosphate and alkalinity of the culture medium were performed weekly using a photocolorimeter (Alfakit™, model AT100P). Total sedimentable solids (TSS) were measured with a Imhoff cone. Mean values were found for temperature, dissolved oxygen and pH, of 25.15 ± 2.20°C, 9.03 ± 0.60 mg/L^{-1} and 6.92 ± 0.25, respectively. Ammonia, nitrite, nitrate, orthophosphate, alkalinity and total sedimentable solids, total values of 0.17 ± 0.09 mg L^{-1}, 0.22 ± 0.07 mg L^{-1}, 10.70 ± 9.11 mg L^{-1}, 13.67 ± 1.20 mg L^{-1} PO_4, 11.49 ± 4.64 mg L^{-1} $CaCO_3$ and 5.99 ± 3.28 mg L^{-1} were observed, respectively. The parameters remained within the recommended range for tilapia throughout the study (El-Sayed, 2006).

2.5 Zootechnical performance

After 53 days, the animals were fasted for 24 hours and were individually weighed (0.01 g precision scale, Marte™, model ML 600, São Paulo, Brazil) and measured to assess the zootechnical parameters: final weight (FW); mean weight gain (MWG) = final weight (g) − initial weight (g); specific growth rate (SGR) = [ln final weight (g) − ln initial weight (g)] / experimental period x 100; (L); Fulton's condition factor (K) = weight / length x 100; feed conversion rate (FCR) = feed intake / weight gain; survival; feed consumption (FC) = food consumed in the trial period (g) and survival (S) = number of dead animals / total fish x 100.

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2.6 Intestinal histomorphometry

For the histomorphometric analysis, intestine fractions (proximal) were collected from the same animals used in the organometric indices (12 animals/treatment). The histological processing of these samples was performed at the Histology Laboratory at UDESC, Campus Lages (Santa Catarina, Brazil), according to routine methods for preparing histological slides (Nunes & Cinsa, 2016). The intestinal fragments were placed in sterile plastic containers with a common formalin solution (100 mL of 37–40% formalin; 900 mL of common water and 9.0 g of sodium chloride), identified and after being fixed for 12–16 hours, preserved in 70% alcohol until evaluation of the morphohistological characteristics of its mucosa. For this purpose, these intestinal fractions were sectioned into slices of approximately 0.3 cm using the technique of histology, diaphanization and embedding in histological paraffin. Three µm-thick sections were prepared on a semi-automatic microtome according to the methodology adapted from Mello *et al.* (2013). Longitudinal sections were prepared, semi-serialized and stained using the Schiff histochemical method (Periodic Acid-Schiff - PAS) (Tolosa, Rodrigues, Behmer & Freitas Neto, 2003).

Three slides were made from each sample and photographed using a digital camera coupled to a microscope (10x objective). The images were digitized and the measures were made using the TCapture software (Laborana® brand, São Paulo, Brazil). After the digitization was completed, ten villi per animal were selected by integrity criterion (Picoli *et al.*, 2019) for the measurements of the intestinal villi, where: i) villi height (VH), that is, distance from the apex of the villi to the beginning of the muscle layer; ii) total villi height (TVH), that is, height from the apex of the villi to the end of the serosa; iii) villi width (VW); and iv) villi thickness (VT), according to the methodology by Mello *et al.* (2013). In the same intestinal villi, goblet cells (GC) count was performed. Each goblet cell was considered as a unit (Picoli *et al.* 2019). All these measurements were performed using ImageJ software (National Institute for Health, Bethesda, Maryland, United States of America).

2.7 Statistical analysis

Normality (Shapiro-Wilk test) and homogeneity of variances (Levene test) were verified and the data were submitted to analysis of variance (ANOVA). When significant, the means were compared using the Tukey test at a 5% probability level. In the case of differences

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between plant-based diets, regression analysis was performed, and R^2 values above 0.8 were presented in the captions of the respective tables.

3. RESULTS

3.1 Zootechnical parameters

In fish fed the diet containing 7% FSBM, the average weight gain did not differ (P >0.05) from that in fish fed the diet containing fish meal (Figure 1). However, the results of this treatment did not differ from the negative control. Similar results (P >0.05) were obtained for final weight, specific growth rate and condition factor (Table 6). In all diets containing FSBM, feed conversion did not differ (P >0.05) from the positive control. In this case there was also no (P <0.05) difference in relation to the plant-based diet.

3.2 Intestinal histomorphometry

The villi of the different treatments can be seen in Figure 2. In the animals fed with 21% and 28% FSBM in the diets, greater (P <0.0001) villi height (Figure 3) and total villi height was observed (Table 7) compared to other treatments. Villi width and thickness (Table 7) were not affected (P >0.05) by the experimental diets. In the inclusion of 28% of FSBM, the goblet cell count (Figure 4) did not differ (P >0.05) from the positive control but was higher compared to the other treatments (P <0.05).

4. DISCUSSION

The water quality parameters remained within expected range for the cultivation of tilapia in a BFT system throughout the experiment (Azim & Little, 2008; Luo *et al.*, 2014; Lima, Souza, Wambach, Silva & Correia, 2015; Lima, Souza, Girao, Braga & Correia, 2018). At the beginning of the experiment, the tanks were inoculated with water from previously matured BFT systems. This is a strategy that can be adopted to prevent the occurrence of unwanted variations in water quality parameters (Krummenauer, Samocha, Poersch, Lara & Wasielesky, 2014; Martins *et al.*, 2020). In addition, the culture macrocosm tanks were connected to a recirculation system, which ensured homogeneity between treatments. Therefore, it is possible

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to infer that the results were obtained due to diet and supplementation supplementation strategy and were not influenced by the conditions in the BFT system.

During soybean meal fermentation there were greater lactic acid bacteria and yeast counts. There was also a reduction in pH, confirming the production of organic acids by microorganisms (Alexander, 1961; Imelda, Raj & Bhantnagar, 2008; Dai *et al.*, 2020). These results confirm that fermentation occurred as expected (Balakrishnan & Pandey, 1996; Sanjukta & Rai, 2016; Dawood & Koshio, 2019). In FSBM, the growth of microorganism colonies resulted in greater microbial protein synthesis and greater crude protein. Similarly, a higher crude protein level was observed in soybean meal fermented with *Bacillus subtilis* (Shiu *et al.*, 2015), *Aspergillus awamori* (Li *et al.*, 2019), *Enteroccocus faecium* (Li *et al.*, 2020) and a mixed culture of microorganisms composed of *B. subtilis, Lactobacillus* and *S. cerevisiae* (He *et al.*, 2020a). Furthermore, soluble protein and sum of amino acids in FSBM were also greater. There was considerably higher soluble protein present in FSBM (40.80 mg g^{-1}) compared to unfermented soybean meal (19.90 mg g^{-1}). This is consistent with a greater availability of low molecular weight peptides (Runho, 2001; Teng *et al.*, 2012; Sanjukta & Rai, 2016) and demonstrates that the nutritional characteristics of the soybean meal were positively affected in the fermentation process.

The growth and body condition of fish fed on plant-based diets containing 7% FSBM did not differ from the diet containing fish meal. These results are unprecedented and demonstrate the feasibility of using low levels of fermentation products as a strategy to optimize the use of plant-based diets. The use of soybean meal fermented with *S. cerevisiae* had previously been evaluated for Nile tilapia, but only a partial replacement of fish meal was viable (Hassaan *et al.*, 2015). In the present study, the utilization of a mixed culture of microorganisms may have favored the positive results due to the synergistic interaction commonly observed in fermentations between lactic acid bacteria and yeasts (Sieuwerts, Bron & Smid, 2018). Lactic acid produced by lactic acid bacteria encourages the growth of yeasts, which also use part of the excreted glucose as a carbon source (Gobbetti & Corsetti, 1997; De Vuyst & Neysens, 2005; Sieuwerts *et al.*, 2018). In contrast, the CO_2 produced by yeasts stimulates the production of these bacteria. Other authors also found favorable results when using a mixed culture microorganisms composed of *Lactobacillus plantarum, B. subtilis* and *S. cerevisiae* as probiotics for Nile tilapia (Essa *et al.*, 2010).

The FSBM inclusion levels of over 14% compromised the growth performance of tilapia cultivated in BFT. For other species, such as the orange-spotted grouper (*Epinephelus coioides*), rockfish (*Sebastes schlegeli*), Japanese sea bass (*Lateolabrax japonicus*), and

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rainbow trout (*Oncorhynchus mykiss*) there was also a reduction in performance with the inclusion of high levels of FSBM (Shiu *et al.*, 2015; Lee *et al.*, 2016; Liang *et al.*, 2017; Choi *et al.*, 2019). Fermented products are rich in low molecular weight peptides (Jiang *et al.*, 2018). At high levels of inclusion, these peptides may contribute to the saturation of intestinal absorption sites and transport mechanisms can occur, compromising the nutrient absorption and feed utilization (Khosravi, Safari, Khodaiyan & Gharibzahedi, 2015; Sanjukta & Rai, 2016; Wei, Liang, Mu, Zheng & Xu, 2016; Siddik, Howieson, Partridge, Fotedar & Gholipourkanani, 2018). Furthermore, an excessive quantity of free peptides from the fermented ingredient can increase amino acid oxidation and reduce dietary protein retention (Wei, Liang & Xu, 2019; Chai, Voo & Chen, 2020).

Fermentation improved the feed efficiency of plant-based diets for Nile tilapia in a BFT system. In all treatments with FSBM, the feed conversion did not differ from the diet containing fish meal. This result is in agreement with the literature, in which the inclusion of 15% to 45% FSBM in fish meal replacement did not affect the feed conversion of Japanese sea bass (*Lateolabrax japonicus*), yellow croaker (*Larimichthys crocea*) and black bass (*Micropterus salmoides*) (Liang *et al.*, 2017; Wang *et al.*, 2019; He *et al.*, 2020a; 2020b). The improvement in feed conversion can be explained by the higher soluble protein in FSBM. Peptides are more easily absorbed by enterocytes and assimilated at higher rates than intact proteins (Ganapathy, Brandsch & Leibach, 1994; Runho, 2001). Furthermore, the improvement in feed conversion can also be attributed to the presence of probiotic microorganisms (Welker & Lim, 2011). The microorganisms inoculated in the FSBM remained active (lactic acid bacteria and yeasts) and their beneficial effects on feed conversion have already been demonstrated (Lara-Flores, Olivera-Novoa, Guzmán-Méndez & López-Madrid, 2003; Lara-Flores, Olivera-Castillo & Olivera-Novoa, 2010; Ghazalah, Ali, Gehad, Hammouda & Abo-State, 2010; Welker & Lim, 2011).

The productive performance results showed that the inclusion of FSBM can enable the use of plant-based diets for tilapia juveniles reared in a BFT system. However, it is important to highlight that even with evidence of the possibility of replacing fish meal, the results with the use of FSBM also did not differ from the plant-based negative control diet. Other factors may be involved and it is still necessary to confirm the results obtained. Long-term studies can highlight the differences between treatments. In addition, BFT system may have positive effects on fish performance (Da Silva *et al.*, 2018; Hisano, Parisi, Cardoso, Ferri & Ferreira, 2019) and may have masked the effects of treatments. The relationship between fermented

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products and production systems still needs to be better studied and understood. More studies are still needed to optimize the use of fermented foods in plant-based fish diets.

The villi height was greater in fish fed with diets containing levels above 21% of FSBM compared to the positive control diet containing fish meal. This result of greater villi height is unprecedented and demonstrates the potential of including fermented soybean meal in feeds to promote fish intestinal health. More intact mucosa with larger villi has a better nutrient absorptive capacity (Clements, Angert, Montgomery & Choat, 2014; Merrifield & Ringo, 2014), which may reflect on fish performance and health (Jobling, 1995; Silva *et al.*, 2010). It is noteworthy that at the lowest levels of FSBM inclusion, 7% and 14%, the villi did not differ from the positive control and were greater when compared to the negative control diet. These results demonstrate that fermentation was effective in protecting against epithelial damage that may occur with unfermented soybean meal supplementation (Lilleeng *et al.*, 2009; Marjara, Chikwati, Valen, Krogdahl & Bakke, 2012). As noted in the negative control, diets with a high plant protein level affect the integrity of the intestinal epithelium and can lead to inflammation.

At the highest level of FSBM inclusion (28%), a higher count of goblet cells was observed in relation to the other plant-based diets, and the values did not differ from the positive control. This result is also unprecedented, and it indicates greater intestinal immunity, since the primary function of these cells is the production of mucus, and consequently, protection and lubrication of the intestinal lining (Clements *et al.*, 2014; Merrifield & Ringo, 2014). Furthermore, they are closely related with good quality local microbiota (Schwarz, Furuya, Natali, Michelato & Gaudezi, 2010). The probiotic microorganisms and the bioactive metabolites present in FSBM may have favorably supported these cells and mitigated possible inflammation caused by the greater inclusion of soybean meal in plant-based diets (Urán *et al.*, 2009; Merrifield, Olsen, Myklebust & Ringø. 2011). This result is confirmed by the improvement in the characteristics of the intestinal epithelium and demonstrates the positive effects of FSBM on intestinal health.

5. CONCLUSION

The utilization of a mixed microbial culture increased the probiotic microorganisms count and improved nutritional characteristics of FSBM. The inclusion of 7% FSBM in the diets allows a total replacement of fish meal without compromising the growth performance of the animals. At all levels of FSBM inclusion, there was an improvement in the food efficiency

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of plant-based diets. In the inclusions above 21% of FSBM, there was also evidence of improvement in the intestinal health of Nile tilapia juveniles.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the Ethics Committee on the use of Animals (CEUA - UDESC) approval has been received (CEUA protocol number 3851120320).

AUTHOR CONTRIBUTIONS

Fernanda Picoli: Conceived and designed the analysis, collected the data, contributed data of analysis tools, performed the analysis and wrote the paper; Suelyn de Oliveira Marques: Collected the data; Alana Duarte de Oliveira: Collected the data; Cristina Gradin Nunes: Collected the data; Suélen Serafini: Contributed data of analysis tools and wrote the paper; Bruna Klein: Contributed data of analysis tools and performed the analysis; Nandara Soares de Oliveira: Collected the data; Nataly Neves Oliveira dos Santos: Contributed data of analysis tools and wrote the paper; Aline Zampar: Conceived and designed the analysis, contributed data of analysis tools and performed the analysis; Diogo Luiz de Alcantara Lopes: Conceived and designed the analysis, collected the data, contributed data of analysis tools, performed the analysis and wrote the paper; Thiago El Hadi Perez Fabregat: Conceived and designed the analysis, collected the data, contributed data of analysis tools, performed the analysis and wrote the paper.

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DATA AVAILABILITY STATEMENT

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The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Tables

Table 1- Warranty levels and basic composition of Aquate Fertilizer®.

Product warranty levels	**Quantity**
Saccharomyces cerevisiae (min.)	8 x 10^7 CFU g^{-1}
Mannans (min.)	26.3 g kg^{-1}
Copper (min.)	2,133 mg kg^{-1}
Zinc (min.)	1,249 mg kg^{-1}
Selenium (min.)	75 mg kg^{-1}
Basic product composition:	
Dry sugar cane yeast, hydrolyzed dehydrated yeast, dry brewery yeast, calcium carbonate, *Saccharomyces cerevisiae*, selenium enriched yeast, copper proteinate, sodium and calcium aluminum silicate, dry fermentation product of *Aspergillus niger*, zinc proteinate, ascorbic acid, *Schizochytrium* sp. seaweed meal, *Enterococcus faecium*, *Lactobacillus acidophilus*, dry fermentation product of *Trichoderma longibrachiatum*, sodium chloride, *Chlorella vulgaris* seaweed meal	

Source: Alltech™, 2021.

Table 2 - Proximal composition, soluble protein, pH and microbiological count of fermented soybean meal (FSBM).

Proximal composition	**Soybean meal***	**FSBM***
DM (%)	88.35	90.82
MM (%)	7.42	7.34
EE (%)	5.23	5.05
GE (kcal kg $^{-1}$)	4,259.09	4,383.85
CP (%)	44.63	47.09
SP (mg g^{-1})	19.90	40.80
	Time	
Parameters	**0 (h)**	**48 (h)**
pH	6.64	6.06
LAB (log CFU g^{-1})	0	6.47
Yeast (log CFU g^{-1})	0	7.43

Source: *Composition analyzed by the author. FSBM: fermented soybean meal; DM – dry matter; MM – mineral matter; EE – ether extract; GE – gross energy; CP – crude protein; SP – soluble protein; LAB – lactic acid bacteria; CFU – colony forming unit; g – grams; pH – hydrogenic potential; h – hours.

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Table 3 - Amino acid composition of soybean meal and fermented soybean meal (FSBM) used for the formulation of experimental diets.

Essential amino acids	**Soybean meal****	**FSBM***
Arginine	3.43	3.09
Histidine	1.23	1.15
Isoleucine	2.18	2.14
Leucine	3.59	3.57
Lysine	2.87	*2.89*
Methionine	0.62	0.51
Phenylalanine	2.39	*2.50*
Threonine	1.82	1.75
Tryptophan	0.66	***
Valine	2.27	*2.28*
Non-essential amino acids	**Soybean meal****	**FSBM***
Alanine	2.08	*2.13*
Aspartic acid	3.30	*5.06*
Glutamic acid	4.55	*8.15*
Cystine	0.69	0.64
Glycine	2.02	1.94
Hydroxyproline	****	0.08
Proline	2.37	2.34
Serine	2.52	2.30
Tyrosine	1.70	1.57
Sum of amino acids	**40.29**	**44.09**

Source: *The authors; **Rostagno *et al.* (2017); ***The tryptophan amino acid analysis for FSBM was not performed. ****No hydroxyproline values for soybean meal s were reported in Rostagno *et al.* (2017). FSBM: fermented soybean meal.

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Table 4 - Formulation, chemical composition and microorganism count of experimental diets (g kg^{-1}) based on dry matter.

	Treatments					
Ingredients (%)	**C+**	**C−**	**7%**	**14%**	**21%**	**28%**
Soybean meal	42.00	65.00	57.00	50.00	42.00	35.00
FSBM	-	-	7.00	14.00	21.00	28.00
Fish meal	20.00	-	-	-	-	-
Corn	21.70	19.50	21.00	21.50	22.50	22.50
Wheat flour	13.00	13.00	13.00	13.00	13.00	13.00
Soy oil	2.00	1.00	0.50	-	-	-
Premix	1.00	1.00	1.00	1.00	1.00	1.00
DL-Methionine	0.30	0.50	0.50	0.50	0.50	0.50
Total (%)	100.00	100.00	100.00	100.00	100.00	100.00

	Treatments					
Analyzed bromatological composition and microorganism count	**C+**	**C−**	**7%**	**14%**	**21%**	**28%**
DM (%)	85.94±4.34	86.49±4.11	84.77±0.46	85.24±0.52	83.90±0.69	83.97±1.81
CP (%)	32.93±0.27	32.85±0.42	32.96±0.06	31.95±0.66	33.23±0.40	34.54±0.38
EE	6.01±0.17	3.80±0.06	3.17±0.09	3.23±0.04	3.10±0.09	3.61±0.09
MM (%)	10.78±0.06	4.84±0.03	4.87±0.02	4.84±0.04	4.79±0.01	5.29±0.02
GE (kcal kg $^{-1}$)	4,200.53	4,397.47	4,416.35	4,261.477	4,338.91	4,411.57
LAB (log CFU g^{-1})	NG	NG	4.73±0.33	4.90±0.37	5.26±0.11	4.11±0.62
Yeast (log CFU g^{-1})	2.45±0.21	2.00±0.00	3.32±0.35	3.25±0.59	3.35±0.33	3.28±0.91

FSBM – fermented soybean meal; C+ – positive control (fish meal diet); C– – negative control (plant-based diet without fish meal); 7% – plant- based diet including 7% FSBM; 14% – plant-based diet with 14% inclusion of FSBM; 21% – plant-based diet with 21% inclusion of FSBM; 28% – plant-based diet including 28% of FSBM. NG – no growth in colony forming units was observed; DM – dry matter; CP – crude protein; GE – *gross energy; MM – mineral matter; LAB – lactic acid bacteria. CFU – colony-forming unit; Premix: folic acid – 2,400 mg, nicotinic acid – 48 g, pantothenic acid – 24 g, biotin – 96 mg, vit. A – 2,400,000 IU, vit. D3 – 400,000 IU, vit. E – 24,000 IU, vit. B1 – 9,600 mg, vit. B2 – 9,600 mg, vit. B6 – 9,600 mg, vit. B12 – 9,600 mg, vit K3 – 4,800 mg, vit. C – 96 g, iron – 100 g, manganese – 40 g, zinc – 6,000 mg, cobalt – 20 mg, iodine – 200 mg, selenium – 200 mg, antioxidant – 19.6 g.

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Table 5 - Required and calculated amino acid composition of experimental diets.

Composition	C+	C−	7%	14%	21%	28%	Requirement*
Arginine	2.11	2.33	2.31	2.31	2.28	2.28	1.38
Histidine	0.89	0.84	0.83	0.83	0.82	0.82	0.57
Isoleucine	1.79	1.55	1.54	1.54	1.52	1.52	1.03
Leucine	2.93	2.73	2.72	2.72	2.70	2.70	1.12
Lysine	2.49	2.10	2.08	2.08	2.05	2.05	1.69
Methionine	0.93	0.88	0.88	0.88	0.88	0.88	0.88
Phenylalanine	1.56	1.48	1.47	1.47	1.46	1.46	1.24
Threonine	1.49	1.40	1.39	1.39	1.37	1.37	1.27
Tryptophan	0.48	0.57	0.57	0.57	0.56	0.56	0.33
Valine	1.98	1.77	1.76	1.76	1.74	1.74	0.92
Sum	**16.65**	**15.65**	**15.55**	**15.55**	**15.38**	**15.38**	**10.43**

*Santiago & Lovell, 1988. C+ – positive control (fish meal diet); C– – negative control (plant-based diet without fish meal); 7% – plant-based diet including 7% FSBM; 14% – plant-based diet with 14% inclusion of FSBM; 21% – plant-based diet with 21% inclusion of FSBM; 28% – plant-based diet including 28% of FSBM.

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Table 6 - Zootechnical parameters (mean ± standard deviation) of Nile tilapia juveniles cultivated in a biofloc system after 54 days of feeding with fermented soybean meal (FSFM).

Parameters	**Treatment**						
	C+	**C−**	**7%**	**14%**	**21%**	**28%**	***p* value**
IW (g)	1.63±0.19	1.63±0.21	1.63±0.20	1.65±0.22	1.63±0.20	1.64±0.19	0.9995 NS
FW (g)	20.29±2.81 a	12.60±3.61 b	14.22±3.22 ab	12.49±1.56 b	12.52±2.26 b	13.41±2.77 b	0.0061**
SGR (% day $^{-1}$)	2.03±0.28 a	1.26±0.36 b	1.42±0.32 ab	1.24±0.16 b	1.25±0.23 b	1.34±0.28 b	0.0061**
K	2.01±0.15 a	1.46±0.26 b	1.56±0.25 ab	1.44±0.12 b	1.46±0.18 b	1.51±0.21 b	0.0071**
FCR	0.92±0.02 b	1.44±0.27 a	1.27±0.24 ab	1.29±0.13 ab	1.40±0.18 ab	1.29±0.31 ab	0.05*
FC (g animal $^{-1}$)	17.19±3.12	15.25±3.26	15.63±2.89	13.89±0.84	15.02±2.31	14.58±1.64	0.5264 NS
S (%)	92.85±14.28	89.28±13.67	89.28±7.14	96.42±7.14	82.14±13.67	85.71±11.66	0.5606 NS

C+ – positive control (fish meal diet); C– – negative control (plant-based diet without fish meal); 7% – plant-based diet including 7% FSBM; 14% – plant-based diet with 14% inclusion of FSBM; 21% – plant-based diet with 21% inclusion of FSBM; 28% – plant-based diet including 28% of FSBM. IW – initial weight; FW – final weight; SGR – specific growth rate; K – Fulton's condition factor; FCR– feed conversion rate; FC – feed consumption; S – survival; g – gram; g/day – gram per day; kg/m³ – kilogram per cubic meter; NS – not significant. *P <0.05; **P <0.01. Means followed by distinct letters in the rows differ from each other by the Tukey test (P <0.05).

Table 7 - Intestinal histomorphometry (mean ± standard deviation) of Nile tilapia juveniles (n = 12 / treatment) cultivated in a biofloc system after 54 days of feeding with fermented soybean meal (FSFM).

	Treatment						
	C+	**C−**	**7%**	**14%**	**21%**	**28%**	***p* value**
TVH (µm)	269.10±45.60 bc	248.72±38.15 c	289.50±73.89 b	290.89±65.28 b	320.38±59.72 a	337.27±61.73 a	<0.0001***
VW (µm)	90.27±13.37	99.18±7.04	86.69±14.63	92.34±17.37	96.12±15.37	97.90±8.36	0.4736 NS
VT (µm)	53.65±4.29	52.04±4.84	45.63±6.87	48.72±7.53	49.96±8.79	51.27±4.32	0.4765 NS

C+ – positive control (fish meal diet); C– – negative control (plant-based diet without fish meal); 7% – plant-based diet including 7% FSBM; 14% – plant-based diet with 14% inclusion of FSBM; 21% – plant-based diet with 21% inclusion of FSBM; 28% – plant-based diet including 28% of FSBM. TVH – total villi height ($y = -0.028x^2 + 3.7622x + 253.03$, $R^2 = 0.9469$); VW – villi width; VT – villi epithelium thickness. NS – not significant. *P <0.05; **P <0.01; ***P <0.0001. Means followed by different letters in the lines differ from each other by the Tukey test (P <0.05). Regression analysis was performed between plant-based diets.

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Figures

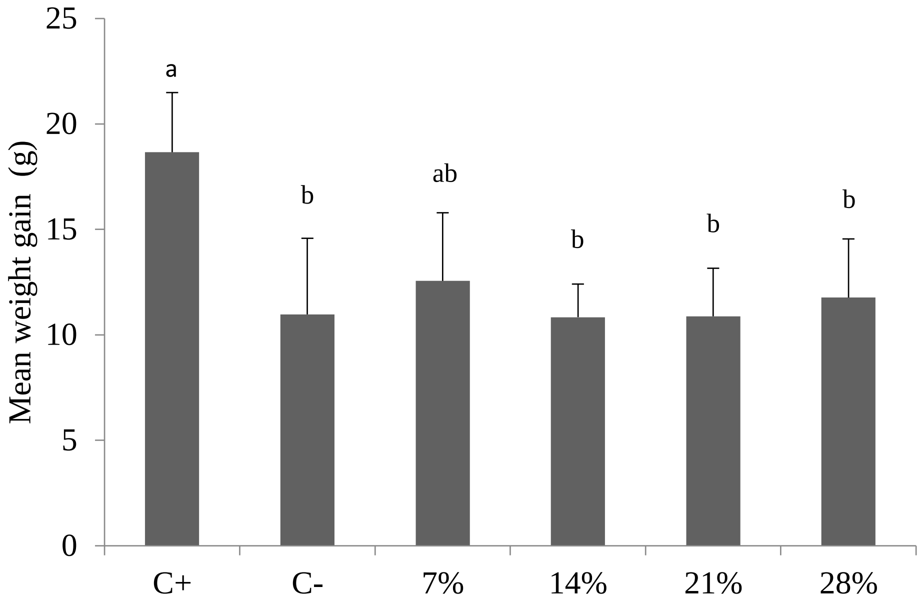

Figure 1 - Mean weight gain (mean ± standard deviation) of Nile tilapia juveniles cultivated in a biofloc system after 54 days of feeding with fermented soybean meal (FSFM).
C+ – positive control (fish meal diet); C– – negative control (plant-based diet without fish meal); 7% – plant-based diet including 7% FSBM; 14% – plant-based diet with 14% inclusion of FSBM; 21% – plant-based diet with 21% inclusion of FSBM; 28% – plant-based diet including 28% of FSBM. Means accompanied by different letters differ from each other by the Tukey test ($P < 0.05$). Regression analysis was performed between plant-based diets, but due to the low R^2 value, the equation was not presented.

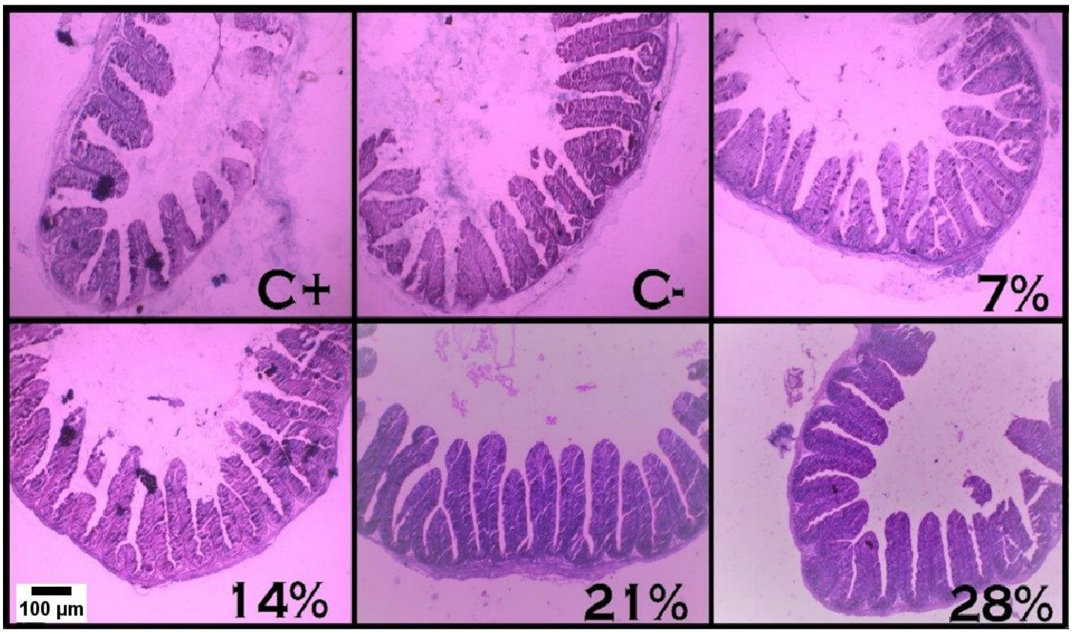

Figure 2 - Intestinal villi of Nile tilapia juveniles after 54 days of feeding with fermented soybean meal (FSBM) in a biofloc system.
Coloration: periodic acid-schiff (PAS). Objective: 10x. C+ – positive control (fish meal diet); C– – negative control (plant-based diet without fish meal); 7% – plant-based diet including 7% FSBM; 14% – plant-based diet with 14% inclusion of FSBM; 21% – plant-based diet with 21% inclusion of FSBM; 28% – plant-based diet including 28% of FSBM.

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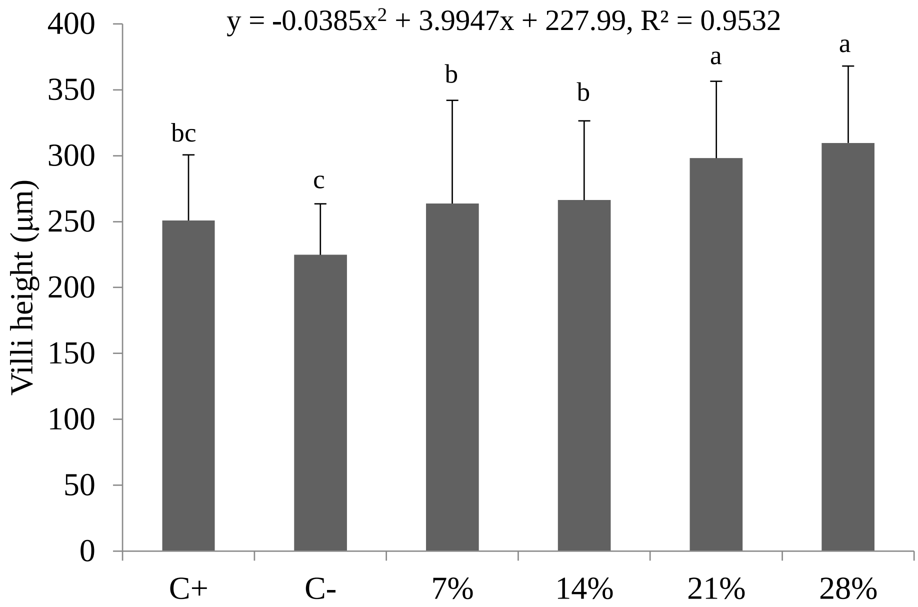

Figure 3 - Villi height of Nile tilapia juveniles cultivated in a biofloc system after 54 days of feeding with fermented soybean meal (FSFM).

C+ – positive control (fish meal diet); C– – negative control (plant-based diet without fish meal); 7% – plant-based diet including 7% FSBM; 14% – plant-based diet with 14% inclusion of FSBM; 21% – plant-based diet with 21% inclusion of FSBM; 28% – plant-based diet including 28% of FSBM. Means accompanied by different letters differ from each other by the Tukey test (P <0.05). Regression analysis was performed between plant-based diets.

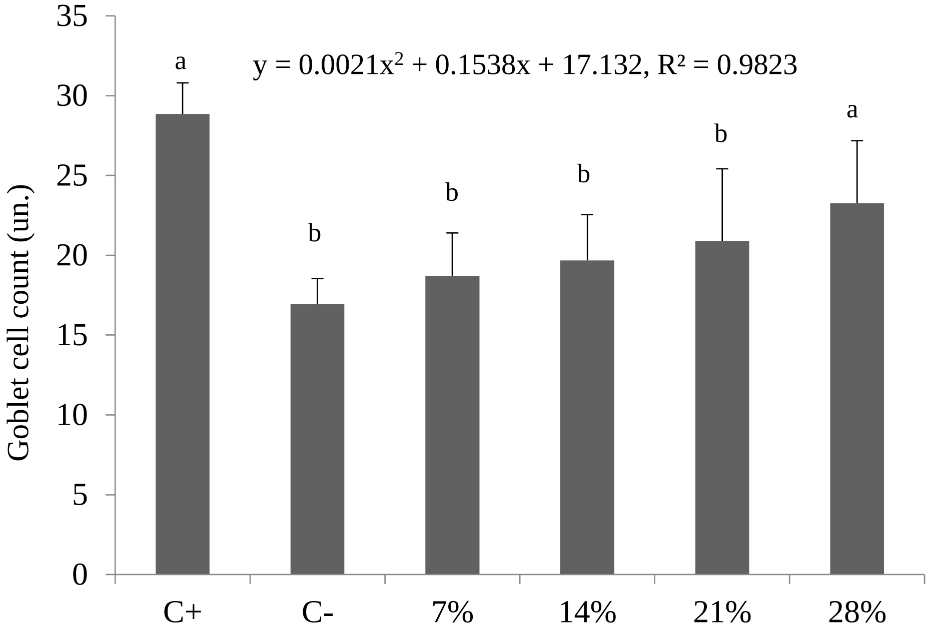

Figure 4 - Goblet cell count of Nile tilapia juveniles cultivated in a biofloc system after 54 days of feeding with fermented soybean meal (FSFM).

C+ – positive control (fish meal diet); C– – negative control (plant-based diet without fish meal); 7% – plant-based diet including 7% FSBM; 14% – plant-based diet with 14% inclusion of FSBM; 21% – plant-based diet with 21% inclusion of FSBM; 28% – plant-based diet including 28% of FSBM. Means accompanied by different letters differ from each other by the Tukey test (P <0.05). Regression analysis was performed between plant-based diets.

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ARTIGO II

Biofloc system avoid the negative effects of diets with suboptimal protein levels on zootechnical performance, intestinal histomorphometry and protein metabolism of Nile tilapia fed *Spirulina* biomass (*Arthrospira platensis*) as alternative protein source

Fernanda Picoli, Alana D. de Oliveira, Suelyn O. Marques, Deise C. Terhorst, Suélen Serafini, Luísa Nora; Aleksandro S. da Silva, Fabio F. Neves, Maurício G. C. Emerenciano, Diogo L. A. Lopes, Thiago E. H. P. Fabregat

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Biofloc system avoid the negative effects of diets with suboptimal protein levels on zootechnical performance, intestinal histomorphometry and protein metabolism of Nile tilapia fed *Spirulina* biomass (*Arthrospira platensis*) as alternative protein source

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Abstract: The objective of this study was to evaluate the effect of the production system (clear water x bioflocs) and the replacement of fish meal with *Spirulina* biomass (*Arthrospira platensis*) on performance, intestinal histomorphometry, serum biochemistry, and oxidative stress of Nile tilapia juveniles (*Oreochromis niloticus*) fed suboptimal levels of protein. The design was completely randomized in a 4 x 2 factorial scheme with four replications, four treatments, and two cultivation systems. Four isoproteic (28% crude protein) and isoenergetic (4,000 crude energy kcal kg $^{-1}$) diets were formulated with 0 (control), 33, 66 and 100% replacement levels of fish meal by *Spirulina* biomass, supplied for 48 days. Juveniles (0.23 ± 0.01 g) were distributed in 16 circular tanks (70 L) at seven fish per tank. The tanks were divided into two independent recirculation systems (clear water x bioflocs) connected to larger tanks (850 L) equipped with a heating and aeration system. No interaction was observed between the evaluated systems and treatments. Rearing in the BFT system avoided the negative effects of diets with suboptimal protein levels on zootechnical performance, intestinal histomorphometry, and protein metabolism. There was evidence of improvements in antioxidant response and lower physiological oxidative stress. *Spirulina* biomass can completely replace fish meal in the diets of Nile tilapia juveniles. Replacing 66% of fish meal with *Spirulina* biomass improved the performance, regardless of the rearing system (clear water x bioflocs).

Keywords: Animal nutrition; *Arthrospira platensis*; Biofloc system; Intestinal histomorphometry; *Spirulina*.

1. Introduction

World aquaculture produced 88 million tons in 2020, most of which were fish (FAO 2022). This significant growth in activity is explained by the increase in the world population and per capita consumption (FAO 2022) associated with increasing demand for healthier animal proteins (Henchion *et al.* 2017; Boer *et al.* 2020). To meet this demand, fish farming tends to intensify (Subashinge 2013; Oddsson 2020). In this context, the demand for quality feed and high food costs create bottlenecks for the continued expansion of fish farming. The ingredients that constitute the protein fraction of fish diets are expensive and contribute significantly to the total expenses (Barbosa *et al.* 2020). In addition, excess protein can increase levels of nitrogen compounds in effluents (Herath and Satoh, 2015). Therefore, using feeds with lower protein levels and searching for alternative protein ingredients are strategies to reduce feed costs and increase the sustainability of the activity.

Fish meal is one of the most commonly used protein ingredients in fish feed. It is characterized by its high protein value, polyunsaturated fatty acids, and considerable mineral and vitamin fractions (Masumoto *et al.* 1996). Their exclusion from diets may decrease zootechnical indices in fish (Gomes *et al.* 1995; Glencross *et al.* 2011; Turchini *et al.* 2019). In addition, its high cost and reduced availability (World Bank 2020) have driven the search for cheaper protein alternatives. The microalgae *Spirulina* (*Arthrospira platensis*) is an alternative source of protein with the potential to replace fish meal (Hussein *et al.* 2013; Raji *et al.* 2018;

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Erdogan 2019; Raji *et al.* 2020; Mansour *et al.* 2021). It is considered a rich source of highly digestible protein and essential amino acids (Andrade *et al.* 2005) and contains various substances with antioxidant properties (fibrous carbohydrates, vitamins, minerals, carotenoid pigments, and polyunsaturated fatty acids) (Promya and Chitmanat 2011; Gutiérrez-Salmeán *et al.* 2015). *Spirulina* improves zootechnical performance more than fish meal (Hussein *et al.* 2013; El-Sheekh *et al.* 2014; Velasquez *et al.* 2015; Roohani *et al.* 2019; Raji *et al.* 2020). Improvements in biochemical (Belal *et al.* 2012; Mansour *et al.* 2021), histological (Abu-Elala *et al.* 2016), and oxidative stress parameters (Cao *et al.* 2018; Salah El-Din *et al.* 2021) have also been reported.

Cultivation in a biofloc system (BFT) is an alternative to reduce protein content in diets and feed costs in intensive fish production (Avnimelech, 2007; Azim and Little 2008). BFT is a closed cultivation system with low water renewal. Water quality is maintained with intensive aeration and management of heterotrophic and autotrophic bacterial populations (Monroy-Dosta *et al.* 2013). An organic carbon source is added to balance the relationship between carbon and nitrogen (Pérez-Fuentes *et al.* 2016; Silva *et al.* 2017). Therefore, the nitrogenous compounds are recycled, and bioflocs are formed, which serve as a food supplement for the fish (Avnimelech 2007; Avnimelech and Kochba 2009; Ekasari 2014). The microbial biomass produced in this system can supply up to 50% of the protein requirement (Avnimelech 2007; Azim and Little 2008; Da Silva *et al.* 2018; Durigon *et al.* 2020). Lower feed conversion rates can also be achieved (Luo *et al.* 2014).

Nile tilapia (*Oreochromis niloticus*) is a prominent species in global fish farming (Prabu *et al.* 2019; FAO 2022). It is a rustic species that quickly adapt to intensive cultivation. The rearing of tilapia in a BFT system has been previously reported with positive results (Lima *et al.* 2018; Martins *et al.* 2020; Picoli *et al.* 2022). Nile tilapia ingests bioflocs, enabling a reduction in the demand for protein (Avnimelech 2007; Avnimelech and Kochba 2009; Ekasari 2014.). *Spirulina* has also been evaluated in tilapia feeding with good results (Belal *et al.* 2012; Hussein *et al.* 2013; Velasquez *et al.* 2015; Abdelkhalek *et al.* 2017). However, its use for the cultivation of this species in a BFT system with suboptimal levels of protein in the diet has not been evaluated. Therefore, the objective of this study was to determine the effect of the culture system and the replacement of fish meal by *Spirulina* biomass (*A. platensis*) on performance, intestinal histomorphometry, serum biochemistry, and oxidative stress in Nile tilapia juveniles fed suboptimal levels of protein.

2. Materials and methods

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2.1 Experimental design

The experiment was carried out at the Aquaculture Laboratory of the State University of Santa Catarina, Campus Chapecó, Santa Catarina, Brazil, for 48 days. The Committee for Ethics in the Use of Animals approved the study under protocol number 6983180521. Four diets were formulated: 0% or control (without *Spirulina* biomass) and 33, 66 and 100% replacement level of fishmeal by *Spirulina* biomass. The design was completely randomized with four replications, in a 4 x 2 factorial scheme, with four treatments (0, 33, 66, and 100%) and two cultivation systems (clear water x BFT).

2.2 *Experimental diets*

The experimental diets were isoenergetic (4,300 GE kcal kg^{-1} – Hafedh 1999) and isoproteic (28% crude protein [CP]). They were formulated using SuperCrac® software to meet protein levels approximately 20% below the required for the species and life stage (35% CP [FAO 2017]). The proximal composition of the diets (Table 1) was analyzed according to methods described in the Association of Official Analytical Chemists (AOAC 2000).

The cultivation of *Arthrospira platensis* was carried out in raceway tanks arranged inside a greenhouse in a semi-continuous system at the Laboratory of Algae Cultivation and Biotechnology – LCBA UDESC, Laguna, Brazil (Table 2). The culture medium contained fresh water and was composed of 10 g L^{-1} of $NaHCO_3$, 1 g L^{-1} of commercial soluble fertilizer with a Nitrogen:Phosphate:Potassium ratio of 18:6:18 and a high amount of NaCl (30 g L^{-1}). The cultivation was carried out under natural temperature and lighting conditions (not controlled) and the *Spirulina* biomass was collected three times a week by filtration with a 20 µm mesh. Subsequently, it was dried in a laboratory oven at 50 ° C for 24 hours and ground in a cryogenic spray mill (MA775, Marconi) before use in the experimental diets. In addition to this ingredient, the formulated diets contained fish and soybean meal as protein sources and soybean and corn oil as energy sources (Table 1). All ingredients were ground in an industrial processor and sieved through a 0.71-mm mesh. Subsequently, they were mixed with the addition of water (50%), pelleted in a 1-mm “spaghetti” mixer (Malta®, Model 1036, nozzle no. 8), and placed in an oven (Cienlab®, Model CE-220/150) at 55 °C for 48 h. Diets were stored in plastic packages and placed in refrigerators (4 °C) until use.

Table 1 - Composition of experimental diets (g kg-1) based on dry matter.

Ingredients	**Replacement percentage of fish meal**

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	0%	33%	66%	100%
Fish meal	36.45	23.26	10.08	-
Spirulina biomass	-	26.25	52.50	72.57
Soybean meal	10.00	10.00	10.00	10.00
Corn	41.59	27.86	14.02	3.43
Soy oil	10.73	9.00	7.29	5.98
Premix	0.40	0.40	0.40	0.40
Limestone	0.64	1.65	2.53	3.20
Dicalcium phosphate	-	1.15	2.53	3.58
Table salt	0.06	0.30	0.53	0.72
Antioxidant	0.10	0.10	0.10	0.10
Total (%)	100	100	100	100
Analyzed composition (%)				
DM	84.33	83.78	84.56	83.10
MM	8.07	8.55	8.80	8.89
EE	10.32	7.09	6.67	6.74
CP	27.64	28.76	27.65	28.26
GE (kcal kg^{-1})	4,596	4,380	4,395	4,425

Composition analyzed (AOAC 2000). DM – Dry Matter; MM – Mineral Matter; EE – Ethereal Extract; CP – Crude Protein. GE – Gross Energy; Premix - Folic Acid – 2,400 mg, Nicotinic Acid – 48 g, Pantothenic Acid – 24 g, Biotin – 96 mg, Vit. A – 2,400,000 IU, Vit. D3 – 400,000 IU, Vit. E – 24,000 IU, Vit. B1 – 9,600 mg, Vit. B2 – 9,600 mg, Vit. B6 – 9,600 mg, Vit. B12 – 9600 mg, Vit K3 – 4800 mg, Vit. C – 96 g, Iron – 100 g, Manganese – 40 g, Zinc – 6,000 mg, Cobalt – 20 mg, Iodine – 200 mg, Selenium – 200 mg, Antioxidant – 19.6 g.

Table 2 - Proximal composition of fish meal and *Spirulina* biomass used in experimental diets.

Proximal composition	**Fish meal**	***Spirulina* biomass**
DM (%)	93.33	84.74
MM (%)	25.79	16.56
EE (%)	7.52	2.60
CP (%)	55.02	32.01

Composition analyzed (AOAC 2000). DM – Dry Matter; MM – Mineral Matter; EE – Ethereal Extract; CP - Crude Protein.

2.3 *Animals and facilities*

Nile tilapia juveniles with an average initial weight of 0.23 ± 0.01 g were purchased from a commercial fish farm. The animals were distributed at seven fish per tank in circular polyethylene tanks (useful volume of 70 L), divided into two independent water recirculation systems (clear water x BFT). Each system consisted of 16 circular boxes interconnected with circular tanks (usable volume: 850 L) called “macrocosms.” The tanks were individually aerated through porous stone (1 x 2 cm) connected to a radial air compressor. Each macrocosm contained a peripheral water pump (Intech® model BP500), responsible for the distribution and recirculation of water in its respective system. Each macrocosm was equipped with a heater

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resistance (Nobre Brasil®, 3000 W) and connected to a digital temperature controller with a sensor (XH-W3001®, 12V, China), aiming to maintain the appropriate temperature for the species (El-Sayed 2006). A biomass of 2.25 kg m^{-3} of tilapia was also maintained in the BFT macrocosm, fed with commercial feed (Supra® Tilapia 32% CP) once a day (1:00 pm; 32% CP). The macrocosm of the clear water system was composed of a filtering system combined with a mechanical filter (60-µm perlon mesh) and biological particulate material (0.2 m^3 of analog of low-density polyethylene bio-balls and 0.05 m^3 of expanded clay stone). These materials served as a substrate for the nitrifying bacteria to carry out nitrification of the culture water.

The animals were fed throughout the experimental period at a rate of 10% of the biomass per day (Workagegn *et al.* 2014), divided into three feedings (08:00 am, 1:00 pm, and 5:00 pm). The biomass monitoring was performed every 16 days using biometrics of all experimental units.

At the beginning of the experiment, all tanks in the BFT system received a previously matured biofloc inoculum (10 L) to prevent unwanted variations in water quality (Krummenauer *et al.* 2014; Martins *et al.* 2020). Subsequently, dechlorinated water was added to complete the useful volume of 70 L. The same proportion (1:6) was used in the macrocosm. For the formation and maintenance of the microbial community and the adequate conditions of the culture medium, a C:N ratio of 10–15:1 was maintained (Pérez-Fuentes *et al.* 2016). Sugarcane molasses was used as an external source of carbon.

The water quality parameters of both cultivation systems were measured daily, including temperature, dissolved oxygen (ALFAKIT® oximeter, model AT170), and pH (pHmeter ALFAKIT®, model AT315). Three times a week, the measurement of total ammoniacal nitrogen (TAN) was performed for both systems, and the measurement of total sedimentable solids using an Imhoff cone for the BFT system. According to the results obtained in these measurements (TAN > 1 mg L^{-1}), molasses was or was not added to the BFT system. Weekly analyses of nitrite, nitrate, orthophosphate, and alkalinity of the culture medium were performed using a photocolorimeter (ALFAKIT®, model AT100P). The parameters remained within the recommended range for tilapia (El-Sayed 2006; Azim and Little 2008; Luo *et al.* 2014; Lima *et al.* 2015; 2018) (Table 3).

Table 3 - Water quality parameters (mean ± standard deviation) of juvenile Nile tilapia grown in two culture systems (clear water x BFT) after 48 days of feeding with *Spirulina* biomass.

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Parameters	Macrocosm	
	AC	**BFT**
T^a (°C)	25.40 ± 1.28	25.90 ± 1.26
OD (mg L^{-1})	7.97 ± 1.10	7.72 ± 1.05
pH	7.53 ± 0.59	7.36 ± 0.30
N-NH_3 (mg L^{-1})	0.24 ± 0.64	0.60 ± 0.67
NO_2^- (mg L^{-1})	0.14 ± 0.15	0.31 ± 0.08
NO_3^- (mg L^{-1})	1.82 ± 0.09	1.32 ± 0.14
ALC (mg L^{-1} $CaCO_3$)	25.8 ± 20.62	41.83 ± 20.18
ORT (PO_4)	0.64 ± 0.03	3.41 ± 1.67
TSS (mg L^{-1})	-	13.35 ± 13.82

T^a = Temperature; °C = Degrees Celsius; pH = Hydrogenionic Potential; N-NH_3 = Ammonia Nitrogen; mg L^{-1} = milligram per liter; NO_2^- = Nitrite; NO_3^- = Nitrate; ALC = Alkalinity; mg L^{-1} $CaCO_3$ = milligram per liter of calcium carbonate; ORT = Orthophosphate; PO4 = Phosphate; TSS = total settling solids; AC = clear water culture system; BFT = Biofloc culture system.

2.4 *Zootechnical performance and hepatosomatic index*

After the experimental period, the animals remained fasting for 24 hours and were individually weighed (0.01 g precision scale, Marte model ML 600, São Paulo, Brazil) and measured for the evaluation of zootechnical parameters: final weight; average weight gain = final weight (g) – initial weight (g); specific growth rate = ln final weight (g) – ln initial weight (g) / experimental period x 100 (% day^{-1}); apparent feed conversion = feed intake/weight gain; feed intake = feed consumed during the experimental period (g) and survival (S) = number of dead animals / total fish x 100 (%). Subsequently, 12 fish per treatment were sedated with eugenol and sacrificed by medullary section to collect the liver to measure the hepatosomatic index (HSI = liver weight / total weight x 100) and the gastrointestinal tract for further analysis.

2.5 *Intestinal and liver histomorphometry*

We collected fractions of the proximal intestine and liver for the histomorphometric analysis. The histological processing of the samples was performed at the UDESC Histology Laboratory, Campus Lages (Santa Catarina, Brazil), according to routine methods for preparing histological slides (Nunes and Cinsa 2016). The intestinal and liver fragments were placed in sterile plastic containers with a 10% formalin solution, identified, and fixed for 12–16 hours in 70% alcohol until the evaluation of the morpho-histological characteristics. The fractions were sectioned at 0.3 cm using the histology technique, diaphanization, and embedding in

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histological paraffin. Fractionation into 3-μm sections was performed using a semiautomatic microtome (Mello *et al.* 2013). Longitudinal sections were semi-serialized and stained using periodic acid-Schiff (Tolosa *et al.* 2003).

The samples were observed using light microscopy (Bioval L-2000[a] microscope, São Paulo, Brazil). Using a camera attached to the microscope, the intestinal (10x) and liver (40x) images were digitized using "TCapture" software (Brand Laborana®, São Paulo, Brazil). After the scans were completed, ten villi per animal were selected by integrity criteria (Picoli *et al.* 2019), as follows: i) VH – villus height (i.e., distance from the apex of the villi to the beginning of the muscle layer); ii) TVH – total height of the villi (i.e., height from the apex of the villi to the end of the serosa); iii) VW – villi width; iv) VT – villus epithelium thickness, according to Mello *et al.* (2013). All measurements were performed using Image J software (National Institute for Health, Bethesda, Maryland, United States of America). An area including 100 hepatocytes per animal was measured. The mean area of hepatocytes (MAH) per treatment was estimated using the same software, according to Picoli *et al.* (2019).

2.6 Serum biochemical and antioxidant analyses

After anesthesia of 12 animals per treatment, blood was collected by puncture of the caudal vein into syringes containing 10% EDTA. The collected blood was centrifuged for 10 min at 3,500 rpm, and supernatants (serum) were stored in microtubes at –20 ºC until analysis. Total proteins (PTNA) and albumin (ALBU) were evaluated in a biochemical analyzer (Bio Plus® BIO-2000) using commercial kits (Gold Analisa®). Globulin levels (GLOBU) were calculated using the formula:

$$GLOBU = PTNA - ALBU$$

Liver samples of 0.5 g were ground/homogenized (Turratec®) with saline solution (0.9%) and centrifuged at 7,000 rpm for 10 min. The supernatants were stored in microtubes at –20 ºC until analysis of the following parameters: thiobarbituric acid reactive substances (TBARS), glutathione S-transferase (GST), and protein thiol (PSH). Lipid peroxidation was estimated in samples by measuring malondialdehyde (MDA). Tissue samples were incubated at 95 °C for 60 minutes in an acid medium containing 8.1% sodium dodecyl sulfate, 0.5 ml acetic acid buffer (500 mM, pH 3.4), and 0.6% TBA (Ohkawa *et al.* 1978). Absorbances were measured at 532 nm and compared to the malondialdehyde standard curve. The results were expressed in nmol MDA/ml (Jentzsch *et al.* 1996).

Glutathione-S-transferase activity was measured kinetically using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The reaction mixture (2 ml) contained 0.1 M phosphate

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buffer, GSH (1 mM), CDNB (1 mM), and TPM (10%). The absorbance was determined at 340 nm at 37 °C, and enzymatic activity was expressed as μmol CDNB/min/mg of protein (Habig *et al.* 1974).

Protein thiols (PSH) were measured using a method based on the use of DTNB (5,5-dithiobis-2-nitrobenzoic acid) (Ellman, 1959; Sedlak and Lindsay 1968), measured after deproteinization of the homogenates with trichloroacetic acid. The sediment formed by the precipitated protein was resuspended with a homogenization buffer to determine the PSH content. Results were expressed as μmol SH g^{-1} of tissue.

2.7 Statistical analysis

Normality (Shapiro-Wilk Test) and homogeneity of variances (Bartlett Test) were verified, and the data were subjected to analysis of variance for a factorial scheme. When not significant, means were compared using Tukey's test at a 5% probability level. In the case of interaction, factorial splitting and means were compared using Tukey's test (5%). The analyses were performed using the R® 4.1.1 statistical analysis program.

3. Results

3.1 Zootechnical parameters and organosomatic indexes

No interaction ($P > 0.05$) was observed between culture systems and levels of replacement of fish meal by *Spirulina* biomass for tilapia performance variables (Table 1). In the BFT system, the performance of the fish was better than those reared in the AC system ($P < 0.05$; Table 1). *Spirulina* biomass completely replaced fish meal without compromising performance ($P > 0.05$). Replacing 66% of fish meal with *Spirulina* biomass resulted in greater growth and improvement in feed conversion than the control treatment (0%; $P < 0.05$). There was no effect of treatments on survival or hepatosomatic index ($P > 0.05$).

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Table 4 - Analysis of variance of performance variables of Nile tilapia juveniles reared in two culture systems (AC x BFT) after 48 days of feeding on *Spirulina* biomass.

		P-value	
Variável	**Systems (S)**	**Fish meal replacement level (T)**	**Interation (S x T)**
FW (g)	*<0.0010**	*0.0029**	0.0853 NS
AWG (g)	*<0.0001**	*0.0029**	0,0851 NS
SGR (% day^{-1})	*<0.0001**	*0.0028**	0.0849 NS
FConv	*0.0002**	*0.0023**	0.0970 NS
FCons (g)	*<0.0001**	0.8360 NS	0.5850 NS
S (%)	0.3640 NS	0.2590 NS	0.2590 NS
HSI (%)	0.1603 NS	0.5107 NS	0.4379 NS

S = Systems (BFT and AC); T = Treatments (0, 33, 66 and 100% replacement of fish meal by *Spirulina*; S x T = Interaction between systems and treatments; FW = Final Weight; AWG = Average Weight Gain; SGR = Specific Growth Rate; FConv = Food Conversion; FCons = Feed Consumption; S = Survival; HSI = Hepatosomatic Index; g = grams; % day^{-1} = % weight gain per day; NS = Not significant; * $P < 0.05$.

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Table 5 - Mean values (± standard deviation; n = 12) of zootechnical parameters of Nile tilapia juveniles reared in two culture systems (AC x BFT) after 48 days of feeding on *Spirulina* biomass.

Variável	**AC**	**BFT**	**0%**	**33%**	**66%**	**100%**
FW (g)	4.50 ± 1.58 B	16.50 ± 0.56 A	9.65 ± 5.99 b	10.03 ± 6.20 b	11.43 ± 6.50 a	10.89 ± 7.17 ab
SGR (% dia $^{-1}$)	0.45 ± 0.05 B	1.65 ± 0.15 A	0.96 ± 0.60 b	1.00 ± 0.62 b	1.14 ± 0.65 a	1.08 ± 0.71 ab
FCons (g)	4.91 ± 0.47 B	15.75 ± 1.60 A	10.56 ± 6.03	10.30 ± 5.77	10.44 ± 6.33	10.01 ± 5.49
S (%)	93.84 ± 0.17	96.93 ± 0.13	90.75 ± 0.20	100.0 ± 0.00	96.93 ± 0.13	93.84 ± 0.18
HSI (%)	1.94 ± 0.66	1.62 ± 0.23	1.75 ± 0.24	1.80 ± 0.78	1.77 ± 0.69	1.80 ± 0.11

AC = Clear water culture system; BFT = Biofloc culture system; T = Treatments (0, 33, 66 and 100% replacement of fish meal by *Spirulina* biomass (*Arthrospira platensis*); FW = Final Weight; SGR = Specific Growth Rate; FCons = Feed Consumption; S = Survival; HSI = Hepatosomatic index; g = grams; % day^{-1} = % weight gain per day; Systems: Means followed by different capital letters in columns, differ from each other by Tukey's test (P <0.05). Fish meal replacement level: Means followed by different lowercase letters in columns differ from each other by Tukey's test (P <0.05).

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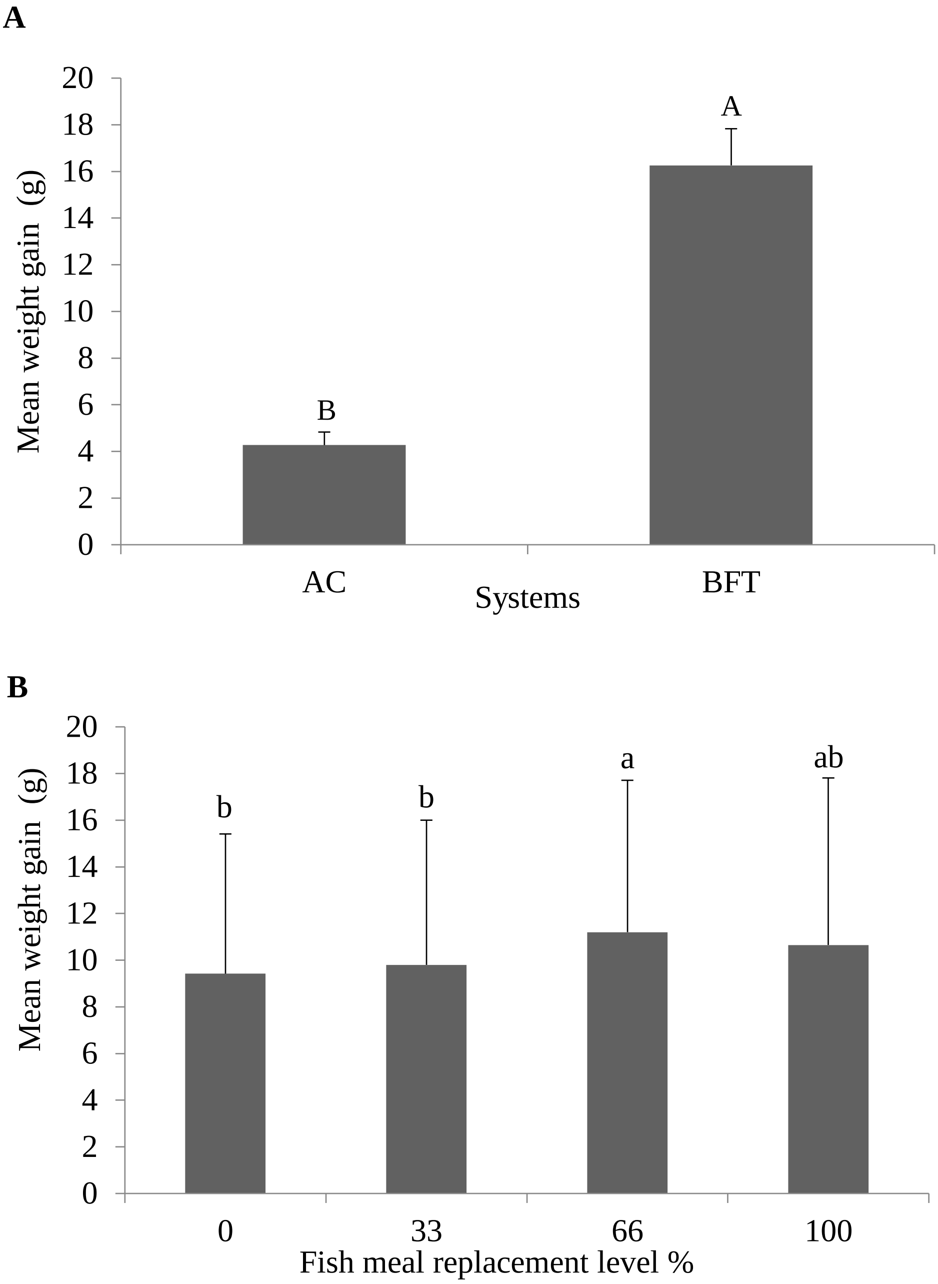

Fig. 1 Average weight gain (mean ± standard deviation) of Nile tilapia juveniles reared in two culture systems (A) with different levels of replacement of fish meal by *Spirulina* biomass (B) after 48 days of feeding.

Means accompanied by distinct letters differ by Tukey's test ($P < 0.05$). AC = Clear water culture system; BFT = Biofloc culture system.

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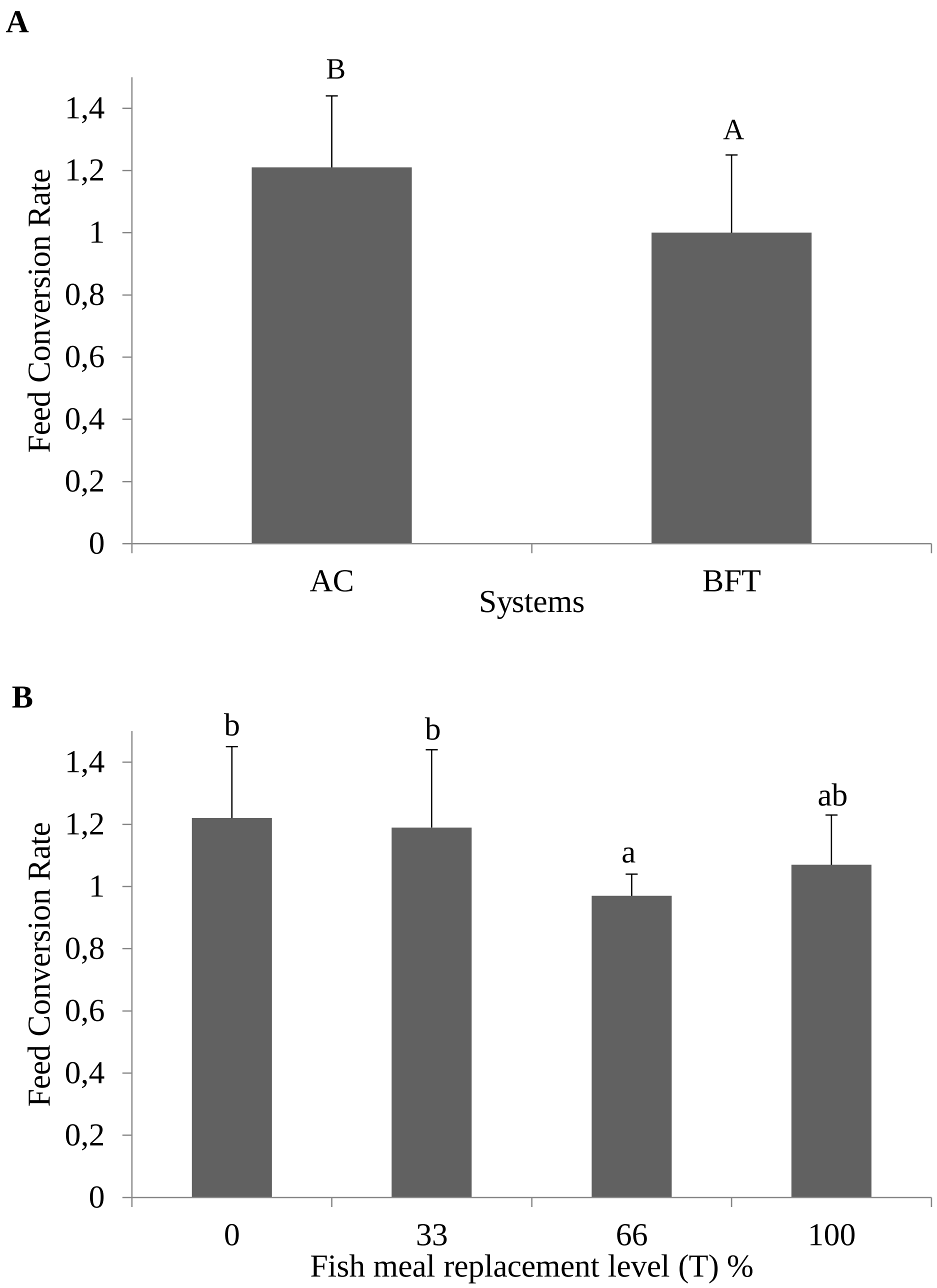

Fig. 2 Feed conversion (mean ± standard deviation) of Nile tilapia juveniles reared in two culture systems (A) with different levels of replacement of fish meal by *Spirulina* biomass (B) after 48 days of feeding.

Means accompanied by distinct letters differ by Tukey's test ($P < 0.05$). AC = Clear water culture system; BFT = Biofloc culture system.

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3.2 Hepato-intestinal histomorphometry

No interaction was observed between the factors in the tilapia hepato-intestinal histomorphometry (P > 0.05; Table 3). In the BFT system, the total villus height (TVH) was higher (P < 0.05) than in the AC system (Figure 3). The treatments did not affect the other evaluated histomorphometric parameters (P > 0.05).

Table 6 - Analysis of variance of hepato-intestinal histomorphometry of Nile tilapia juveniles grown in two culture systems (AC x BFT) after 48 days of feeding with *Spirulina* biomass.

	P-value		
	Systems (S)	**Fish meal replacement level (T)**	**Interaction (S x T)**
TVH (µm)	*0.048**	0.871 NS	0.623 NS
VH (µm)	0.105 NS	0.728 NS	0.549 NS
VW (µm)	0.219 NS	0.950 NS	0.630 NS
VT (µm)	0.217 NS	0.868 NS	0.614 NS
MAH (µm²)	0.700 NS	0.211 NS	0.667 NS

S = Systems (BFT and AC); T = Treatments (0, 33, 66, and 100% replacement of fish meal by *Spirulina* biomass; S x T = Interaction between systems and treatments; TVH = Total Villi Height; VH = Villi Height; VW = Villi Width; VT = Villi Epithelium Thickness; MHA = Mean Area of Hepatocytes; NS = Not Significant; * = P < 0.05.

Table 7 - Mean values (± standard deviation; n= 12) of hepato-intestinal histomorphometry of Nile tilapia juveniles cultivated in two culture systems (AC x BFT) after 48 days of feeding with *Spirulina* biomass.

	AC	**BFT**	**0%**	**33%**	**66%**	**100%**
VH (µm)	454.79 ± 208.32	592.35 ± 220.98	459.25 ± 171.29	515.92 ± 217.42	542.45 ± 239.84	584.67 ± 272.83
VW (µm)	158.07 ± 55.39	184.17 ± 53.72	172.67 ± 59.32	161.73 ± 46.91	171.38 ± 65.20	178.70 ± 58.30

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VET (µm)	77.77 ± 30.62	95.43 ± 42.57	95.41 ± 58.69	79.83 ± 24.17	82.92 ± 32.79	88.23 ± 31.40
MAH (µm²)	502.51 ± 36.50	515.08 ± 91.79	470.34 ± 33.85	492.81 ± 35.69	529.81 ± 88.27	536.22 ± 86.40

AC = Clear water culture system; BFT = Biofloc culture system; S = Systems (AC x BFT); T = Treatments (0, 33, 66, and 100% replacement of fish meal by *Spirulina* biomass; SxT = Interaction between systems and treatments; VH = Villi Height; VW = Villi Width; VET = Villi Epithelium Thickness; MHA = Mean Area of Hepatocytes; NS = Not Significant; * = P <0.05. Systems: Means followed by different capital letters in the columns differ from each other by Tukey's test (P <0.05). Fish meal replacement level: Means followed by different lowercase letters in the columns differ from each other by Tukey's test (P <0.05).

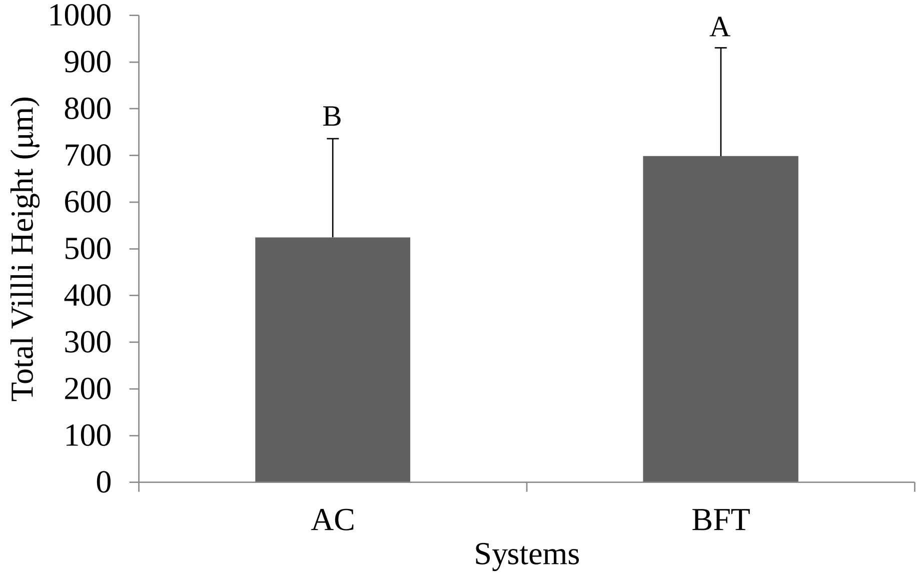

Fig. 3 Total villi height (mean ± standard deviation) of Nile tilapia juveniles reared in two culture systems (AC x BFT) with diferent levels of replacement of fish meal by *Spirulina* biomass after 48 days of feeding.

Means accompanied by distinct letters differ by Tukey's test (P <0.05). AC = Clear water culture system; BFT = Biofloc culture system

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3.3 Serum biochemistry and oxidative stress

For the biochemical and oxidative stress parameters, no interactions (P >0.05) were observed between the cultivation systems and the replacement levels of fish meal by *Spirulina* biomass (Table 5). In the BFT system, ALBU and GLOBU were higher in relation to AC (P<0.05). (Table 6). Lower values for thiobarbituric acid reactive substances (TBARS) were observed in the BFT system compared to the AC system (Figure 4). The opposite was observed for the enzyme GST (Figure 4). Serum total protein (PTNA) and protein thiol (PSH) levels were unaffected by treatments and systems (P >0.05) (Table 6).

Table 8 - Analysis of variance of biochemical parameters and oxidative stress of Nile tilapia juveniles cultivated in two culture systems (AC x BFT) after 48 days of feeding with *Spirulina* biomass.

	P-value		
	Systems (S)	**Fish meal replacement level (T)**	**Interaction (S x T)**
PTNA (g dL^{-1})	0.189 NS	0.870 NS	0.150 NS
ALBU (g dL^{-1})	*<0.000****	0.826 NS	0.987 NS
GLOBU (g dL^{-1})	*0.0475***	0.067 NS	0.129 NS
TBARS	*<0.0001*	0.987 NS	0.830 NS
GST	*<0.0001*	0.983 NS	0.600 NS
PSH	0.326 NS	0.676 NS	0.247 NS

S = Systems (BFT and AC); T = Treatments (0, 33, 66, and 100% replacement of fish meal by *Spirulina* biomass; S x T = Interaction between systems and treatments; PTNA = Total proteins; ALBU = Albumin; GLOBU = Globulins; g/dL = gram per deciliter; TBARS = Thiobarbituric acid reactive substances (nmol MDA/mL); GST = Glutathione S-transferase (µmolCDNB/min/mg protein); PSH = Protein thiols (µmol SH/mg protein); NS = Not significant; * = P < 0.05.

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Table 9 - Mean values (mean ± standard deviation; n = 12) of biochemical parameters and oxidative stress of Nile tilapia juveniles grown in two culture systems (AC x BFT) after 48 days of feeding with *Spirulina* biomass.

	AC	**BFT**	**0%**	**33%**	**66%**	**100%**
PTNA (g dL^{-1})	3.53 ± 0.57	4.37 ± 1.33	4.31 ± 0.78	3.86 ± 0.97	3.60 ± 0.42	4.07 ± 1.35
ALBU (g dL^{-1})	0.88 ± 0.25 B	1.42 ± 0.47 A	1.27 ± 0.50	1.13 ± 0.59	1.09 ± 0.39	1.12 ± 0.40
GLOBU (g dL^{-1})	2.65 ± 0.44 B	2.95 ± 0.47 A	3.04 ± 0.28	2.73 ± 0.48	2.51 ± 0.46	2.95 ± 0.52
PSH	1.33 ± 0.18	1.40 ± 0.21	1.36 ± 0.18	1.42 ± 0.22	1.30 ± 0.18	1.38 ± 0.24

S = Systems (BFT and AC); T = Treatments (0, 33, 66, and 100% replacement of fish meal by *Spirulina* biomass; S x T = Interaction between systems and treatments; PTNA = Total proteins; ALBU = Albumin; GLOBU = Globulins; g/dL – gram per deciliter; PSH = Protein thiols (µmol SH/mg protein); Systems: Means followed by different capital letters in the columns, differ from each other by Tukey's Test ($P < 0.05$).

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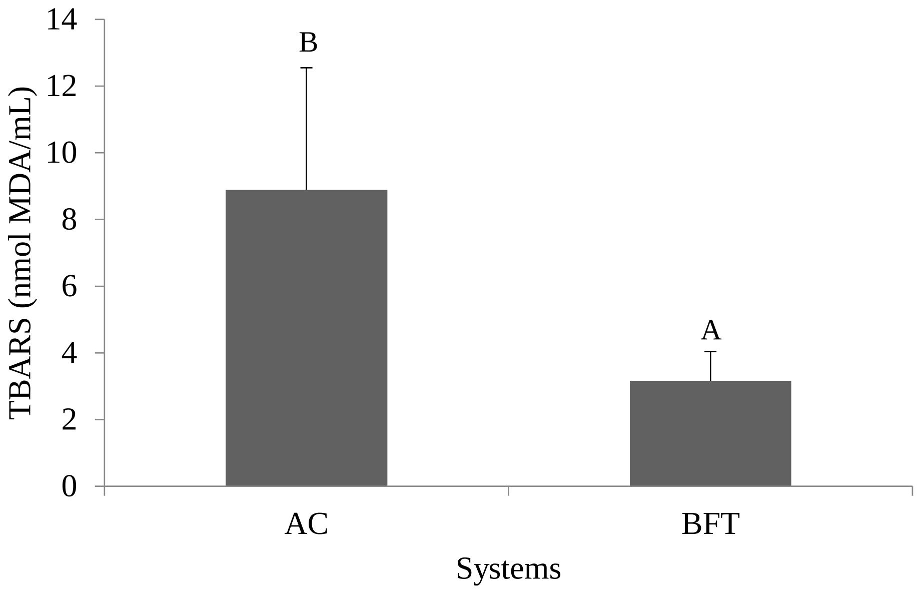

Fig. 4 Thiobarbituric acid reactive substances (TBARS) (mean ± standard deviation) of Nile tilapia juveniles reared in two culture systems (AC x BFT) with different levels of replacement of fish meal by *Spirulina* biomass after 48 days of feeding.
Means accompanied by distinct letters differ by Tukey's test ($P < 0.05$). AC = Clear water culture system; BFT = Biofloc culture system.

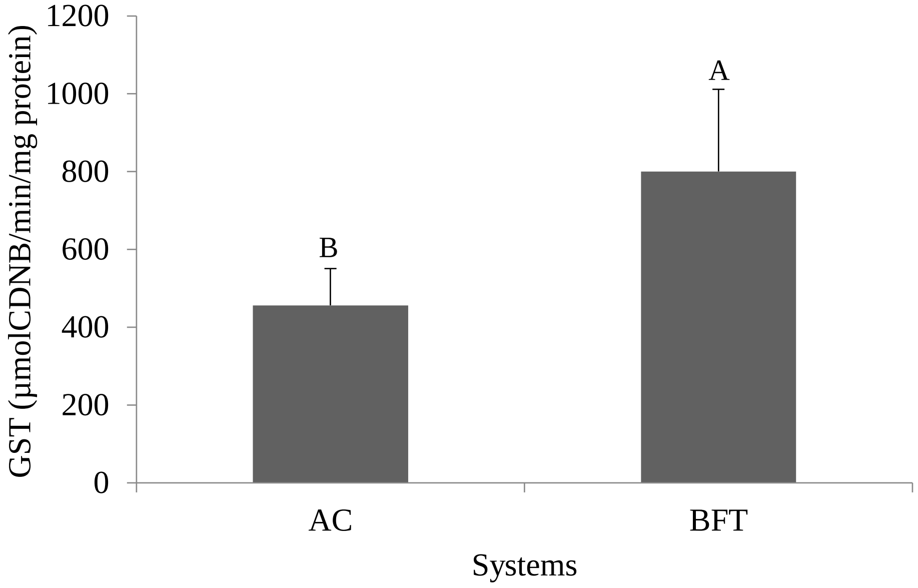

Fig. 5 Glutathione S-Transferase (GST) (mean ± standard deviation) of Nile tilapia juveniles grown in two culture systems (AC x BFT) with different levels of replacement of fish meal by *Spirulina* biomass after 48 days of feeding.
Means accompanied by distinct letters differ by Tukey's test ($P < 0.05$). AC = Clear water culture system; BFT = Biofloc culture system.

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4. Discussion

4.1 Zootechnical parameters and organosomatic indexes

In the clear water system, the supply of suboptimal levels of protein reduced fish growth. The specific growth rate was much lower (0.45% day^{-1}) than observed in other studies (5.01% day^{-1}, 1.9% day^{-1}, 1.31% day^{-1}, and 4.42% day^{-1}), in Gullian-Klanian and Arámburu-Adame 2013, Luo *et al.* 2014, Tanjung *et al.* 2019 and Akter *et al.* 2021, respectively. This finding suggests that the nutrient supply was inadequate. By contrast, for fish reared in BFT the growth was not impaired, confirming that reduced protein levels in this system did not affects the perfomance (Azim and Little 2008; Luo *et al.* 2014; Durigon *et al.* 2020). Nile tilapia is able to supplement the diet by ingesting the bioflocs rich in nutrients, which can avoid possible deficiencies problems (Tacon 2002; Hargreaves 2006; Azim and Little 2008; Crab *et al.* 2012).

The performance results suggest that it is possible to replace fish meal completely with *Spirulina* biomass in Nile tilapia juvenile diets. Previous studies have demonstrated the viability of replacing fish meal with *Spirulina* biomass for tilapia and other species (Nandeesha *et al.* 1998; 2001; El-Sheekh *et al.* 2014; Velasquez *et al.* 2015; Cao *et al.* 2018; Raji *et al.* 2020). The protein content, amino acid profile, and polyunsaturated fatty acid contents of this microalga are excellent (Albuquerque 2014; Zhang *et al.* 2020; Rutar *et al.* 2022). Its attraction-palatability properties can also explain the observed results (Jaime-Ceballos *et al.* 2005; 2007; Silva-Neto *et al.* 2011; Ali 2014). In the present study, replacing fish meal with *Spirulina* biomass did not affect consumption. Generally, diets without animal-based proteins reduces feed intake by tilapia (Fontaínhas-Fernandes *et al.* 1999; Zhou and Yue 2010; Hernández *et al.* 2013), suggesting adequate palatability of this ingredient.

The 66% level of fish meal replacement by *Spirulina* biomass (52.50% inclusion) improved feed efficiency, and there was a growth-promoting effect. Other authors observed that *Spirulina* biomass could be used as a growth promoter (Abdel-Tawwab; Ahmad 2009; Elsayed *et al.* 2012; Hernández *et al.* 2012; Jana *et al.* 2014). Supplementation with *Spirulina* biomass in the diets improved feed conversion in tilapia (Elsayed *et al.* 2012; El-Sheekh *et al.* 2014; El-Ward *et al.* 2016) and other species such as *Poecilia reticulata* (Dernekbasi *et al.* 2010) and *Puntius gelius* (Hajiahmadian *et al.* 2012). *Spirulina* is rich in various substances with bioactive properties, including fibrous carbohydrates, vitamins, minerals, carotenoid pigments, and polyunsaturated fatty acids (Promya and Chitmanat 2011; Gutiérrez-Salmeán *et al.* 2015). Further studies are needed to explain how one or more of these compounds may positively impact performance.

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4.2 Hepato-intestinal histomorphometry

The animals cultivated in the BFT system presented larger villi than those in the clear water system. The BFT system may improve gut health (Ahmad *et al.* 2017; Pilotto *et al.* 2018; Deng *et al.* 2022). The rich microbial community in bioflocs competes directly with pathogenic microorganisms in the environment and can mitigate possible health challenges (Monroy-Dosta *et al.* 2013; Ferreira *et al.* 2015). Furthermore, beneficial microorganisms can colonize the digestive tract and provide a possible probiotic effect, which may help explain the improvement in villus height (Aguilera-Rivera *et al.* 2014; Pilotto *et al.* 2018). Studies on the microbiology of the digestive tract of fish cultured in a BFT system demonstrated the multiplication of beneficial bacteria and colonization of the digestive tract necessary to confirm this result. In addition to the benefits of the BFT system, it should be mentioned that in the clear water system, there was a deficit of nutrients, which may have affected the integrity of the intestinal epithelium of the animals (Dawood 2020; Zarkasi *et al.* 2016; Zhu *et al.* 2020).

The replacement of fish meal by *Spirulina* biomass did not compromise intestinal histomorphometry. This result corroborates the performance results and reinforces the viability of its use as a substitute for fish meal in tilapia diets. Abu-Elala *et al.* (2016) observed larger intestinal villi in tilapia supplemented with *Spirulina* in the diet. In the present study, *Spirulina*'s lack of positive effects on the villi may be related to the suboptimal levels of protein used. More studies are needed to confirm these results.

4.3 Serum biochemistry

Albumin and globulin values were lower in fish cultivated in the clear water system. Albumin and globulins are plasma proteins directly linked to protein metabolism. In addition, they are essential markers of the nutritional status of animals (Sampaio *et al.* 2012). Notably, in this system, the levels of albumin and globulins were lower (0.88 ± 0.25 g dL^{-1} and 2.65 ± 0.44 g dL^{-1}, respectively) than those normally observed for tilapia (1.20 g dL^{-1} and 4.0 g dL^{-1}, respectively; Pereira *et al.* 2016). Protein deficiency in fish cultivated in a clear water system may have decreased these markers, as observed in the performance.

The protein source did not alter the animal serum levels of albumin and globulin. However, in all treatments, the values obtained for these markers differed from those commonly observed for tilapia (Pereira *et al.* 2016; Hastuti and Subandiyono 2020; Fujimoto *et al.* 2019). As previously reported, protein deficits may have altered these biomarkers. Previous studies showed the beneficial effects of *Spirulina* on biochemical parameters in tilapia (Belal *et al.*

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2012; Abdekhalek *et al.* 2017; Abdel-Daim *et al.* 2020; Mansour *et al.* 2021; Soliman *et al.* 2021). Further studies using *Spirulina* in conventional diets are needed to confirm the possible effects of the protein source on markers of protein metabolism in tilapia grown in a BFT system.

4.4 Oxidative stress

Lower values of TBARS (lower lipid peroxidation) and higher values of GST were observed in the BFT system than in the clear water system. These parameters are commonly used to assess cell damage (TBARS) and antioxidant capacity (GST) (Bacou *et al.* 2021). The BFT system avoided the negative effects of physiological oxidative stress and improved the antioxidant responses of the animals. These results might be explained by bioactive compounds (essential fatty acids, carotenoids, free amino acids and chlorophylls, among others), minerals, and vitamins (Tacon *et al.* 2002; Ju *et al.* 2008; Crab *et al.* 2012) in microbial aggregates. Collaborating with this hypothesis, in the clear water system, the lower efficiency in the antioxidant defense mechanism (GST) was insufficient to prevent the increase in lipid peroxidation (TBARS). Further evaluations of the composition of bioflocs are necessary to correlate the presence of bioactive compounds with the antioxidant response of animals.

The replacement of fish meal with *Spirulina* did not change lipid peroxidation levels or antioxidant capacity. These findings can be explained by the nutritional and functional properties of fish meal and *Spirulina* biomass (Henrikson 1994; Masumoto *et al.* 1996; Wang *et al.* 2007; Kumar and Sibi 2020). Several authors observed an increase in the antioxidant response of tilapia supplemented with *Spirulina* biomass (Abdelkhalek *et al.* 2017; Awed *et al.* 2020; Elabd *et al.* 2020; Abdel-Daim *et al.* 2020; Salah El-Din *et al.* 2021; Soliman *et al.* 2021). Similarly, fish meal is rich in polyunsaturated fatty acids with antioxidant properties (Pike 1999; Cho and Kim 2011), which may explain differences in results. More studies are needed to confirm these results.

5. Conclusion

Cultivation in the BFT system avoided the negative effects of diets with suboptimal protein levels on zootechnical performance, intestinal histomorphometry, and protein metabolism of Nile tilapia juveniles. In fish grown in this system, there was evidence of improvements in antioxidant responses and lower levels of physiological oxidative stress.

Spirulina biomass can completely replace fish meal in the diets of Nile tilapia juveniles. Replacing 66% of fish meal with *Spirulina* biomass (52.50% inclusion) improved performance regardless of the culture system (clear water or BFT).

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6. CONSIDERAÇÕES FINAIS

A busca por maior eficiência e sustentabilidade da atividade aquícola, tem tornado imprescindível a procura por ingredientes alternativos à farinha de peixe, bem como, sistemas de cultivo mais intensivos e biosseguros. Neste sentido, ambos os experimentos conduzidos nessa tese, permitiram reforçar o grande potencial de ingredientes, como o farelo de soja fermentado (FSBM) e a biomassa de *Spirulina* (*Arthrospira platensis*) em dietas aquícolas. Esses ingredientes permitiram a substituição (parcial ou total) da farinha de peixe nas dietas para juvenis de tilápia-do-Nilo. Além disso, foi possível validar os inúmeros benefícios advindos do sistema de bioflocos (BFT) em comparação ao sistema água clara. Benefícios que fundamentam seu uso no cultivo desses animais.

Importantes descobertas para o setor aquícola foram alcançadas com este estudo. No entanto, mais pesquisas são necessárias para avaliar, bem como, validar o potencial desses ingredientes e sistemas, para a tilápia-do-Nilo em outras fases de vida.

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