ED CHRISTIAN SUZUKI DE LIMA

REVISÃO SISTEMÁTICA SOBRE O USO DE β-GLUCANAS NA DIETA DE PEIXES E EFEITO DA INCLUSÃO DIETÉTICA DE β-1,3/1,6-GLUCANAS EM ACARÁ-BANDEIRA (*Pterophyllum scalare*)

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Tese apresentada ao Programa de Pós-graduação em Ciência Animal da Universidade Estadual de Londrina - UEL, como requisito parcial para a obtenção do título de Doutor.

Orientador: Prof. Dr. Nelson Mauricio Lopera Barrero

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2021

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

L732r Lima, Ed Christian Suzuki de.

Revisão sistemática sobre o uso de β-glucanas na dieta de peixes e efeito da inclusão dietética de β-1,3/1,6-glucanas em acará-bandeira (*Pterophyllum scalare*) / Ed Christian Suzuki de Lima. - Londrina, 2021.
104 f. : il.

Orientador: Nelson Mauricio Lopera Barrero.
Tese (Doutorado em Ciência Animal) - Universidade Estadual de Londrina, Centro de Ciências Agrárias, Programa de Pós-Graduação em Ciência Animal, 2021.
Inclui bibliografia.

1. Aditivo aquícola β-glucanas - Tese. 2. Peixe ornamental *Pterophyllum scalare* - Tese. 3. Desempenho produtivo de peixes - Tese. 4. Microbiota intestinalde peixes - Tese. I. Lopera Barrero, Nelson Mauricio . II. Universidade Estadual deLondrina. Centro de Ciências Agrárias. Programa de Pós-Graduação em Ciência Animal. III. Título.

CDU 639

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Biorigin

Londrina, 26 de fevereiro de 2021.

Dedico este trabalho *à* minha família, amigos e *todos aqueles que confiaram e me acompanharam durante não apenas essa etapa, mas durante toda essa longa jornada que me trouxe até aqui, pois foram quem me impulsionaram a continuar caminhando.*

.

AGRADECIMENTOS

Primeiramente agradeço à Universidade Estadual de Londrina pela possibilidade de ter cursado a Graduação, Mestrado e Doutorado, pois sem tudo isso eu não estaria aqui hoje.

Ao Programa de Pós Graduação em Ciência Animal por ter possibilitado a obtenção do título de mestre, e agora o de doutor, além de tudo que foi disponibilizado pelo programa durante todo esse longo periodo.

À CAPES e Fundação Araucária pelo auxílio financeiro permitido através da concessão da bolsa de estudos.

Ao meu orientador, professor Dr. Nelson Maurício Lopera Barrero, não apenas por todo auxilio prestado durante a orientação no mestrado e doutorado, mas pelo convivio e amizade durante todo esse periodo, além de ter me dado a oportunidade de trabalhar com peixes ornamentais, um sonho que tinha há muito tempo.

À minha mãe Alice, meu pai Edenil e meu irmão Petrie por todo apoio durante toda a caminhada até aqui.

À minha irmã Ariadne, a pessoa que mais me acompanhou e me ajudou durante toda a minha vida.

Ao meu amigo Felipe Pinheiro de Souza, que além de todo o auxílio prestado no planejamento, preparo e realização do experimento, coletas, analises, revisão sistemática e escrita dos artigos, foi um grande amigo durante todo o período do doutorado, em todos os momentos nessa trajetória.

Aos amigos Victor César Freitas Pandolfi e Andrei Lincoln Yamachita, por toda ajuda prestada no experimento e também pelos bons momentos de descontração nas horas vagas.

Às amigas Angela Maria Urrea Rojas e Natália Gonçalves Leite, por toda ajuda no experimento e pela amizade durante esses quatro anos.

À Pâmela Juliana Furlan Murari, por todo o auxílio durante o experimento e também por ter compartilhado o gosto por peixes ornamentais, que foi um impulsionador para seguir em frente com o sonho de trabalhar com peixes ornamentais.

Ao meu amigo Filipe Alexandre Nunes Amaral, pela grande ajuda em várias atividades do experimento e pelo compartilhamento de conhecimentos sobre peixes ornamentais.

Às amigas Caroline Baratela Alves e Cindy Namie Seino Leal pelo grande auxílio durante o experimento, além da amizade e bons momentos compartilhados.

À professora Dra. Angela Rocio Poveda Parra pelo grande auxílio prestado durante o decorrer do doutorado, por aceitar participar da banca de qualificação deste trabalho, e também pelas valiosas sugestões e correções.

Ao professor Dr. Ricardo Pereira Ribeiro, por aceitar participar da banca de qualificação desta tese, além das correções e sugestões que auxiliaram no enriquecimento do trabalho como um todo.

À professora Dra. Amalia Pérez Jiménez, por aceitar participar da banca de defesa, que acredito, ajudará a melhorar muito a tese.

Ao Dr. João Fernando Albers Koch da empresa Biorigin, por todo auxílio em diversos detalhes da elaboração do experimento, resolução de diversas dúvidas e por aceitar participar da banca de defesa da tese.

Ao professor Dr. Paulo Cesar Meletti por ter aceitado participar da banca de defesa da tese, mas também por ter sido a pessoa que me apresentou ao “mundo científico dos peixes” na universidade.

Ao professor Ulisses de Pádua Pereira pelo auxilio prestado durante a realização do experimento e das análises, e por possibilitar a utilização das instalações dos seus laboratórios para realização de análises.

À Raffaella Meneguetti Mainardi e Roberta Torres Chideroli pelo auxílio nas coletas do experimento e na realização das análises.

À empresa Biorigin pela doação das β-glucanas utilizadas na elaboração das dietas do experimento.

À empresa Nutricon pela doação da ração usada no experimento.

À Zootecnista Samara Mostafa, da empresa Nutricon, por toda ajuda com detalhes e adequação da ração para seu uso no experimento.

“O homem livre, no que pensa menos é na morte, e a sua sabedoria é uma meditação, não da morte, mas da vida.”
(**Baruch Espinoza**)

LIMA, Ed Christian Suzuki de. **Revisão sistemática sobre o uso de β-glucanas na dieta de peixes e efeito da inclusão dietética de β-1,3/1,6-glucanas em acará-bandeira (*Pterophyllum scalare*)**. 2021. 104 f. Tese (Doutorado em Ciência Animal) – Universidade Estadual de Londrina, Londrina, 2021.

RESUMO

O acará-bandeira (*Pterophyllum scalare*) é uma espécie de peixe ornamental de elevada beleza e facilidade de criação. Contudo, há necessidade do uso de estratégias que visem a sua maior produtividade, dentre estas, o uso de imunoestimulantes, como as β-glucanas. As β-glucanas dietéticas, além do seu potencial imunoestimulante, podem também influenciar o desempenho produtivo e a microbiota intestinal em peixes. Contudo, há poucos estudos avaliando os efeitos das β-glucanas dietéticas em peixes ornamentais. Com base nisso, a presente tese é composta de dois estudos: 1- Revisão sistemática de literatura avaliando os efeitos da suplementação dietética de β-glucanas de leveduras sobre os parâmetros de desempenho produtivo em peixes; 2- Avaliação da suplementação dietética de β-1,3/1,6-glucanas em *P. scalare*. No primeiro estudo, a revisão sistemática teve como objetivo avaliar pesquisas publicadas entre 2000 e 2020, que analisaram os efeitos das β-glucanas de leveduras dietéticas sobre o desempenho produtivo, processo digestivo e microbiota intestinal em peixes. As buscas foram feitas nas bases de dados PubMed, Scielo, Science Direct e Scopus, e também com uso de *backward snowballing*. Foram encontrados 47 estudos, com efeitos da suplementação observada em 29 deles. As concentrações de β-glucanas com efeitos variaram de 0,01% a 2,00%. As durações da suplementação com efeitos variaram de 14 a 240 dias. Foi demonstrado que os efeitos sobre a digestão, absorção e microbiota intestinal podem ter relação com os efeitos no desempenho produtivo. Apesar das evidências da eficiência das β-glucanas de leveduras na modulação dos parâmetros avaliados, alguns aspectos precisam ser melhor compreendidos. Se espera que os dados aqui compilados possam orientar futuros estudos e possibilitem a aplicação dessas informações na piscicultura comercial. No segundo estudo, foi avaliada a suplementação dietética de β-1,3/1,6-glucanas de *Saccharomyces cerevisiae* (0%, 0,05%, 0,1% e 0,2%) em juvenis de *P. scalare* alimentados por 42 dias. Foram analisados os efeitos sobre os parâmetros de desempenho, parâmetros sanguíneos e microbiota intestinal. Para o desempenho, foi observado efeito apenas para o fator de condição, que foi maior para 0,2%. Não foram observados efeitos em nenhum dos parâmetros sanguíneos avaliados. Na microbiota intestinal, houve o efeito de todas as concentrações de β-glucanas sobre a maior abundância de bactérias benéficas do gênero *Phascolarctobaterium*. Foram também obtidos maior Sobs (riqueza de espécies observada) e número de UTOs (unidades taxonômicas operacionais) para 0,2%. Esses resultados demonstram que as β-glucanas, ao modular positivamente a microbiota intestinal, possivelmente proporcionaram melhores condições nutricionais, aumentando o fator de condição. Assim, os resultados gerais obtidos podem ser úteis para o uso mais eficiente das β-glucanas de leveduras na dieta de peixes.

Palavras-chave: crescimento; imunoestimulante; peixes ornamentais; prebióticos; revisão sistemática.

LIMA, Ed Christian Suzuki de. **Systematic review on the use of β-glucans in the fish diet and effect of dietary inclusion of β-1,3/1,6-glucans in angelfish (*Pterophyllum scalare*).** 2021. 104 p. Thesis (Doctorate in Animal Science) – Universidade Estadual de Londrina, Londrina, 2021.

ABSTRACT

The angelfish (*Pterophyllum scalare*) is an ornamental fish species of high beauty and ease of rearing. However, there is a need to use strategies that aimed at its greater productivity, among them, the use of immunostimulants, such as β-glucans. Dietary β-glucans, in addition to their immunostimulant potential, can also influence productive performance and intestinal microbiota in fish. However, there are few studies evaluating the effects of dietary β-glucans on ornamental fish. Based on this, the present thesis consists of two studies: 1- Systematic literature review evaluating the effects of dietary supplementation of yeast β-glucans on the productive performance parameters in fish; 2- Evaluation of the dietary supplementation of β-1,3/1,6-glucans in *P. scalare*. In the first study, the systematic review aimed to evaluate research published between 2000 and 2020, which analyzed the effects of dietary yeast β-glucans on the productive performance, digestive process and intestinal microbiota in fish. The searches were made in the PubMed, Scielo, Science Direct and Scopus databases, and also with the use backward snowballing. Forty-seven studies were found, with the effects of supplementation observed in 29 of them. The concentrations of β-glucans with effects ranged from 0.01% to 2.00%. The durations of supplementation with effects ranged from 14 to 240 days. It has been shown that the effects on digestion, absorption and intestinal microbiota may be related to the effects on productive performance. Despite the evidence of the efficiency of yeast β-glucans in modulating the evaluated parameters, some aspects need to be better understood. It is expected that the data compiled here can guide future studies and enable the application of these information in commercial fish farming. In the second study, dietary supplementation of β-1,3/1,6-glucans from *Saccharomyces cerevisiae* (0%, 0.05%, 0.1% and 0.2%) in *P. scalare* juveniles fed for 42 days was evaluated. The effects on performance parameters, blood parameters and intestinal microbiota were analyzed. For performance, the effect was observed only for the condition factor, which was greater for 0.2%. No effects were observed on any of the blood parameters evaluated. In the intestinal microbiota, there was the effect of all concentrations of β-glucans on the greater abundance of beneficial bacteria of the genus *Phascolarctobacterium*. Were also obtained greater Sobs (observed species richness) and number of OTUs (operational taxonomic units) for 0.2%. These results demonstrate that β-glucans, when positively modulating the intestinal microbiota, possibly provided better nutritional conditions, increasing the condition factor. Thus, the general results obtained may be useful for the more efficient use of yeast β-glucans in the fish diets.

Key-words: growth; immunostimulant; ornamental fish; prebiotics; systematic review.

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LISTA DE ABREVIATURAS E SIGLAS

BL	Body length
BW	Body weight
CF	Condition factor
CR3	Complement receptor 3
FCR	Feed conversion ratio
FER	Feed efficiency ratio
FI	Feed intake
FW	Final weight
IW	Initial weight
MCH	Mean corpuscular hemoglobin
OTUs	Operational taxonomic units
PER	Protein efficiency ratio
PMAPs	Padrões moleculares associados a patógenos
RBC	Red blood cells
RRPs	Receptores de reconhecimento de padrões
SGR	Specific growth rate
SL	Standard length
Sobs	Observed species richness
SR	Survival rate
TL	Total length
TLRs	Toll-like receptors
UTOs	Unidades taxonômicas operacionais
WG	Weight gain

SUMÁRIO

1 INTRODUÇÃO

A criação de peixes ornamentais é uma atividade amplamente distribuída pelo mundo, (DEY, 2016), envolvendo os setores do comércio internacional, pesca e aquicultura (GHOSH, SINHA, SAHU, 2008). O cultivo em cativeiro destes peixes é uma atividade antiga que se confunde com o início da própria aquicultura (RIBEIRO *et al.*, 2009). No Brasil, foi iniciada na década de 1920, mas ainda não teve seu potencial produtivo totalmente explorado (FARIA *et al*., 2016).

Neste contexto o acará-bandeira (*Pterophyllum scalare*) é uma das espécies ornamentais nativas da América do sul de grande beleza e demanda no mercado (RIBEIRO; RODRIGUES; FERNANDES, 2007; RIBEIRO; PRETO; FERNANDES, 2008), apresentando características físicas e comportamentais peculiares (NAGATA *et al*., 2010; PEREIRA *et al*., 2016), diversas linhagens (RIBEIRO; RODRIGUES; FERNANDES, 2007) e facilidade de criação (KOCA *et al*., 2009; RIBEIRO; PRETO; FERNANDES, 2008; RODRIGUES; FERNANDES, 2006; TAKAHASHI *et al*., 2010). No entanto, como ocorre com outras espécies de peixes ornamentais, alguns fatores podem comprometer seu desenvolvimento e qualidade de vida.

Durante o cultivo e/ou a manutenção em aquários domésticos, os peixes ornamentais estão dispostos a diversas fontes de estresse, como baixa qualidade da água, iluminação inadequada, manipulações, elevadas densidade de cultivo, ambiente social inadequado, má nutrição, doenças, entre outros (STEVENS *et al.*, 2017). Diferente do que acontece com espécies utilizadas para o consumo, a exposição ao estresse não é finalizada ao término do período produtivo, pois são expostos ao estresse também durante todo processo de comercialização e no destino final em aquários domésticos (DAGAR *et al*., 2010). Certas condições estressantes podem levar a piora de desempenho, aumento da incidência de doenças e morte (STEVENS *et al.*, 2017), visto que podem comprometer a resposta imunológica e levar ao aumento da suscetibilidade a patógenos (FAST *et al*., 2008; RAMSAY *et al*., 2009; ESLAMLOO *et al*., 2014). Dentre os fatores relacionados à produção, a nutrição pode ser um dos principais que podem ser manejados para aumentar a resistência aos fatores estressantes (AZEVEDO *et al.*, 2016a; DAGAR *et al.*, 2010; GHOSH; SINHA, SAHU, 2008; LIM *et al*., 2002), sendo os imunoestimulantes dietéticos utilizados de forma eficaz com esta finalidade, dentre os quais há destaque para as β-glucanas.

As β-glucanas são polissacarídeos de glicose unidas por ligações β-glicosídicas, encontrados em plantas, leveduras, bactérias, cogumelos e algas marinhas (MEENA et al., 2013). Um dos seus diferenciais é o seu reconhecimento por receptores específicos presentes nas células do sistema imune, que, ao serem estimulados, proporcionam a melhoria das funções imunológicas (MEENA *et al*., 2013; RODRIGUES *et al*., 2020). Entre as várias formas de administração, a via oral através da dieta é uma das mais práticas e efetivas (PETIT; WIEGERTJES, 2016), sendo por isso amplamente utilizada. Dentre as diversas fontes, as β-glucanas das leveduras *Saccharomyces cerevisiae* estão entre as melhor estudadas e mais utilizadas (MEENA *et al*., 2013; PETIT; WIEGERTJES, 2016).

Diversos estudos com peixes de produção têm demonstrado a influência positiva da suplementação dietética de β-glucanas de leveduras sobre a resposta imunológica, resistência a doenças e ao estresse (ABREU *al*., 2014; EL-BOSHY *et al*., 2010; JUNG-SCHROERS *et al*., 2018; TALPUR *et al*., 2014). Os poucos estudos realizados com peixes ornamentais têm demonstrado esses mesmos efeitos (ABREU *et al*., 2014; LIN *et al*., 2011; RUSSO; YANONG; MITCHELL, 2006). Além disso, para os peixes no geral, há também evidências dos efeitos positivos dessa suplementação sobre o desempenho produtivo (LIN *et al*., 2011; MUNIR *et al*., 2016; TALPUR *et al*., 2014), o que demonstra ainda mais seu potencial para uso na piscicultura comercial. Também já foram observados os efeitos da suplementação dietética sobre a atividade de enzimas digestivas (GÚZMAN-VILLANUEVA *et al*., 2014), digestibilidade (MUNIR *et al*., 2016), morfologia da superfície absortiva intestinal (LIRANÇO *et al*., 2013) e modulação de bactérias intestinais (DO-HUU; SANG; THUY, 2016; JAMI *et al*., 2019), juntamente com a melhoria do desempenho.

Tais evidências demonstram a necessidade da realização de uma revisão sistemática de literatura para compilar informações a respeito dos efeitos da suplementação dietética de β-glucanas de leveduras sobre o desempenho produtivo em peixes, uma vez que não foram encontradas revisões de literatura com esse foco. Também é relevante verificar se esses efeitos sobre o desempenho produtivo podem estar relacionados aos efeitos sobre os parâmetros intestinais (digestibilidade, morfologia da superfície absortiva e microbiota). Além disso, é de extrema importância a realização da avaliação da suplementação dietética de β-glucanas de *S. cerevisiae* em espécies de peixes ornamentais de importância, como o *P. scalare*, que possam proporcionar a otimização da criação comercial dessa espécie.

2 REVISÃO DE LITERATURA

2.1 A AQUICULTURA ORNAMENTAL NO BRASIL

A criação dos peixes ornamentais é uma atividade presente em mais de 125 países, que envolve mais de 2500 espécies, das quais 60% são peixes de água doce, com o mercado sendo dominado por poucas espécies como os vivíparos, neon (*Paracheirodon innesi*), acará-bandeira (*Pterophyllum scalare*), kinguio (*Carassius auratus*), zebrafish (*Danio rerio*) e acará-disco (*Symphysodon* spp.) (DEY, 2016). A indústria envolvida neste setor é um componente global do comércio internacional, da pesca e do desenvolvimento da aquicultura, constituindo um dos setores mais lucrativos da piscicultura (GHOSH; SINHA; SAHU, 2008).

A produção de peixes ornamentais em cativeiro é uma atividade antiga, tendo a piscicultura possivelmente se originado da vontade do homem de manter peixes coloridos para ornamentação, visto que os kinguios e carpas koi (*Cyprinus carpio koi*) foram os primeiros peixes produzidos pela aquicultura (RIBEIRO *et al*., 2009). Um dos aspectos positivos do cultivo é a diminuição do impacto da pesca sobre as espécies ameaçadas de extinção (ZUANON; SALARO; FURUYA, 2011). Contudo, os principais motivos do surgimento da produção em cativeiro foram a necessidade de se manter espécies de clima tropical em países de clima temperado e o desenvolvimento de variedades com maior apelo visual (RIBEIRO *et al.*, 2009).

A aquicultura ornamental no Brasil teve início em 1920, sendo impulsionada apenas na década de 1970. Na atualidade o potencial das espécies de peixes cultivadas nos estados de Minas Gerais, Rio de Janeiro, São Paulo, Pernambuco e Paraná ainda não foi totalmente explorado (FARIA *et al.*, 2016). Contudo, para otimização do cultivo seria necessário o estabelecimento de índices zootécnicos, desenvolvimento de dietas específicas para as diversas espécies, formação de mão de obra qualificada, estabelecimento de sistemas de apoio aos produtores e desenvolvimento de sistemas de cultivo que minimizem a poluição ambiental, dentre outros aspectos relacionados à produção (ZUANON; SALARO; FURUYA, 2011).

Além disso, são inexistentes dados produtivos precisos referentes a

aquicultura ornamental, devido à falta de organização da cadeia produtiva (FARIA *et al*., 2016). Uma consequência disso é a dificuldade de quantificar quais as espécies envolvidas no mercado interno de peixes ornamentais, devido à carência de dados do comércio varejista (ASSIS; CAVALCANTE; BRITO, 2014). Nesse aspecto, as espécies de peixes ornamentais encontradas com maior facilidade nas lojas brasileiras são aquelas que apresentam manejo mais simples, como: Guppy (*Poecilia reticulata*), Platy (*Xiphophorus maculatus*), Espada (*Xiphophorus hellerii*), Molinésia (*Poecilia sphenops*), Betta (*Betta splendens*), Colisa (*Colisa lalia*), Tricogaster (*Trichogaster trichopterus*), Kinguio (*C. auratus*), Carpa (*C. carpio*), Barbos (*Puntius sp.*), Paulistinha ou Zebrafish (*D. rerio*), Oscar (*Astronotus ocelaltus*) e Acará bandeira (*P. scalare*) (FARIA *et al*., 2016).

2.2 O ACARÁ-BANDEIRA (*Pterophyllum scalare*)

O acará-bandeira (*P. scalare*) é uma das espécies ornamentais de água doce nativas da América do Sul de grande beleza e elevada demanda no mercado (RIBEIRO; RODRIGUES; FERNANDES, 2007; RIBEIRO; PRETO; FERNANDES, 2008), representando uma das mais importantes espécies de ciclídeos ornamentais (KASIRI; FARAHI; SUDAGAR, 2011). Esta espécie possui origem na Bacia Amazônica, estando amplamente distribuída, ocorrendo no Peru, Colômbia, Guianas e Brasil (PEREIRA *et al.*, 2016). Possui o corpo comprimido lateralmente, de forma triangular, que é gerada pelas nadadeiras dorsal e anal que são fortes e alongadas; apresenta cor prateada contrastando com listras verticais pretas, nadadeiras ventrais finas e modificadas, e atinge comprimento corporal de até 15 cm (NAGATA *et al*., 2010).

No seu habitat natural, geralmente é encontrado junto a madeiras e vegetação submersa, que servem de abrigo contra predadores, preferindo localidades de águas com baixa dureza e levemente ácida (RIBEIRO; RODRIGUES; FERNANDES, 2007). É uma espécie pacífica, nadando em cardumes principalmente quando jovem; no entanto, na fase adulta alguns indivíduos são subjugados por outros mais desenvolvidos, devido ao comportamento hierárquico (PEREIRA *et al*., 2016). De uma forma geral, são calmos e territorialistas (RIBEIRO; RODRIGUES; FERNANDES, 2007), podendo ser criados em aquários como espécie única ou com outras espécies de peixe (KASIRI; FARAHI; SUDAGAR, 2011). Quanto ao processo

reprodutivo, a desova é realizada em um substrato previamente limpo por ambos os pais, e assim como para outros peixes da família Cichlidae, ocorre o cuidado parental, sendo observado na reprodução em cativeiro que os machos apresentam proteção mais intensa dos ovos, e as fêmeas apresentam maior envolvimento com a proteção das larvas (CACHO; YAMAMOTO; CHELLAPPA, 1999).

O acará-bandeira pode ser criado em diversos sistemas de criação (RIBEIRO; PRETO; FERNANDES, 2008) e aceita diversos tipos de alimentos (KOCA *et al*., 2009; RODRIGUES; FERNANDES, 2006; TAKAHASHI *et al*., 2010), fatores que facilitam seu cultivo. Além disso, estão disponíveis diversas linhagens de acará-bandeira, podendo ser citadas: marmorato, ouro, siamês, Koi, leopardo, negro, fumaça e palhaço (RIBEIRO; RODRIGUES; FERNANDES, 2007). Esses dentre outros aspectos garantem a grande popularidade e disponibilidade desta espécie.

Em pesquisas avaliando a cadeia de peixes ornamentais no Brasil, há a confirmação da popularidade desta espécie. Em Aracaju- SE, em pesquisa realizada entre 2009 e 2010, *P. scalare* foi a quinta espécie mais comercializada nas lojas avaliadas, sendo registrados 3029 indivíduos de um total de 54.981 indivíduos pertencentes a 143 diferentes espécies (ASSIS; CAVALCANTE; BRITO, 2014). Cardoso *et al*. (2012), ao avaliarem a cadeia produtiva na Zona da Mata Mineira – MG, maior polo produtor de peixes ornamentais do Brasil, constataram que em 2009 a produção de *P. scalare* foi de 168.400 indivíduos, sendo a 11ª espécie mais produzida. Coe, Freitas e Araújo (2011), em pesquisa realizada em Fortaleza -CE e região metropolitana, constataram que *P. scalare* estava entre as quatro espécies de peixes ornamentais mais comercializadas nas lojas avaliadas, que representavam juntas mais de 50% do volume total comercializado. Apesar de serem dados regionais é possível observar a importância desta espécie para a indústria nacional de peixes ornamentais.

Devido à grande importância dessa espécie, há a necessidade do entendimento de aspectos inerentes a sua criação em cativeiro, sendo que alguns estudos têm sido realizados de forma a gerar informações para a otimização dos manejos produtivos. Entre esses, Nagata *et al*. (2010) observaram para peixes (peso inicial médio de 0,80 g) criados em aquários com aeração constante e troca de água diária, que os parâmetros de desempenho não diferiram entre as densidades de 0,33, 0,67 e 1,00 peixe L^{-1}, mas as maiores densidades resultaram no aumento da amônia total. Para pós-larvas (peso inicial médio de 0,0012 g) criadas em recipientes plásticos

de 1 L sem aeração em diferentes densidades (5, 10, 15, 20 e 25 pós-larvas L^{-1}), Gonçalves Júnior *et al*. (2013) encontraram maior crescimento para a densidade de 5 pós-larvas L^{-1}, apesar do pior aproveitamento do espaço ao se comparar com a produtividade obtida para a criação de 15 pós-larvas L^{-1}. Adicionalmente, Ribeiro, Preto e Fernandes (2008) observaram que o desempenho produtivo de acarás-bandeira (peso inicial médio de 480,58 mg) criados em sistema semi-intensivo em hapas alocadas em viveiros escavados (1,6 peixes 10 L^{-1}), em monocultivo ou policulitvo com o camarão-da-Amazônia (*Macrobrachium amazonicum*), foi superior ao de peixes criados em sistema intensivo em aquários com filtragem e aeração (3,2 peixes 10 L^{-1}). Ao avaliarem a criação de *P. scalare* (peso médio inicial de 0,002 g) em gaiolas confeccionadas em malha inseridas em caixas d´água sob diferentes densidades, Deon *et al*. (2017) observaram melhor conversão alimentar, uniformidade de peso e comprimento e sobrevivência para o cultivo de 2,33 peixe L^{-1} em relação a 4,66 e 9,33 peixes L^{-1}.

Além disso, especificamente para a alimentação e nutrição, pesquisas realizadas têm gerado importantes informações no que se refere à melhoria dos manejos com foco no desempenho produtivo. Nesse aspecto, foi observada por Rodrigues e Fernandes (2006) a melhoria dos parâmetros de crescimento e consumo de alimento de acarás-bandeira em duas classes de peso inicial (0,30 g a 0,50 g; 0,51 g a 0,80 g), alimentados com dietas extrusadas e peletizadas, em relação aos alimentados com dieta farelada, contudo, a conversão alimentar foi melhor para a dieta extrusada em relação as demais. Ribeiro *et al*. (2012) avaliaram o efeito de diferentes níveis (30, 60 e 90 g de ração/kg de peso vivo ao dia) e frequências alimentares (1 e 2 vezes por dia) em peixes em duas classes de peso inicial (0,7 a 1,2 g; 1,3 a 1,7 g), e constataram que o fornecimento de 60 g de ração por kg de peso vivo em duas alimentações diárias proporcionou o melhor desempenho produtivo. Para a fase de larvicultura, foi observado por Pereira *et al*. (2016) que a alimentação conjunta (fornecimento de meta-náuplios de *Artemia* spp. juntamente com a ração) por 3, 4 e 5 dias antes do fornecimento exclusivo de ração proporcionou o aumento da sobrevivência em relação a 1 e 2 dias; e o fornecimento de meta-náuplios de *Artemia* spp. por 20 e 25 dias antes do início da alimentação conjunta proporcionou o melhor crescimento em relação a 5, 10 e 15 dias.

Quanto às exigências nutricionais, em especial de proteínas, alguns estudos têm gerado informações a respeito dos níveis adequados. Ribeiro, Rodrigues

e Fernandes (2007), ao avaliarem diferentes níveis de proteína bruta (26, 28, 30, 32%) na dieta de peixes em duas faixas de peso inicial médio (150 a 150 mg; 151 a 200 mg), verificaram a melhoria dos parâmetros de desempenho para aqueles que receberam dieta contendo 32% de proteína bruta, e com os valores obtidos pela análise de regressão polinomial os autores sugeriram que níveis superiores a 32% de proteína bruta poderiam melhorar o desempenho produtivo. Adicionalmente, De Franca *et al*. (2017) ao avaliarem dietas com diferentes níveis de proteína digestível (30, 32, 34, 36 e 38%) para *P. scalare* com peso inicial médio de 0,39 g, observaram melhores valores dos parâmetros de desempenho para os peixes alimentados com dietas contendo 34, 36 e 38%, que não diferiram entre si. Para peixes com peso inicial médio de 2,33 g, Zuanon *et al.* (2009) ao testarem diferentes níveis de proteína bruta (26, 30 e 34%) em conjunto com diferentes níveis de energia digestível (3100 e 3300 kcal/kg), não encontraram diferenças para peso final, comprimento final, ganho em peso, consumo de ração, conversão alimentar, taxa de crescimento específico e fator de condição, mas para a taxa de eficiência proteica os valores foram superiores para 26% em relação a 34% de proteína bruta, sem diferenças para os níveis de energia, indicando que a ração com 26% de proteína bruta e 3100 kcal/kg seria suficiente para promover um crescimento adequado.

Outro ponto importante em relação ao manejo nutricional de *P. scalare* é a suplementação de alguns aditivos na dieta, que como demonstrado em alguns estudos, podem possibilitar além da melhoria do desempenho, efeitos positivos em outros parâmetros de importância na criação de peixes ornamentais. Confirmando isso, Sousa *et al*. (2020) observaram em larvas (peso inicial médio de 1,10 mg) que o fornecimento de náuplios de artêmia enriquecidos com um probiótico comercial resultou no aumento do crescimento, aumento da sobrevivência, redução de bactérias heterotróficas totais e aumento de bactérias benéficas (bactérias ácido láticas) no trato intestinal. Para peixes com peso inicial médio de 6,5 a 7,7 g, apesar de não encontrarem efeitos sobre o crescimento, Kouba *et al*. (2013) obtiveram o aumento da intensidade da coloração com a suplementação dietética da microalga *Haematococcus pluvialis* (fonte de carotenoides). Kasiri, Farahi e Sudagar (2011) constataram que a administração dietética do extrato da planta medicinal *Echinacea purpurea* para acarás-bandeira com peso inicial médio variando entre 3,79 g a 3,80 g, proporcionou o maior crescimento dos peixes, e após a reprodução, ocasionou na maior sobrevivência das larvas oriundas de reprodutores que receberam a dieta

suplementada.

Mesmo com esses resultados demonstrando os efeitos positivos e relevantes dos manejos alimentares e nutricionais, esses dados ainda são escassos, não apenas para o *P. scalare*, mas para os peixes ornamentais de uma forma geral.

2.3 A INFLUÊNCIA DE COMPONENTES DA DIETA SOBRE A RESISTÊNCIA A DOENÇAS E ESTRESSE EM ESPÉCIES DE PEIXES ORNAMENTAIS

Os peixes ornamentais estão expostos a diversos fatores estressantes durante a sua criação em pisciculturas e a sua manutenção em aquários. A baixa qualidade da água, fotoperíodo inadequado, tamanho do tanque/aquário, procedimentos que envolvem a manipulação, elevada densidade de estocagem, ambiente social inadequado, predisposições genéticas e nutrição, estão entre os fatores que podem ocasionar estresse aos peixes ornamentais durante o cultivo e manutenção em aquários (STEVENS *et al*., 2017). Na aquicultura ornamental, diferentemente do cultivo de peixes utilizados na alimentação humana, o estresse não termina com o fim do período produtivo, visto que os peixes são expostos a diversas mudanças até que cheguem ao seu destino, geralmente em aquários domésticos (DAGAR *et al*., 2010). Apesar do estresse ser uma resposta geralmente adaptativa, quando crônico, severo ou repetitivo pode prejudicar a saúde e o bem-estar, desencadeando em deficiência no crescimento, surgimento de doenças e morte (STEVENS *et al*., 2017). Neste contexto, há evidências da influência de determinadas condições estressantes sobre a piora da resposta imunológica (FAST *et al*., 2008; ESLAMLOO *et al*., 2014), e sua influência sobre a maior suscetibilidade a patógenos (FAST *et al*., 2008; RAMSAY *et al*., 2009) em algumas espécies de peixes.

O desenvolvimento de medidas efetivas para aumentar a resistência ao estresse e doenças são de extrema importância, sendo que dietas que proporcionem uma melhor adaptação e maior sobrevivência constituem ferramentas valiosas (DAGAR *et al.*, 2010). Concordando com este fato, estudos têm demonstrado os efeitos positivos de alguns aditivos dietéticos sobre a otimização da resposta imunológica, resistência a doenças e condições estressantes, bem como maior sobrevivência. Neste âmbito, Lim *et al*. (2002) avaliando a suplementação de vitamina C na dieta de Guppy (*P. reticulata*) submetidos a estresse osmótico, observaram maior resistência nos grupos que receberam suplementação em relação ao controle, com

menor mortalidade. Anusha *et al*. (2014) constataram que a administração do extrato da erva *Ixora coccínea* na dieta de kinguio (*C. auratus*) desafiados com *Aeromonas hydrophila*, uma bactéria conhecidamente prejudicial à saúde de peixes, proporcionou o aumento da concentração de proteínas, albumina, globulinas, hemoglobina, número de eritrócitos, número de macrófagos, atividade fagocítica, atividade bactericida, atividade da lisozima e sobrevivência após desafio. Avaliando o fornecimento do extrato aquoso das folhas de *Avicennia marina* na dieta de peixe-palhaço (*Amphiprion sebae*) infectados com *Vibrio alginolyticus*, Dhayanithi *et al*. (2015) obtiveram a otimização dos parâmetros imunológicos e aumento da taxa de sobrevivência. Azevedo *et al.* (2016b) observaram que a suplementação de mananoligossacarídeo (MOS) na dieta proporcionou o aumento da atividade da lisozima em larvas de *B. splendens*, demonstrando sua possível ação imunoestimulante. Para acarás-bandeira, Azimirad *et al.* (2016) observaram o efeito do enriquecimento de alimento vivo (*Artemia fransciscana*) com prebiótico (frutooligossacarídeos), probiótico (*Pediococcus acidilacti*) e simbiótico (*Pediococcus acidilacti* e frutooligossacarídeos) sobre o aumento do crescimento, resposta imunológica, resistência ao estresse (baixa temperatura (exceto para o prebiótico) e alta salinidade), bem como sobre o aumento da população de bactérias intestinais benéficas (bactérias ácido láticas). Contudo, apesar da importância destas informações, elas ainda são escassas, tornando necessária a realização de estudos adicionais sobre a incorporação destas substâncias na dieta de espécies de peixes ornamentais com importância econômica.

Neste contexto, a suplementação de imunoestimulantes na dieta é uma forma eficaz de proteger os peixes de doenças ao melhorar seus mecanismos de defesa, gerando aumento produtivo (PRABU *et al*., 2016). Diversos polissacarídeos de várias fontes possuem a capacidade de estimular o sistema imune, atuando dessa forma como imunoestimulantes, dos quais se destacam as β-glucanas (RINGØ *et al.*, 2012).

2.4 β-GLUCANAS NA ALIMENTAÇÃO DE PEIXES

As β-glucanas são polissacarídeos naturais compostos de glicose unidas por ligações β-glicosídicas, fazendo parte da parede celular de diversas plantas, leveduras, bactérias, cogumelos e algas marinhas, apresentando diferenças estruturais entre as diversas fontes, diferenças que determinam a sua atividade

biológica (MEENA *et al*., 2013; SOLTANIAN *et al*., 2009). As subunidades de glicose são principalmente ligadas por ligações glicosídicas (1,3)-β, (1,4)-β ou (1,6)-β, com a maioria das β-glucanas apresentando uma estrutura base uniforme de diversos comprimentos, com cadeias laterais de D-glicose unidas por ligações (1,4)-β ou (1,6)-β (STIER; EBBESKOTTE; GRUENWALD, 2014). As β-glucanas de cevada e aveia são lineares apresentando ligações β-(1,4) e (1,3), já as de cogumelos apresentam ramificações β-(1,6) curtas ligadas à estrutura principal β-(1,3) (MEENA *et al*., 2016). As leveduras *Saccharomyces cerevisiae* apresentam as β-1,3/1,6-glucanas (que possuem ramificações β-1,6 com regiões adicionais β-1,3), que são as β-glucanas melhor estudadas e mais utilizadas (MEENA *et al*., 2013; PETIT; WIEGERTJES, 2016). Dentre as principais funções das β-glucanas estão um importante papel na ativação tanto das atividades da imunidade inata quanto da adquirida, proporcionando o aumento da resistência a doenças (MEENA *et al*., 2013; VETVICKA; VANNUCCI; SIMA, 2013). Os estudos iniciais com esses polissacarídeos ocorreram nas décadas de 1960 e 1970, com investigações sobre os efeitos biológicos das β-glucanas extraídas de *S. cerevisiae* e do cogumelo shitake (*Lentinula edodes*) (NOVAK; VETVICKA, 2009).

Uma das vantagens das β-glucanas em relação a outros imunoestimulantes, é a existência de receptores específicos que possibilitam o seu reconhecimento pelo sistema imunológico. Como são estruturas presentes em microrganismos, são classificadas como padrões moleculares associados a patógenos (PMAPs), que podem ser reconhecidos por meio de receptores de reconhecimento de padrões (RRPs) nas células do sistema imune (MEENA *et al*., 2013; SAMUELSEN; SCHREZENMEIR; KNUTSEN, 2014). Assim, todos os vertebrados, desde peixes à humanos, possuem esses receptores de reconhecimento de β-glucanas (entre eles: *Complement receptor* 3 (CR3), *Dectin*-1 e Toll-like receptors (TLRs)) presentes em macrófagos, neutrófilos, monócitos e células *natural killer*, que quando estimulados pela presença de β-glucanas, modulam a imunidade inata, melhorando as funções de fagocitose, processamento de antígenos e liberação de citocinas (que estimulam a formação de novos leucócitos) (MEENA *et al*., 2013; RODRIGUES *et al*., 2020).

Apesar de estudos com peixes terem demonstrado a eficiência da administração via inclusão na água (SOUZA *et al*., 2020; UDAYANGANI *et al*., 2017; ZHANG *et al*., 2009) e via injetável (KIM; KE; ZHANG, 2009; RODRIGUEZ *et al*., 2009;

SELVARAJ; SAMPATH; SEKAR, 2005), o fornecimento via dieta é uma das formas de administração mais práticas e efetivas (PETIT; WIEGERTJES, 2016), podendo ser fornecidas facilmente para um grande número de peixes. Neste contexto, estudos com diversas espécies de peixes têm demonstrado tanto em animais criados em condições normais quanto naqueles submetidos a desafios, a influência das β-glucanas dietéticas sobre a melhoria das defesas do organismo. Entre estes, foi obtida por Talpur *et al*. (2014) a otimização dos parâmetros hematológicos e imunológicos (após 56 e 84 dias de alimentação, antes e após desafio com *A. hydrophila*) em *snakehead* (*Channa striata)* para a suplementação de 0,1% de β-glucanas de *S. cerevisiae*. Marel *et al*. (2012) observaram que a administração de 0,1% de β-glucanas de *S. cerevisiae* na dieta de carpa-comum (*Cyprinus carpio*) por 14 dias proporcionou o aumento da expressão de 2 genes de β-defensinas (β-defensina 1 e 2) e um gene de mucina (Muc5B), sendo estes relacionados com a resposta imunológica. Foi constatada por El-Boshy *et al*. (2010) em tilápias-do-Nilo (*Oreochromis niloticus*) submetidas a dose sub letal de cloreto de mercúrio e posteriormente desafiadas com *A. hydrophila*, melhoria da resposta imunológica e diminuição da mortalidade naquelas que receberam dieta suplementada com 0,1% de β-glucanas de *S. cerevisiae* por 21 dias.

Além disso, estudos avaliando a suplementação de β-glucanas na dieta de peixes ornamentais têm demonstrado efeitos positivos sobre a imunidade e resistência a patógenos e ao estresse. Para carpas Koi (*C. carpio koi*) alimentadas com dieta contendo 0,5% de β-glucanas de *S. cerevisiae* por 56 dias, Lin *et al*. (2011) observaram efeito da inclusão sobre a otimização de diversos parâmetros imunológicos e diminuição da mortalidade em peixes desafiados com *Aeromonas veronii*. Para Labeo bicolor (*Epalzeorhynchos bicolor*) desafiados com *Streptococcus iniae*, Russo, Yanong e Mitchell (2006) observaram que a administração dietética de 0,1% de um produto comercial à base de β-glucanas de *S. cerevisiae* por 24 dias proporcionou a diminuição da mortalidade. Adicionalmente, Abreu *et al*. (2014) constataram que a suplementação dietética de β-glucanas de *S. cerevisiae* por 14 dias para o peixe-lápis (*Nannostomus trifasciatus*) proveniente de captura na natureza, acarretou em manutenção do equilíbrio de sódio (para suplementação de 0,1% e 0,5%) e potássio (para suplementação de 0,01%, 0,1% e 0,5%) durante o transporte, agindo de forma positiva sobre as alterações iônicas que ocorreram durante o procedimento. No entanto, apesar do grande número de estudos existentes quanto à ação das β-glucanas sobre a resposta imunológica e efeitos relacionados em peixes,

ainda não há total entendimento de como esses processos ocorrem (RODRIGUES *et al*., 2020), o que demonstra a necessidade da realização de estudos adicionais para melhorar a compreensão acerca desses efeitos.

Alguns estudos também têm constatado outros efeitos resultantes da suplementação dietética de β-glucanas em peixes, que incluem melhoria do crescimento, aproveitamento da dieta, composição corporal, aspectos relacionados ao processo digestivo e microbiota intestinal. Em *Pacific red snapper* (*Lutjanus peru*), Gúzman-Villanueva *et al*. (2014), observaram efeito da suplementação de β-glucanas de *S. cerevisiae* sobre o aumento da atividade antioxidante (atividade da superóxido dismutase (para inclusão de 0,1% aos 28 e 42 dias)) e da atividade de enzimas digestivas (tripsina (para inclusão de 0,1% aos 14 dias), quimotripsina (para inclusão de 0,2% aos 28 dias) e aminopeptidase (para inclusão de 0,1% e 0,2% aos 28 dias)). Ao realizar enriquecimento de rotíferos com β-glucanas de *S. cerevisiae* (0,5 g L^{-1}) fornecidas para larvas de turbot (*Scophthalmus maximus*) dos 3 aos 24 dias pós-eclosão, Miest *et al*. (2016) observaram redução da mortalidade e aumento da expressão de genes relacionados à resposta imune, desenvolvimento e digestão. Para pompano (*Trachinotus ovatus*), Do-Huu, Lam e Nguyen (2018) constataram que a administração de 0,05% e 0,1% de β-glucanas de *S. cerevisiae* por 56 dias proporcionou o aumento do crescimento e aproveitamento da dieta, além de levar ao aumento das proteínas e diminuição dos lipídeos corporais. Ao avaliarem os efeitos da suplementação de 0,2% de β-glucanas de *S. cerevisiae* fornecida por 112 dias, Munir *et al*. (2016) observaram em *snakehead* melhoria do crescimento, índices corporais, aproveitamento da dieta, digestibilidade, atividade de enzimas digestivas e menor acúmulo de gordura intraperitoneal. Além disso, Carda-Dieguez, Mira e Fouz (2014) ao avaliarem a administração de 0,1% de um produto comercial à base de β-glucanas de *S. cerevisiae* na dieta de robalo (*Dicentrarchus labrax*) por 56 dias, obtiveram modulação da microbiota intestinal autóctone, havendo influência da inclusão sobre a modulação dos gêneros dominantes na microbiota. De forma similar, para carpas-comum alimentadas por 14 dias com dieta contendo 1,0% de β-glucanas de *S. cerevisiae*, Jung-Schroers *et al*. (2018) observaram após intubação com *A. hydrophila*, a diminuição do número de bactérias intestinais, acompanhada pelo aumento do número de espécies, além do menor número de células caliciformes contendo mucina no epitélio intestinal, o que poderia estar indicando que o muco foi liberado pelas células e então eliminado do intestino juntamente com um alto número

de bactérias.

Existem evidências demonstrando que os efeitos da suplementação dietética de β-glucanas em peixes são influenciados por fatores como a concentração de β-glucanas utilizada e a duração da suplementação. Demonstrando a influência das concentrações, Aramli, Kamangar e Nazari (2015) observaram que a suplementação com 0,2% e 0,3% de β-glucanas de *S. cerevisiae* por 42 dias para esturjão-persa (*Acipenser persicus*) acarretaram no aumento da contagem de leucócitos, porcentagem de linfócitos, atividade da lisozima sérica e atividade da via alternativa do sistema complemento no soro, o que não foi constatado para a suplementação com 0,1%. Adicionalmente, Zhu e Wu (2018) observaram em *large-scale loach* (*Paramisgurnus dabryanus*), que apesar da suplementação com 0,05 e 0,2% de β-glucanas (fonte não informada) por 60 dias ter proporcionado a melhoria no ganho em peso, conversão alimentar, taxa de crescimento específico, fator de condição e atividade de enzimas digestivas (protease, lipase e amilase), os efeitos para 0,1% foram superiores aos dos demais níveis. Quanto à influência da duração da suplementação, Welker *et al*. (2012) observaram em tilápias-do-Nilo que a suplementação de 0,1% de β-glucanas de *S. cerevisiae* por 30 dias não influenciou o consumo de ração e a eficiência alimentar, mas influenciou o aumento da atividade respiratória dos leucócitos, ao passo que a suplementação por 42 dias influenciou o aumento do consumo de ração e a eficiência alimentar, mas não a atividade respiratória dos leucócitos. Para a mesma espécie de peixe, Liranço *et al*. (2013) obtiveram para administração de 0,03% de β-glucanas de *S. cerevisiae* diferentes efeitos ao decorrer do tempo para os parâmetros de morfologia intestinal, com influência da suplementação sobre a maior espessura das vilosidades (não incluindo a camada muscular) aos 30 e 60 dias; sobre a maior altura das vilosidades aos 60 e 90 dias; e sobre o aumento da altura total das vilosidades (incluindo a camada muscular) apenas aos 60 dias. Além disso, Misra *et al*. (2006) observaram para os parâmetros sanguíneos que a suplementação de 0,01%, 0,025% e 0,05% de β-glucanas (fonte não informada) na dieta de rohu (*Labeo rohita*) proporcionaram respostas variadas no decorrer da suplementação, com diferentes efeitos observados aos 14 dias (aumento da albumina (para 0,025% e 0,05%) e proporção de albumina: globulina (para 0,01% e 0,025%); e diminuição da glicose (para todas as concentrações)), 28 dias (aumento das proteínas séricas totais (para 0,025% e 0,05%), albumina (para 0,025% e 0,05%) e globulinas (para 0,025% e 0,05%); e

diminuição da glicose (para todas as concentrações)), 42 dias (aumento das proteínas séricas totais (para todas as concentrações), albumina (para todas as concentrações) e globulinas (para todas as concentrações); e diminuição da glicose (para 0,025%)) e 56 dias (diminuição da glicose (para todas as concentrações). De forma adicional, demonstrando a variação dos efeitos das diferentes concentrações no decorrer do tempo, Do-Huu, Sang e Thuy (2016) observaram em pompano, que a suplementação com 0,05%, 0,1% e 0,2% de β-glucanas de *S. cerevisiae* ocasionaram o aumento do coeficiente de crescimento diário (% dia^{-1}) aos 14 dias de suplementação, mas aos 28, 42 e 56 dias esse efeito só foi ocorreu em peixes alimentados com dietas contendo 0,1%.

Esses resultados demonstram que apesar da eficiência da suplementação dietética de β-glucanas (com destaque para as extraídas de *S. cerevisiae*) na melhoria de parâmetros de desempenho produtivo, imunológicos, hematológicos, bioquímicos, digestivos e microbiota intestinal, há uma variação nos efeitos, que ocorre principalmente devido às diferentes quantidades utilizadas e a duração da administração. Há evidências também de que essas diferenças podem ocorrer de forma distinta entre as espécies de peixes. A influência desses e de outros possíveis fatores demonstram a grande complexidade do entendimento dos efeitos proporcionados pela suplementação dietética de β-glucanas em peixes, mesmo com a grande quantidade de informação disponível. Isso evidencia a necessidade da realização de estudos adicionais e revisões de literatura, de forma a ampliar e avaliar o conhecimento acerca dos efeitos desse aditivo.

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4 HIPÓTESE

A realização de uma revisão sistemática de literatura possibilitará o entendimento dos efeitos da suplementação dietética de β-glucanas de leveduras sobre o desempenho produtivo, digestão intestinal, morfologia da superfície absortiva intestinal e microbiota intestinal em peixes. A suplementação de β-glucanas de *S. cerevisiae* na dieta de juvenis de acará-bandeira (*P. scalare*) deverá proporcionar melhoras em parâmetros de crescimento, sanguíneos e microbiota intestinal nesses animais.

5 OBJETIVOS

5.1. Objetivo geral

Avaliar a literatura disponível buscando o entendimento dos efeitos da suplementação dietética de β-glucanas de leveduras sobre o desempenho produtivo em peixes. Além disso, avaliar os efeitos da suplementação dietética de β-glucanas purificadas sobre os parâmetros de crescimento, sanguíneos e sobre a microbiota intestinal em juvenis de *P. scalare*.

5.2. Objetivos específicos

- Por meio de uma revisão sistemática de literatura avaliar em estudos publicados entre os anos de 2000 e 2020 os efeitos da suplementação dietética de β-glucanas de leveduras sobre o consumo, aproveitamento da dieta, crescimento e composição corporal em peixes. Além disso, verificar se esses efeitos podem ser resultantes da modulação dos parâmetros relacionados ao processo de digestão intestinal, morfologia da superfície absortiva intestinal e microbiota intestinal.
- Avaliar o efeito da suplementação dietética de β-glucanas de *S. cerevisiae* sobre os parâmetros de crescimento em juvenis de *P. scalare*.
- Avaliar o efeito da suplementação dietética de β-glucanas de *S. cerevisiae* sobre os parâmetros hematológicos, imunológicos e bioquímicos do sangue em juvenis de *P. scalare*.
- Avaliar o efeito da suplementação dietética de β-glucanas de *S. cerevisiae* sobre a microbiota intestinal em juvenis de *P. scalare*, por meio de análise metagenômica.

6 ARTIGO A – DIETARY SUPPLEMENTATION OF YEASTS β-GLUCANS ON PRODUCTIVE PERFORMANCE IN FISH: A SYSTEMATIC LITERATURE REVIEW

Reviews in Fisheries Science & Aquaculture

Abstract

β-glucans are polysaccharides from different sources, being those extracted from yeasts the most used. Its dietary administration, in addition to immunostimulant effects, can also optimize productive performance in fish. Thus, this systematic literature review aimed to verify in studies published between 2000 and 2020, the effects of dietary yeast β-glucans on productive performance, digestion, absorption and intestinal microbiota, also verifying the relationship between these effects. The compiled studies demonstrate the effectiveness of this supplementation on the positive modulation of growth, feed utilization, body composition, digestive enzyme activity, intestinal absorptive surface and intestinal microbiota. One aspect demonstrated is that the improvement in growth can occur together or not with the modulation of feed utilization and body composition. The results also demonstrate that one of the possible causes of these effects would be the optimization of digestive and absorptive capacity and modulation of intestinal microbiota. Despite the efficiency of dietary yeast β-glucans in optimizing these parameters, there are still some aspects that need to be better understood. Thus, it is hoped that the information compiled here can guide future studies in order to enable the application of these data to optimize the productive performance in commercial fish farming.

Keywords: Body composition, Digestion, Feed utilization, Growth, Intestinal microbiota, Nutrient absorption

1. Introduction

The global fish production reached an estimated amount of 179 million tons in 2018, with the amount generated by aquaculture representing 46% of the total production and 52% of production for human consumption (FAO, 2020). However, the existence of numerous diseases that cause great economic losses is one of the main threats to the development of global aquaculture (Srivastava and Pandey 2015). As a result of the need to reduce or eliminate the use of antibiotics due to their side effects, the focus on safe alternatives for the control of these

diseases has gained attention, with emphasis on immunostimulants (Wang et al. 2017). Polysaccharides from different sources have the ability to stimulate the immune system, thus acting as immunostimulants, among them, the β-glucans (Ringø et al. 2012). β-glucan is the denomination of a heterogeneous group of glucose polymers containing β bonds, presenting a great diversity of structures, which are related to their biological functions (Barsanti et al. 2011; Novak and Vetvicka 2008; Soltanian et al. 2009). These substances can be extracted from several unicellular and multicellular fungi, plants, seaweed, and even bacteria, with the yeast *Saccharomyces cerevisiae* the most common source (Meena et al. 2013), being among the best studied and most applied (Petit and Wigertjes 2016).

Due to their immunostimulant capacity, β-glucans are used in aquaculture mainly due to their effects on improving immunity and related physiological responses (Meena et al. 2013; Rodrigues et al. 2020; Vetvicka et al. 2013). Regarding the administration routes, the efficiency of administration by injection and immersion has been demonstrated, however, the oral administration via diet, besides presenting efficiency, is one of the most practical (Petit and Wigertjes 2016). In this context, studies have demonstrated the efficiency of including yeast β-glucans in the fish diet on improving the immune response and related physiological responses (Aramli et al. 2015; El-Boshy et al. 2010; Ghaedi et al. 2015; Montoya et al. 2018; Sánchez-Martínez et al. 2017), and the decrease in mortality after exposure to pathogens (Lin et al. 2011; Kumari and Sahoo 2006; Siwicki et al. 2010).

However, in addition to the immunostimulant effects, there is evidence of the influence of dietary supplementation with yeast β-glucans on the improvement of productive performance in fish. Among these effects, an increase in body weight, weight gain and specific growth rate has been observed (Ai et al. 2007; Lee et al. 2018; Munir et al. 2016a). The improvement in the feed utilization has also been verified, through the evaluation of feed conversion, feed efficiency and protein efficiency rate (Do-Huu et al. 2018; Jami et al. 2019; Talpur et al. 2014;

Welker et al. 2012). In addition, there is evidence of effects on body composition (Munir et al. 2016a; Rufchaie and Hoseinifar 2014).

In oral administration, part of the non-digestible β-glucans can induce changes in the composition of the intestinal microbiota (Rodrigues et al. 2020), which has been shown by some research evaluating dietary supplementation of yeast β-glucans in fish (Do-Huu et al. 2016; Harris et al. 2020; Munir et al. 2018; Rufchaie and Hoseinifar 2014). One of the possible consequences of modulating the intestinal microbiota is the increase of bacteria related to the development of intestinal epithelial cells (Ren et al. 2019). In this regard, there is evidence of the influence of dietary yeast β-glucans on the modulation of the intestinal microbiota together with the increase in the intestinal absorptive surface (Kühlwein et al. 2013; Munir et al. 2018). Another effect observed in the intestine is the increase in activity of digestive enzymes (Cao et al. 2019; Guzmán-Villanueva et al. 2014). Considering these effects of dietary yeast β-glucans in the intestine of fish, some studies have shown their occurrence accompanied by an improvement in productive performance (Guzmán-Villanueva et al. 2014; Jami et al. 2019; Liranço et al. 2013; Munir et al. 2016b; Rufchaie and Hoseinifar 2014). Such results indicate that the improvement in digestion and absorption and the modulation of the intestinal microbiota may be some of the causes of the improvement in the feed utilization and growth provided by dietary supplementation of yeast β-glucans.

There are some literature reviews evaluating the effects of β-glucans on the modulation of the immune response, resistance to disease and stress and related physiological responses in fish (Ching et al. 2021; Dalmo and Bøgwald, 2008; Do-Huu 2019; Meena et al. 2013; Petit and Wiegertjes 2016; Pogue et al. 2021; Rodrigues et al. 2020; Vetvicka et al. 2013). However, none of them had as main objective to evaluate the effects on productive performance and related characteristics, or even focused only on the administration of yeast β-glucans via diet. Therefore, due to the evidence of these relevant effects on productive performance, intestinal

characteristics related to the digestion and absorption process and intestinal bacteria populations, it would be extremely important to compile and evaluate the data available in the literature. As a result, these data can be used in a practical way to enhance the use of yeast β-glucans in fish diets, generating an increase in scientific knowledge related to these effects and possible positive consequences in the commercial fish farming. Thus, the present systematic literature review was carried out with the aim of answering the following questions: '1- Are the studies published between 2000 and 2020 able to demonstrate the influence of dietary supplementation of yeast β-glucans on consumption, feed utilization, growth and body composition in fish?' '2-Can the effects on the activity of intestinal digestive enzymes, digestibility, absorptive surface and intestinal microbiota be some of the causes for the improvement in productive performance?'

2. Materials & methods

2.1 Strategies used in the search for publications

To obtain the publications used in this systematic literature review, searches were performed in the PubMed, Scielo, Science Direct and Scopus databases, as they are the most representative for publications related to aquaculture. Initially, searches were performed in the databases to test different combinations of keywords related to the topic. After evaluating the results, the following string was chosen: ("Fish") AND ("Beta-glucan" OR "β-glucan" OR "Glucans"). The search was performed for the first time in February 2019, with an update being carried out in September 2020 to obtain all works published by the end of 2019, and another update in January 2021 to obtain all works published in 2020.

2.2 Stages of publications selection

The study selection process was carried out in accordance with the PRISMA recommendation, in four stages: identification, selection, eligibility and inclusion, according to methodologies proposed by Kitchenham and Brereton (2013) and Galvão et al. (2015), with adaptations for the research area of this review. At identification (stage 1), all studies found in the databases were grouped, and duplicate studies were subsequently excluded. Publications that were identified in the reference list of selected studies were also submitted to this process (*backward snowballing*). In the selection of search results (stage 2), the titles and abstracts of all the studies found were read. For this stage, the following inclusion criteria were applied: 1-Studies evaluating the inclusion of β-glucans-based products in the fish inert diets (diets where the inclusion was carried out together with other additives were not considered); 2-Publications in "scientific article" and "short communication" formats, written in Portuguese, Spanish and English, published between 2000 and 2020. At this stage, all publications that had evaluated the dietary inclusion of β-glucans in the diet of fish were selected, regardless of the β-glucans source and the analyzes performed. These first two stages were performed using the StArt software version 2.3.4 (UFSCar, São Carlos, SP, Brazil).

The next procedure was the eligibility (stage 3), when the results were analyzed by reading the publications in full. The availability of access to each publication was also verified at this stage. The first three stages were carried out independently by two researchers to ensure non-interference in the selection. To assess the level of agreement between researchers for the results obtained in the selection process, the statistical method Kappa test was used (Cohen 1960). A $\kappa = 0.92$ value was obtained, which represents an "almost perfect" agreement ($\kappa =$ 0.81 to 1.00), according to the classification proposed by Landis and Koch (1977). This result demonstrates high reliability in the publications selection process. The publications in disagreement were later defined jointly by the researchers.

The final step was the inclusion process (Stage 4), performed by screening all studies that had evaluated the dietary supplementation of yeast β-glucans, not being considered β-glucans from other sources. Only studies that evaluated the effects on productive performance (growth, feed utilization and body composition) were selected at this stage. Some studies evaluating the effects on intestinal parameters, but without evaluating productive performance, were found. Since one of the objectives was to verify the effects together, these publications were not included in the review. Furthermore, studies where no statistical analyzes were performed, or whose data did not allow comparison with the others, were not considered. Subsequently, the 61 selected studies were submitted to a bias assessment based on the methodology proposed by Mori and Smith (2019) and on Mixed Methods Appraisal Tool (MMAT) version 2018 (Hong et al. 2018), with some adaptations for the present review. For this, the following questions were applied:

1- Is the evaluation of productive performance mentioned in the study objectives?

2- Were other parameters evaluated in addition to those related to growth?

3- Was the purity level of the source of β-glucans used informed?

4- Are the groups being compared actually comparable, and if not, do the authors address this?

5- Are biometrics/samplings presented in a clear and detailed way?

6- Is the experiment replicated, or is there redundancy in the experimental setup?

7- Were the fish acclimated to the experimental conditions prior to the start of the experiment?

8- Is there an explanation of which results are considered significant?

9- Are the experimental conditions explained well enough to be replicated?

10- Is there any group that was not fed diets containing β-glucans (control group)?

The studies received one point for each of the unmet or unreported criteria, and those that totaled five or more points were excluded. After all stages of selection and bias assessment process, 53 publications were included in the qualitative synthesis of the systematic review.

Summarized data from all stages of the selection process are shown in figure 1.

2.3 Extraction of data from selected studies

From the studies included in the synthesis of the systematic review, the following data were extracted: fish species, initial weight of fish, purity of the product used (% of β-glucans), β-glucans concentration (s) and administration duration (s). Then, the results of the following analyzes were extracted: body weight (BW), body length (BL), weight gain (WG) (including indices based on weight gain), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER), and body composition (moisture, proteins, lipids, and ash). Results of digestive enzymes activity in the intestine (proteases, lipases and amylase), digestibility, morphology of the intestinal absorptive surface and intestinal microbiota (populations of beneficial and/or harmful bacteria, microbiota richness and diversity) were also extracted. Based on the extracted data, it was verified which of the evaluated parameters were influenced by the dietary yeast β-glucans, taking into account the fish species, concentration (s) used and duration (s) of supplementation. The effects were considered when comparing the group(s) fed diet(s) containing β-glucans with the group(s) fed diet(s) without supplementation.

3. Results

3.1 General data from the studies evaluated in the systematic literature review

Data from the 53 studies included in the systematic review were extracted and categorized according to publication details, experimental aspects and results of the analyzes

performed. First, in relation to the year of publication (Figure 2), most studies were published after 2010, with the greatest amount for the years 2020 (8), 2014 (6), 2017(5) and 2019 (5). No publications were found for the years 2000, 2001, 2002, 2003, and 2004. The selected studies involved 28 species (1 hybrid), which are shown in table 1.

In relation to the yeast species from which the β-glucans were extracted, *Saccharomyces cerevisiae* was the most used (45 studies, 84.9% of the total), with effects observed in most studies (31 studies, 68.89% of which used this species) (Table 2). β-glucans from *S. uvarum* were used in one study (Nguyen et al. 2017). Yeast species were not specified in seven studies. Furthermore, in one study (Revina et al. 2020) β-glucans from *S. cerevisiae* and also from a not mentioned yeast species were used (Table 2).

Of the 53 evaluated studies, the effect of dietary yeast β-glucans was obtained in 37 of them (Table 2). Regarding the β-glucans concentrations, they ranged from 0.005% to 2.0%, with 0.05% (11 studies), 0.1% (28 studies), 0.2% (16 studies) and 1.0% (10 studies) being the most used. The purity levels of the β-glucans sources used were mentioned by only nine studies, ranging from 10% to >99%. As for the duration of administration, it ranged from seven to 240 days, with supplementation for 14 days (7 studies), 28 days (5 studies), 30 days (5 studies), 42 days (8 studies), 56 days (12 studies) and 60 days (6 studies) being the most used. Effects were obtained in 19 of the 28 species used in the studies. No effects were observed for *C. batrachus*, *C. macropomum*, *D. labrax*, *I. punctatus*, *M. chrysops* x *M. saxatilis*, *P. mesopotamicus*, *R. quelen*, *S. lucioperca* and *S. asotus* (Table 2).

3.2 Effects of dietary yeast β-glucans on productive performance and intestinal parameters in fish

Summary data on the effects on growth, feed utilization, body composition, digestive

enzyme activity, digestibility, intestinal absorptive surface morphology, and intestinal microbiota are compiled in table 3. For the growth analyzes, weight gain (WG) and specific growth rate (SGR) were the most used parameters, with effects in most of the evaluated studies. Feed intake (FI) was evaluated by a smaller number of studies, with effects seen in few of them. For the parameters related to feed utilization, feed conversion ratio (FCR) was the most used, however, a greater percentage of studies with effect was obtained for protein efficiency ratio (PER). For body composition, effects on moisture, protein, lipid, and ash were found, but only in a few of the studies evaluating these parameters. For the analyzes of digestive enzyme activity in the intestine, the effect of dietary yeast β-glucans was verified in most studies (except for lipase activity), with effects for all studies that evaluated the proteases activity. The effect on digestibility was obtained in only one study, for protein digestibility. Regarding the analyzes of the intestinal absorptive surface morphology, effects were obtained in most of the studies that performed them. Finally, for the intestinal microbiota analyzes, effects were observed in all studies that evaluated these parameters.

Detailed data on the effects on growth, feed utilization, and body composition are presented in table 4. The effects on these parameters were obtained by 32 studies, in 16 fish species. The influence of supplementation on growth was verified in 29 of the 53 studies that evaluated it, with effects on at least one parameter. None of the studies obtained the growth worsening. Feed utilization was evaluated in 40 studies, with effects observed for at least one of the parameters evaluated in 18 of them. Of these, 16 obtained effects on growth and feed utilization together, and two obtained effects only on feed utilization (Refstie at al. 2010; Sealey et al. 2008). Furthermore, a worsening on feed utilization was observed, which occurred together with the improvement (Aramli et al. 2015) or the absence of effects on growth (Refstie et al. 2010; Sealey et al. 2008). The influence on feed intake (FI) was observed in five studies, of which two obtained its increase. The remaining three studies found the decrease in FI,

accompanied by the improvement (Do-Huu et al. 2018; Jami et al. 2019) or by the absence of effects on growth and diet utilization (Shelby et al. 2009). For body composition, the influence of β-glucans was obtained in four studies, with these effects accompanied by the improvement of growth and/or feed utilization in all of them.

Regarding the concentrations of β-glucans used and the durations of supplementation, its influence was different among the parameters evaluated. For growth parameters (BW, BL, WG, and SGR), effects were observed for supplementation with concentrations ranging from 0.01% to 2.0% and durations ranging from 14 to 240 days. For feed intake (FI) effects were observed for concentrations ranging from 0.01% to 0.3% and durations ranging from 14 to 60 days. As for feed utilization (FCR, FER, and PER), the effects were obtained for concentrations ranging from 0.025% to 1.0% and durations ranging from 21 to 112 days. For body composition (moisture, protein, lipid and ash) effects were observed for concentrations ranging from 0.05% to 1.0% and durations ranging from 56 to 112 days.

Detailed data on the effects on digestive enzyme activity, digestibility, intestinal morphology and intestinal microbiota are presented in table 5. Despite the smaller proportion of studies evaluating these characteristics, it was possible to verify that the improvement in productive performance can occur along with the increase in the activity of digestive enzymes (Guzmán-Villanueva et al. 2014; Munir et al. 2016b), digestibility (Munir et al. 2016b), intestinal absorptive surface (Liranço et al. 2013) and intestinal microbiota (Do-Huu et al. 2016; Jami et al. 2019; Rufchaie and Hoseinifar 2014). However, it was also verified an improvement in the growth and feed utilization without changes in the intestinal absorptive surface (Kühlwein et al. 2014). Additionally, some studies found effects on activity of digestive enzymes (Cao et al. 2019; Nieves-Rodríguez et al. 2018), intestinal absorptive surface (Cao et al. 2019; Kazún et al. 2020) and intestinal microbiota (Jung-Schroers et al. 2016; Xu et al. 2020), without verifying the influence on productive performance. Due to the smaller proportion of studies

evaluating these parameters, a smaller variation in β-glucans concentrations and durations of supplementation were tested and generated effects, with effects only being verified in a small number of fish species.

4. Discussion

The first objective of this systematic review was to compile data on the influence of dietary yeast β-glucans on productive performance (growth, feed utilization, consumption and body composition) of fish. From these data, we sought to verify the influence of supplementation on growth and how these effects may be related to the better use of nutrients provided in the diet. It was possible to confirm that the improvement in growth can occur together with the greater use of the diet. However, some studies did not verify this relationship, since this variation may be due to factors such as species of fish, β-glucans concentration (s) and/or duration (s) of supplementation.

Additionally, some studies obtained the improvement on growth and feed utilization along with modification of body composition. Among the observed effects was the increase in body protein in kutum (Rufchaie and Hoseinifar 2014), pompano (Do-Huu et al. 2018) and snakehead (Munir et al. 2016a). It was also found that the increase in body protein may occur together with the decrease in body lipids (Do-Huu et al. 2018; Munir et al. 2016a) and ash (Munir et al. 2016a). Furthermore, in all of these studies the increase in body proteins was obtained together with the increase in PER. In this regard, most studies that evaluated PER obtained the effect of supplementation, indicating the effectiveness of yeast β-glucans in improving the use of dietary protein. Among the goals in animal nutrition are improving the conversion of dietary inputs into lean mass growth rather than adipose tissue, in order to benefit producers and consumers (Dumas et al. 2010). Thus, such effects resulting from

supplementation of β-glucans can be used in order to improve the quality of fish produced, combined with better use of feed.

However, the effect of dietary yeast β-glucans on body composition was quite variable. For sea trout, an increase in body minerals was observed, together with an improvement in growth and diet utilization, with no effects on body lipids and proteins (Jami et al. 2019). Furthermore, no effects on body composition were obtained for Amur catfish (Amoah et al. 2017), common carp (Kühlwein et al. 2014), Crucian carp (Cao et al. 2019), hybrid striped bass (Yamamoto et al. 2020a), and rainbow trout (Sealey et al. 2008). These data demonstrate that there may be variations between species, as well as the effects of different β-glucans concentrations and durations of supplementation. However, as few studies have assessed body composition, more evidence is needed to better understand these effects.

As mentioned, the effects on growth may or may not be related to improved feed utilization and changes in body composition. This demonstrates that the increase in muscle mass can be the result of other effects besides the more efficient use of dietary nutrients. In this aspect, there are different hypotheses regarding increased growth in fish provided by supplementation with β-glucans. One of them suggests that the increase in growth could be a result of the resistance against pathogens, induced by the occurrence of a local intestinal inflammatory response provided by the administration of β-glucans (Dalmo and Bøgwald 2008). Another hypothesis would be that the use of β-glucans as an energy source after degradation by β-glucanases in the digestive tract allows a greater proportion of dietary protein to be used for growth (López et al. 2003). Also, specifically for yeast β-glucans, there is the possibility that the increased growth is a result of the presence of other compounds that remain after the extraction process, such as nucleotides and prebiotics (Yamamoto et al. 2020b).

Additionally, one possibility evaluated by this review is that the increase in feed utilization, and consequently in the growth, result from the improvement of aspects related to

digestion, absorption and intestinal microbiota. The compiled data demonstrate the influence of dietary yeast β-glucans on the modulation of these parameters. First, in relation to the activity of digestive enzymes, the compiled results demonstrate the positive effects of yeast β-glucans in all studies that performed these analyses. In the intestine of fish are present alkaline proteases (mainly trypsin and chymotrypsin), lipases and carbohydrases (mainly amylase) (Moraes and Almeida 2014). In this regard, the studies evaluated demonstrated the effects of the supplementation on the activity of all these enzymes. Among them, it was observed in Pacific red snapper an increase in the activity of proteases (trypsin, chymotrypsin and aminopeptidase) together with an increase in BW, WG, and SGR (Guzmán-Villanueva et al. 2014). For snakehead, the effect on the increase of protease, lipase and amylase activities was accompanied by the improvement on protein digestibility, FCR, PER, WG, and SGR (Munir et al. 2016b). Effects on the increase of trypsase and amylase activity in Crucian carp (Cao et al. 2019) and increase of chymotrypsin activity in tropical gar (Nieves-Rodríguez et al. 2018) were also obtained, however, without the occurrence of effects on the productive performance. Despite these positive results, few studies have used these analyzes in a limited number of species, which makes an in-depth understanding of these effects impossible.

For the morphological analysis of the intestinal absorptive surface, the effectiveness of modulation by yeast β-glucans was also confirmed. In the intestine of fish, the existence of villi with microvilli increases the surface area for absorption, allowing greater contact between cells and nutrients (Moraes and Almeida, 2014). The effect on the increase of the intestinal absorptive surface together with the improvement of the growth was only observed in Nile tilapia (Liranço et al. 2013). For common carp, an improvement in growth and feed utilization without changes in the intestinal absorptive surface was observed (Kühlwein et al. 2014). In contrast, effects were observed on the intestine of Crucian carp (Cao et al. 2019) and roach (Kazún et al. 2020) without the optimization of productive performance. However, as well as

for the activity of digestive enzymes, the small number of studies makes it impossible to better understand this effect. Furthermore, an increase in digestive enzyme activity together with an increase in the intestinal absorptive surface was observed in Crucian carp (Cao et al. 2019), indicating effects on both digestion and absorption improvement. Although these results were obtained without changes on productive performance, these data demonstrate the possibility that these effects are one of the causes of better feed utilization, improvement in growth and changes in body composition.

For the intestinal microbiota, positive effects of dietary yeast β-glucans were found in all evaluated studies. Among them, it was observed for common carp (Jung-Schroers et al. 2016) an increase in the diversity of the intestinal microbiota together with a decrease in the proportion of *Shewanella putrefaciens* and *Vibrio* spp. For Nile tilapia reared in brackish water (Xu et al. 2020) a decrease in the richness and diversity of the intestinal microbiota was found, but with an increase in the abundance of beneficial bacteria, such as *Lactobacillus*, *Phycicoccus*, and *Rikenellaceae*. Both mentioned studies did not obtained effects on productive performance. On the other hand, the other studies obtained the modulation of the abundance of certain bacteria together with the improvement of the productive performance. It was obtained for sea trout (Jami et al. 2019) and kutum (Rufchaie and Hoseinifar 2014) an increase in lactic acid bacteria, together with the improvement of growth and feed utilization. The lactic acid bacteria are able to prevent the establishment of pathogenic bacteria (Ringø et al. 2010), which indirectly may have contributed to the improvement in performance. Additionally, it was verified for pompano (Do-Huu et al. 2016) a decrease of pathogenic bacteria of the genus *Vibrio* in the intestine together with the improvement of growth.

However, none of the studies evaluated showed evidence of how the use of dietary yeast β-glucans by the intestinal microbiota could result in an improvement in productive performance. In this regard, in a study not evaluated in this review, Ren et al. (2019) obtained

for Taimen (*Hucho taimen*) fed diets containing β-glucans from the mushroom *Lentinula edodes*, increase of abundance and diversity in the intestinal microbiota, increase in the proportion of Lactobacillaceae and Clostridiaceae, bacteria related to energy metabolism and intestinal epithelial cell development, in addition to an increase in the proportion of the phylum Bacteroidetes. The effects of dietary yeast β-glucans on the modulation of the intestinal microbiota together with the increase in the intestinal absorptive surface were observed by studies not evaluated in this review (Kühlwein et al. 2013; Munir et al. 2018), that were not included because they did not evaluate the productive performance. In relation to Bacteroidetes phylum, there is evidence that these bacteria have a high capacity to degrade several glucans, including yeast β-glucans (Lapébie et al. 2019; Temple et al. 2017). Although the modulation of these taxa and the modulation of microbiota and intestinal morphology together were not found in this review, it is possible that such effects, as well as their influence on the growth and use of the diet, also occur by the dietary supplementation of yeast β-glucans.

As already mentioned, some studies demonstrate the connection between the modulation of digestive capacity, absorptive capacity and intestinal microbiota with the improvement of productive performance, with these data showed in table 6. Such evidences confirm that the optimization of these parameters can be one of the explanations for the improvement in growth and feed utilization provided by dietary yeast β-glucans, which satisfactorily answers one of the questions of this systematic literature review. However, this relationship of effects did not occur in all evaluated studies. This variation possibly occurs mainly due to the influence of fish species, β-glucans concentration and duration of administration, as well as by the effect of the interaction between these factors.

According to Dalmo and Bøgwald (2008), the increase in growth provided by β-glucans depends on factors such as the amount incorporated in the diet, duration of feeding and the species of fish evaluated. In this aspect, the compiled data demonstrate the influence of β-

glucans concentrations and durations of supplementation in different fish species. Regarding the concentrations, it was verified that the productive performance was modulated in studies that used 0.01% to 2.0%, with 0.05%, 0.1%, 0.2% and 1.0% being the most used and with effects observed in a large proportion of researches. However, it is not possible to affirm which concentrations are the most efficient, since these effects may also be related to the duration of feeding and the species of fish. An important aspect related to the β-glucans concentrations is the purity level of the source used, which was reported by only nine of the 53 studies evaluated. This makes it impossible to know the actual quantity of β-glucans used in most studies. Demonstrating the influence of the purity level, Pilarski et al. (2017) observed greater effects on growth (increase in BW, WG and SGR) for a product based on β-glucans with 55.70% purity compared to another with 77.30% (only increase in SGR), both supplemented at 0.01% (sources not informed) for 30 days. For products based on β-glucans extracted from the yeast *S. cerevisiae*, Yamamoto et al. (2020b) comment that they may contain remaining compounds with growth-promoting properties. Agreeing with this, Revina et al. (2020) when evaluating two products based on yeast β-glucans in the sea trout diet, they obtained a better effect on growth for the less pure product, which contained nucleotides, amino acids, peptides and polypeptides. This demonstrates the importance of further exploring the effects of different purity levels, in order to provide greater understanding of the actual quantity of β-glucans that promote the modulation of productive performance.

Regarding the duration of supplementation, the effects were observed for periods ranging from 14 to 240 days, with most of the effects obtained for analyzes performed after 14, 28, 30, 42, 56 and 60 days after the beginning of feeding. An aspect verified was the variation of effects on productive performance during supplementation, which was observed in Nile tilapia (Liranço et al. 2013), rainbow trout (Sealey et al. 2008), and pompano (Do-Huu et al. 2016). This variation was also found for the intestinal absorptive surface in Nile tilapia (Liranço

et al. 2013) and protease activity in the intestine of Pacific red snapper (Guzmán-Villanueva et al. 2014). Furthermore, Do-Huu et al. (2016) observed in pompano, the effect of supplementation with 0.05, 0.1 and 0.2% on growth after 14 days of feeding, an effect that after 28, 42 and 56 days was verified only for 0.1%. These results demonstrate that the effects of yeast β-glucan levels can vary during the feeding period, which may indicate the combined effect of dose and duration of supplementation.

5. Conclusions and future perspectives

The compiled data demonstrate the effects of dietary yeast β-glucans on productive performance in 16 fish species, with effects on growth, feed utilization, body composition, digestion and absorption process and intestinal microbiota. The results presented here can serve to guide future studies that will investigate these effects, in order to enable a deeper understanding of how this supplementation can result in the improvement of productive performance. This is extremely important, since the available data are insufficient for such an understanding. In this context, the activity of digestive enzymes in the intestine, digestibility, intestinal absorptive surface morphology and intestinal microbiota were evaluated in few studies. In addition, these analyzes were only performed in studies published from the year 2010, which demonstrates a recent tendency to verify these effects. As discussed, there is evidence that the modulation of these intestinal parameters may be related to the improvement in productive performance provided by the dietary yeast β-glucans. Therefore, it is extremely important that future research carry out additional investigations to broaden the understanding of how the modulation of these parameters influence growth, feed utilization and body composition. Additionally, it is also important that more researches evaluate how the effects on digestion, absorption and intestinal microbiota relate to each other, and how this relationship

influences the improvement of productive performance, which has been evaluated in a small number of studies.

In addition to the evaluated parameters, other ways to verify the effects of dietary yeast β-glucans on production performance can be used. According to Dalmo and Bøgwald (2008), the assessment of the effects of increased growth provided by immunostimulants must be accompanied by other analyses, such as the assessment of gene expression. Related to this, Dawood et al. (2020) observed for Nile tilapia that the dietary inclusion of β-glucans from an unknown source provided the increase in the gene expression of the insulin-like growth factor I (IGF-I). This result demonstrates the possibility of evaluating the effects of dietary yeast β-glucans on the expression of genes related to traits of productive interest. The results of future studies using this type of analysis may provide a more detailed understanding of how the effects of dietary yeast β-glucans on nutrient utilization, and consequently on increased growth, occur.

For the different species of fish and evaluated parameters, it was verified that a wide range of concentrations and durations of supplementation provided the effects. Such data can also help to guide future studies. However, as the purity of the products used was not reported in most of the studies, it is not possible to confirm what were the actual quantities of β-glucans that provided the effects. Therefore, it is extremely important that future studies, in addition to presenting these data, also carry out evaluations of the effects of products with different purity levels. It was also observed that the effectiveness of β-glucans concentrations on the improvement of growth may vary over the period of supplementation, which, however, was assessed in only one study (Do-Huu et al. 2016). Therefore, it is important that future researches evaluate this variation, to provide a better understanding of how the concentrations and durations of supplementation together can affect the productive performance in different fish species.

Regarding the application of these data in commercial fish farming, the vast majority of

studies were carried out under laboratory conditions. Due to the possible existing gap between the effects of feed additives found in the laboratory researches and their application in commercial aquaculture, it is necessary to develop methodologies that enable that this information can be applied efficiently (Amenyogbe et al. 2020). Despite these obstacles, it is hoped that the information presented here can help to understand the effects of dietary yeast β-glucans on productive performance in fish, as well as serving to guide future studies. Furthermore, this systematic literature review can serve as a basis for the development of meta-analyses on the evaluation of the effects not only of β-glucans, but also of other feed additives with a focus on optimizing productive performance in fish. This could enable the development of protocols for the application of this knowledge in the fish farming on a commercial scale.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

The first author was supported by a doctoral fellowship provided by CAPES (Coordination for the Improvement of Higher Education Personnel) and by Araucária Foundation.

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Table 1. Fish species used in the evaluated studies followed by the number of studies.

Scientific/popular name	Scientific/popular name
Acipenser persicus/Persian sturgeon (1)	*Oncorhynchus mykiss*/Rainbow trout (6)
Ancherythroculter nigrocauda/None (1)	*Oreochromis niloticus*/Nile tilapia (7)
Atractosteus tropicus/Tropical gar (1)	*Pangasianodon hypophthalmus*/Striped catfish (2)
Carassius auratus/ Crucian carp (1)	*Paralichthys olivaceus*/ Olive flounder (1)
Channa striata/Snakehead (3)	*Piaractus mesopotamicus*/Pacu (2)
Clarias batrachus/Asian catfish (1)	*Pseudosciaena crocea*)/ Large yellow croaker (1)
Colossoma macropomum/Tambaqui (1)	*Rhamdia quelen*/Silver catfish (1)
Cyprinus carpio/Common carp (5)	*Rutilus frisii*/Kutum (1)
Dicentrarchus labrax/Sea bass (1)	*Rutilus rutilus*/Roach (1)
Epinephelus fuscoguttatus/ Brown-marbled grouper (2)	*Salmo salar*/Atlantic salmon (1)
Morone chrysops x *Morone saxatilis*/ Hybrid striped bass (2)	*Salmo trutta*/Sea trout (2)
Ictalurus punctatus/Channel catfish (1)	*Sander lucioperca*/ Pikeperch (1)
Lutjanus guttatus/ Spotted rose snapper (1)	*Silurus asotus*/Amur catfish (1)
Lutjanus peru/ Pacific red snapper (1)	*Trachinotus ovatus*/Pompano (4)

Table 2. Studies included in the systematic literature review with details of aspects related to dietary supplementation of yeast β-glucans.

Reference	Species	Yeast species, purity and concentration (s) of the product (%) used	Duration (s) of supplementation (days)	Supplementation Effects
Abdelhamid et al. (2020)	*O. niloticus*	*S. cerevisiae*. NA. 0.1	60	Yes
Adloo et al. (2015)	*P. hypophthalmus*	*S. cerevisiae*. NA. 0.5, 1.0 and 2.0	63	No
Ai et al. (2007)	*P. crocea*	*S. cerevisiae*. 18%. 0.5 and 1.0	56	Yes
Amoah et al. (2017)	*S. asotus*	Yeast. NI. 0.05	56	No
Apines-Amar et al. (2012)	*E. fuscoguttatus*	*S. cerevisiae*. NA. 1.0	84	Yes
Apines-Amar et al. (2013)	*E. fuscoguttatus*	*S. cerevisiae*. NA. 1.0	56	Yes
Aramli et al. (2015)	*A. persicus*	*S. cerevisiae*. NA. 0.1, 0.2, and 0.3	42	Yes
Bagni et al. (2005)	*D. labrax*	*S. cerevisiae*. NA. 0.1	15 days with/45 days without (4 cycles)	Yes
Cao et al. (2019)	*C. auratus*	Yeast. 85%. 0.1	70	Yes
Cerozi et al. 2017	*P. mesopotamicus*	*S. cerevisiae*. NA. 0.024%	59	No
Chagas et al. (2013)	*C. macropomum*	*S. cerevisiae*. NA. 0.1, 0.2, 0.4, and 0.8	60	No
Del Rio-Zaragoza et al. (2011)	*L. guttatus*	*S. cerevisiae*. NA. 0.05	14 and 35	Yes
Do-Huu (2020)	*T. ovatus*	*S. cerevisiae*. NA. 0.1, 0.2, and 0.4	56	Yes
Do-Huu et al. (2016)	*T. ovatus*	*S. cerevisiae*. NA. 0.05, 0.1, 0.2, and 0.4	56	Yes
Do-Huu et al. (2018)	*T. ovatus*	*S. cerevisiae*. NA. 0.05, 0.1, 0.2, and 0.4	56	Yes
Do-Huu et al. (2019)	*T. ovatus*	*S. cerevisiae*. NA. 0.1 and 0.2	21	Yes
Domenico et al. (2017)	*R. quelen*	*S. cerevisiae*. NA. 0.01 and 0.1	42	No
Ghaedi et al. (2015)	*O. mykiss*	*S. cerevisiae*. NA. 0.1 and 0.2	60	Yes
Guzmán-Villanueva et al. (2014)	*L. peru*	*S. cerevisiae*. NA. 0.1 and 0.2	42	Yes
Jami et al. (2019)	*S. trutta*.	*S. cerevisiae*. NA. 0.3	56	Yes
Ji et al. (2017)	*O. mykiss*	*S. cerevisiae*. NA. 0.05, 0.1, and 0.2	42	Yes
Ji et al. (2019)	*O. mykiss*	*S. cerevisiae*. 0.1 and 0.2	30	Yes
Jung-Schroers et al. (2016)	*C. carpio*	*S. cerevisiae*. >68%. 1.0	14	Yes
Kazún et al. (2020)	*R. rutilus*	*S. cerevisiae*. NA. 1.0	14	Yes
Kühlwein et al. (2014)	*C. carpio*	*S. cerevisiae*. NA. 0.1, 1.0, and 2.0	56	Yes

Kumari and Sahoo (2006)	*C. batrachus*	*S. cerevisiae*. NA. 0.1	7, 14, and 21	No
Lee et al. (2018)	*P. olivaceus*	*S. cerevisiae*. 10.00%. 0.01, 0.05, and 0.1 (total β-glucans concentration)	30	Yes
Li et al. (2009)	*M. chrysops* x *M. saxatilis*	*S. cerevisiae*. NA. 0.05, 0.1, and 0.2	28	No
Lin et al. (2011)	*C. carpio*	*S. cerevisiae*. 18%. 0.5	56	Yes
Liranço et al. (2013)	*O. niloticus*	*S. cerevisiae*. 70%. 0.03	90	Yes
Munir et al. (2016a)	*C. striata*	*S. cerevisiae*. NA. 0.2	112	Yes
Munir et al. (2016b)	*C. striata*	*S. cerevisiae*. NA. 0.2	112	Yes
Neamat-Allah et al. (2020)	*O. niloticus*	*S. cerevisiae*. NA. 0.05	60	Yes
Nguyen et al. (2017)	*P. hypophthalmus*	*S. uvarum*. 93%. 0.005, 0.01, and 0.02	45	Yes
Nguyen et al. (2019)	*C. carpio*	*S. cerevisiae*. NA. 0.025	63	No
Nieves-Rodríguez et al. (2018)	*A. tropicus*	*S. cerevisiae*. NA. 0.5, 1.0, 1.5, and 2.0	62	Yes
Ohtani et al. (2020)	*O. mykiss*	*S. cerevisiae*. NA. 0.014 (total β-glucans concentration)	28	No
Ramzani et al. (2014)	*O. mykiss*	Yeast. NA. 0.05, 0.1 and 0.15	77	Yes
Refstie et al. (2010)	*S. salar*	*S. cerevisiae*. NA. 0.05 and 0.1	70	Yes
Revina et al. (2020)	*S. trutta*	*S. cerevisiae* (source 1) and yeast (source 2). NA. 0.1, 0.3 (source 1), 0.6, and 1.4% (source 2)	240	Yes
Rufchaie and Hoseinifar (2014)	*R. frisii*	Yeast. NA. 0.1, 1.0, 1.5, and 2.0	60	Yes
Schorer et al. (2009)	*P. mesopotamicus*	*S. cerevisiae*. 85%. 0.1, 0.2, and 0.3	90	No
Sealey et al. (2008)	*O. mykiss*	*S. cerevisiae*. NA. 0.2	63	Yes
Shelby et al. (2009)	*O. niloticus*	*S. cerevisiae* (sources 1 and 2). NI. 0.01 (source 2), 0.1 and 1.0% (sources 1 and 2)	14 and 28	Yes
Siwicki et al. (2009)	*S. lucioperca*	*S. cerevisiae*. NA. 0.1 and 0.2	21 and 42	No
Talpur et al. (2014)	*C. striata*	*S. cerevisiae*. NA. 0.1	56	Yes
Welker et al. (2007)	*I. punctatus*	*S. cerevisiae* (sources 1 and 2). NA. 0.01 (source 1) and 0.1% (source 2)	28	No
Welker et al. (2012)	*O. niloticus*	*S. cerevisiae*. NA. 0.1	30 and 42	Yes
Whittington et al. (2005)	*O. niloticus*	*S. cerevisiae*. NA. 0.005, 0.01, and 0.02	70	No
Xu et al. (2020)	*O. niloticus*	Yeast. NI. 0.2 and 0.4	53	Yes
Yamamoto et al. (2020a)	*M. chrysops* × *M. saxatilis*	*S. cerevisiae*. NA. 0.005 and 0.01.	56	No
Ye et al. (2011)	*C. carpio*	Yeast. NA. 0.15 and 0.50	60	No
Yin et al. (2014)	*A. nigrocauda*	Yeast. >99%. 0.025, 0.05, 0.075, and 0.1	50	Yes

NA: not available.

Table 3. Effects of dietary yeast β-glucans on growth, feed utilization, body composition, digestive enzyme activity, digestibility, intestinal morphology and intestinal microbiota.

Parameter	Total of studies	Studies with effect	Studies with effect (%)
Body weight	29	14	48.28
Body length	7	3	42.86
Weight gain	39	21	53.85
Specific growth rate	38	21	55.26
Feed intake	13	5	38.46
Feed conversion ratio	26	13	50.00
Feed efficiency ratio	15	6	40.00
Protein efficiency ratio	11	8	72.73
Body moisture	7	1	14.29
Body protein	9	3	33.33
Body lipid	9	2	22.22
Body ash	8	2	25.00
Protease activity	4	4	100.00
Lipase activity	3	1	33.33
Amylase activity	3	2	66.67
Protein/nitrogen digestibility	2	1	50.00
Intestinal absorptive surface	5	3	60.00
Intestinal microbiota	5	5	100.00

Table 4. Detailed effects of dietary yeast β-glucans on growth, feed utilization and body composition.

Reference	Species	Effects of supplementation
Abdelhamid et al. 2020	*O. niloticus*	↑ BW, ↑ WG, ↑ SGR, ↓ FCR, and ↑ PER (0.1% for 60 days) for fish exposed to diazinon
Ai et al. 2007	*P. crocea*	↑ SGR (0.5% for 56 days)
Apines-Amar et al. 2012	*E. fuscoguttatus*	↑ WG, ↑ SGR, ↑ FER (1.0% for 84 days), ↔ FI, and ↔ FW
Apines-Amar et al. 2013	*E. fuscoguttatus*	↑ WG (1.0 % for 56 days), ↔ FI, ↔ SGR, and ↔ FER
Aramli et al. 2015	*A. persicus*	↑ BW, ↑ SGR and ↑ FCR (0.1, 0.2 and 0.3% for 42 days)
Del Rio-Zaragoza et al. 2011	*L. guttatus*	↑ WG (0.05 and 0.1% for 35 days) and ↑ SGR (0.1% for 35 days)
Do-Huu 2020	*T. ovatus*	↑ BW, ↑ BL, and ↑ SGR (0.1% for 56 days)
Do-Huu et al. 2016	*T. ovatus*	↑ WG (daily growth coefficient) (0.1% for 14, 28, 42, and 56 days; 0.05 and 0.2% for 14 days)
Do-Huu et al. 2018	*T. ovatus*	↓ daily FI, ↑ BW, ↑ WG, ↑ daily WG, ↑ SGR, ↑ FER, ↑ body protein, ↑ PER, ↓ FCR (0.05 and 0.1% for 56 days), ↓ body lipids (0.05, 0.1, 0.2 and 0.4% for 56 days)
Do-Huu et al. 2019	*T. ovatus*	↑ SGR (0.1% for 21 days)
Ghaedi et al. 2015	*O. mykiss*	↑ BW, ↑ WG, ↑ SGR (0.2% for 60 days), and ↔ FCR
Guzmán-Villanueva et al. 2014	*L. peru*	↑ BW (0.1% for 42 days), ↑ WG (0.1 and 0.2 % for 42 days), and ↑ SGR (0.1 and 0.2% for 42 days)
Jami et al. 2019	*S. trutta*	↑ BW, ↑ WG, ↔ SGR, ↓ FI, ↓ FCR, ↑ PER, ↑ body ash (0.3% for 56 days), ↔ body moisture, ↔ body protein, and ↔ body lipids

Ji et al. 2017	*O. mykiss*	↑ WG, ↑ SGR, and ↑ FER (0.1 and 0.2% for 42 days)
Ji et al. 2019	*O. mykiss*	↑ WG, ↑ SGR, and ↑ FER (0.2% for 30 days)
Kühlwein et al. 2014	*C. carpio*	↑ BW, ↑ WG, ↑ SGR (1.0 and 2.0% for 56 days), ↓ FCR (1.0% for 56 days), ↔ PER, ↔ body moisture, ↔ body protein, ↔ body lipid, and ↔ body ash
Lee et al. 2018	*P. olivaceus*	↑ BW, ↑ SGR (0.05 and 0.1% for 30 days), and ↔ FCR
Lin et al. 2011	*C. carpio*	↑ BW, ↑ SGR (0.5% for 56 days) and ↔ FCR
Liranço et al. 2013	*O. niloticus*	↑ BW (0.03% for 60 and 90 days), ↑ BL (0.03% for 60 and 90 days), ↔ FI, ↔ daily WG, ↔ FCR, ↔ SGR, and ↔ PER
Munir et al. 2016a	*C. striata*	↑ WG, ↑ SGR, ↓ FCR, ↑ PER, ↓ body moisture, ↑ body protein; ↓ body lipid, and ↓ body ash (0.2% for 112 days)
Munir et al. 2016b	*C. striata*	↑ WG, ↑ SGR, ↓ FCR, and ↑ PER (0.2% for 56 and 112 days)
Neamat-Allah et al. 2020	*O. niloticus*	↑ BW (for fish exposed and not exposed to atrazine), ↑ WG (for fish exposed and not exposed to atrazine), ↑ FI (for fish exposed to atrazine) and ↓ FCR (for fish exposed and not exposed to atrazine) (0.05% for 60 days)
Nguyen et al. 2017	*P. hypophthalmus*	↑ BW, ↑ WG, and ↑ SGR (0.01 and 0.02% for 45 days)
Ramzani et al. 2014	*O. mykiss*	↑ WG (0.15% for 77 days), ↑ SGR (0.1 and 0.15% for 77 days), and ↓ FCR (0.05, 0.1, and 0.15% for 77 days)

Refstie et al. 2010	*S. salar*	↓ FER (0.05% for 70 days) for fish fed a diet containing 32% soybean meal, ↔ FI, ↔ BW, ↔ WG (thermal-unit growth coefficient), and ↔ SGR
Revina et al. 2020	*S. trutta*	↑ BW (0.3% source 1 for 180 and 210 days; 0.6% source 2 for 120, 150, 180, 210, 240 days; 1.4% source 2 for 90, 120, 150, 180, 210 and 240 days), and ↑ BL (0.6 e 1.4% source 2 for 240 days).
Rufchaie and Hoseinifar 2014	*R. frisii*	↑ WG, ↑ SGR, ↑ PER, ↓ FCR (0.5 and 1.0% for 60 days), ↑ body crude protein (0.5, 1.0 and 1.5% for 60 days), ↔ body moisture, ↔ body lipid, and ↔ body ash
Sealey et al. 2008	*O. mykiss*	↑ FCR (0.2% for 21 days), ↔ WG, ↔ body moisture, ↔ body protein, ↔ body lipid, and ↔ body ash
Shelby et al. 2009	*O. niloticus*	↓ FI (source 1, 0.1% for 14 days), ↑ FI (source 2, 0.01% for 28 days), ↔ WG, and ↔ FER.
Talpur et al. 2014	*C. striata*	↑ WG, ↓ FCR, and ↑ PER (0.1% for 56 days)
Welker et al. 2012	*O. niloticus*	↑ WG (0.1% for 30 and 42 days), ↑ FI, and ↑ FER (0.1% for 42 days), after 42 days of feeding
Yin et al. 2014	*A. nigrocauda*	↑ WG, ↑ SGR, ↑ PER, and ↓ FCR (0.025, 0.05 and 0.075% for 50 days)

Effects in fish fed yeast β-glucans diets compared to fish fed control diet. ↑: significant increase, ↓: significant decrease, ↔: no significant effects
BL: body length, BW: body weight, FCR: feed conversion ratio, FER: feed efficiency ratio, FI: feed intake, PER: protein feed efficiency ratio, SGR: specific growth rate, WG: weight gain.

Table 5. Detailed effects of dietary yeast β-glucans on digestive enzyme activity, digestibility, intestinal absorptive surface and intestinal microbiota.

Reference	Species	Effects
Cao et al. 2019	*C. auratus*	↑ trypsase activity, ↑ amylase activity, ↑ intestinal fold height, (0.1% for 70 days), ↔ lipase activity, ↔ enterocyte height, and ↔ microvillus height
Do-Huu et al. 2016	*T. ovatus*	↓ total *Vibrio* counts in the intestine (0.05, 0.1, and 0.2 % for 56 days)
Guzmán-Villanueva et al. 2014	*L. peru*	↑ trypsin activity (0.1% for 14 days), ↑ chymotrypsin activity (0.2% for 28 days), and ↑ aminopeptidase activity (0.1 and 0.2% for 28 days)
Jami et al. 2019	*S. trutta*	↑ intestinal load of *Lactobacillus plantarum* (0.3% for 56 days)
Jung-Schroers et al. 2016	*C. carpio*	↑ Shannon-Winer index, ↑ Simpson index, ↓ intestinal bacteria, ↓ *Shewanella putrefaciens*, ↓ *Vibrio* spp. (1.0% for 14 days)
Kazún et al. 2020	*R. rutilus*	↓ anterior intestine fold length (1.0% for 14 days), ↔ midgut fold length, and ↔ posterior intestine fold length
Liranço et al. 2013	*O. niloticus*	↑ height of the villi (0.03% for 60 and 90 days), ↑ total height of the villi (0.03% for 60 days), and ↑ thickness of epithelium of the villi (0.03% for 30 and 60 days)
Munir et al. 2016b	*C. striata*	↑ protein digestibility, ↑ protease activity, ↑ lipase activity and ↑ amylase activity (0.2% for 56 and 112 days)
Nieves-Rodríguez et al. 2018	*A. tropicus*	↑ chymotrypsin activity (1.0 and 1.5% for 62 days), ↔ total alkaline proteases activity, ↔ trypsin activity, ↔ leucine peptidase activity, ↔ lipase activity, ↔ α-amylase activity

Rufchaie and Hoseinifar 2014	*R. frisii*	↑ total heterotrophic autochthonous bacteria (0.5, 1.0 and 1.5% for 60 days), ↑ lactic acid bacteria levels (0.5 and 1.0% for 60 days), and ↑ % lactic acid bacteria (0.5% for 60 days)
Xu et al. 2020	*O. niloticus*	↓ richness (Chao 1 and ACE) and diversity (Shannon and Simpson) of the intestinal microbiota (0.4% for 53 days), and ↑ beneficial bacteria (*Lactobacillus, Phycicoccus* and *Rikenellaceae*) (0.2 and 0.4% for 53 days) in fish under brackish water

Effects in fish fed yeast β-glucans diets compared to fish fed control diet. ↑: significant increase, ↓: significant decrease, ↔: no significant effects

Table 6. Studies that obtained the intestinal effects together with the improvement in productive performance provided by dietary yeast β-glucans.

References	Species	Effects
Do-Huu et al. 2016	*T. ovatus*	↓ *Vibrio* counts in the intestine ↑ WG (% day^{-1})
Guzman-Villanueva et al. 2014	*L. peru*	↑ trypsin, chymotrypsin, and aminopeptidase activities ↑ BW, ↑ WG, and ↑ SGR
Jami et al. 2019	*S. trutta*	↓ FI ↑ intestinal load of *Lactobacillus plantarum* ↓ FCR and ↑ PER ↑ BW, ↑ WG, and ↔ SGR ↑ body ash, ↔ body moisture, ↔ body protein, and ↔ body lipids
Liranço et al. 2013	*O. niloticus*	↔ FI ↑ intestinal absorptive surface ↔ FCR and ↔ PER ↑ BW, ↑ BL, and ↔ SGR
Munir et al. 2016b	*C. striata*	↑ protease activity, ↑ lipase activity, and ↑ amylase activity ↑ protein digestibility ↓ FCR and ↑ PER ↑ WG and ↑ SGR
Rufchiae and Hoseinifar 2014	*R. frisii*	↑ total bacteria and ↑ lactic acid bacteria in the intestine ↓ FCR and ↑ PER ↑ body protein, ↔ body moisture, ↔ body lipid, and ↔ body ash ↑ WG and ↑ SGR

Effects in fish fed yeast β-glucans diets compared to fish fed control diet. ↑: significant increase, ↓: significant decrease, ↔: no significant effects.
BL: body length, BW: body weight, FCR: feed conversion ratio, FI: feed intake, PER: protein feed efficiency ratio, SGR: specific growth rate, WG: weight gain.

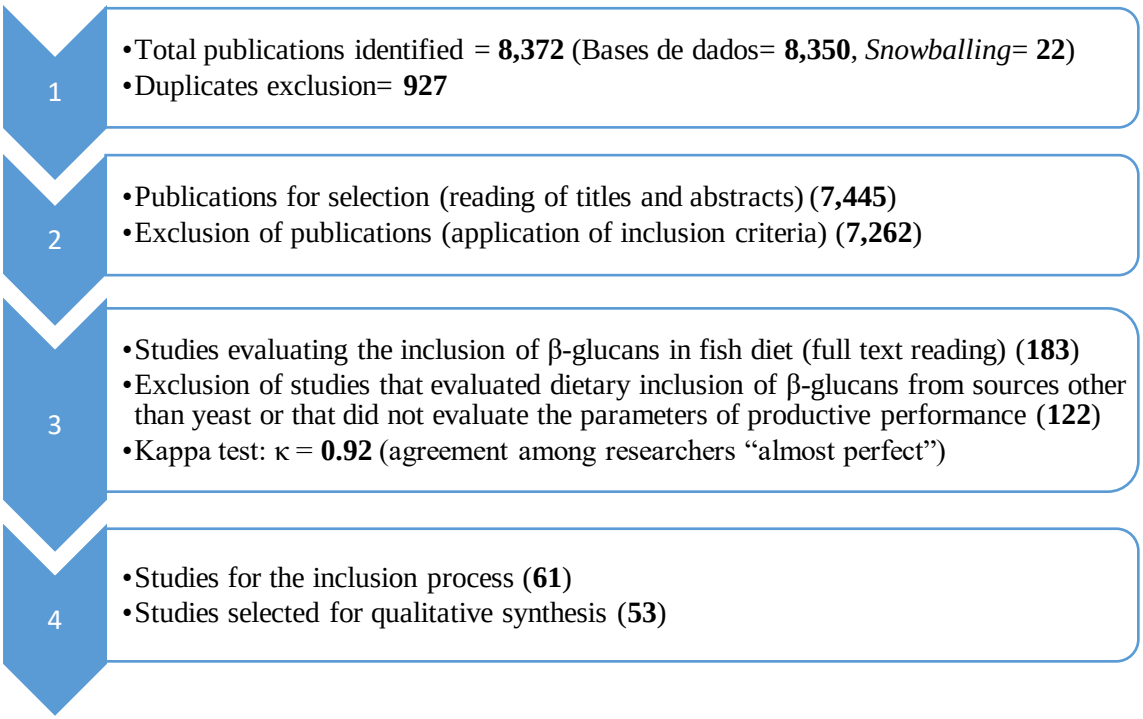

Figure 1. Stages in the selection process of studies evaluating dietary supplementation of yeast β-glucans on productive performance in fish.

1- Identification, 2- Selection, 3- Eligibility, 4- Inclusion

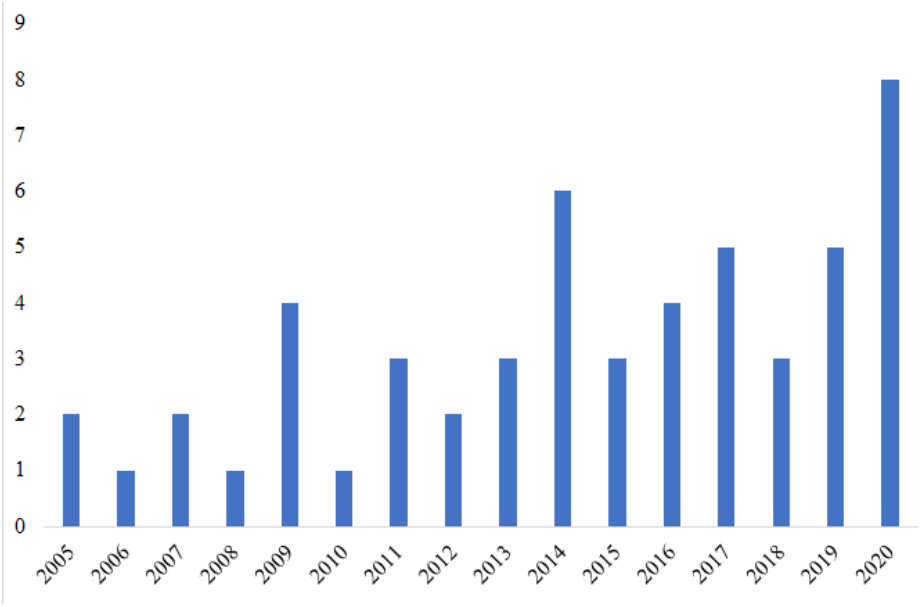

Figure 2. Number of studies included in the systematic review by year of publication.

7 ARTIGO B – EFFECTS OF DIETARY SUPPLEMENTATION OF β-GLUCANS ON THE PERFORMANCE, BLOOD PARAMETERS, AND INTESTINAL MICROBIOTA OF ANGELFISH (*Pterophyllum scalare*) JUVENILES

Aquaculture Research

Abstract

Among the potential feed additives, β-glucans are known to positively affect the growth performance, blood parameters, and intestinal microbiota of fish, even the ornamental species. Therefore, the present study evaluated the effects of the dietary supplementation of different *Saccharomyces cerevisiae* β-glucans concentrations (0, 0.05, 0.1, and 0.2%) in juvenile angelfish (*Pterophyllum scalare*) over a 42-day period. Regarding growth performance, no effects were observed on most parameters. However, 0.2% β-glucans supplementation produced higher condition factor values, indicating a better nutritional status. Furthermore, β-glucans supplementation did not affect blood parameters. Regarding intestinal microbiota, β-glucans supplementation increased the abundance of the potentially beneficial bacterial genus *Phascolarctobacterium*. The high abundance of bacteria from the phylum *Bacteroidetes*, which can degrade β-glucans, may be attributed to the increased abundance of *Phascolarctobacterium* spp. In addition, 0.2% β-glucans supplementation produced more operational taxonomic units and higher Sobs (observed species richness), indicating effects on the overall bacterial community structure. These results demonstrate the potential application of β-glucans as a dietary supplement to improve the performance and modulate the intestinal microbiota of angelfish.

KEYWORDS: Condition factor, Growth, Immunostimulants, Ornamental fish, *Phascolarctobacterium*, Prebiotics

1. INTRODUCTION

In the overall global trade of live fish, the trade of ornamental fish involves smaller quantities but higher economic value than the trade of fish destined for human consumption (FAO, 2016). Furthermore, the aquarium industry is a rapidly developing sector, and aquarium keeping is no longer simply a hobby (Karadal, Güroy, & Türkmen, 2017). In this context, freshwater angelfish (*Pterophyllum scalare*), a cichlid native to the Amazon Basin, is one of

the most popular ornamental fish species worldwide, mainly because of the body and fin shape, availability of several varieties, peaceful behavior, relative rusticity, excellent adaptability to captivity, easy reproduction, omnivorous eating habit, and acceptance of artificial foods (Azimirad, Meshkini, Ahmadifard, & Hoseinifar, 2016; Ikeda et al., 2011; Fujimoto, Vendruscolo, Schalch, & Moraes, 2006; Ribeiro, Rodrigues, & Fernandes, 2007). Regarding nutritional management in aquaculture, the use of feed additives can optimize productivity and increase profitability. For this purpose, immunostimulants, such as β-glucans, can be supplied.

β-glucans are polysaccharides composed of glucose linked by β-glycosidic bonds, which are found in the cell wall of several plants, yeasts, mushrooms, seaweeds, and bacteria (Meena et al., 2013). In particular, among the best studied and most applied are β-glucans derived from the cell wall of yeast *Saccharomyces cerevisiae* (Petit & Wiegertjes, 2016). Although studies in fish have demonstrated the efficiency of β-glucans administration through water (Souza et al., 2020a; Zhang, Swain, Bøgwald, Dalmo, & Kumari, 2009) and injection (Rodriguez, Chamorro, Novoa, & Figueras, 2009; Selvaraj, Sampath, & Sekar, 2005), dietary supplementation is a more practical route of administration (Petit & Wiegertjes, 2016). However, only a few studies have explored the dietary inclusion of β-glucans in ornamental fish and demonstrated its positive effects on stress resistance, pathogen resistance, immunity, and hematologic response (Abreu et al., 2014; Lin, Pan, Luo, & Luo, 2011; Russo, Yanong, & Mitchell, 2006; Türnal, Schimidt, Kürzinger, & Böhm, 2000).

Although most previous studies primarily focused on the use of β-glucans for improving immunity and pathogen resistance (Meena et al., 2013; Petit & Wiegertjes, 2016), these polysaccharides can also improve growth and other aspects related to productive performance. As such, previous studies have reported the positive effects of dietary β-glucans on parameters related to growth and feed utilization in fish (Ji et al., 2017; Liranço, Ciarlini, Moraes, Camargo, & Ramagosa, 2013; Talpur, Munir, Mary, & Hashim, 2014; Welker, Lim, Yildirim-Aksoy, &

Klesius, 2012), including ornamental species (Lin et al., 2011). Additionally, there is evidence of the efficacy of β-glucans in modulating the intestinal microbiota of fish (Carda-Diéguez, Mira, & Fouz, 2014; Harris et al. 2020; Jung-Schroers et al., 2016), including ornamental species (Jung-Schroers, Harris, Adamek, Jung, & Steinhagen, 2019). Based on these reports, β-glucans show a great potential for application in commercial fish farming intended for human consumption and ornamental purposes.

To date, however, no study has evaluated the effectiveness of β-glucans supplementation in *Pterophyllum scalare*. Therefore, the present study evaluated the effects of dietary supplementation of different β-glucans concentrations on the growth, feed utilization, blood parameters (hematological, immunological, and biochemical), and intestinal microbiota of angelfish juveniles.

2. MATERIALS AND METHODS

2.1 Ethics statement

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 appropriate ethical review committee approval has been received. Brazil's National Council for the Control of Animal Experimentation (CONCEA) guidelines for the care and use of animals in teaching or scientific research activities were followed. The procedures performed in the present study were approved by the Ethics Committee on the Use of Animals at the State University of Londrina (CEUA/UEL) (protocol CEUA no. 13903.2018.86).

2.2 Animals and experimental conditions

The experiment was performed in the laboratory of the Center for Study and Research in Aquaculture and Genetics (NEPAG) at the State University of Londrina (UEL). Angelfish (*Pterophyllum scalare*) juveniles of the marble strain were purchased from local suppliers and housed in laboratory facilities. Prior to the initiation of the experiment, the fish were acclimatized to the experimental conditions for 28 days. The experimental units were aquariums with a total volume of 60 L, connected to a recirculation system, with additional aeration performed directly at the filter. Adequate temperature was maintained using a space heater and a thermostat in the filtration system. The fish were fed to apparent satiation twice a day at 09:00 and 16:00. To maintain water quality, feces and feed remains were removed daily, and 25% of the system volume was renovated twice a week. Temperature (°C), pH, and dissolved oxygen (DO) (mg L^{-1}) were measured daily. The photoperiod was maintained at 12 h of light and 12 h of dark. All procedures were performed during the acclimatization and the experimental period. Prior to the beginning of the experiment, all fish were individually weighed to obtain the initial weight (mean weight ± SD: 5.28 ± 0.91 g).

2.3 Diet preparation

To evaluate the effects of dietary inclusion of β-glucans, a specific commercial diet for discus fish (*Symphysodon* spp.) and angelfish (Nutricon, Araçoiaba da Serra, SP, Brazil) (12% moisture, 38% protein, 3.5% lipids, 2.5% fiber, and 12% minerals) was used; all feed additives that could compromise the effects of β-glucans were removed from the formulation. The same commercial feed was used during the acclimatization period. As the source, a commercial product (MacroGard®, Biorigin, Lençóis Paulista, Brazil) containing a minimum of 60% β-

1,3/1,6-glucans extracted from *S. cerevisiae* was used. As additives, the respective concentrations of β-glucans for each diet (0.0, 0.05, 0.1, and 0.2%) were diluted in distilled water, homogenized, and distributed evenly over the feed. Then, the mixture was blended to ensure a homogeneous distribution. To ensure β-glucans fixation, 40 mL of an agglutinating feed additive (Vansil Saúde Animal, Descalvado, São Paulo, Brazil) was added per kilogram of feed, which was also evenly distributed. Thereafter, the feeds were dried at room temperature under ventilation for 24 h. The control diet (0.0%) was prepared using the same procedures, except for the addition of β-glucans.

2.4 Experimental design and performance evaluation

The juvenile fish were distributed in 16 aquariums (n = 10 fish each) following a completely randomized design, comprising four treatments (β-glucans concentrations) with four replicates each. The experimental diets were provided until apparent satiety twice a day at 09:00 and 16:00 h. The amount of feed consumed in each aquarium throughout the experimental period was recorded. During the feeding period, the values of temperature, DO, and pH were 27.51 ± 0.70°C, 9.54 ± 0.83 $mg \cdot L^{-1}$, and 6.94 ± 0.10 (mean ± SD), respectively.

Following 42 days of feeding the diets containing different β-glucans concentrations, biometrics of all fish were obtained after fasting for 24 h to assess growth and other zootechnical parameters. To minimize stress during the measurements and as a prerequisite for subsequent procedures, the fish were anesthetized with benzocaine (0.1 g L^{-1}) and then immobilized with wet towels. All fish were weighed and measured individually to obtain the final weight (g), total length (from the anterior end of the head to the end of the caudal fin) (cm), and standard length (from the anterior end of the head to the beginning of caudal fin insertion) (cm). Based on these data, the following parameters were calculated: weight gain (g): mean final weight −

mean initial weight; weight gain (%): (mean final weight − mean initial weight/mean initial weight) × 100; specific growth rate (% day $^{-1}$): [(ln final weight − ln initial weight)/experimental period (days)] × 100; feed intake (g): amount of feed consumed per aquarium/number of fish; feed conversion ratio: mean feed intake/mean weight gain; protein efficiency ratio: mean weight gain/mean protein intake; and survival rate (%): (final fish number/initial fish number) × 100. The condition factor (CF) was calculated using both total length (TL) [CF (TL) = (final weight/total length^3) × 100)] and standard length (SL) [CF (SL) = (final weight /standard length^3) × 100)], as described in other studies on *Pterophyllum scalare* (Nagata, Takahashi, Gimbo, Kojima, & Biller, 2010; Ribeiro, Preto, & Fernandes, 2008).

2.5 Blood parameter analyses

Following anesthesia administration with benzocaine (0.1 g L^{-1}), blood samples were collected from the caudal vein. For analyses using whole blood, samples were collected with 3 mL syringes containing ethylenediaminetetraacetic acid (EDTA) for preservation (two fish per aquarium and eight fish per treatment). To obtain serum samples, blood was collected using 3 mL syringes without EDTA (pool of blood from three fishes, two pools per aquarium and eight pools per treatment) and centrifuged for 10 min at 1400 ×*g* for serum separation.

Red blood cells (RBC, 10^6 μL^{-1}) were counted using Neubauer chamber following dilution (1:200) in Dacie's solution (Blaxhall & Daisley, 1973). Total hemoglobin concentration (g dL^{-1}) was determined using the hemoglobincyanide method (Collier, 1944) with a commercial kit (Labtest, Lagoa Santa, MG, Brazil). Mean corpuscular hemoglobin (MCH) concentration was also calculated (Ranzani-Paiva, Pádua, Tavares-Dias, & Egami, 2013). Plasma glucose concentration (mg dL^{-1}) was evaluated using a drop of blood introduced on a glucose test strip, and the dosage was determined by the FreeStyle Optium Neo glucometer

(Abbott, Maidenhead, BRK, England) immediately after blood collection. For plasma lactate (mmol L^{-1}), blood samples were centrifuged for 10 min at 1400 ×*g* for plasma separation and the concentration was determined using an enzymatic colorimetric assay (Interkit, Belo Horizonte, MG, Brazil).

Serum lysozyme concentration (μg mL^{-1}) was assessed according to the methodology described by Ellis (1990). Standard solutions of chicken egg lysozyme L6876 (Sigma-Aldrich Chemical Co., Saint Louis, MO, USA) were prepared to generate a standard curve. Subsequently, 90 μL of serum was used to measure the initial and final absorbance using spectrophotometry, and the serum lysozyme activity was determined based on the lysis of the gram-positive bacterium *Micrococcus lysodeikticus* (Sigma-Aldrich Chemical Co., Saint Louis, MO, USA). The reduction in sample absorbance was converted to an estimate of lysozyme concentration (μg mL^{-1}) using the linear equation of the standard lysozyme curve.

Total serum protein (g dL^{-1}) was quantified using a colorimetric method (Analisa, Belo Horizonte, MG, Brazil). Serum albumin (g dL^{-1}) and total cholesterol (mg dL^{-1}) concentrations were measured using enzymatic colorimetric assays (Analisa, Belo Horizonte, MG, Brazil). Total globulin concentration was obtained by subtracting albumin concentration from total protein concentration. Absorbance was measured at 540 nm for lactate, 492 nm for lysozyme, 545 nm for total serum proteins, 630 nm for albumin, and 500 nm for cholesterol, on a Coleman 33D digital spectrophotometer.

2.6 Metagenomic analysis of the intestinal microbiota

For the intestinal microbiota analysis, DNA was extracted from the stool pools of six individuals from the same aquarium (three pools per treatment) at the end of the feeding trial. For sample collection, the fish were euthanized through a medullary section, the ventral surface

of the abdomen was opened, and stool was removed aseptically from the entire intestine and immediately stored at −80°C. For bacterial DNA extraction, the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) was used, and the manufacturer's recommendations were followed. Following extraction, DNA integrity was confirmed using 1% agarose gel electrophoresis.

Subsequently, the DNA samples were sent to NGS Soluções Genômicas (Piracicaba, SP, Brazil) for sequencing (paired-end library) on the Illumina MiSeq platform. For this, primers for the V3–V4 regions containing adapters for Illumina MiSeq sequencing were used for PCR amplification of the 16S rRNA gene. A first PCR (16S rRNA V3-V4) was performed under the following conditions: 95°C for 3 min, followed by 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 5 min. A second PCR was subsequently performed using the index sequences under the following conditions: 95°C for 3 min, followed by 12 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 5 min. The PCRBio Ultra Mix (PCR Biosystems, London, United Kingdom) was used both reactions, and the AMPure XP beads (Beckman Coulter, Brea, CA, USA) were used for purification. The samples were then grouped into sequencing libraries. Amplicons were sequenced on the Illumina MiSeq platform using a paired-end 250-cycles V3 MiSeq reagent kit.

All bioinformatic analyses were performed on Mothur software (v.1.36.1) following the methodologies described by Kozich, Westcott, Baxter, Highlander, & Schloss, (2013) and Schloss et al. (2009), with some modifications. The obtained sequences were aligned with the SILVA database, and homopolymers, nonspecific amplifications, redundancies, and chimeras were removed using VSEARCH algorithm. The sequences were classified into operational taxonomic units (OTUs) for taxonomic comparison. To reduce the bias caused by non-uniform sequence numbers, a subsample of 70,787 reads per sample was created for data normalization,

and the Shannon and Simpson indices were calculated.

2.7 Statistical analysis

For the statistical analysis of productive performance, blood parameters, and diversity indices of gut microbiota, after verifying the homogeneity of the variances and normality of the residues, the data were subjected to the analysis of variance; for parameters that showed significant differences, the means were compared using Duncan's test at a significance level of 5%. When the assumptions of the homogeneity of variances and normality of the residues were not met, the data were subjected to Kruskal–Wallis nonparametric test, and the means compared using the Dunn test at a significance level of 5%. All analyses were performed using R software (R Core Team, 2017).

Analysis of molecular variance (AMOVA) was used for the statistical comparison of the structure of the microbial communities, performed using Mothur software (v.1.36.1). The Metastats tools of Mothur were used to determine the differentially represented OTUs between groups. A Venn diagram was generated to display microbial assemblages common to the four treatments.

3. RESULTS

3.1 Growth parameters

Regarding growth parameters, there were no differences ($P > 0.05$) in final weight, total length, standard length, weight gain (g and %), specific growth rate, feed intake, feed conversion ratio, protein efficiency ratio, and survival rate among the treatments (Table 1).

However, β-glucans supplementation affected the condition factors calculated based on both total [CF (TL)] and standard [CF (SL)] length. The CF (TL) values of fish that received the diet supplemented with 0.2% β-glucans ($P < 0.05$) were higher than those of fish that received the control diet (Table 1). Conversely, the CF (SL) values of fish that received the diet supplemented with 0.2% β-glucans were higher than those of fish that received diets supplemented with the remaining three β-glucans concentrations ($P < 0.05$) (Table 1).

TABLE 1. Growth parameters of *Pterophyllum scalare* juveniles fed diets supplemented with different β-glucans concentrations (mean ± standard deviation).

	β-glucans concentrations			
Parameter	Control	0.05%	0.1%	0.2%
IW (g)	5.29 ± 0.96	5.30 ± 0.95	5.22 ± 0.99	5.24 ± 0.78
FW (g)	9.04 ± 1.76	8.94 ± 1.66	8.77 ± 1.59	9.16 ± 1.48
TL (cm)	7.61 ± 0.46	7.51 ± 0.56	7.45 ± 0.50	7.50 ± 0.47
SL (cm)	5.88 ± 0.36	5.84 ± 0.46	5.79 ± 0.38	5.78 ± 0.35
WG (g)	3.79 ± 0.70	3.65 ± 0.26	3.59 ± 0.85	3.92 ± 0.32
WG (%)	71.42 ± 9.93	69.16 ± 8.13	68.54 ± 13.76	75.00 ± 8.12
SGR	1.26 ± 0.42	1.21 ± 0.48	1.20 ± 0.41	1.30 ± 0.39
FI (g)	4.01 ± 0.30	4.16 ± 0.14	3.97 ± 0.35	4.21 ± 0.13
FCR	1.10 ± 0.08	1.14 ± 0.10	1.16 ± 0.11	1.08 ± 0.06
PER	2.40 ± 0.18	2.31 ± 0.19	2.28 ± 0.23	2.45 ± 0.14
CF (TL)	2.03 ± 0.18^{b}	2.10 ± 0.17^{ab}	2.10 ± 0.18^{ab}	2.16 ± 0.21^{a}
CF (SL)	4.42 ± 0.42^{b}	4.47 ± 0.44^{b}	4.48 ± 0.41^{b}	4.75 ± 0.51^{a}
SR (%)	97.50 ± 5.00	100.00 ± 0.0	97.50 ± 5.00	100.00 ± 0.0

IW: initial weight, FW: final weight, WG: weight gain, FI: feed intake, FCR: feed conversion ratio, TL: total length, SL: standard length, SGR: specific growth rate, CF: condition factor, PER: protein efficiency ratio, SR: survival rate. Different letters in the same row indicate significant differences ($P < 0.05$) among treatments.

3.2 Blood parameters

RBC count and hemoglobin, MCH, lysozyme, total protein, albumin, globulin, total cholesterol, glucose, and lactate concentrations (mean ± SD) are presented in Table 2. After 42 days of feeding, β-glucans supplementation did not affect blood parameters at any concentration ($P > 0.05$).

TABLE 2. Red blood cells (RBC) count and hemoglobin, mean corpuscular hemoglobin (MCH), lysozyme, total proteins, albumin, globulins, total cholesterol, glucose and lactate concentrations (mean ± standard deviation) in the blood samples of *Pterophyllum scalare* juveniles fed diets supplemented with different β-glucans concentrations.

	β-glucans concentrations			
Parameters	Control	0.05%	0.1%	0.2%
RBC ($\times 10^6$ μL^{-1})	1.41 ± 0.45	1.13 ± 0.37	1.63 ± 0.40	1.23 ± 0.32
Hemoglobin (g dL^{-1})	5.17 ± 1.04	4.41 ± 0.82	4.63 ± 1.75	4.62 ± 0.83
MCH (g dL^{-1})	39.24 ± 11.95	40.28 ± 6.56	28.55 ± 4.84	38.73 ± 7.09
Lysozyme concentration (µg mL^{-1})	3.17 ± 1.88	2.99 ± 1.69	3.58 ± 1.48	3.58 ± 0.31
Total serum proteins (g dL^{-1})	4.30 ± 0,69	4.47 ± 0.50	3.96 ± 0.83	4.64 ± 0.44
Serum albumin (g dL^{-1})	1.36 ± 0.57	1.20 ± 0.22	1.10 ± 0.19	1.12 ± 0.22
Globulins (g dL^{-1})	2.95 ± 0.70	3.28 ± 0.41	2.83 ± 0.45	3.57 ± 0.35
Total serum cholesterol (mg dL^{-1})	177.96 ± 42.11	170.84 ± 13.60	177.74 ± 11.40	166.71 ± 22.49
Plasma glucose (mg dL^{-1})	52.86 ± 16.64	47.63 ± 20.74	38.63 ± 8.14	40.13 ± 14.42
Plasma lactate (mmol L^{-1})	2.36 ± 0.76	2.05 ± 0.36	1.95 ± 0.49	1.91 ± 0.71

3.3 Intestinal microbiota

A total of 1,005,662 contigs were generated from the sequence reads. Following quality control, a total of 962,686 contigs were generated and aligned in the SILVA database to obtain

information on OTUs present in the samples. The subsample yielded the coverage higher than 99.9%, indicating good representativeness of the total microbial population. Based on all sequences obtained from the intestinal microbiota of *Pterophyllum scalare* that received diets supplemented with different β-glucans concentrations, 260 genera belonging to 20 phyla were identified. Of these 260, respectively 157, 143, 154, and 194 genera were recorded in samples from the control, 0.05, 0.1, and 0.2% groups. Among the identified phyla, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, and *Bacteroidetes* were the most abundant in samples from all β-glucans groups (Figure 1). At the genus level, *Cetobaterium* was the most abundant in the control, 0.05%, and 0.1% groups, and *Phascolarctobacterium* was the most abundant in the 0.2% group (Figure 2). However, the abundance of *Phascolarctobacterium* (*Firmicutes*) was significantly higher ($P < 0.05$) in all β-glucans groups than in the control group. Moreover, the abundance of Lachnospiraceae_unclassified (*Firmicutes*) differed between the 0.1% and 0.2% β-glucans groups (Table 3).

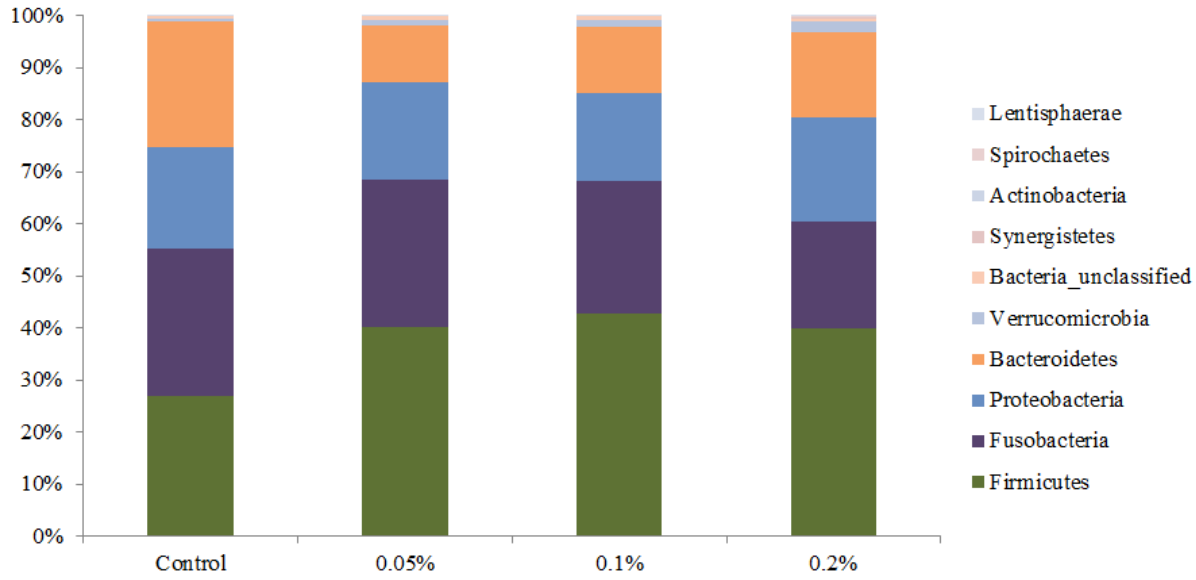

FIGURE 1. Ten most abundant phyla in the intestinal microbiota of *Pterophyllum scalare* juveniles fed diets supplemented with different β-glucans concentrations.

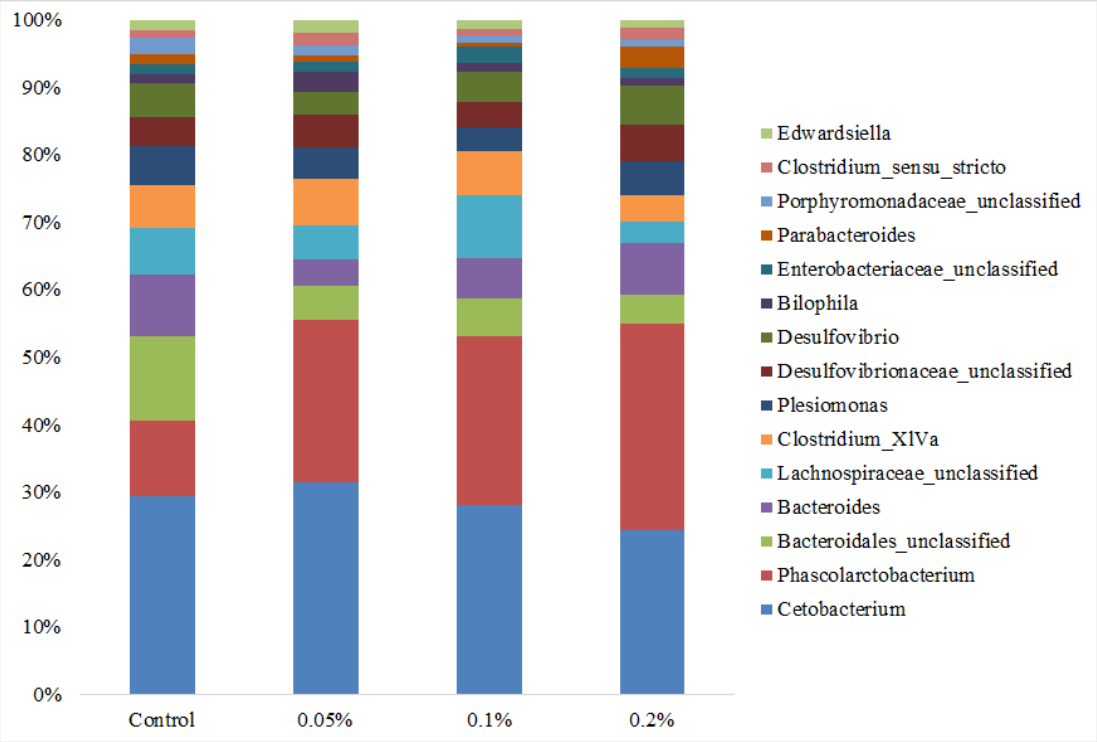

FIGURE 2. Fifteen most abundant genera in the intestinal microbiota of *Pterophyllum scalare* juveniles fed diets supplemented with different β-glucans concentrations.

TABLE 3. Number of sequences for the five most abundant genera in the intestinal microbiota of *Pterophyllum scalare* juveniles fed diets supplemented with different β-glucans concentrations (mean ± standard deviation).

	β-glucans concentrations			
Genus	Control	0.05%	0.1%	0.2%
Cetobacterium (Fusobacteria)	22,103 ± 11,507	21,665 ± 2,313	18,961 ± 7,737	16,482 ± 7,275
Phascolarctobacterium (Firmicutes)	8,436 ± 6,771[b]	16,687 ± 1,489[a]	16,922 ± 1,185[a]	20,541 ± 6,506[a]
Bacteroidales_unclassified (Bacteroidetes)	9,406 ± 8,756	3,443 ± 323	3,760 ± 4,190	2,863 ± 979
Bacteroides (Bacteroidetes)	6,965 ± 8,444	2,668 ± 348	3,992 ± 2,446	5,134 ± 3,383
Lachnospiraceae_unclassified (Firmicutes)	5,156 ±	3,429 ±	6,354 ±	2,181 ±

	2,387[ab]	1,904[ab]	2,262[a]	357[b]

Different letters in the same row indicate significant differences (P < 0.05) among treatments.

The Shannon and Simpson indices did not differ among the groups (Table 4). However, the observed species richness (Sobs) significantly differed among the treatments, with a higher value in the 0.2% β-glucans group than in the other groups (Table 4). The rarefaction curve (Figure 3) demonstrated that the composition of the microbial community in fish that received the diet supplemented with 0.2% β-glucans was different from that in fish that received other diets. The Venn diagram (Figure 4) demonstrated that the number of OTUs shared between the groups was similar, although there was a greater overlap between the 0.2% and the other groups. Furthermore, the number of exclusive OTUs was higher for the 0.2% β-glucans group than for the other groups.

TABLE 4. Shannon index, Simpson index, and observed species richness (Sobs) of the gut microbiota of *Pterophyllum scalare* juveniles fed diets supplemented with different β-glucans concentrations (mean ± standard deviation).

	Control	0.05%	0.1%	0.2%
Shannon	2.53 ± 0.32	2.57 ± 0.11	2.59 ± 0.17	2.70 ± 0.23
Simpson	0.14 ± 0.06	0.14 ± 0.03	0.14 ± 0.03	0.13 ± 0.04
Sobs	107.33b ± 6.03	107.33b ± 7.57	107.00b ± 10.39	134.33a ± 15.50

Different letters in the same row indicate significant differences (P < 0.05) among treatments.

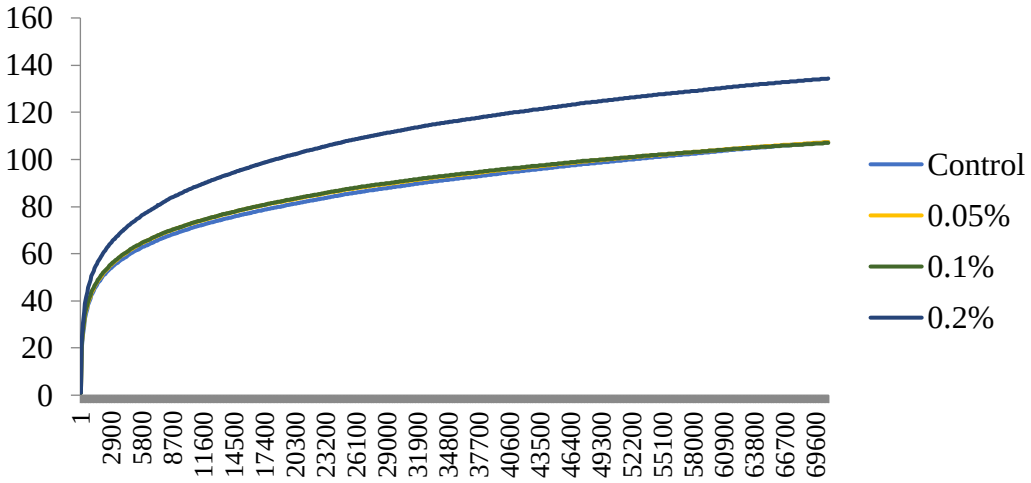

FIGURE 3. Rarefaction curve for each β-glucans concentration presenting the number of reads (x-axis) relative to the number of operational taxonomic units (OTUs) (y-axis).

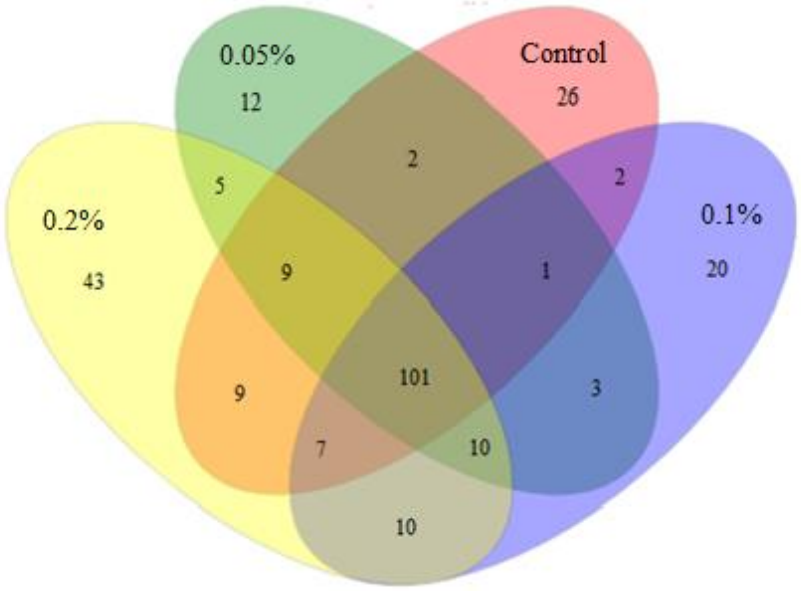

FIGURE 4. Venn diagram showing the overlap between operational taxonomic units (OTUs) for the intestinal microbiota of *Pterophyllum scalare* juveniles fed diets supplemented with different β-glucans concentrations.

4. DISCUSSION

4.1 Effects of β-glucans on growth parameters

Among the evaluated growth parameters, only CF was affected by β-glucans supplementation. Previously, the effects of dietary *S. cerevisiae* β-glucans on CF increase have already been reported in some fish species, such as pompano (*Trachinotus ovatus*) (Do-Huu, 2020), snakehead (*Channa striata*) (Munir, Hashim, Manaf, & Nor, 2016), Nile tilapia (*Oreochromis niloticus*) (Liranço et al., 2013), and Caspian trout (*Salmo trutta caspius*) (Jami, Kenari, Paknejad, & Mohseni, 2019). Therefore, CF can be applied as an indirect measure of energy reserves (Camara, Caramaschi, & Petry, 2011), indicating the significance of the observed effect. For a given length (higher CF), a fish with a greater weight is considered healthier than the one with a lower weight, since the extra weight indicates extra energy reserves, allowing less susceptibility to environmental stressors (Morado, Araújo, & Gomes, 2017). Thus, CF can be used as an indicator of fish welfare as it can offer information on chronic stresses, diseases, water contamination, and nutritional status (Lemos et al., 2015; Rocha et al., 2005). Previous studies have already demonstrated the efficiency of CF as an indicator of food availability (Morado et al., 2017), proper feed types (Takahashi, Silva, Fernandes, Biller, & Sandre, 2010), and proper diet composition (Ighwela, Ahmed, & Abol-Munafi, 2011). Therefore, despite the lack of effects on other growth parameters, higher CF values in the 0.2% group indicates a better nutritional status of fish that received this diet.

Furthermore, the positive effects of dietary *S. cerevisiae* β-glucan supplementation for 42 days on growth parameters other than CF have already been reported. For instance, in a study by Aramli, Kamangar, and Nazari (2015), supplementation with 0.1%, 0.2%, and 0.3% β-glucans improved the FW and SGR of Persian sturgeon (*Acipenser persicus*) juveniles, with

the highest values recorded in the 0.2% group. In another study by Ji et al. (2017), the WG and SGR of rainbow trouts (*Oncorhynchus mykiss*) receiving diets supplemented with 0.1% and 0.2% β-glucans were higher than those of fish receiving the control diet and the diet supplemented with 0.05% β-glucans, with the highest values recorded in the 0.2% group. Additionally, Guzmán-Villanueva, Ascencio-Valle, Macías-Rodríguez, and Tovar-Ramírez (2014) reported that in Pacific red snapper (*Lutjanus peru*) juveniles, supplementation with 0.1% and 0.2% β-glucans increased WG and SGR and supplementation with 0.1% β-glucans also improved FW. As the present study used the same feeding duration and β-glucans concentrations as the previous studies, the effects of β-glucans may be species-specific. In the culture of most ornamental fish species, including angelfish, the target of selection is not growth, as in the culture of fish used for human consumption, which was likely reflected in less intense growth and less evident effects on most parameters. Furthermore, studies evaluating the effects of dietary inclusion of *S. cerevisiae* β-glucans in Nile tilapia (Liranço et al., 2013; Welker et al., 2012) and pompano (Do-Huu, Sang, & Thuy, 2016) have demonstrated changes in growth performance during the supplementation period. Thus, additional studies on angelfish involving evaluations during the feeding period and experiments over longer durations are warranted to demonstrate the efficiency of β-glucans in improving other performance parameters.

4.2 Effect of β-glucans on hematological, immunological, and biochemical parameters

Previous studies have shown that the source, concentration, and period of β-glucans supplementation (Aramli et al., 2015; El-Boshy, El-Ashram, Abdel Hamid, & Gadalla, 2010; Welker et al., 2012) determine the presence of effects on blood parameters. In the present study, MacroGard®, a commercial product extracted from *S. cerevisiae* containing a minimum of 60%

β-1,3/1,6- glucans, was the source used. Some studies using the same supplementation concentrations as the present study have demonstrated the effect of dietary yeast β-glucans on the modulation of RBC counts and hemoglobin, lysozyme, total protein, albumin, globulin, total cholesterol, and glucose concentrations (Cao et al., 2019; Ghaedi, Keyvanshokooh, Azarm, & Akhlaghi, 2015; Montoya, Favero, Zanuzzo, & Urbinati, 2018; Talpur et al., 2014). Meanwhile, some studies have also reported the lack of effects on hematological, immunological, and biochemical blood parameters at the same concentrations (Cao et al., 2019; Del Rio-Zaragoza, Fajer-Ávila, & Almazán-Rueda, 2011; Kühlwein, Merrifield, Rawling, Foey, & Davies, 2014; Siwicki et al., 2010; Welker et al., 2012).

Overall, the effects of different β-glucans concentrations vary widely, likely depending on the duration of supplementation. As such, variations in effects on RBC count and lysozyme activity, hemoglobin, serum total protein, globulin, albumin, and glucose over the supplementation period have been reported (Amphan, Unajak, Printrakoon, & Areechon, 2019; Liranço et al., 2013; Misra, Das, Mukherjee, & Pattnaik, 2006; Sánchez-Martínez et al., 2017). Therefore, such effects could have been verified in the present study if blood samples were also collected during the supplementation period. However, this was not possible because of the small volume of blood in *Pterophyllum scalare* juveniles, which allows only a single collection.

4.3 Effects of β-glucans on intestinal microbiota

Our results demonstrated the efficiency of the dietary inclusion of *S. cerevisiae* β-glucans in modulating the intestinal microbiota composition by shaping the dominance of certain taxa and diversity of bacterial populations; our findings are consistent with previous reports (Carda-Diéguez et al., 2014; Harris et al., 2020; Jung-Schroers et al., 2016; Jung-Schroers et al., 2019). In the present study, *Firmicutes*, *Fusobacteria*, *Proteobacteria* and

Bacteroidetes were observed to be the dominant phyla in *Pterophyllum scalare*, similar to the reports in Nile tilapia (*Oreochromis niloticus*) (Souza et al., 2020a,b), discus fish (*Symphysodon haraldi*) (Zhang, Wen, Meng, Gao, & Chen, 2021), and several African cichlids (Baldo, Riera, Tooming-Klunderud, Albà, & Salzburger, 2015). For instance, in a study by Baldo, Riera, Salzburger, and Barluenga (2019), *Proteobacteria*, *Fusobacteria*, *Firmicutes*, *Bacteroidetes*, and *Planctomycetes* constituted the core microbiota (taxonomic components shared by at least 90% of the individuals) of several African and Central American cichlid species. In the present study, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, and *Bacteroidetes* together accounted for 97.93% of the total reads obtained. At the genus level, *Cetobacterium* (Fusobacteria) was the most abundant in the control, 0.05% β-glucan, and 0.1% β-glucans groups. Similarly, *Cetobacterium* was one of the most abundant genera in other omnivorous cichlids, such as *Oreochromis niloticus* and *Astatotilapia burtoni* (Faber-Hammond et al., 2019; Souza et al., 2020a,b), as well as other cichlids from the Amazon basin, such as *Symphysodon haraldi* (Zhang et al., 2021). Although bacteria from the gastrointestinal tract of *Pterophyllum scalare* have already been isolated and identified (Monroy-Dosta et al., 2012; Ramirez & Dixon, 2003), no study has evaluated the composition of intestinal microbiota in this species. Thus, the present study is the first to report the dominance of these taxa in *Pterophyllum scalare*; nonetheless, further studies are required to consolidate all information.

Some beneficial intestinal microbes use indigestible substances, generating metabolites that can be used as the energy sources by fish (Yukgehnaish et al., 2020). In the present study, Bacteroidales_unclassified (*Bacteroidetes*) and *Bacteroides* (*Bacteroidetes*) were some of the most abundant taxa. Bacteria from the phylum *Bacteroidetes* possess an excellent polysaccharide degradation capacity, producing several enzymes for the breakdown of various glycans, including yeast β-glucans (Lapébie, Lombard, Drula, Terrapon, & Henrissat, 2019; Temple et al., 2017). In addition to *Bacteroides*, *Parabacteroides* (*Bacteroidetes*) and

Phascolarctobacterium (*Firmicutes*) were also some of the most abundant genera in the present study. *Bacteroides* and *Parabacteroides* are among the major succinate producers (Wu et al., 2017), while *Phascolarctobacterium* spp. use succinate as an energy source (Tran et al., 2020; Watanabe, Nagai, & Morotomi, 2012; Wu et al., 2017). Therefore, the coexistence of *Bacteroides* and *Phascolarctobaterium* may be beneficial for both taxa (Ikeyama et al., 2020). Thus, following supplementation, the degradation of β-glucans by bacteria of the phylum *Bacteroidetes* likely created conditions suitable for a significant increase in the abundance of *Phascolarctobacterium*.

Phascolarctobacterium has been detected in some studies evaluating the intestinal microbiota of fish, albeit not as one of the most abundant taxa (Bao et al., 2020; Basili et al., 2020; Meng et al., 2018). In the present study, *Phascolarctobacterium* was abundant in the intestine of fish fed the β-glucans-supplemented diets, possibly characterizing it as a genus forming the core microbiota of the species. To the best of our knowledge, the present study is the first to record *Phascolarctobacterium* as one of the most abundant genera in the intestinal microbiota of fish. The intestinal microbiome may be shaped by various factors, such as host genetics and intestinal physiology as well as the symbiotic relationships among the gut bacteria themselves (Tarnecki, Burgos, Ray, & Arias, 2017). Such symbiotic relationships may explain the abundance of specific taxa in *Pterophyllum scalare* but not in other species. The presence of bacteria of the genus *Phascolarctobacterium* has been linked to the decrease in the body weight of zebrafish (*Danio rerio*) exposed to the fungicide carbendazim (Bao et al., 2020) as well as to lipid metabolism in common carp (*Cyprinus carpio*) exposed to copper (Meng et al., 2018). However, further studies are required to verify these relationships in fish under normal non-stressful conditions. Simultaneously, bacteria of the *Lachnospiraceae* family have been implicated in increased blood glucose levels, decreased plasma insulin levels, and increased liver and mesenteric adipose tissue weights in mice genetically predisposed to obesity

(Kameyama & Itoh, 2014). However, the lower proportion of these bacteria in fish fed the diet supplemented with 0.2% β-glucans than in those fed the diet supplemented with 0.1% β-glucans in the present study presented no link with any of the evaluated parameters.

As mentioned earlier, the supplementation of 0.2% β-glucans increased CF. There is evidence that CF is a reliable measure for estimating energy reserves in juveniles which store energy as proteins (Schloesser & Fabrizio, 2017). Specifically, Munir et al. (2016) observed in snakehead juveniles an increase in CF accompanied by an increase in proteins and a decrease in body lipids in fish fed diets supplemented with *S. cerevisiae* β-glucans. However, as the body composition was not evaluated in the present study, we could not determine whether the increase in CF was a result of lipid or protein accumulation. Thus, the data generated in the present study do not demonstrate any association between intestinal microbiota and energy metabolism in *Pterophyllum scalare*. Owing to their proximity to humans, ornamental fish, similar to other pets, can live longer but are at a greater risk of obesity (Sicuro, 2018). Therefore, further studies are warranted to better understand the involvement of intestinal microbiota in energy metabolism in ornamental fish; this information can be useful to optimize the quality of life of these fish.

Furthermore, compared with fish receiving the other diets, fish receiving the diet supplemented with 0.2% β-glucans showed a higher observed species richness (Sobs), suggesting better conditions for the development of a greater number of taxa. Consistently, the rarefaction curve, which is the representation of species richness plotted against the number of sequences (species density) (Dias & Bonaldo, 2012), demonstrated differences in intestinal microbiota between fish fed the diet supplemented with 0.2% β-glucans and those fed the other diets. Similarly, the Venn diagram showed differences in gut microbial composition between fish fed the diet supplemented with 0.2% β-glucans and those fed the other diets. These results demonstrated that only the highest of the tested concentrations of β-glucans could modulate the

intestinal microbiota of *Pterophyllum scalare* in a more complex manner. In a study by Jung-Schroers et al. (2016), common carps fed diets containing β-glucans exhibited increased bacterial diversity and decreased *Vibrio* spp. abundance in the intestine; according to the authors, a more diverse microflora possesses a greater ability to exclude pathogenic bacteria through competition for adhesion sites and nutrients. Therefore, changes in the composition of intestinal microbiota may be related to conditions that are more advantageous for certain taxa and indirectly affect other taxa. Thus, the dietary supplementation of 0.2% β-glucans promoted the formation of a distinct bacterial community by benefiting certain taxa while indirectly harming or benefiting other bacteria. To our best knowledge, the present study is the first to evaluate and record the intestinal microbiota of *Pterophyllum scalare*. Additional research is warranted to better understand gut microbial composition in this species. Additionally, the effects of β-glucans on gut microbes must be further elucidated.

5. CONCLUSION

Under the experimental conditions of the present study, the dietary supplementation of 0.2% β-glucans increased the CF and positively modulated the intestinal microbiota of juvenile angelfish, without affecting other performance parameters and blood parameters.

ACKNOWLEDGMENTS

The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES) and the Araucária Foundation for financial support during the study. The authors also thank Biorigin, Brazil, for providing β-glucan used in the experiment (MacroGard®). Finally, we thank Nutricon for donating the feed used in the experiment.

DATA AVAILABILITY STATEMENT

All data are available in the manuscript.

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8 CONSIDERAÇÕES FINAIS

Na presente revisão sistemática de literatura foi demonstrada a eficiência da inclusão de β-glucanas de leveduras na dieta de peixes em otimizar o crescimento, aproveitamento da dieta e composição corporal. Foi também observado que a melhoria do processo de digestão e absorção, bem como a modulação da microbiota intestinal, podem ser algumas das causas para a melhora do desempenho produtivo. Outro aspecto verificado foi que os efeitos podem variar de acordo com as espécies de peixes, quantidades de β-glucanas empregadas e durações da suplementação. Apesar do grande volume de dados obtido, existem certos aspectos dos efeitos da suplementação dietética de β-glucanas de leveduras que precisam ser melhor compreendidos. Dessa forma, os dados aqui compilados podem servir para orientar futuros estudos, e além disso, para possibilitar o desenvolvimento de protocolos que permitam a aplicação dessas informações na piscicultura comercial.

Com base nos resultados obtidos foi possível afirmar que a suplementação dietética de 0,2% de β-glucanas de *S. cerevisiae* foi eficiente em influenciar positivamente o fator de condição e a microbiota intestinal em juvenis de *Pterophyllum scalare*, com efeitos de todas as concentrações de β-glucanas sobre o aumento de bactérias do gênero *Phascolarctobacterium*. Contudo, novos estudos são necessários para averiguar os efeitos sobre os demais parâmetros de crescimento e sobre os parâmetros hematológicos, imunológicos e bioquímicos do sangue, tendo como estratégias a utilização de diferentes períodos de administração ou amostragens no decorrer do período experimental. A utilização dessas estratégias aumentaria a probabilidade de obtenção de efeitos para os demais parâmetros avaliados.