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Benefits of Adding Probiotic Based on *Lactobacillus fermentum* to tambaqui (*Colossoma macropomum*)

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ABSTRACT

This study aimed to evaluate the histomorphometric characteristics of the intestinal mucosa of juvenile tambaqui (*Colossoma macropomum*) after adding the probiotic *Lactobacillus fermentum* to their diet, from sources both internal and external to the host. A randomized block design was used, with two treatments and a control in four replicates, as follows: T1, fish fed with feed supplemented with autochthonous *L. fermentum* (LMUFT10) (10 mL kg^{-1} of feed; 5 x 109 CFU mL^{-1}); T2, fish fed with diet supplemented with commercial *L. fermentum* (10 mL kg^{-1} of feed; 5 x 109 CFU mL^{-1}); and T3, control: fish that only received feed. Supplementation with probiotic (autochthonous *L. fermentum*) had no effect on the height and length of the tambaqui intestinal villi. However, there were increases in the numbers of goblet cells in both treated groups (T1 and T2). Thus, it can be concluded that *L. fermentum*, regardless of its source, contributes to defense of the intestinal mucosa of tambaqui through increased production of goblet cells.

Keywords: Brazilian amazon. Fish farm. Histomorphometric characteristics. Supplemented probiotics.

Benefícios da adição de probiótico à base de *Lactobacillus fermentum* ao tambaqui (*Colossoma macropomum*)

RESUMO

O estudo teve como objetivo avaliar as características histomorfométricas da mucosa intestinal de juvenis de tambaqui (*Colossoma macropomum*) após adição do probiótico *Lactobacillus fermentum* à sua dieta, proveniente de fontes internas e externas ao hospedeiro. O delineamento experimental utilizado foi blocos casualizados, com dois tratamentos e uma testemunha em quatro repetições, sendo T1, peixes alimentados com ração suplementada com *L. fermentum* autóctone (LMUFT10) (10 mL kg^{-1} de ração; 5 x 109 UFC mL^{-1}); T2, peixes alimentados com dieta suplementada com *L. fermentum* comercial (10 mL kg^{-1} de ração; 5 x 109 UFC mL^{-1}); e T3, controle: peixes que receberam apenas ração. A suplementação com probiótico (*L. fermentum* autóctone) não teve efeito sobre a altura e comprimento das vilosidades intestinais do tambaqui. No entanto, houve aumentos no número de células caliciformes em ambos os grupos tratados (T1 e T2). Assim, pode-se concluir que *L. fermentum*, independente de sua origem, contribui para a defesa da mucosa intestinal do tambaqui através do aumento da produção de células caliciformes.

Palavras-chave: Amazônia brasileira. Características histomorfométricas. Piscicultura. Probióticos suplementados. Tambaqui.

INTRODUCTION

Worldwide, there have been significant increases in aquaculture production, possibly through improvements of modern sustainable techniques. These have contributed not only to growth of activity, although also to reduction of the pressure on natural fish stocks (SARTORI; AMANCIO, 2012; BOYD et al., 2022). According to FAO (2022), the estimated worldwide production of fish in captivity has reached the mark of 54.279 million tons with an estimated per capita consumption of 20.5 kg^{-1} inhabitant. In Brazil, the volume of farmed fish increased by 5.93% compared with 2019, with an estimated production of 802,930 tons in year 2020. Of this amount, native fish contributed 34.7% of the total and thus form an important proportion of Brazilian fish farming (PEIXE BR, 2022). The tambaqui (*Colossoma macropomum* Cuvier, 1818) is the main native species produced in Brazil. This is because this species has characteristics favorable to cultivation, such as acceptance of artificial feed, good growth rates and feed conversion, easy acquisition of fingerlings and tolerance of low concentrations of dissolved oxygen. Moreover, there is increasing demand for this fish, especially in regional markets (PEIXE BR, 2022; AZEVEDO et al., 2016).

With the intensification of production systems, there is a need to use techniques aimed at improving the resistance of animals that are kept in confinement and subjected to adverse effects caused by stress. Good zootechnical indices also need to be attained (LEE; KIM, 2020; CASTRO et al., 2021). Use of probiotics in aquaculture has frequently been investigated. This has been shown to be a promising tool for health management in intensive farming (NEWAJ-FYZUL; et al., 2014; WEI et al., 2022). When these live microorganisms are properly added to the diet and administered in satisfactory quantities, they confer benefits to the hosts' health (NEWAJ-FYZUL et al, 2014). There is evidence shown that the effects caused by probiotics occur through colonization by bacteria in the digestive tract, especially in the epithelium of the gastrointestinal mucosa (NEWAJ-FYZUL et al., 2014; YOUSEFI et al., 2019; DIAS et al., 2020). Incorporation of probiotics into diets can provide better feed conversion and enables therapeutic, prophylactic and growth-promoting functions in fish (TORRES; MANCILHA, 2020).

Probiotic *Lactobacillus fermentum* is live microbial food additive and commercially unenforceable. Nowadays probiotic is widely used in aquaculture practices to improve disease resistance, productive performance and feed efficiency of fish (GOU et al., 2022). *Lactococcus lactis subsp. lactis* I2 was a potential probiotic, which increased growth of olive flounder compared to the untreated group and could be used an alternative source for the synthetic antibiotic for controlling streptococcosis in aquaculture. Similarly, Yamashita et al. (2022) reported that olive flounder fed the diet containing the mixture of probiotics of *L. plantarum* and *L. lactis* BFE920 improved weight gain the adhesion of all rainbow trout pathogens (*A. hydrophila, A. salmonicida and Yersinia ruckeri*), except for *V. anguillarum* with intestinal mucus was reduced by *L. fermentum* CLFP 242 and the mixture of three Lactic acid bacterial strains (*L. lactis* CLFP 101, *L. plantarum* CLFP 238 and *L. fermentum* CLFP 242). However, various biotic and abiotic factors influenced the production of the antibiotics in bacteria (RAAIJMAKERS et al., 2022).

In the group of probiotics that has been most studied, the genera Bacillus and Lactobacillus can be highlighted (TORRES; MANCILHA, 2020; WEI et al., 2022). Strains of these genera are almost always obtained from sources that are exogenous to the target species. However, in selecting a functional food, it is preferable to isolate strains from the host, because these present greater chances of colonizing the intestinal tract of the target species (BALCÁZAR et al., 2016). Studies have addressed the effects of use of probiotics on the intestinal morphology of fish and have highlighted their benefits for the structure of microvilli and for absorption surfaces (CASTRO; BALCÁZAR, 2006; FERREIRA et al., 2014).

Thus, the aim of this study was to evaluate the histomorphometric characteristics of the intestinal mucosa of tambaqui (*Colossoma macropomum*) after use of a probiotic based on autochthonous *Lactobacillus fermentum* that was added to the diet.

MATERIAL AND METHODS

Experimental design

This experiment was conducted in the Laboratory of Applied Agromicrobiology and Biotechnology, which forms part of the Business Incubator af the Universidade Federal do Tocantinis (UFT), Gurupi campus, Brazil. The methods used in this study were approved by the institution’s ethics committee (CEUA/UFT), under procedural no. 23.101.007555/2018-87.

The specimens used were purchased through a local fingerling supplier. Before removing the fish from the tanks where they have been kept, they were kept fasting for 24 hours and then after capture for another 24 hours, in order to empty the intestinal tract and minimize stress during transportation. These juvenile tambaquis were transported to the laboratory in plastic bags with a capacity of 60 L, which were filled with oxygen and water in proportions of 5:1, respectively, at a density of 80 g of fish per liter of water (YOUSUF et al., 2022).

In the laboratory, a total of 120 specimens of tambaqui with an average weight of 16.33 ± 3.29g were acclimatized for 30 minutes and stored in water boxes with a usable volume of 30 L, respecting a density of 10 individuals per box (0.33 fish L^{-1}), with constant aeration and daily renewal corresponding to 10% of the volume. These fish were fed with extruded feed corresponding to 5% of their live weight, over a 15-day adaptation period.

The experiment lasted for 45 days, we opted for a randomized block design consisting of two treatments and a control, in four replicates, as follows: T1, fish that received feed supplemented autochthonous *L. fermentum* (LMUFT10), which was obtained from the collection of the Laboratory of Applied Agromicrobiology and Biotechnology; T2, fish that received feed supplemented with lyophilized allochthonous *L. fermentum* that was acquired commercially, originating in China); and T3, control fish that only received the feed. Throughout the experiment, there was constant aeration daily renewal of water corresponding to 10% of its volume and daily removal of waste that had accumulated at the bottoms of the water boxes.

Pathological conditions such as shock, trauma, stress, and inflammation damage the intestinal barrier to varying degrees, aggravating the primary disease. Intestinal probiotics are a type of active microorganisms beneficial to the health of the host and an essential element of human health. Reportedly, intestinal probiotics can affect the renewal of intestinal epithelial cells, and also make cell connections closer, increase the production of tight junction proteins and mucins, promote the development of the immune system, regulate the release of intestinal antimicrobial peptides, compete with pathogenic bacteria for nutrients and living space, and interact with the host and intestinal commensal flora to restore the intestinal barrier (GOU et al., 2022).

In fish, there is evidence of the role of probiotics in regulating intestinal epithelial function by promoting mucus layer formation. A study with gilthead sea bream found that supplementing with probiotic combination of *B. licheniformis, L. plantarum,* and *B. subtilis* along with fenugreek increased hepatic superoxide dismutase and catalase, which are antioxidants that protect against oxidative stress (NIMALAN et al., 2022).

Preparation of diets

The diet consisted of an extruded commercial diet for fish (Table 1) composed of 40% crude protein (CP), with grain size of 2 mm, which is the ideal size for the phase of life of these fish.

Table 1 - Nutritional composition of the commercial diet used in the experiment.

Nutrients	Specification	Quantity
Macronutrients	Humidity (max.)	110 g kg^{-1}
	Crude protein (min.)	400 g kg^{-1}
	Ether extract (min.)	50 g kg^{-1}
	Mineral matter (max.)	110 g kg^{-1}
	Fibrous matter (max.)	70 g kg^{-1}
Macrominerals and microminerals	Calcium (min.)	20 g kg^{-1}
	Calcium (max.)	30 g kg^{-1}
	Phosphorus (min.)	15 g kg^{-1}
	Sodium (min.)	370 mg kg^{-1}
	Cobalt (min.)	0.75 mg kg^{-1}
	Iron (min.)	75 mg kg^{-1}
	Iodine (min.)	1.5 mg kg^{-1}
	Manganese (min.)	7.5 mg kg^{-1}
	Copper (min.)	4.5 mg kg^{-1}
	Selenium (min.)	0.3 mg kg^{-1}
Vitamins	Folic acid (min.)	5.1 mg kg^{-1}
	Pantothenic acid (min.)	17 mg kg^{-1}
	Biotin (min.)	0.85 mg kg^{-1}
	Vitamin A (min.)	6800 IU kg^{-1}
	Vitamin B1 (min.)	17 mg kg^{-1}
	Vitamin B12	25.5 mg kg^{-1}
	Vitamin B2 (min.)	17 mg kg^{-1}
	Vitamin B6 (min.)	17 mg kg^{-1}
	Vitamin C (min.)	300 mg kg^{-1}
	Vitamin E (min.)	2550 IU kg^{-1}
	Vitamin K3 (min.)	3.4 mg kg^{-1}
	Niacin (min.)	34 mg kg^{-1}
	Choline (min.)	850 mg kg^{-1}

In preparing the inoculum, the strains of *L. fermentum* (LMUFT10 and commercial) were activated separately in 400 mL of sterilized MRS broth, sterilized. After growth, the strains were plated in Man, Rogosa & Sharpe (MRS) agar and incubated at 37 °C for 48 hours. After this period, serial dilutions (factor 1:10) were performed and the dilutions (10^4, 10^6 and 10^8) were plated to characterize the numbers of cells and were aliquoted to obtain a concentration close to 10^{10} CFU mL^{-1} (SANTOS et al., 2023).

The feed that was used in the diets of the treatments (T1 and T2) was sprinkled with 10 mL of the bioproduct kg^{-1} of feed, containing the probiotic at the concentration of 5 x 10^9 CFU mL^{-1}. The feed was then mixed with soybean oil at a proportion of 2% of the biomass of the feed: this technique was used to prevent dispersion of cells in the water at the time when the feed was offered to the fish. After preparation, the diets were packed in plastic bags and stored in closed containers, at room temperature. For treatment T3, the same commercial diet was used without addition of the probiotic.

Histomorphological analysis on intestinal villi

In order to evaluate the histomorphometric structure of the intestine, one fish specimen per replication was used, thus totaling four per treatment, including the control. These fish were euthanized by means of thermal shock, using a mixture of water and ice at the ratio of 1:1. They were subjected to a longitudinal incision in the ventrum, to remove the intestines aseptically.

After collection, the intestines were fixed in 10% formalin for 24 hours and then in alcohol 70% for an indefinite period until preparation of slides. The material collected was sent to the Laboratório de Aquicultura e Análises de Água (LAQUA), at the Centro de Engenharia e Ciências Agrárias, at the Universidade Federal de Alagoas (UFAL), for histological analysis.

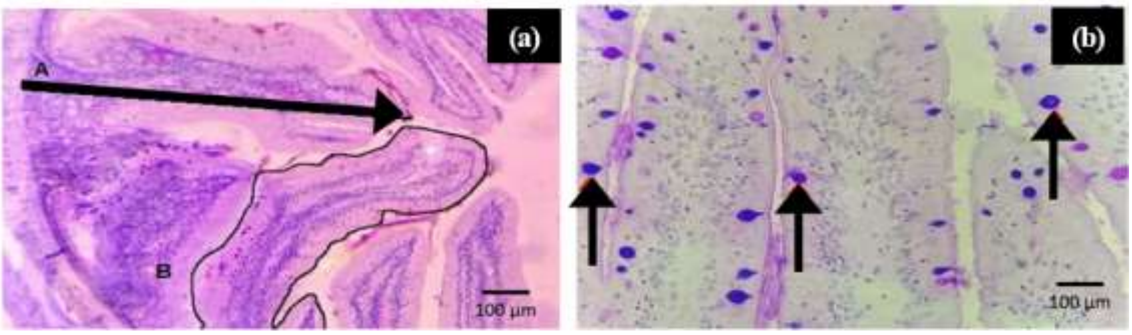
The morphohistological characteristics of the intestinal mucosa (height and length) were evaluated through the following procedure. Fragments of the middle part of the intestine (2.0 cm) were collected and dehydrated in an increasing alcoholic series at 80 and 90% (10 minutes each) and then in three 10-minute baths each in 99% ethyl alcohol.

The samples were infiltrated, at room temperature, firstly in a two-hour bath with a mixture of historesin (Leica®) and alcohol (1:1) and were then kept immersed in pure historesin for 24 hours. The material was embedded in historesin and was transferred to polyethylene molds (‘histomolds’). It was then kept in a desiccator, at room temperature, until the blocks had become completely polymerized. Semi-thin sections of thickness 5 µm were obtained by using a rotary microtome with stainless steel blades.

These sections were transferred to histological slides, which were placed on a plate heated at 40 °C for 15 minutes so that the cuts would become distended and would adhere to the slide. Subsequently, the sections were stained with Harris hematoxylin for 30 minutes, washed in running water for 5 minutes, stained with eosin for 30 seconds and washed under running water. To protect the material, coverslips were attached to the slides using Canada balsam as the mounting medium.

The material was then analyzed under a light microscope that was coupled to a digital camera. The villi were analyzed and measured in microns (µm): height from the apex to the base; and circumference. Two villi were measured per histological section, as prescribed by (FERREIRA et al., 2014). Thus, a total of 72 villi were analyzed, i.e. 24 per treatment (Figure 1). Histochemical analysis was performed using the Periodic acid-Schiff (PAS) reagent. A total of 10 images were captured from each treatment, including the control group, and goblet cells with positive reaction were counted. This was done on all villi within the capture field. All photos were taken using the 40x lens of the microscope.

Figure 1 - Intestinal villi of tambaqui (*Colossoma macropomum*). (a): height of villus, surface amplification A and basal B; (a): circumference of villus. Subtitle: Hematoxylin-eosin; objective lens 40x; this figure is in color in the electronic version.



Analysis on cultivation water quality

The water quality parameters of dissolved oxygen (mg L^{-1}), temperature (ºC) and pH were evaluated once a week. There was a need to correct the pH of the water supply, with the addition of 4% Acetic acid at a concentration of one drop per liter of water. This correction was performed in the reservoir before the water was taken to the boxes. To measure dissolved oxygen and temperature, a multiparameter probe (HI 9146; Hanna Instruments) was used; and for pH, a digital pH meter (HI 98103; Hanna Instruments, USA).

Statistical analyses

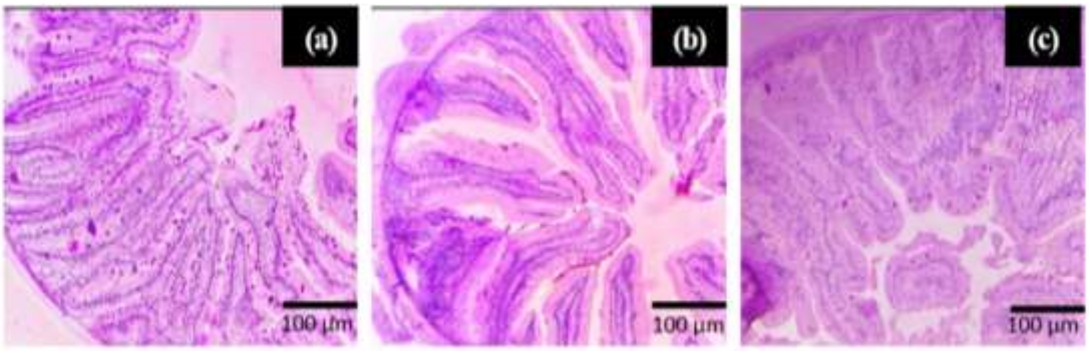
The variables relating to the intestinal villi (height, circumference and number of goblet cells) and the water quality parameters were subjected to analysis of variance (ANOVA). If significant differences were found between the treatments, the Tukey's test was then used at the significance level of 5%. The Minitab software, version 20.4, was used for the analyses.

RESULTS AND DISCUSSION

There were no significant differences in water quality parameters were observed between the different treatments ($p > 0.05$). During the experiment, the average dissolved oxygen concentrations recorded were 6.1 mg L^{-1} ± 1.4, 6.2 mg L^{-1} ± 1.8 and 6.0 mg L^{-1} ± 1.4 for T1, T2 and T3, respectively. Regarding dissolved oxygen, the recommendation for tropical fish species implies values above 5.0 mg L^{-1} (BARBOSA, 2022), which is what was observed in the current study.

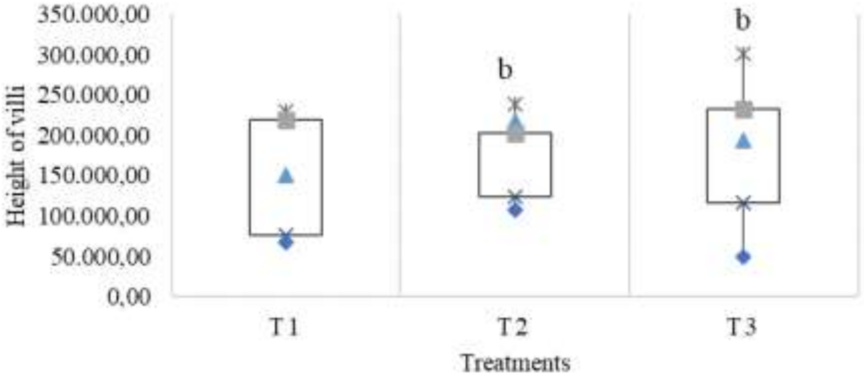
For pH, the median was 7.5 for all treatments, i.e. close to neutrality. The pH values found in the current study were within the ranges recommended in the literature. In general, tropical fish species tolerate a range between 6.5 and 8.5, while values lower or higher than these can cause several physiological problems (AMANJÁS et al., 2022). However, the Amazonian tambaqui is a biologically resilient species of fish that withstands extremes of oxygen, temperature and pH (VAL; OLIVEIRA, 2021) and when compared to other species, the tambaqui starts to present physiological and biochemical disturbances only when exposed to pH 3.5. The survival of the individuals during the experimental period was 100% (Figure 2) demonstrates the morphological structure of the intestinal villi, for the different treatments analyzed.

Figure 2 - Villi of the intestine middle region of juvenile tambaqui (*Colossoma macropomum*). (a) Treatment with autochthonous *L. fermentum*. (b) Treatment with allochthonous commercial *L. fermentum*. (c) Control treatment. Subtitle: Hematoxylin-eosin; objective lens 40x. This figure is in color in the electronic version.



In the histomorphometric analysis on the intestine, it was found that the mean height of the intestinal villi of the group of fish that received autochthonous probiotic *L. fermentum* (T1), showed a significant difference ($p < 0.05$) in relation to T2 and control, which had smaller averages (Figure 3). However, no significant differences were found between the treatments regarding the circumference of the villi ($p > 0.05$).

Figure 3 - Average and standard deviation of villus heights in the intestine middle region of juvenile tambaqui (*Colossoma macropomum*). Subtitle: Different letters (a,b) differ from each other according to the Tukey test ($p < 0.05$).



The main function of the intestine is to complete the digestive process that began in the stomach, with absorption of nutrients. The structure of the intestine encompasses a series of prominent and morphologically distinct folds, such as the intestinal villi. In addition to enabling colonization by bacteria, the villi have beneficial effects regarding digestibility (CASTRO et al., 2021; CHUNG et al., 2021). Silva et al., (2022) evaluated the intestinal villi (area and length) of wild and farmed tambaquis and found significant differences between the groups tambaquis natural/wild environment and fish farm (RAY; RINGO, 2014; LAUZON et al., 2014). They attributed the dissimilarity to possible differences in the diets available in each environment, such that the cultivated fish showed greater lengths and areas of villi than those of the wild tambaquis, as a result of possible adaptation due to the regular offer and the quality of the diet in the confinement environment. According to results in the current study, the characteristics of area and length of the villi showed differences between the fish farming and natural environment groups (AVS = 13,690.71 ± 12,309.46 µm; AVP = 44,771.93 ± 23,793.75 µm; CVS = 303.93 ± 164.75 µm; CVP = 505.89 ± 190.03 µm).

The influence of probiotics on the morphological alterations of villi has been demonstrated in several studies. However, different results have been seen in farmed fish.

The effects of probiotics on the histomorphometry of the intestine was evaluated by Mello et al., (2013), in juvenile tilapias that received a diet containing *Bacillus cereus* and *Bacillus subtilis* for 80 days. This resulted in increased total height, width and thickness of the villus epithelium. However, Castro et al. (2021) found that the diet supplemented with Bacillus only influenced the width of the villi and the thickness of the tunica in post-larval fish of the same species.

Regarding temperature, the values recorded for the experimental units corresponded to those suitable for the cultivation of Amazonian species. The average temperature was 27.4 ºC for T1 and 27.5 ºC for T2 and T3. These values are considered appropriate for cultivation of Amazonian species, such as tambaqui, which has a preference for temperatures close to 28 ºC (AMANJÁS et al., 2022).

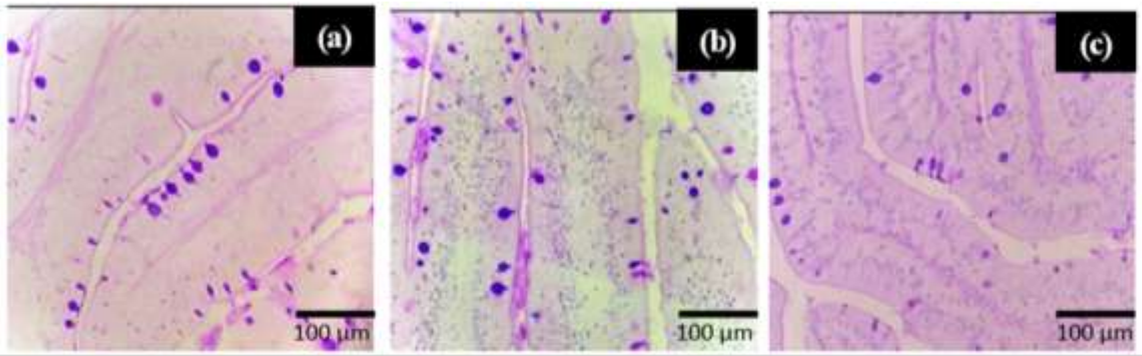
With the rapid growth of the aquaculture production since in year 2016 in Rondonia state, there has been a concomitant increase in disease outbreaks to native species who tambaqui in Amazon. The injudicious and/or incorrect use of antimicrobial agents against diseases of farmed aquatic species poses a considerable threat to the development and growth of a successful and sustainable aquaculture industry (BONDAD-REANTASO et al., 2023). An increase in antimicrobial resistance is an important consequence, resulting to the difficulty in treating common bacterial diseases in populations of aquatic organisms, combined with the presence of antibiotic residues in food fish and their products, leading to import refusals and negative impacts on international trade. To reduce the frequency of antimicrobial resistance, good aquaculture and effective biosecurity practices should include the prudent and responsible use of antibiotics and consider the use of alternatives to antibiotics, in addition to disease prevention management (DANG, 2021).

Effect of dietary inclusion of yacon (YC), ginger (GG) and blueberry (BB) on growth, feed utilization and body composition and plasma chemistry of sole (*Paralichthys olivaceus*) and immunity test against Streptococcus iniae compared to a commercial probiotic (*Lactobacillus fermentum*). No differences were observed in husbandry performance, feed utilization, body composition and plasma chemistry at the end of the 8-week feeding trial (KIM et al., 2019). Mortality was observed in fish fed the CON diet from 42h until the end of 8 days. Therefore, YC, GG and BB were effective as an immunostimulant against *S. iniae* rather than a growth promoter of olive sole.

Results in the literature attest to the influence that probiotics exert on the anatomical characteristics of the gastrointestinal tract. Probiotics promote increased mucosal absorption surface in the intestine and contribute to increased bowel length, which leads to a response of improved host performance (RAY; RINGO, 2014; LAUZON et al., 2014).

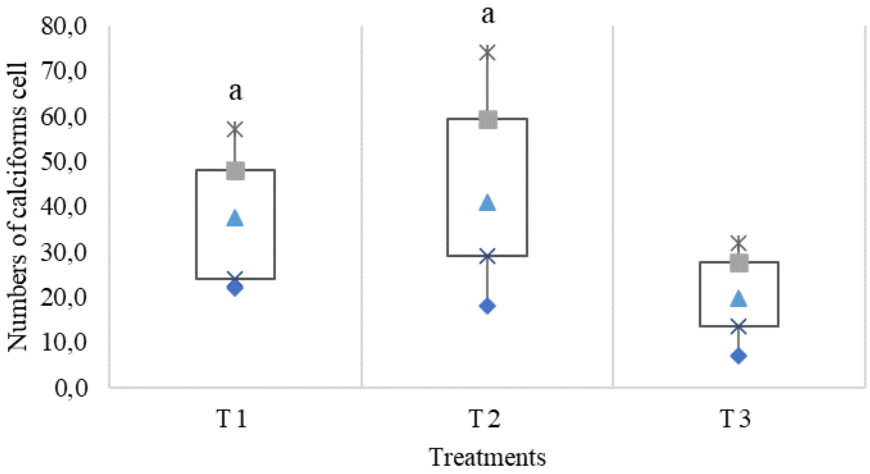
Probiotics restore the intestinal mechanical barrier by regulating the expression of genes and proteins involved in tight junction (TJ) signaling in intestinal epithelial cells (IECs), regulating their apoptosis or inducing IECs proliferation (ZHANG et al., 2015). TJs between IECs can selectively transport substances, prevent pathogenic bacteria and harmful substances from entering the intestinal lumen, and maintain normal intestinal barrier function (SHARMA et al., 2010). Furthermore, the mixture of *Lactobacillus fermentum* GOS57 and *L. plantarum* GOS42 was able to reduce the number of Enterobacteriaceae, increase the abundance of Lactobacillus, adjust the intestinal flora balance and improve the intestinal barrier function (LINNINGE et al., 2019). In the villi analyzed, presence of goblet cells was observed in all treatments (Figure 4).

Figure 4. Villi of the intestine middle region of juvenile tambaqui (*Colossoma macropomum*), shown goblet cells (blue dots). (a) Treatment with autochthonous *L. fermentum*, (b) Treatment with allochthonous commercial *L. fermentum*, and (c) Control treatment. Subtitle: Periodic acid-Schiff; objective lens 40x. This figure is in color in the electronic version



Regarding the total number of cells, there was no significant difference between the treatments that used the probiotic, i.e. between autochthonous *L. fermentum* and allochthonous commercial *L. fermentum* ($p > 0.05$) (Figure 5).

Figure 5 - Average and standard deviation of the number of goblet cells present in villi in the intestine middle region of juvenile tambaqui (*Colossoma macropomum*). Subtitle: Periodic acid-Schiff, Different letters differ (a,b) from each other according to the Tukey test ($p < 0.05$).



As observed in the current study, the significant difference in the length of the intestinal villi that was found between the treatments using autochthonous and allochthonous *L. fermentum* did not influence their circumference. This result corroborates what was reported Ferreira et al. (2014), who observed that there were no significant differences in the height and width of the villi of the intestinal mucosa of juvenile tambaquis that received a diet based on *Bacillus* spp. for 60 days.

According to Nguyen et al. (2021), differences in the width and height of villi may be related to the presence of microorganisms in the intestinal biota. When a pathogen comes into contact with the intestine, this can cause an imbalance that interferes with cell renewal, such that the height, width and thickness of the villi become modified. According to (WEI et al., 2022) and Stojanović et al., (2021), changes to the length of the intestine that occur can be compensated by variations in area of the intestinal mucosa and by the different types of cells present in the epithelium.

It is already scientific knowledge that the size of villi is maintained by constant cell renewal because of loss versus renewal of epithelial cells, after successive mitoses for formation of the epithelial cells of the villus (FERREIRA et al., 2014). According to Zhang et al. (2022), increased mitosis at the bases of villi causes greater cell proliferation in the mucosa, which causes an increase in the height of the villi and thus improves the organism’s absorption and performance. Nonetheless, studies with approaches focusing on information on digestive tract physiology and morphology of neotropical fish species remain at an early stage (GONÇALVES et al., 2012).

Goblet cells are specialized cells that produce mucins and glycoproteins with important functions relating to defense of the intestinal mucosa against the actions of digestive enzymes, chemical and physical damage, and invasion of potential pathogens (FERREIRA et al., 2014; CONRAD; STÖCKER, 2014; BÜYÜKDEVECI et al., 2023;). Counts on the numbers of these cells in the current study showed a significant difference, such that they were higher in fish that received treatments with probiotics, regardless of whether these were autochthonous or allochthonous (WEI et al., 2022).

The results from the current study were similar to those of Büyükdeveci et al., (2020), who evaluated juvenile tilapias that received probiotics based on *B. cereus* and *B. subtilis*, which showed higher numbers of goblet cells. According to Rogers et al. (2023), goblet cells proliferate to increase mucus production and thus provide a protective barrier when aggression by pathogens occurs.

Regarding the mucus layer secreted by goblet cells prevents contact between microorganisms and epithelial cells and also has bactericidal action due to the presence of lysozyme and low molecular weight fatty acids (BONDAD-REANTASO et al., 2023). Therefore, addition of probiotic to the diet contributes towards increasing the number of goblet cells, thus favoring defense against the action of bacteria that are harmful to the intestinal mucosa (BÜYÜKDEVECI et al., 2023).

In the scientific literature, the importance of isolating lineages of probiotics that are internal to the target species has been highlighted because these are more likely to colonize the intestinal tract of the host and remain viable (BALCÁZAR et al., 2006; LEE; KIM, 2019). Ferreira et al., (2018) mentioned that the host-probiotic relationship seemed to be species-specific, after observing that strains of allochthonous *Bacillus* did not influence the histomorphometry of the tambaqui intestine. Thus, the importance of isolation and selection of beneficial bacteria specific to the intestinal tract of the species, as an important strategy for development of probiotics for tambaquis, was highlighted. Ray and Ringo (2014) and Lauzon et al. (2014), highlighted the importance of the length of time for which probiotics were provided in the diet. These authors identified significant changes to the intestinal microbiota after the 66th day of supplementation with *Lactobacillus* spp.

A variety of positive results from use of probiotics in aquaculture have been reported in recent studies, with emphasis on growth promotion (AZEVEDO et al., 2016; TANG et al., 2017), modulation of the intestinal microbiota (LUIS-VILLASEÑOR et al., 2017), stimulation of the immune response (TORRES; MANCILHA, 2020) and even combat of toxicity effects (ZHAI et al., 2017). Use of probiotics is thus a promising alternative for promoting sustainability of aquaculture activity.

In the systematic review developed by Hasan and Banerjee (2020), demonstrated the various beneficial aspects of probiotics in aquaculture sectors. Probiotics are considered as novel functional agents that have potential implications in influencing the gut microbiota of any aquatic organism. Researchers have already documented that probiotics play a wide spectrum function (such as decrease diseases and stress, enhance immunity, modulate gut microbiota, helps in nutrition, improve water quality, etc.) in host body. Furthermore, the beneficial effects of probiotics contribute to increase feed value and growth of the animal and improve spawning and hatching rate in aquaculture system. Here, we have discussed each and every function of probiotics and tried to correlate with the previous knowledge. Hasan and Banerjee (2020) concluded in their study that the efficacy of probiotics and its detailed mechanism of action are scarce. Till date, several probiotics have been reported; however, their commercial use has not been implicated. Most of the studies are based on laboratory environment and thus the potentiality may vary when these probiotics will be used in natural environments (pond and lakes). The effects singular or combined administration of fermentable fiber and probiotic on mucosalimmune parameters, digestive enzyme activity, gut microbiota, and growth performance of Caspian white fish (*Rutilus frisii kutum*) fingerlings.

Important factors to mention, the remedial probiotics provide an extension of the intestinal absorption area, through the enlargement of the villi, in addition to avoiding pathogenic microorganisms due to the predominance of Gram (+) beneficial microorganisms (CLAES et al., 2012; SANTOS et al., 2021). Diseases in intensively farmed fish are a major concern, so the use of bioremediating can be a good option to reduce the economic losses caused by diseases and increase productivity (ZHANG et al., 2019). According to Hasan and Banerjee (2020), probiotics are considered functional agents and their applications are more efficient in developing fish, influencing the formation of the intestinal microbiota. According to Ferreira et al. (2014) documented the performance of broad-spectrum probiotics in juvenile tambaqui. The main functions being to reduce diseases and stress, increase immunity, modulate the intestinal microbiota, and assist in nutrition. Consequently, they optimize the growth of the fish and in the future improve the spawning rate.

Therefore, for the significant effect of bioremediating probiotics, in addition to the correct dosage to be applied according to the time of year, rearing phase and cultivation strategy, the pond sediment must be free of excess organic matter and water transparency at least minus 40 cm. Because the beneficial microorganisms are able to settle and remain activated (NAGHMOUCHI et al., 2019). Otherwise, the application of the probiotic may cause microalgae blooms such as cyanobacteria, a potential toxicological risk for cultured fish (LIMA-PINHEIRO et al., 2023). An alternative to avoid these blooms and the surplus of organic matter without the need for continuous water renewal is the adoption of artificial aeration. Therefore, the effect of the probiotics can be attractive for fish farming, although to be economically viable, an efficient control of water quality is necessary, especially in relation to the ammonia, nitrite, transparency, alkalinity and pH (ZHANG et al., 2019; SOLTANI et al., 2019).

In addition, bioremediation proposes other important roles in aquaculture, in addition to the zootechnical and physiological benefits to fish, although also with an environmental plan it is possible to reduce metabolites in effluents, as they are practically all metabolized by microorganisms within the culture system (BHAKTA et al., 2022). So, the other proposed solution is for remediators to optimize water organic matter decomposition and oxidation and other water systems (ZHANG et al., 2019).

CONCLUSION

The results found in the current study suggest that supplementation of the diet for juveniles of tambaqui (*Colossoma macropomum*) by means of a probiotic based on *Lactobacillus fermentum*, independent of the rain origin, contributes to defense of the intestinal mucosa through increased mucus production by goblet cells.

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