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AQUACULTURE

Diet supplementation formulated with *Bacillus* sp. SMIA-2 and its enzymes for Nile tilapia: zootechnical performance and effects on intestinal morphometry

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ABSTRACT. The use of probiotics and exogenous enzymes in fish feed is a promising alternative to improve animal performance. This study evaluated the feasibility of applying *Bacillus* sp. SMIA-2 and its enzymes as supplements in the diet for juvenile tilapia. The effect of adding different concentrations of *Bacillus* sp. SMIA-2 and its enzymes in food on zootechnical development, intestinal morphometry of animals was analyzed. The bacteria could be recovered from the intestines of animals, demonstrating its ability to survive gastric and bile acids. The comparative study of SMIA-2 with commercial products showed a significant effect on individual food intake, final weight and weight gain in all treatments. Gut length, villus height and intestinal coefficient were an advantage of SMIA-2 compared to commercial products and the control group. Therefore, the inclusion of Bacillus sp. SMIA-2 and its enzymes in fish feed may represent a viable alternative to improve animal development and significantly increase intestinal villi, contributing to nutrient absorption and animal health.

Keywords: aquaculture; *Bacillus*; enzymes; animal nutrition; *Oreochromis niloticus*.

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Introduction

Tilapia is currently the main fish species farmed in Brazil (Figueiredo et al., 2022). In 2020, 486,155 tons of the species were produced, making the country the fourth largest tilapia producer globally (Peixe BR, 2021).

Expansion of tilapia farming requires high amounts of sustainable ingredients for nutritionally balanced fish feed (Furuya, Cruz, & Gatlin III, 2023). Plant-based feed, such as flours, oilseeds, legumes, and cereal by-products, is the main ingredient in formulations. Nevertheless, there are limitations in their inclusion levels due to the presence of antinutritional factors, such as phytin, non-starch polysaccharides, and protease inhibitors, which may impair the use of nutrients, fish development, and overall health (Castillo & Gatlin, 2015). Adding enzymes in feed formulation is an alternative to remove or reduce antinutritional factors, improving nutrient digestion and, consequently, the zootechnical performance of animals (Xu, Zheng, Dong, Ai, & Mai, 2022). Microbial enzymes, especially those produced by the genus *Bacillus*, have been widely explored for this aim.

Bacillus SMIA-2, an aerobic, thermophilic, and spore-forming bacterium (Souza & Martins, 2001), can produce thermostable enzymes, such as proteases (Silva, Delatorre, & Martins, 2007), amylases (Carvalho, Corrêa, Silva, Viana, & Martins, 2008), pectinases (Andrade, Delatorre, Ladeira, & Martins, 2011), and cellulases (Ladeira, Cruz, Delatorre, Barbosa, & Leal Martins, 2015; Cruz, Moraes, Costa, Barbosa, & Martins, 2019). Bernardo et al. (2020) recently sequenced the SMIA-2 genome and detected no virulence gene. According to the authors, this strain is 100% similar to *Bacillus licheniformis* Gibson 46 (ATCC 14580T).

Due to the spore-forming ability of the genus *Bacillus*, which resists gastric and biliary acids, it can survive in the gastrointestinal tract and remain in high concentration in the intestine (Lee & Kim, 2011). Therefore, *Bacillus* species are currently widely studied for the human, animal, and aquaculture nutrition as growth promoters and agents of competitive exclusion (Latorre et al., 2016).

This study evaluated the feasibility of using *Bacillus* sp. SMIA-2 and its enzymes as supplement in diets formulated for tilapia (*Oreochromis niloticus*).

Material and methods

The experiment was conducted in the Laboratory of Nutrition and Production of Ornamental Species (Laboratório de Nutrição e Produção de Espécies Ornamentais – LNPEO) in the Campus of Alegre, state of Espírito Santo, between March and May 2021. The study was approved by the Animal Research Ethics Committee of the Federal Institute of Espírito Santo (Instituto Federal do Espírito Santo), registered under the number 23149.001859/2021-56.

The synbiotic product used in the experiments was supplied by the Food Technology Laboratory from the State University of Northern Rio de Janeiro (Universidade Estadual do Norte Fluminense Darcy Ribeiro - UENF). It contained 7.3 Log spores mL^{-1} of *Bacillus* sp. SMIA-2 and the following enzymes (U mL^{-1}): 11.46 proteases, 1.10 avicelases (avicel hydrolyzing enzymes – insoluble cellulose), 0.13 carboxymethyl cellulose (carboxymethyl cellulose hydrolyzing enzymes - soluble cellulose), 0.30 xylanases, 0.29 amylases, and 0.23 polygalacturonases.

Initially, an experiment was conducted to check the effect of adding different concentrations of *Bacillus* sp SMIA-2 in the feed on the zootechnical development and intestinal morphometry of tilapia juveniles. The experimental design adopted was the completely randomized design with five concentrations of SMIA-2 (g kg^{-1} feed): 0.0; 0.383 g; 0.765 g; 1.147 g; 1.531 g, and five replications of 15 juveniles in each treatment, resulting in 25 experimental units. The experimental units (EU) contained 15 juveniles and the aquarium, with a volume of 50 L.

After, the zootechnical development of tilapia juveniles using feed supplemented with SMIA-2 and its enzymes was compared to two commercial products tested in the same conditions. A completely randomized design (CRD) with four treatments was adopted: Treatment 1 (T1): control feed; Treatment 2 (T2): feed with spores and enzymes of *Bacillus* sp. SMIA-2; Treatment 3 (T3): feed with a commercial product containing *Bacillus licheniformis* and enzymes (0.250 g kg^{-1}); Treatment 4 (T4): feed including a commercial product containing *Bacillus coagulans*, *Bacillus subtilis*, *Bacillus licheniformis*, and enzymes (0.100 g kg^{-1}).

The doses of the commercial products followed the instructions of manufacturers. The inclusion dose of *Bacillus* sp. SMIA-2 was 0.765 g kg^{-1}.

Each treatment had six replications, with 15 tilapia juveniles each, resulting in 24 experimental units (EU) with a 50 L volume each. Each EU had water recirculation, individual water input and output, physical, chemical, and biological filtering, an overflow box with a water pump of 3/4 hp, and heaters with thermostats. The experimental units were siphoned for partial water exchange (PWE) with approximately 20% of the useful olume every three days.

Feed was supplied three times a day until satiation. The experimental feeds were formulated for tilapia juveniles, containing 36% crude protein (CP) and 3,100 Kcal digestible energy (DE). Each treatment received the same basal feed but added with *Bacillus* sp. SMIA-2 in different concentrations.

Feed ingredients were ground and mixed in the proportions mentioned above (Table 1). After mixing, ingredients were moistened with water at 55°C and mixed. Only after this process, the feed was pelleted. Later, ingredients were dried outdoors in the shade and duly stored in a refrigerator or freezer to be used during the experiment. The additive was included on the top of the feed.

Table 1. Composition percentage and nutritional value of the basal experimental feed used in the fish diet.

Ingredients	
Soybean meal	64.85 %
Cornmeal	20.14 %
Fish meal	10.00 %
Wheat bran	3.00 %
Soybean oil	2.00 %
Nutritional value	
Crude protein	36%
Digestible energy	3,101.2 Kcal

After the adaptation period, tilapia juveniles were selected according to their initial biometrics. Afterwards, total length, standard length, and height were measured with a caliper and weighed on a digital scale with four decimal places and three precision numbers. All fish were sedated with 0.25 mL eugenol per liter of water. Fish had, on average, an initial weight of 1.14 g and a mean total length of 4.32 cm. After initial biometrics, fish were evenly distributed in the EUs.

For the control and monitoring of water quality in the experimental systems, the following water quality parameters were measured daily: Dissolved oxygen, Higher water temperature, Lower water temperature, and the temperature at the time. Dissolved oxygen and temperature were measured with an oximeter and a digital thermometer. Values for pH and conductivity were measured with a digital pH meter and an electrical conductivity meter, respectively, and ammonium nitrite and nitrate with a digital microprocessor photocolorimeter every two days. All devices were calibrated before each measurement.

Zootechnical performance

For the final biometrics of the experiments, animals were euthanized following the Guidelines for Euthanasia Practices of the National Council for the Control of Animal Experimentation – CONCEA – for bony and cartilaginous fish, using eugenol at the dose of 5 mL/liter of water.

Variables used to quantify the development of juveniles were weight, total length, standard length, height, weight gain, apparent food conversion, specific growth rate, condition factor, protein efficiency ratio, energy efficiency ratio, survival rate, and batch uniformity, as follows:

Weight Gain: $WG = Wf - Wi$ (*g*). Wf = average final weight (g), and Wi = average initial weight (g).

•Feed consumption: *FC* (*g*) *in the period* = (*Feed supplied – Feed leftovers*) / *number of animals*.

•Feed Conversion ratio: $FCR = FC / WG$.

•Specific Growth rate: $SGR = ((\ln Wf - \ln Wi)) / Tta) \times 100$.

Ta= feed period (days).

•Condition Factor: $K = (W \times 100) / L\ 3$. W= weight (g), and L= length (cm).

•Survival Rate: $SR = (Nf / Ni) \times 100$. Nf = final number of fish, and Ni= initial number of fish/experimental unit.

•Protein efficiency ratio: *PER*= (*WG/ (FC X %RP of the diet*)) *X* 100. WG= weight gain, and FC = feed consumption.

•Energy efficiency ratio: *EER*= (*WG/ (FC X %RP of the diet*)) *X* 100. WG= weight gain, and FC = feed consumption.

Histopathological analysis

For villi analysis, intestinal fragments with around 3 cm length and 5 mm thickness were taken with a cross section in two different intestinal segments (duodenum and ileum).

Intestinal samples were fixed in neutral buffered formalin at 10%, and stored until processing. Cleavage was conducted, and fragments were placed into histology cassettes for paraffin embedding. The material processing consists of the following steps: sample dehydration in increasing alcohol series (70, 85, 95%, absolute alcohol I, II, and III) during one hour for each sample. After dehydration, samples were cleared in xylol solutions I, II, and III, where they remained for an hour in each concentration. Then, they were immersed in paraffin I and II for an hour in each. Soon after, samples were embedded in paraffin mixed with beeswax, and stored in a freezer at -20°C. Blocks were cross sectioned with a microtome to 4 μm thickness. Slides were stained with hematoxylin and eosin and observed under a light microscope. Villi height was observed and measured with an ocular micrometer (Figure 1).

Recovering Bacillus sp SMIA-2 from the intestine

After euthanasia, fish were externally decontaminated with 70% alcohol by immersion in a glass container for five minutes. Intestines were removed, weighed, and macerated with a solution of 0.1% (w/v) sterile peptone water (Jatobá et al., 2008).

To counter SMIA-2 spores, samples were submitted to thermal shock (80ºC 10 min.^{-1}) (Rabinovitch & Oliveira, 2015) in a water bath and immediately cooled in an ice bath. Then, serial dilutions were conducted using 0.1% peptone water (w/v), and 0.1 mL were seeded on the surface of Petri dishes containing tryptic soy agar (TSA). Dishes were incubated at 50°C for 48 hours for posterior counting of colonies. The results were expressed as the number of spores per mL.

Figure 1. Photomicrography of the intestine section of fish with the measurement of villus height.

Results and discussion

Effect of adding different concentrations of Bacillus sp SMIA-2 in the diet on the zootechnical development and intestinal morphometry of tilapia juveniles

Mean values of quality parameters measured during the experimental period were temperature – 26.77°C; dissolved oxygen – 3.207 mg L^{-1}; ammonium 0.062 mg L^{-1}, and potential of hydrogen (pH) – 8.055.

Water quality monitoring is essential for aquaculture productivity, even when farming more rustic species, such as tilapia. According to Freitas et al. (2022), tilapia presents thermal comfort at temperatures between 27 and 30°C. Higher temperatures (above 38°C) can lead to mortality due to the reduced levels of dissolved oxygen in water. When the temperature is low, there is lower resistance, and the animal is susceptible to illnesses, besides increasing cortisol levels (Falcon, Barros, Pezzato, Solarte, & Guimarães, 2008).

Although the dissolved oxygen is below the recommended for most species, between 5 and 6 mg L^{-1}, tilapia can tolerate low concentrations. Wambua, Home, Raude, and Ondimu. (2021) state that these animals can support oxygen levels between 0.4 and 0.7 mg L^{-1}, but the development may be compromised.

High ammonia levels may affect osmoregulation, excretion, and oxygen transport; therefore, levels below 0.24 mg L^{-1} are recommended (Harsij, Kanani, & Adineh, 2020). Thus, the ammonia level found in our study is within the recommended range.

The pH remained within the recommended range for the species, which are between 6.0 and 8.5 (Mili et al., 2023). The main reasons for pH alterations are breathing, fertilizing, liming, photosynthesis, and pollution. The pH value below 4.0 and above 9.0 stress animals, which can cause death (Makled, Hamdan, & El-Sayed, 2019).

Regarding the recovery of *Bacillus* sp SMIA-2 from the intestine (Figure 2), its presence was not detected in the treatment in which it was not added to the feed. This demonstrates that there was no contamination of aquaria or feed. Therefore, the bacterium was not present in the system. It was possible to recover the bacterium from the intestine in the other treatments. The number of colony-forming units increased with increasing concentrations of SMIA-2 in the feed. Such results demonstrate their survival ability due to resistance of their spores to gastric and biliary acids. Therefore, they are viable as a probiotic.

In tilapia post-larvae fed a feed containing between 5 and 10 g kg^{-1} *Bacillus subtilis*, between $1.15x10^4$ and $4.74x10^5$ CFU g^{-1} bacteria were recovered from the intestine (Tachibana et al., 2011).

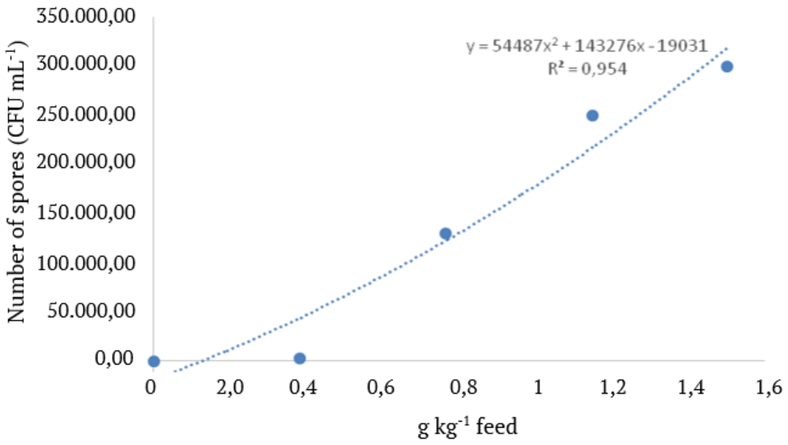

Figure 2. Effect of different concentrations of *Bacillus* sp. SMIA-2 and its enzymes on the number of spores recovered from the intestines of tilapias.

Animal performance

Among the variables statistically analyzed, those with significance were final weight (FW), weight gain (WG), food conversion ratio (FCR) (Table 2), individual feed consumption (IFC), survival rate (SR), specific growth rate (SGR), protein efficiency ratio (PER), viscera weight (VW), hepatosomatic index (HSI), and viscerosomatic index (VSI) (Table 3).

Regarding final weight, weight gain, and food conversion, the animals had results statistically similar in all treatments.

Table 2. Mean values of performance characteristics in tilapia juveniles fed different inclusion levels of SMIA-2.

Inclusion levels of smia-2 (g kg^{-1} feed)	Performance Characteristics		
	FW (g)	WG (g)	FCR
Control	19.656	18.952	1.237
0.383	23.859	23.156	1.121
0.765	19.380	18.678	1.339
1.147	20.305	19.601	1.186
1.53	20.687	19.984	1.209

At the end of the experimental period, the livers of the animals had a standard coloration and satisfying integrity. The increase in this organ size might be due to a low-protein but high-carbohydrate and high-fat diet, resulting in a yellowish-brown color. As the feed supplied to animals was the same in the five treatments, it is improbable that the liver increase is due to the feed quality. Thus, we suggest that the liver weight increase might be caused by the energy reserves of animals, which can be beneficial because the reserves have an essential role as a glucose source for animals in the pre- and postprandial period (Da Silveira, Logato, & Da Conceição Pontes, 2009). As Table 3 shows, the inclusion of 1.147 g kg^{-1} *Bacillus* sp. SMIA-2 in the feed promoted the best response regarding the variable LW. Regarding HPI, VW, and VSI, the concentration of SMIA-2 of 1.147 g kg^{-1} c presented a better response.

Table 3. Mean values of liver weight, viscera weight, hepatosomatic index, and viscerosomatic index in tilapia juveniles fed different inclusion levels of *Bacillus* sp. SMIA-2.

Inclusion levels OF SMIA-2 (g kg^{-1} of feed)	Performance Characteristics			
	LW (g)	VW (g)	HSI	VSI
Control	0.392ab	1.436abc	1.998ab	7.379a
0.383	0.362ab	1.666a	1.572ab	7.101a
0.765	0.242b	1.094c	1.253b	5.729a
1.147	0.408a	1.558ab	2.039a	7.701a
1.53	0.255ab	1.232bc	1.240b	5.969a

Means followed by different letters (columns) statistically differ by Tukey's test at 5% significance. Liver weight (LW), viscera weight (VW), hepatosomatic index (HSI), and viscerosomatic index (VSI).

VSI is a factor that affects fish farming. An increase in fat deposition in the viscera can reduce the commercial value of fish (Wang et al., 2016). As tilapia is a species with high popularity and the second most farmed fish species globally (Islam, Rohani, & Shahjahan, 2021), the maximal use of the animal is desirable. According to Moraes et al. (2018), waste of fishing (head, entrails, skin, and dorsal spine) may represent 62.5% animal weight and cause environmental contamination. Thus, the lower the weight of viscera, the better the use by the animal.

Histomorphometric analysis

The statistical analysis evidenced that the villi height decreased with increasing levels of SMIA-2 in the feed, as illustrated in Figure 3.

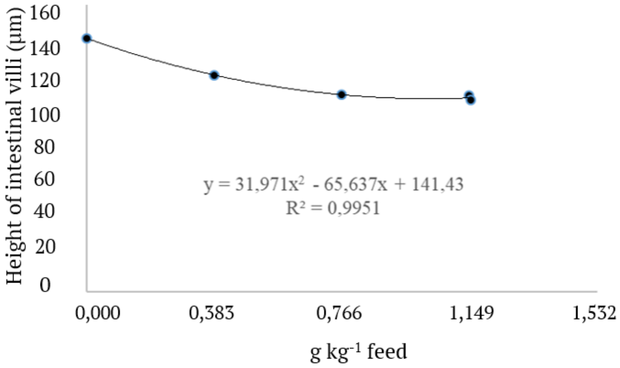

Figure 3. Intestinal villi height of tilapia according to different levels of SMIA-2 in the feed.

The feed without symbiotics (control) resulted in higher intestinal villi height. Such results are similar to those reported by Cechim et al. (2013), who investigated the addition of prebiotic mannan oligosaccharide (MOS) in the concentration of 4.0 g kg^{-1} to tilapia feed, and observed a reduction in intestinal villi height compared to the control. When environmental and sanitary conditions are favorable, the use of probiotics may have no effect (Dawood, Koshi, Abdel-Daim, & Van Doan, 2019). As tilapia is rustic and easily adaptable, the action of additives may be masked.

Effects of adding SMIA-2 and different commercial products in the diet on the zootechnical development of tilapia juveniles.

Mean values of the quality parameters measured during the experimental period were temperature – 26.94°C; dissolved oxygen – 3.87 mg L^{-1}; ammonium 0.00205 mg L^{-1}, and potential of hydrogen (pH) – 6.93.

Regarding animal performance, the variables with statistical significance were final weight, weight gain, feed conversion ratio (Table 4), individual food consumption, survival rate, specific growth rate, protein efficiency ratio, viscera weight, intestine length, and intestinal coefficient (Table 5). According to the results, there was a significant effect on the individual food consumption, final weight, and weight gain when the symbiotics were added to the feed. In addition, feed conversion ratio did not present significant differences between the treatments, the groups with symbiotics showed a better response in these development characteristics. Such results corroborate Cornélio et al. (2013) and Pezzato, Menezes, Barros, Guimarães, and Schich (2006), which added probiotic bacteria to tilapia fish feed. Based on the principle that one of the action mechanisms of probiotics is the competition for absorption, its inclusion in the feed might have prevented the colonization by prejudicial bacteria, resulting in a better nutrient absorption efficiency. According to Marengoni et al. (2010), probiotics in the feed can decrease production costs because microorganisms can decompose macronutrients, transforming them into simpler compounds, which results in better food use.

Although the use of bacteria from genus *Bacillus* in aquaculture can be related to good survival indexes, the survival rate with the use of *Bacillus* sp. SMIA-2 in this experiment was lower than the treatments with commercial symbiotics. To permit the symbiotics to express their beneficial effects, many factors must be considered, such as the presence of stressors, low water quality, high storage densities, or diseases (Silva, Salomao, Mareco, Dal Pai, & Santos al., 2021). Marengoni et al. (2010) highlight that it is not always possible to evidence the positive effects of additives as they may depend on the diet ingredients, the stress levels of animals, and each the characteristics of products.

Table 4. Mean values of performance characteristics in tilapia juveniles with the addition of different commercial symbiotics and *Bacillus* sp. SMIA-2.

Treatments	Performance Characteristics			
	IFC (g)	FW (g)	WG (g)	FCR
Control	26.801b	25.43b	24.29a	1.10a
SMIA-2	35.617a	35.45a	34.31a	1.04a
Commercial product 1	36.653a	35.39a	34.25a	1.07a
Commercial product 2	36.315a	36.28a	35.15b	1.03a

Means followed by different letters (columns) statistically differ by the Tukey test at 5% significance. Individual food consumption (IFC), final weight (FW), weight gain (WG), and feed conversion ratio (FCR).

Table 5. Mean values of performance characteristics in tilapia juveniles fed different commercial symbiotics and *Bacillus* sp. SMIA-2.

Treatments	Performance Characteristics					
	SR (%)	SGR (%)	PER (%)	VW	IL	IC
Control	64ab	6.716b	2.5109a	1.1416b	47.60b	5.8458b
SMIA-2	42b	7.472a	2.6749a	2.0204ab	44.80b	5.6325b
Commercial product 1	82a	7.457a	2.5909a	2.6609a	58.20a	7.3616a
Commercial product 2	76a	7.529a	2.6881a	2.6798a	57.74a	7.2167a

Means followed by different letters (columns) statistically differ by Tukey's test at 5% significance. Survival rate (SR), specific growth rate (SGR), protein efficiency ratio (PER), viscera weight (VW), intestinal length (IL), and intestinal coefficient (IC).

The specific growth rate (SGR) and the protein efficiency ratio (PER) presented similar results in the groups fed different symbiotics. In this sense, *Bacillus* sp. SMIA-2 was more similar to the commercial products as a growth-promoting additive than the control group. Marengoni et al. (2010) reported that SGR is directly proportional to the sanitary conditions of the medium. In this study, water quality indicators remained within the recommended range for the species. Therefore, they did not compromise the normal physiological activities of animals.

Both in the treatments with commercial products and the treatments with *Bacillus* sp. SMIA-2, there was an efficient use of proteins by fish, suggesting that including symbiotics in feed influenced the animal metabolism. Further, the major residues in the excretion of fish are phosphorus and nitrogen compounds, which in excessive quantities might degrade the farming environment, resulting in low water quality (Macedo & Sipaúba-Tavares, 2010). Thus, additives in the feed can increase digestibility and promote better use of nutrients, impeding the accumulation of nutrients in the water (Gomes et al., 2016).

Although the viscera weight (liver, gallbladder, stomach, pancreas, and intestine) was higher with the inclusion of commercial products, its value was higher in the treatment with *Bacillus* sp. SMIA-2 than in the control group. Considering that the meat of fish is the main product of interest, fillet yield depends on many factors, including the percentage of residues. In this aspect, the lower the weight of entrails, fins, skin, and head, the higher the animal yield (De Moraes Gonçalves, De Almeida, & Santo Borges, 2003). Considering that tilapia is the most farmed fish species in Brazil, a lower viscera weight is desirable to produce fillets with a higher yield. Therefore, *Bacillus* sp. SMIA-2 has a slight advantage compared to the commercial products tested, considering that FW in the three treatments was statistically similar.

Intestinal length (IL) and intestinal coefficient (IC) also presented an advantage for *Bacillus* sp. SMIA-2 compared to the commercial products and the control group. The IC of omnivorous animals ranges between 0.6 and 8.0, and animals with smaller intestines have a higher number of villi, increasing the surface, for example, with a higher development of pyloric caeca, expanding the absorption surface without increasing the IL (Ferreira et al., 2014). Thus, if it is intended to enhance the fillet yield of these animals, *Bacillus* sp. SMIA-2 has an advantage compared to the commercial products, although tests are still in an initial phase regarding aquatic animals.

Intestinal histomorphometric analysis

The height of intestinal villi of the fish was higher in the treatment with *Bacillus* sp SMIA-2 in the feed, as shown in Figures 4 and 5.

Considering that the intestinal mucosa is more intact, the higher the villi and the nutrient absorption capacity (Zhaxi et al., 2020). Such results are promising for fish nutrition, because there is an association between intestinal villi importance and the nutrition and health of animals.

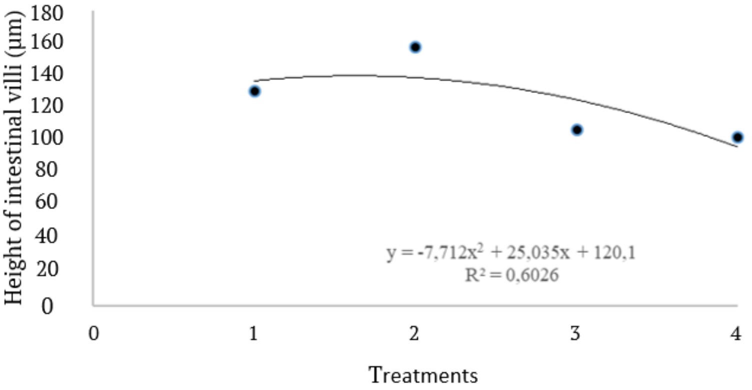

Figure 4. Intestinal villi height of fish according to the diet 1 (Control), 2 (SMIA-2), 3 (Commercial product 1), 4 (Commercial product 2).

Figure 5. Photomicrography of the intestinal portion of a fish fed inclusion levels of *Bacillus* sp. SMIA-2 with the measurement of the villus height.

Such results are promising for fish nutrition, since there is an association between intestinal villi and the nutrition and health of animals. The inclusion of microorganisms in the diet can influence the intestinal microbiota of animals, acting in the villi necessary for intestinal functioning. Villi expand intestinal surface, enhancing water, ion, and nutrient absorption. Therefore, they have a key role in the development and health of animals, which may be altered according to the diet (de Souza & Ferreira, 2022).

Conclusion

The inclusion of *Bacillus* sp. SMIA-2 showed great potential as an additive for fish nutrition, even compared to the commercial products tested. It demonstrated the capacity to enhance the performance of animals and caused a significant increase in intestinal villi, contributing to better nutrient absorption and animal health.

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References

Andrade, M. V. V. D., Delatorre, A. B., Ladeira, S. A., & Martins, M. L. L. (2011). Production and partial characterization of alkaline polygalacturonase secreted by thermophilic Bacillus sp. SMIA-2 under submerged culture using pectin and corn steep liquor. *Food Science and Technology*, *31*, 204-208. DOI: https://doi.org/10.1590/S0101-20612011000100031

Bernardo, S. P. C., Rosana, A. R. R., de Souza, A. N., Chiorean, S., Martins, M. L. L., & Vederas, J. C. (2020). Draft genome sequence of the thermophilic bacterium Bacillus licheniformis SMIA-2, an antimicrobial- and thermostable enzyme-producing isolate from Brazilian soil. *Microbiology Resource Announcements*, *9*(17), e00106-20. DOI: https://doi.org/10.1128/MRA.00106-20

Carvalho, R. V. D., Corrêa, T. L. R., Silva, J. C. M. D., Viana, A. P., & Martins, M. L. L. (2008). Otimização das condições de cultivo para a produção de amilases pelo termofílico Bacillus sp. e hidrólise de amidos pela ação da enzima. *Food Science and Technology*, *28*(2), 380-386. DOI: https://doi.org/10.1590/S0101-20612008000200017

Castillo, S., & Gatlin, D. M. (2015). Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: A review. *Aquaculture*, *435*, p. 286-292. DOI: https://doi.org/10.1016/j.aquaculture.2014.10.011

Cornélio, F. H. G., Cargnin-Ferreira, E., Borba, M. R. D., Mouriño, J. L. P., Fernandes, V. A. G., & Fracalossi, D. M. (2013). Crescimento, digestibilidade e resistência à infecção por patógeno em tilápia-do-nilo alimentada com probióticos. *Pesquisa Agropecuária Brasileira*, *48*(8), 863-870. DOI: https://doi.org/10.1590/S0100-204X2013000800008

Cruz, E., Moraes, L. P. D., Costa, E. A., Barbosa, J. B., & Martins, M. L. L. (2019). Optimization of food-waste based culture medium for cellulase production by thermophilic Bacillus sp SMIA-2 and effect of divalent metal ions on activity and stability of the enzyme at higher temperatures. *International Journal of Advanced Engineering Research and Science (IJAERS). 6*(7) 331-337. DOI: https://dx.doi.org/10.22161/ijaers.6741

Da Silveira, U. S., Logato, P. V. R., & da Conceição Pontes, E. (2009). Utilização e metabolismo dos carboidratos em peixes. *Revista Eletrônica Nutritime*, *6*(1), 817-836.

Dawood, M. A., Koshio, S., Abdel-Daim, M. M., & Van Doan, H. (2019). Probiotic application for sustainable aquaculture. *Reviews in Aquaculture*, *11*(3), 907-924. DOI: https://doi.org/10.1111/raq.12272

De Moraes Gonçalves, T., de Almeida, Á. J. L., & Santo Borges, E. D. E. (2003). Características de carcaça de tilápias do Nilo (Oreochromis niloticus) em quatro classes de peso ao abate. *Acta Scientiarum. Animal Sciences*, *25*, 25-29. DOI: https://doi.org/10.4025/actascianimsci.v25i1.2069

De Souza, F. L., & Ferreira, M. W. (2022). Inclusão de probióticos na piscultura para o estímulo à resposta imune inata e enriquecimento fisiológico-Revisão de literatura: Inclusion of probiotics in pisculture to stimulate the innate immune response and physiological enrichment-Literature review. *Brazilian Journal of Development*, *8*(11), 71766-71775. DOI: https://doi.org/10.34117/bjdv8n11-068

Falcon, D. R., Barros, M. M., Pezzato, L. E., Solarte, W. V. N., & Guimarães, I. G. (2008). Leucograma da tilápia-do-nilo arraçoada com dietas suplementadas com níveis de vitamina ce lipídeo submetidas a estresse por baixa temperatura. *Ciência Animal Brasileira/Brazilian Animal Science*, *9*(3), 543-551.

Ferreira, C. M., Antoniassi, N. A., Silva, F. G., Povh, J. A., Potença, A., Moraes, T. C., ... & Abreu, J. S. (2014). Características histomorfométricas do intestino de juvenis de tambaqui após uso de probiótico na dieta e durante transporte. *Pesquisa Veterinária Brasileira*, *34*(12), 1258-1264. DOI: https://doi.org/10.1590/S0100-736X2014001200020

Figueiredo, H. C. P., Tavares, G. C., Dorella, F. A., Rosa, J. C. C., Marcelino, S. A. C., Pierezan, F., & Pereira, F. L. (2022). First report of infectious spleen and kidney necrosis virus in Nile tilapia in Brazil. *Transboundary and Emerging Diseases*, *69*(5), 3008-3015. DOI: https://doi.org/10.1111/tbed.14217

Freitas, J. M. A. D., Peres, H., Carvalho, P. L. P. F. D., Furuya, W. M., Sartori, M. M. P., Pezzato, L. E., & Barros, M. M. (2022). Interactive effects of digestible protein levels on thermal and physical stress responses in Nile tilapia. *Revista Brasileira de Zootecnia*, *51*, e20200183 DOI: https://doi.org/10.37496/rbz5120210067

Furuya, W. M., Cruz, T. P. D., & Gatlin III, D. M. (2023). Amino Acid Requirements for Nile Tilapia: An Update. *Animals*, *13*(5), 900. DOI: https://doi.org/10.3390/ani13050900

Gomes, V. D. S., Silva, J. H. V. D., Cavalcanti, C. R., Fonseca, S. B. D., Jordão Filho, J., Silva Neto, M. R. D., & Silva, F. B. D. (2016). Utilização de enzimas exógenas na nutrição de peixes-revisão de literatura. *Arquivos de Ciências Veterinárias e Zoologia da UNIPAR*, *19*(4), 259-264. DOI: https://doi.org/10.25110/arqvet.v19i4.2016.6106

Harsij, M., Kanani, H. G., & Adineh, H. (2020). Effects of antioxidant supplementation (nano-selenium, vitamin C and E) on growth performance, blood biochemistry, immune status and body composition of rainbow trout (Oncorhynchus mykiss) under sub-lethal ammonia exposure. *Aquaculture*, *521*, 734942. DOI: https://doi.org/10.1016/j.aquaculture.2020.734942

Islam, S. M., Rohani, M. F., & Shahjahan, M. (2021). Probiotic yeast enhances growth performance of Nile tilapia (Oreochromis niloticus) through morphological modifications of intestine. *Aquaculture Reports*, *21*, 100800. DOI: https://doi.org/10.1016/j.aqrep.2021.100800

Ladeira, S. A., Cruz, E., Delatorre, A. B., Barbosa, J. B., & Leal Martins, M. L. (2015). Cellulase production by thermophilic Bacillus sp: SMIA-2 and its detergent compatibility. *Electronic journal of biotechnology*, *18*(2), 110-115. DOI: http://dx.doi.org/10.1016/j.ejbt.2014.12.008

Latorre, J. D., Hernandez-Velasco, X., Wolfenden, R. E., Vicente, J. L., Wolfenden, A. D., Menconi, A., ... & Tellez, G. (2016). Evaluation and selection of Bacillus species based on enzyme production, antimicrobial activity, and biofilm synthesis as direct-fed microbial candidates for poultry. *Frontiers in Veterinary Science*, *3*, 95. https://doi.org/10.3389/fvets.2016.00095

Lee, H. J., & Kim, H. Y. (2011). Lantibiotics, class I bacteriocins from the genus Bacillus. *Journal of microbiology and biotechnology*, *21*(3), 229-235. DOI: https://doi.org/10.4014/jmb.1010.10017

Macedo, C. F., & Sipaúba-Tavares, L. H. (2010). Eutrofização e qualidade da água na piscicultura: consequências e recomendações. *Boletim do instituto de Pesca*, *36*(2), 149-163.

Makled, S. O., Hamdan, A. M., & El-Sayed, A. F. M. (2019). Effects of dietary supplementation of a marine thermotolerant bacterium, Bacillus paralicheniformis SO-1, on growth performance and immune responses of Nile tilapia, Oreochromis niloticus. *Aquaculture Nutrition*, *25*(4), 817-827. DOI: https://doi.org/10.1111/anu.12899

Marengoni, N. G., Albuquerque, D. M., Mota, F. L. S., Passos Neto, O. P., Silva Neto, A. A., Silva, A. I. M., & Ogawa, M. (2010). Desempenho e proporção sexual de tilápia vermelha sob à inclusão de probiótico em água mesohalina. *Archivos de Zootecnia*, *59*(227), 403-414.

Mili, S., Ennouri, R., Fatnassi, M., Zarrouk, H., Thabet, R., & Laouar, H. (2023). Nile Tilapia "Oreochromis niloticus" farming in fresh and geothermal waters in Tunisia: A comparative study. In *Intensive animal farming-a cost-effective tactic*. IntechOpen. DOI: https://doi.org/10.5772/intechopen.106646

Moraes, A. C., Prado, E. J., Foz, E. P., Barbuio, R., Faria, V. P., & Belo, M. A. (2018). Esteatose hepática altera acúmulo celular em tilápias do Nilo durante aerocistite infecciosa. *Pesquisa Veterinária Brasileira*, *38*(8), 1570-1576. DOI: https://doi.org/10.1590/1678-5150-PVB-5533

Peixe BR. (2021). *Anuário PeixeBR da Piscicultura 2020*. São Paulo, SP: Associação Brasileira da Piscicultura.

Pezzato, L. E., Menezes, A., Barros, M. M., Guimarães, I. G., & Schich, D. (2006). Levedura em dietas para alevinos de tilápia do Nilo. *Veterinária e Zootecnia*, *13*, 84-94.

Rabinovitch, L., & de Oliveira, E. J. (2015). *Coletânea de procedimentos técnicos e metodologias empregadas para o estudo de Bacillus e gêneros esporulados aeróbios correlatos* (p. 160). Rio de Janeiro, RJ: Montenegro Comunicação.

Silva, C. R. D., Delatorre, A. B., & Martins, M. L. L. (2007). Effect of the culture conditions on the production of an extracellular protease by thermophilic Bacillus sp and some properties of the enzymatic activity. *Brazilian Journal of Microbiology*, *38*(2), 253-258. DOI: https://doi.org/10.1590/S1517-83822007000200012

Silva, V. V., Salomao, R. A. S., Mareco, E. A., Dal Pai, M., & Santos, V. B. (2021). Probiotic additive affects muscle growth of Nile tilapia (Oreochromis niloticus). *Aquaculture Research*, *52*(5), 2061-2069. DOI: https://doi.org/10.1111/are.15057

Souza, A. N. D., & Martins, M. L. L. (2001). Isolation, properties and kinetics of growth of a thermophilic Bacillus. *Brazilian Journal of Microbiology*, *32*(4), 271-275. DOI: https://doi.org/10.1590/S1517-83822001000400003

Tachibana, L., Dias, D. C., Ishikawa, C. M., Correa, C. F., Leonardo, A. F. G., & Ranzani-Paiva, M. J. T. (2011) Probiótico na alimentação da tilápia-do-nilo (Oreochromis niloticus Lineu, 1758): desempenho zootécnico e recuperação da bactéria probiótica intestinal. *Bioikos*, *25*, 25-31. Retrieved from https://seer.sis.puc-campinas.edu.br/bioikos/article/view/555

Wambua, D. M., Home, P. G., Raude, J. M., & Ondimu, S. (2021). Environmental and energy requirements for different production biomass of Nile tilapia (Oreochromis niloticus) in recirculating aquaculture systems (RAS) in Kenya. *Aquaculture and fisheries*, *6*(6), 593-600. DOI: https://doi.org/10.1016/j.aaf.2020.07.019

Wang, L., Lu, Q., Luo, S., Zhan, W., Chen, R., Lou, B., & Xu, D. (2016). Effect of dietary lipid on growth performance, body composition, plasma biochemical parameters and liver fatty acids content of juvenile yellow drum Nibea albiflora. *Aquaculture reports*, *4*, 10-16. DOI: https://doi.org/10.1016/j.aqrep.2016.05.002

Xu, S. D., Zheng, X., Dong, X. J., Ai, Q. H., & Mai, K. S. (2022). Beneficial effects of phytase and/or protease on growth performance, digestive ability, immune response and muscle amino acid profile in low phosphorus and/or low fish meal gibel carp (Carassius auratus gibelio) diets. *Aquaculture*, *555*, 738157. DOI: https://doi.org/10.1016/j.aquaculture.2022.738157

Zhaxi, Y., Meng, X., Wang, W., Wang, L., He, Z., Zhang, X., & Pu, W. (2020). Duan-Nai-An, A Yeast probiotic, improves intestinal mucosa integrity and immune function in weaned piglets. *Scientific reports*, *10*, 4556. |DOI: https://doi.org/10.1038/s41598-020-61279-6