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Probiotic in diets for Nile tilapia (Oreochromis niloticus) fingerlings

Probiótico em dietas para alevinos de tilápia do Nilo (*Oreochromis niloticus)*

Probioticos en las dietas de los alevinos Nilo (*Oreochromis niloticus)*

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Abstract
The objective was to evaluate the effects of the probiotics Bacillus subtilis and Bacillus cereus (4x1011 CFU kg−1) (BSC) in diets for Nile tilapia fingerlings. A total of 450 fish (average weight of 2.81 ± 0.06 g) were distributed in a completely randomized design, in 15 aquariums of 210 liters, consisting of five treatments and three replications. The isoproteic and isoenergetic experimental rations were formulated as follows: BSC1: 0% (control); BSC2: 0.6%; BSC3: 0.8%; BSC4: 1.0%; BSC5: 2.0% of probiotics per kg of feed at a concentration of 4x1011 CFU kg−1. The animals were fed the experimental diets for 30 days. At the end of the study, the productive performance was determined, and bromatological and histological analyzes of the fish were carried out. It was concluded that the probiotic did not influence the zootechnical performance of Nile tilapia fingerlings. The use of 0.8% of BSC appeared as a dietary supplementation strategy for Nile tilapia fingerlings, due to the efficacies found in the main results of body centesimal composition and in adaptations in the histomorphometry of the intestinal villi.
Keywords: Aquaculture; Microbiota; Intestinal permeability.

Resumo
Objetivou-se avaliar os efeitos dos probióticos Bacillus subtilis e Bacillus cereus (4x1011 UFC Kg-1) (BSC) em dietas para alevinos de tilápia do Nilo. Foram distribuídos 450 peixes (peso médio de 2,81±0,06 g) em um delineamento inteiramente casualizado, em 15 aquários de 210 litros, composto por cinco tratamentos e três repetições. As rações experimentais isoproteicas e isoenergéticas foram formuladas da seguinte maneira: BSC1: 0% (controle); BSC2: 0,6%; BSC3: 0,8%; BSC4: 1,0%; BSC5: 2,0% de probióticos por kg de ração na concentração de 4x1011 UFC Kg-1. Os animais foram alimentados com as dietas experimentais por 30 dias. Ao final do estudo, determinou-se o desempenho produtivo, realizou-se análises bromatológicas e histológicas dos peixes. Conclui-se que o probiótico não influenciou no desempenho zootécnico de alevinos de tilápia do Nilo. A utilização 0,8% de BSC surge como estratégia de suplementação dietética para alevinos de tilápia do Nilo, devido as eficácias encontradas nos

principais resultados de composição centesimal corpórea e em adaptações na histomorfometria das vilosidades intestinais.
Palavras-chave: Aquicultura; Microbiota; Permeabilidade intestinal.

Resumen

El objetivo era evaluar los efectos de los probióticos Bacillus subtilis y Bacillus cereus (4x1011 UFC Kg-1) (BSC) en dietas para alevinos de tilapia del Nilo. Un total de 450 peces (peso medio 2,81±0,06 g) se distribuyeron en un diseño totalmente aleatorizado, en 15 tanques de 210 litros, compuestos por cinco tratamientos y tres réplicas. Las dietas experimentales isoproteicas e isoenergéticas se formularon como sigue: BSC1: 0% (control); BSC2: 0,6%; BSC3: 0,8%; BSC4: 1,0%; BSC5: 2,0% de probióticos por kg de alimento a una concentración de 4x1011 UFC Kg-1. Los animales fueron alimentados con las dietas experimentales durante 30 días. Al final del estudio se determinó el rendimiento productivo y se realizaron análisis bromatológicos e histológicos de los peces. Se concluyó que el probiótico no influyó en el rendimiento zootécnico de los alevinos de tilapia del Nilo. El uso de 0,8% de BSC aparece como una estrategia de suplementación dietética para alevinos de tilapia del Nilo, debido a las eficacias encontradas en los principales resultados de composición centesimal corporal y en las adaptaciones en la histomorfometría de las vellosidades intestinales.
Palabras clave: Acuicultura; Microbiota; Permeabilidad intestinal.

1. Introduction

Aquaculture is the fastest growing animal protein sector to meet the demands of the world population (DOAN et al., 2019). Allied to its high growth, there are intensifications in the production process and, with this, significant impacts on the breeding environment have been observed (Martínez Cruz et al., 2012). As a consequence, high outbreaks of infectious diseases that adversely affect the sustainability of this industry (Mardones et al., 2018) have become recurrent. These inappropriate factors arise due to high densities of fish per unit area practiced, stress resulting from the management, nutritional failures, and low water quality, leading to the loss of homeostasis of the animals (Favero et al., 2019), favoring the opportunistic pathogenic organisms.

So, to face these adversities, the aquaculture industry becomes heavily dependent on the use of antibiotics and chemotherapeutics in the control and prevention of diseases (Fe Ckaninova et al., 2017; Romero et al., 2012). However, excess of these chemicals has led to the rapid spread of drug-resistant pathogens in aquaculture environments and residual antibiotics in aquatic products (Liu et al., 2017).

Thus, it is noteworthy that the indiscriminate use of antibiotics as prophylactic and therapeutic measures in fish causes intestinal dysbiosis and changes resistant bacterial populations, which can result in compromised fish immunity and reduced resistance to diseases (Limbu, 2018).

Therefore, preventive measures as a way to protect the health of fish in stressful environments are being implemented, among which is the use of probiotics in their food, especially in the early life stages, when immunity is reduced (Brito et al., 2019).

Probiotics are additives based on live microorganisms capable of inhabiting, forming and multiplying in the host's intestine, developing the balance of the microbiota (Ferreira et al., 2018) and preventing and controlling enteric diseases (Diepers et al., 2017).

The main probiotic microorganisms used in aquaculture include species belonging to the lactic acid bacteria (LAB) group (Beck et al., 2015; Liu et al., 2016; Liu et al., 2017) and Bacillus spp. (Chai et al., 2016; Giatsis et al., 2016; HE et al., 2013; SUN et al., 2011).

Research indicates that the probiotics act on fish by stimulating immune responses (Galagarza et al., 2018); improving productive performance and disease resistance (Liu et al., 2017); promoting intestinal colonization and improving survival (SR) without negatively influencing food intake, total biomass, gross income, operating costs and net income (Nilton et al., 2015); generating an immune barrier, competing with pathogenic microorganisms for adhesion sites and minimizing their action (Vieira & Pereira, 2016); producing antimicrobial substances in the gastrointestinal (GI) lumen in order to prevent the

growth of opportunistic pathogenic microorganisms; promoting competition for nutrients essential to the growth of pathogens and stimulation of the host's immune system (Zorriehzahra et al., 2016); generating inhibitory compounds, competing for available chemicals or energy, improving water quality, interacting with phytoplankton as a source of macro and micronutrients and contributing to enzymatic digestion (Verschuere, 2000; Reid 2016); and, lastly, collaborating with enzymes that help in digestion and even have antiviral effects (Loh, 2017).

Thus, the present research aimed to evaluate the productive performance of Nile tilapia (Oreochromis niloticus) fingerlings fed diets containing the probiotics Bacillus subtilis and Bacillus cereus (BSC).

2. Methodology

Experimental design

The research was conducted at the Aquaculture Laboratory of the Grupo de Estudos de Manejo na Aquacultura (GEMAq), located at the State University of Western Paraná (UNIOESTE) – Toledo/PR campus, with a duration of 30 days. The procedures adopted in conducting this experiment were approved by the Institution's Committee on Ethics in the Use of Animals (CEUA), under protocol number of animal tests n° 61/19.

The experiment was carried out under laboratory conditions, using 450 fingerlings of Oreochromis niloticus with an average initial weight of 2.81 ± 0.06 g. The specimens were distributed in a completely randomized design (CRD) in 15 polyethylene boxes with a useful volume of 210 liters, in five treatments with three replications, totaling 30 animals per experimental unit.

Experimental diets and food management

Five experimental diets were formulated to be isoproteic (40.00% crude protein) and isoenergetic (3,462 kcal of digestible energy/kg of feed) in order to meet the recommended nutritional values for the species in the development phase in which the animals were (Table 1). The treatments consisted of: basal diet, CONT: 0% (without the addition of the test ingredient); BSC1: 0.6%; BSC2: 0.8%; BSC3: 1.0%; BSC4: 2.0% of Bacillus subtilis and Bacillus cereus at a concentration of 4x1011 CFU kg−1 in the feed.

All ingredients were initially ground (0.3 mm) in a hammer mill (model MCs 280, Vieira Moinhos e Martelo, Tatuí–SP, Brazil). Afterwards, a homogenization procedure was performed for 15 minutes in a “Y” type mechanical mixer (model MA 200, Marconi Equipamentos Laboratórios, Piracicaba–SP, Brazil). The diets were then extruded, with 20% moisture, in a 1.0 mm diameter matrix in an Ex-Micro® model equipment with capacity of 10 kg h−1 (Exteec Máquinas, Ribeirão Preto–SP, Brazil).

After extrusion, the rations were placed in a ventilated incubator for 72 hours at 55°C (model TE-394/3-D, Tecnal Equipamentos Científicos para Laboratórios, Piracicaba–SP, Brazil). The procedure for the inclusion of the probiotic in the diet was carried out for 15 minutes by the spray method, mixing the probiotic to be added to the diet with soybean oil to improve stability. Then, the experimental diets were stored in a refrigerator throughout the entire experimental trial.

Table 1. Formulation of diets containing different levels of Bacillus subtilis and Bacillus cereus probiotics offered to Nile tilapia (Oreochromis niloticus) fingerlings.

Ingredients (kg)	Levels of probiotic supplementation (%)				
	0	0.6	0.8	1.0	2.0
Cornmeal	25.24	25.24	25.24	25.24	25.24
Soy protein concentrate	24.07	24.07	24.07	24.07	24.07
Rice grits	10.00	10.00	10.00	10.00	10.00
Poultry offal meal	10.00	10.00	10.00	10.00	10.00
Feather meal	6.80	6.80	6.80	6.80	6.80
Blood meal	5.00	5.00	5.00	5.00	5.00
Fish meal 55% CP	5.00	5.00	5.00	5.00	5.00
Corn gluten 60% CP	5.00	5.00	5.00	5.00	5.00
Soy oil	3.62	3.62	3.62	3.62	3.62
Dicalcium phosphate	1.34	1.34	1.34	1.34	1.34
Limestone	0.88	0.88	0.88	0.88	0.88
Premix[1]	0.60	0.60	0.60	0.60	0.60
L-lysine hcl	0.56	0.56	0.56	0.56	0.56
L-threonine	0.55	0.55	0.55	0.55	0.55
Common salt	0.50	0.50	0.50	0.50	0.50
Dl – methionine	0.38	0.38	0.38	0.38	0.38
Vitamin C	0.20	0.20	0.20	0.20	0.20
Choline chloride	0.15	0.15	0.15	0.15	0.15
Antifungal	0.10	0.10	0.10	0.10	0.10
Antioxidant	0.02	0.02	0.02	0.02	0.02
Probiotic	-	6.00	8.00	10.00	20.00
Nutrient (%)[2]					
Starch	24.05	24.05	24.05	24.05	24.05
Total arginine	2.63	2.63	2.63	2.63	2.63
Calcium	1.50	1.50	1.50	1.50	1.50
Digestible energy (kcal/kg)	3,462.00	3,462.00	3,462.00	3,462.00	3,462.00
Total phenylalanine	2.07	2.07	2.07	2.07	2.07
Raw fiber	1.20	1.20	1.20	1.20	1.20
Available phosphorus	0.83	0.83	0.83	0.83	0.83
Total phosphorus	1.00	1.00	1.00	1.00	1.00
Fat	7.19	7.19	7.19	7.19	7.19
Total histidine	1.05	1.05	1.05	1.05	1.05
Total isoleucine	1.60	1.60	1.60	1.60	1.60
Total leucine	3.43	3.43	3.43	3.43	3.43
Total methionine	1.00	1.00	1.00	1.00	1.00
Crude protein	40.00	40.00	40.00	40.00	40.00
Digestible protein	34.19	34.19	34.19	34.19	34.19
Total threonine	2.20	2.20	2.20	2.20	2.20
Total tryptophan	0.43	0.43	0.43	0.43	0.43
Total valine	2.26	2.26	2.26	2.26	2.26
Total lysine	2.60	2.60	2.60	2.60	2.60

1 Warranty levels per kilogram of product: Vit. A, 1,750,000 IU; Vit. D3, 375,000 IU; Vit. E, 20,000 IU; Vit. K3, 500 mg; Vit. B1, 2000 mg; Vit. B2, 2,500 mg; Vit. B6, 2,500 mg; Vit. B12, 5,000 mg; Folic acid, 625 mg; Pantothenate Ca, 7,500 mg; Vit. C, 37,500 mg; Biotin, 50 mg; Inositol, 12,500 mg; Niacin, 8,750 mg; Co, 50 mg; Cu, 1,250 mg; Fe, 15,000 mg; I, 100 mg; Mn, 3,750 mg; Se, 75 mg; Zn, 17,500 mg. 2Values calculated in the formulation. Source: Authors.

Feeding was performed six times a day (8 am, 10 am, 12 pm, 2 pm, 4 pm, and 6 pm), until apparent satiety. The experimental system was equipped with water recirculation, mechanical filter, aeration, constant heating and daily siphoning. The water quality parameters were measured weekly using the portable multiparameter (YSI professional).

The physical and chemical variables of water such as temperature (25 ± 2.09°C), dissolved oxygen (3.06 ± 0.19 mg/L) and pH (6.96 ± 0.02) remained within the range recommended for Nile tilapia (BOSCOLO et al., 2006; SIPAÚBA-TAVARES et al. 2006).

Data collection

After the experimental period, all specimens were fasted for 24 hours to empty the gastrointestinal tract. Then, a portion of the fish were euthanized in a 250 mg L−1 benzocaine solution (OKAMURA et al., 2010) to determine the individual measurements of weight (g) and total length (cm).

The zootechnical performance calculations were determined by the following equations: Weight gain (WG) = (Wf − Wi); Daily weight gain (DWG) = (Wf − Wi) / T; Apparent food conversion (AFC) = FI / WG; Specific growth rate (SGR) = [(ln Wf − ln Wi) × 100] / T; Protein efficiency rate (PER) = WG / CPI; and Survival (S) = 100 × (Initial number of fish − Final number of fish) / Initial number of fish; where Wf = final weight; Wi = initial weight; T = experimental time; WG = weight gain; FI = feed intake; CPI = crude protein intake.

Bromatological analyzes

Five fish from each experimental unit were submitted to centesimal composition analysis (humidity - HU, protein - CP, ethereal extract - EE, mineral matter - MM), according to the methodology described by Instituto Adolfo Lutz (2008). All analyzes were performed at the Food Quality Laboratory (LQA) of GEMAq.

The dry matter content was calculated using an incubator at 105°C until constant weight (Tecnal, model TE-394/2), the mineral matter by calcination of the samples at 550°C (TRADELAB, model TLA 200D), while the lipid content was obtained by solvent extraction (petroleum ether) in a specific device for lipid determination (Tecnal, model TE-044-5/50). Protein content was determined using the Kjedahl method, using a digester (Tecnal, model TE-018) and a distiller (Tecnal, model TE-0363).

Somatic indices

For the somatic indices, the organs (stomach, intestine and liver) of four fish from each experimental unit were collected, in order to calculate the eviscerated yield [(eviscerated fish weight × 100 / total fish weight]; the hepatosomatic index [(weight of liver / body weight) × 100]; the coefficient of the intestine [(Intestine length / Fish length)]; and the relative weight of the intestine [(Intestine weight / body weight) × 100].

Histological analyzes

For the histomorphometric analysis of the intestinal villi, three specimens of each experimental unit were collected and a transverse segment of the medial portion of the intestines was removed, which were then fixed in Alfac solution for 24 hours, and subsequently preserved in 70º alcohol to remove the fixative until processing.

Tissues were dehydrated in increasing concentrations of alcohols, clarified in xylene and embedded in paraffin. The histological samples were obtained by serial sections of 7 µm and stained in Hematoxilia-Eosin (HE). The slides were analyzed under light microscopy, measuring the height of the villi (distance from the top of the villi to the beginning of the muscular layer), width of the villi (distance from the top of an enterocyte to the top of the enterocyte on the opposite side),

tunica thickness (total distance of the circular and longitudinal muscle layer), and total number of villi, in the 40X objective lens, by the cellSens Standard 1.15® software.

Statistical analysis

The data regarding productive performance and bromatological and histological analyzes, taking into account the assumptions of normality and homoscedasticity, were submitted to ANOVA analysis of variance to verify the interaction between treatments, and when significant, the means were compared by the Tukey test at 5% with the aid of the R software.

3. Results and Discussion

There was no significant effect (P > 0.05) on the parameters of WG, DWG, AFC, PER, SGR and S (%) of fingerlings fed diets containing 0.6, 0.8, 1.0, and 2.0 (%) of Bacillus subtilis and Bacillus cereus at a concentration of 4x1011 CFU kg−1 in the diet (Table 2).

Table 2. Mean values and standard deviations of the zootechnical performance of Nile tilapia fingerlings fed diets containing different levels of *Bacillus subtilis and Bacillus cereus* probiotic.

	TREATMENTS*				
Variables	CONT	BSC1	BSC2	BSC3	BSC4
[1]WG (g)	14.7 ± 1.56	13.1 ± 1.80	16.0 ± 1.61	13.6 ± 0.42	14.3 ± 1.84
[2]DWG (g)	0.45 ± 0.04	0.41 ± 0.05	0.50 ± 0.04	0.42 ± 0.01	0.45 ± 0.06
[3]AFC	1.09 ± 0.33	1.00 ± 0.11	1.18 ± 0.20	1.25 ± 0.20	1.14 ± 0.02
[4]PER	42.1 ± 4.49	37.6 ± 5.15	45.9 ± 4.62	39.0 ± 1.19	41.0 ± 5.27
[5]SGR	8.57 ± 0.29	8.28 ± 0.43	8.82 ± 0.23	8.44 ± 0.10	8.54 ± 0.35
[6]S (%)	96.6 ± 5.77	100 ± 0.00	95.5 ± 7.69	86.6 ± 5.77	92.2 ± 8.39

1Weight gain (WG); 2Daily weight gain (DWG); 3Apparent feed conversion (AFC); 4Protein efficiency rate (PER); 5Specific growth rate (SGR); 6Survival (S). *BSC (*Bacillus cereus and Bacillus subtilis*). Source: Authors.

According to some studies, the effect of the probiotic on the host may be related to the feeding time (MELLO et al., 2013). Silva et al. (2021), when evaluating the inclusion of a complex of bacteria and probiotic B. subtilis for a period of 53 days, observed that it did not affect the performance of juvenile Nile tilapia, on the other hand, Nakandakare et al. (2013) observed differences in growth performance of Nile tilapia juveniles fed B. subtilis and B. toyoi over 63 days. Prolonged exposure can lead to the establishment of probiotic colonization, resulting in an improvement in digestive and enzymatic processes that help extract nutrients from food, thus positively interfering with productive performance (Albuquerque et al., 2013).

The performance of animals kept in good management conditions is hardly influenced by the ingestion of probiotics (Lima et al., 2003), because in these conditions the contact with harmful microorganisms is minimal. Ferreira et al. (2018) when using probiotics (B. licheniformis, B. cereus, B. subtilis and yeasts — Saccharomyces cerevisiae and Saccharomyces boulardi) in diets for Nile tilapia during the fingerling and juvenile stages submitted to domestic sewage water treated as sanitary challenge, observed an improvement in the specific growth parameter.

Thus, the zootechnical performance responses observed in the study can be attributed to the rusticity and apparent ability of tilapia fingerlings to adapt to such experimental conditions and efficiently use the ration offered, even without the probiotic (Carvalho et al., 2011). Another important factor would be the use of good management conditions and dosage of probiotics. However, if the animals are not submitted to some type of sanitary challenge, the probiotic effect will possibly not be proven (Moringo et al., 2010).

The probiotic supplementation in the diets demonstrates that the values of fish centesimal body composition showed significant differences in the levels of protein, lipids and ash ($p < 0.05$) with no significant difference in moisture ($p > 0.05$) (Table 3).

Table 3. Mean values and standard deviations of centesimal body composition of Nile tilapia fingerlings fed diets containing different levels of *Bacillus subtilis and Bacillus cereus probiotic*. Values are expressed on the basis of dry matter.

	TREATMENTS*				
Parameter (%)	CONT	BSC1	BSC2	BSC3	BSC4
Moisture	74.99 ± 0.17	75.13 ± 0.23	75.43 ± 0.58	74.49 ± 0.17	75.43 ± 0.58
Protein	52.56 ± 0.09^{d}	56.15 ± 0.08^{b}	57.26 ± 0.23^{a}	55.1 ± 0.03^{c}	55.37 ± 0.03^{c}
Lipids	16.11 ± 1.12^{ab}	16.35 ± 1.5^{ab}	14.07 ± 1.35^{b}	15.05 ± 1.65^{ab}	16.89 ± 1.47^{a}
Ashes	14.35 ± 0.06^{b}	15.78 ± 0.50^{a}	15.69 ± 0.64^{a}	14.78 ± 0.38^{ab}	15.24 ± 0.22^{ab}

Mean values followed by a different letter on the same line indicate a significant difference by Tukey's test ($P < 0.05$). *BSC (Bacillus cereus and Bacillus subtilis). Source: Authors.

The inclusion of 0.8% (BSC2) of probiotic in the diet showed a higher protein content and lower lipid content in the carcass. A better use of protein was presented, but the same does not apply to the lipid content. Therefore, the probiotic can allow an increase of protein and a decrease of lipids in the carcass. This may be related to the different mechanisms of action of the probiotic. According to Martínez et al. (2017), probiotics can increase the production of enzymes that induce the absorption of nutrients, and similar results were observed by Mello et al. (2013) who found an increase in the percentage of crude protein and a decrease in the values of ether extract in the carcasses of fish fed with the addition of *B. cereus* and *B. subtilis*, which seems to have occurred in the present study. Thus, there were advantages and positive effects on the body composition of animals that received probiotics *B. cereus* and *B. subtilis* in the diets compared to the control group.

No significant differences ($P > 0.05$) were observed in the somatic values of the specimens, indicating that the inclusion of probiotics (*B. cereus* and *B. subtilis*) does not compromise these indices in this cultivation phase (Table 4).

Table 4. Mean values and standard deviations of somatic indices of Nile tilapia fingerlings fed diets containing different levels of the probiotic *Bacillus subtilis* and *Bacillus cereus*.

	TREATMENTS*				
Parameter	CONT	BSC1	BSC2	BSC3	BSC4
[1]RBW (g)	4.61 ± 1.86	5.20 ± 0.92	5.27 ± 1.16	5.45 ± 1.30	5.16 ± 0.84
[2]IC	4.85 ± 0.99	5.14 ± 0.67	5.35 ± 0.64	5.31 ± 0.88	5.11 ± 0.78
[3]HSI (%)	2.13 ± 0.57	2.50 ± 0.24	2.33 ± 0.51	2.35 ± 0.43	2.21 ± 0.51
[4]EY (%)	77.52 ± 7.05	79.11 ± 4.99	81.89 ± 2.07	80.61 ± 1.47	81.86 ± 7.03

1Relative bowel weight (RBW); 2Intestinal coefficient (IC); 3Hepatosomatic index (HSI); 4Eviscerated yield (EY); 5Intestinal length (IL). *BSC (Bacillus subtilis and Bacillus cereus). Source: Authors.

The hepatosomatic index (HSI) is related to the nutritional status of the fish, where diets with high protein content directly influence the liver, which can retain or spend its lipid source (BUSACKER et al., 1990). Ferreira et al. (2015) and Faria et al. (2001), when providing high levels of protein (35% and 40% of CP in the diet, respectively), did not observe significant differences in the HSI, unlike some authors who worked with levels of up to 20% of CP in the diet.

For intestinal villi histomorphometry, significant differences ($P < 0.05$) were observed in the variable villus width (VW) where, in the treatment with 0.6% (BSC1), the best result was observed (Table 5).

Table 5. Mean values and standard deviations of intestinal villi histomorphometry of Nile tilapia fingerlings fed diets containing different levels of the probiotic *Bacillus subtilis* and *Bacillus cereus.*

	TREATMENTS*				
Variables	CONT	BSC1	BSC2	BSC3	BSC4
[1]VH	166.4 ± 22.04	153.9 ± 22.98	184.0 ± 24.52	173.0 ± 16.69	158.19 ± 20.52
[2]VW	76.56 ± 8.13^{b}	89.44 ± 6.85^{a}	83.75 ± 5.24^{ab}	84.33 ± 11.6^{ab}	76.23 ± 7.85^{b}
[3]TT	24.59 ± 5.38	20.47 ± 2.27	23.63 ± 3.48	22.34 ± 5.35	18.66 ± 1.29
[4]VT	34.28 ± 1.69	35.58 ± 1.91	36.93 ± 4.63	38.10 ± 4.04	35.02 ± 2.81

Mean values followed by a different letter on the same line indicate a significant difference by Tukey's test ($P < 0.05$). 1Villus height (VH); 2Villus width (VW); 3Tunic thickness (TT); 4Villus thickness (VT). *BSC (Bacillus subtilis and Bacillus cereus). Source: Authors.

The intestinal musculature can act as an indicator of nutritional performance according to the changes in the tissue that reveal the ability of the fish to absorb nutrients (JUNQUEIRA & CARNEIRO, 2005). The use of probiotics in the diet had a positive influence on the villus width when compared to the control diet, as observed by Castro et al. (2021), who found similar results for the use of B. cereus var. toyoi and Bacillus subtilis, in tilapia post-larvae, demonstrating an improvement in the nutrient absorption capacity. According to Junqueira & Carneiro (2005), the greater the size of the intestinal villus, the greater the capacity of nutrient absorption by the animal.

Although the bacteria may not have fully colonized the animals' gastrointestinal tract, this colonization, although initial, had beneficial effects on Nile tilapia fingerlings, promoting an improvement in the centesimal and histomorphometric composition of the intestinal villi.

4. Conclusion

The inclusion of BSC in the diet did not influence the zootechnical performance of the animals. The use of 0.8% of the probiotic Bacillus subtilis and Bacillus cereus (4x1011 CFU kg−1) in the feed appears as a dietary supplementation strategy for Nile tilapia in fingerling stage due to the efficacies found in the main results of body centesimal composition and in the histomorphometry of intestinal villi.

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