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ANIMAL SCIENCE

Effects of dietary β-glucans on the productive performance, blood parameters, and intestinal microbiota of angelfish (*Pterophyllum scalare*) juveniles

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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.

Key words: Freshwater angelfish, Immunostimulants, Ornamental fish, Phascolarctobacterium, Prebiotics, *Saccharomyces cerevisiae*.

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 et al. 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 et al. 2016, Ikeda et al. 2011, Fujimoto et al. 2006, Ribeiro et al. 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 et al. 2009) and injection (Rodríguez et al. 2009, Selvaraj et al. 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 et al. 2011, Russo et al. 2006, Türnal et al. 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 et al. 2013, Talpur et al. 2014, Welker et al. 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 et al. 2014, Harris et al. 2020, Jung-Schroers et al. 2016), including ornamental species (Jung-Schroers et al. 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 dietary β-glucans supplementation in *Pterophyllum scalare*. There is only one study evaluating the effectiveness of β-glucans in *P. scalare* larvae cultivation water (Sushila et al. 2022). 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.

MATERIALS AND METHODS

Animals and experimental conditions

The procedures performed in the present study were approved by the Ethics Committee on the Use of Animals at the Universidade Estadual de Londrina (CEUA/UEL) (protocol CEUA no. 13903.2018.86).

The experiment was performed in the laboratory of the Núcleo de Estudos e Pesquisa em Aquicultura e Genética (NEPAG) at the Universidade Estadual de 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 using an oximeter (Hanna Instruments, Barueri, SP, Brazil) and a pH meter (Akso, São Leopoldo, RS, Brazil). Total ammonia levels were measured three times a week with a colorimetric kit (Labcon Tests, Camboriu, Brazil). During the experimental period, the values of temperature, DO, and pH were 27.51 ± 0.70°C, 9.54 ± 0.83 mg·L^{-1}, and 6.94 ± 0.10 (mean ± standard deviation), respectively. Total ammonia levels ranged from 0.0 to 0.25 mg L^{-1}. 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 ± standard deviation: 5.28 ± 0.91 g).

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 (sugar-cane yeast, spirulina, yeast extract, multienzyme additive, prebiotic, canthaxanthin, and yucca extract) were removed from the feed formulation and manufacturing process by the manufacturer at the request of our research group. 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 MacroGard® 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 MacroGard®. The concentrations of MacroGard® used were chosen because research using these concentrations has already obtained positive effects on productive performance, hematological, immunological, blood biochemical, and intestinal microbiota parameters (Aramli et al. 2015, Do-Huu et al. 2016, Ghaedi et al. 2015, Harris et al. 2020, Talpur et al. 2014). The fixation of the additive in the feed was carried out based on the methodologies proposed by Furlan-Murari et al. (2022) and Siwicki et al. (2015).

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.

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}) (Souza et

al. 2020a) 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 mean final weight – ln mean initial weight)/experimental period (days)] × 100; feed intake (g): amount of feed consumed per aquarium/number of fish; feed conversion ratio: feed intake (g)/weight gain (g); protein efficiency ratio: weight gain (g)/ protein intake (g); 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 et al. 2010, Ribeiro et al. 2008).

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 et al. 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) (Barham & Trinder 1972, Shimojo et al. 1989, Trinder 1969).

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) (Gornall et al. 1949). Serum albumin (g dL^{-1}) and total cholesterol (mg dL^{-1}) concentrations were measured using enzymatic colorimetric assays (Analisa, Belo Horizonte, MG, Brazil) (Allain et al. 1974, Doumas et al. 1971). 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.

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. Stool collection was performed according to Suphoronski et al. (2019). 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 et al. (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.

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 (Kruskal & Wallis 1952), 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.

RESULTS

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 I). 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 I). 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 control diet and diets supplemented with the other concentrations of β-glucans ($P < 0.05$) (Table I).

Blood parameters

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

Table I. 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%**	**P-value**
IW (g)	5.29 ± 0.96	5.30 ± 0.95	5.22 ± 0.99	5.24 ± 0.78	0.9767
FW (g)	9.04 ± 1.76	8.94 ± 1.66	8.77 ± 1.59	9.16 ± 1.48	0.6146
TL (cm)	7.61 ± 0.46	7.51 ± 0.56	7.45 ± 0.50	7.50 ± 0.47	0.5694
SL (cm)	5.88 ± 0.36	5.84 ± 0.46	5.79 ± 0.38	5.78 ± 0.35	0.6496
WG (g)	3.79 ± 0.70	3.65 ± 0.26	3.59 ± 0.85	3.92 ± 0.32	0.8598
WG (%)	71.42 ± 9.93	69.16 ± 8.13	68.54 ± 13.76	75.00 ± 8.12	0.8068
SGR	1.28 ± 0.14	1.25 ± 0.11	1.24 ± 0.19	1.33 ± 0.11	0.6780
FI (g)	4.01 ± 0.30	4.16 ± 0.14	3.97 ± 0.35	4.21 ± 0.13	0.4996
FCR	1.10 ± 0.08	1.14 ± 0.10	1.16 ± 0.11	1.08 ± 0.06	0.5572
PER	2.40 ± 0.18	2.31 ± 0.19	2.28 ± 0.23	2.45 ± 0.14	0.5813
CF (TL)	2.03 ± 0.18^{b}	2.10 ± 0.17^{ab}	2.10 ± 0.18^{ab}	2.16 ± 0.21^{a}	0.0244
CF (SL)	4.42 ± 0.42^{b}	4.47 ± 0.44^{b}	4.48 ± 0.41^{b}	4.75 ± 0.51^{a}	0.0057
SR (%)	97.50 ± 5.00	100.00 ± 0.0	97.50 ± 5.00	100.00 ± 0.0	0.5433

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

Table II. 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%**	**P-value**
RBC ($\times 10^6$ μL^{-1})	1.41 ± 0.45	1.13 ± 0.37	1.63 ± 0.40	1.23 ± 0.32	0.0725
Hemoglobin (g dL^{-1})	5.17 ± 1.04	4.41 ± 0.82	4.63 ± 1.75	4.62 ± 0.83	0.6161
MCH (g dL^{-1})	39.24 ± 11.95	40.28 ± 6.56	28.55 ± 4.84	38.73 ± 7.09	0.1927
Lysozyme concentration (µg mL^{-1})	3.17 ± 1.88	2.99 ± 1.69	3.58 ± 1.48	3.58 ± 0.31	0.9173
Total serum proteins (g dL^{-1})	4.30 ± 0,69	4.47 ± 0.50	3.96 ± 0.83	4.64 ± 0.44	0.2151
Serum albumin (g dL^{-1})	1.36 ± 0.57	1.20 ± 0.22	1.10 ± 0.19	1.12 ± 0.22	0.6546
Globulins (g dL^{-1})	2.95 ± 0.70	3.28 ± 0.41	2.83 ± 0.45	3.57 ± 0.35	0.2365
Total serum cholesterol (mg dL^{-1})	177.96 ± 42.11	170.84 ± 13.60	177.74 ± 11.40	166.71 ± 22.49	0.9189
Plasma glucose (mg dL^{-1})	52.86 ± 16.64	47.63 ± 20.74	38.63 ± 8.14	40.13 ± 14.42	0.2759
Plasma lactate (mmol L^{-1})	2.36 ± 0.76	2.05 ± 0.36	1.95 ± 0.49	1.91 ± 0.71	0.4808

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 (Table III). Moreover, the abundance of Lachnospiraceae_unclassified (*Firmicutes*) differed between the 0.1% and 0.2% β-glucans groups (Table III).

The Shannon and Simpson indices did not differ among the groups (Table IV). 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 IV). 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.

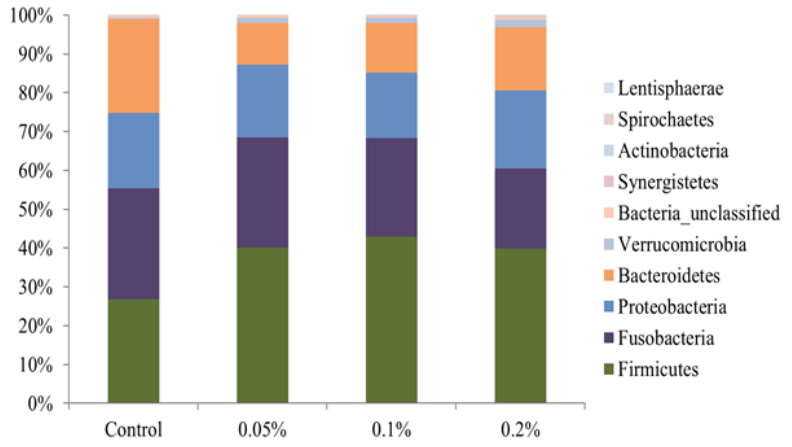

Figure 1. Ten most abundant phyla in the intestinal microbiota of *Pterophyllum scalare* juveniles fed diets supplemented with different β-glucans concentrations.

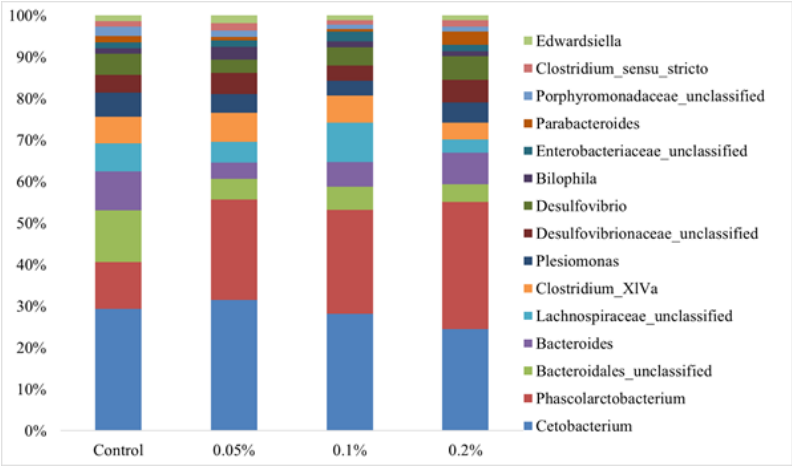

Figure 2. Fifteen most abundant genera in the intestinal microbiota of *Pterophyllum scalare* juveniles fed diets supplemented with different β-glucans concentrations.

Table III. 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 ± 2,387[ab]	3,429 ± 1,904[ab]	6,354 ± 2,262[a]	2,181 ± 357[b]

Different letters in the same row indicate significant differences (P < 0.05) among treatments. P-value generated by Metastats for the comparison between different diets for each of the five most abundant genera: Cetobacterium: Control x 0.05%: 0.8158; Control x 0.1%: 0.8908; Control x 0.2%: 0.6833; 0.05% x 0.1%: 0.5808; 0.05% x 0.2%: 0.2481; 0.1% x 0.2%: 0.7403. Phascolarctobacterium: Control x 0.05%: 0.0439; Control x 0.1%: 0.0416; Control x 0.2%: 0.0363; 0.05% x 0.1%: 0.9262; 0.05% x 0.2%: 0.5596; 0.1% x 0.2%: 0.5683. Bacteroidales_unclassified: Control x 0.05%: 0.3126; Control x 0.1%: 0.5234; Control x 0.2%: 0.2326; 0.05% x 0.1%: 0.9012; 0.05% x 0.2%: 0.3400; 0.1% x 0.2%: 0.7611. Bacteroides: Control x 0.05%: 0.5088; Control x 0.1%: 0.7416; Control x 0.2%: 0.8307; 0.05% x 0.1%: 0.5168; 0.05% x 0.2%: 0.2855; 0.1% x 0.2%: 0.8190. Lachnospiraceae_unclassified: Control x 0.05%: 0.4911; Control x 0.1%: 0.5908; Control x 0.2%: 0.1045; 0.05% x 0.1%: 0.0662; 0.05% x 0.2%: 0.2161; 0.1% x 0.2%: 0.0117.

Table IV. 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%	P-value
Shannon	2.53 ± 0.32	2.57 ± 0.11	2.59 ± 0.17	2.70 ± 0.23	0.8176
Simpson	0.14 ± 0.06	0.14 ± 0.03	0.14 ± 0.03	0.13 ± 0.04	0.9890
Sobs	107.33 ± 6.03[b]	107.33 ± 7.57[b]	107.00 ± 10.39[b]	134.33 ± 15.50[a]	0.0307

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

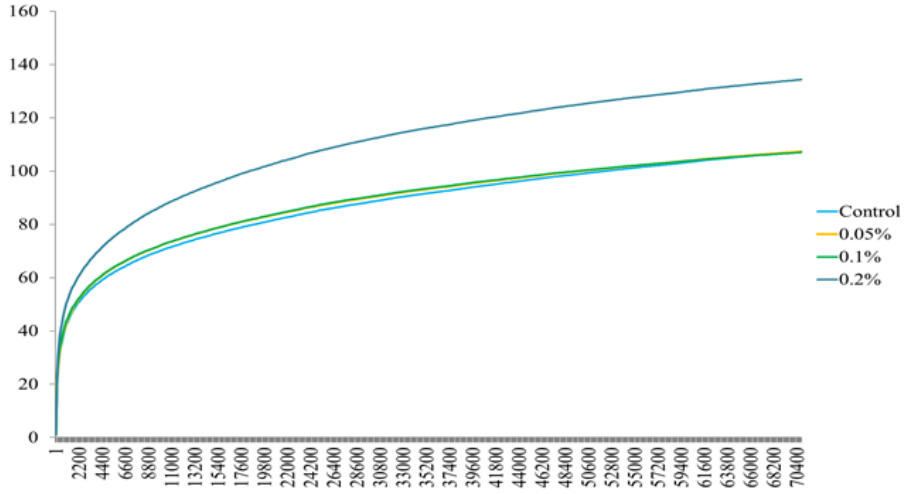

Figure 3. Rarefaction curve for each β-glucans concentration presenting the number of reads (x-axis) relative to the number of operational taxonomic units (OTUs) (y-axis).

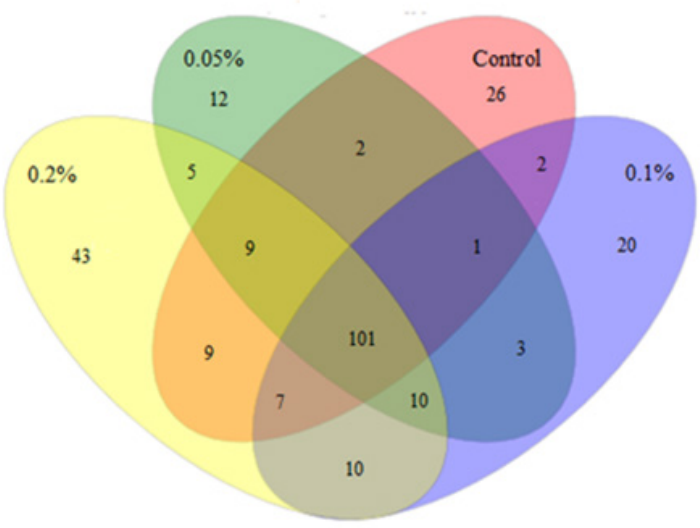

Figure 4. Venn diagram showing the overlap between operational taxonomic units (OTUs) for the intestinal microbiota of *Pterophyllum scalare* juveniles fed diets supplemented with different β-glucans concentrations.

DISCUSSION

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 et al. 2016), Nile tilapia (*Oreochromis niloticus*) (Lirança et al. 2013), and

Caspian trout (*Salmo trutta caspius*) (Jami et al. 2019). Therefore, CF can be applied as an indirect measure of energy reserves (Camara et al. 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 et al. 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 et al. 2010), and proper diet composition (Ighwela et al. 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 et al. (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 et al. (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 et al. 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.

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 et al. 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 et al. 2015, Montoya et al. 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 et al. 2011, Kühlwein et al. 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. In this regard, some studies demonstrate that these effects may vary over the experimental period. Among them, Sánchez-Martínez et al. (2017) observed for channel catfish (*Ictalurus punctatus*) the effect of β-glucans on reducing RBC counts during the five weeks of feeding. Amphan et al. (2019), observed the effects of supplementation on lysozyme activity in Nile tilapia (*O. niloticus*) in the first, second and third weeks of feeding, which did not occur in the following five weeks. Also for Nile tilapia, Liranço et al. (2013) found that the administration of β-glucans provided higher hemoglobin concentrations at 30 and 90 days compared to 60 days of feeding. For Rohu (*Labeo rohita*), Misra et al. (2006) observed a large variation in the influence of supplementation throughout the feeding period, with effects on total serum protein and globulin at 28 and 42 days; on albumin at 14, 28 and 42 days; and on glucose at 14, 28, 42 and 56 days. Such evidence demonstrates that the duration of supplementation is an extremely important factor when using β-glucans as a feed additive for fish. 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.

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, 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 et al. 2021), and several African cichlids (Baldo et al. 2015). For instance, in a study by Baldo et al. (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* (Souza et al. 2020a,b) and *Astatotilapia burtoni* (Faber-Hammond et al. 2019), 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 et al. 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 et al. 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 et al. 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.

CONCLUSIONS

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.

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