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# PRODUCTIVE PERFORMANCE AND MORPHOLOGICAL ANALYSES OF JUNDIARAS HYBRIDS SUPPLEMENTED WITH PHYTOBIOTICS AND COMMERCIAL PROBIOTICS

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**ABSTRACT**

O uso de aditivos alimentares na piscicultura tem sido amplamente estudado em relação à sua eficácia na melhoria das condições de saúde, manejo alimentar e modulações morfológicas. O objetivo desta pesquisa foi comprovar a capacidade de alimentos funcionais influenciarem na biometria, no desempenho produtivo e na morfofisiologia de jundiaras (*Pseudoplatystoma reticulatum x Leiarius marmoratus),* com e sem desafio sanitário com fezes de capivaras *(Hydrochoerus hydrochaeris - Rhodentia*: Caviidea). Para o experimento, foram utilizados 120 animais, totalizando 10 grupos, dois controles e oito tratamentos, com doze repetições. Os animais foram alimentados com dietas contendo probióticos comerciais (DBAqua® e Aquaplus®) e fitobióticos em pó (alho, canela e alecrim). Avaliou-se aos 60 e 90 dias, os comprimentos e pesos finais dos animais bem como o índice de eficiência alimentar e conversão alimentar aparente. Ao final do experimento, os animais foram necropsiados, o trato intestinal foi coletado e fixado em formol para a confecção de lâminas histológicas. Ao exame microscópico, observou-se que as vilosidades intestinais ocorreram em maior quantidade nos animais dos grupos controle sem aditivos e com desafio sanitário, com probiótico DBAqua com desafio e com probiótico DBAqua sem desafio. A quantidade de células caliciformes mostrou-se maior nos grupos com suplementação de prebióticos fitogênicos e com desafio sanitário, controle sem aditivos e sem desafio sanitário e com probiótico DBAqua e desafio. Concluiu-se que a utilização de dietas com probióticos comerciais mostrou-se eficiente nas modulações morfofisiológicas intestinais de jundiaras e sendo uma alternativa inovadora e ambientalmente sustentável na nutrição e produção destes animais.



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# INTRODUCTION

The environment establishes direct relationships with the intestinal tract and plays an important role in productive performance, morphophysiological and immunological modulations (Bakke *et al*., 2010; Gioda *et al*. 2017; González-Félix *et al*., 2018; Kamaszewski *et al*., 2020). Histomorphological studies of teleost fish have demonstrated the relationship between the morphological, qualitative and quantitative morphometric characteristics of the gastrointestinal tract with nutritional and physiological habits. Despite the effectiveness ofthe use of food additives (probiotics and phytogenic prebiotics) for the maintenance ofthe intestinal integrity and well-being of fish reared in captivity (Ng & Koh, 2016),technologies must be associated to food management in order to reduce the use of antibiotics, since they can accumulate in the environment, in the food chain and in humans (Kubitza, 1999; 2004; 2011; Gonçalves *et al*., 2012; Ng & Koh, 2016). Consolidated researchhas reported new technologiessuch as the use of functional foods in the nutrition of farming fish, with significant improvements in the modulation of environmental conditions, external and internal microbiota and morphophysiological characteristics adapted to captive conditions (Vieira and Pereira, 2016; Hayatgheib *et al*., 2020; Curvo *et al*., 2021). Among the bioactive products used in commercial fish farming, phytogenic prebiotics (phytobiotics) (Keating *et al*., 2021) and probiotics (Bhat *et al*., 2019; Fernandes *et al*., 2019; Nataraj *et al*., 2020; Curvo *et al*., 2021), which aredefined as non-digestible foods and substances (Hayatgheib *et al*., 2020; Keating *et al*., 2021) associated or not with living organisms (Hayatgheib *et al*., 2020; Curvo *et al*., 2021), respectively, stand out. The additives promote the necessary conditions to maintain animal physiology through the interactions between the recolonization of the water microbiotain the intestinal tract and the morphophysiological structure, as well as favoring the best flexibility in relation to the adaptability to changes imposed by the environment (Suphoronski *et al*., 2019; Hayatgheib *et al*., 2020). In this scenario, the production of interspecific hybrids is encouraged, aiming at the improvement of species of productive/commercial and nutritional interest to obtain faster desirable physical and/or physiological results. In this context, the jundiaras are hybrid fish of thenative Pimeloidae family, and result of the cross between the cachara (*Pseudoplatystoma reticulatum*) and the jundiá-da-Amazônia (*Leiarius marmoratus*), which have been widely bred in Brazil (Tavares *et al*., 2018) and have great acceptance in the national and international market, due to their characteristics such as greater productive performance, better reproductive, nutritional and rustic management (Silva *et al*., 2015; Tavares *et al*., 2018; Yabu *et al*., 2017). Thus, the objective of the present study was to evaluate the influences of the addition of phytogenic prebiotics and commercial probiotics on the productive performance and on the intestinal morphology of jundiarasunder sanitation challenge.

# MATERIAL AND METHODS

### Ethical aspects, design, and experimental diets

***Water quality:*** This research This research was carried out in the area of Fisheries Production and Health and in the Pathological Anatomy Laboratories of the Dom Bosco Catholic University, located in the Center-West region and in the State of Mato Grosso do Sul, Brazil. All the experiments were approved by the Ethics Committee of the University protocol number 015/2019, according to the legislation in force in Brazil (2015). The study and experimental tests, with a total duration of 90 total days, following a randomized design, using 120 jundiaras (*Pseudoplatystoma reticulatum* x *Leiaurius marmoratus*), weighing 37.08± 7.21g and measuring17.45 ± 1.09 cm. The experimental essays were carried out with one hundred twentyspecimens of jundiaras, from the Grupo Acorci Fish Farming Station (Brazil).The specimens were allocated in ten 100-liter polyethylene (L) boxes, acclimated for seven days, in 10 groups, being 2 controls ($C_1$ and $C_2$), 8 treatments ($T_1$ to $T_8$) and 12 repetitions, five under sanitation challenge and five without challenge (watery solution 83, 33g /L with 360 ml/day of wild capybara - *Hydrochoerus hydrochaeris* - feces Rhodentia: Caviidea) (Table 1), adapted from Ferreira *et al*. (2018); Meurer *et al*. (2007); Tapia-Paniagua *et al*. (2014); Brito *et al*. (2019). The experimental diets were prepared with the inclusion of food additives at 150g of feed (Guabi® Aqua Tech) and homogenized in soy oil (7.5mL), for every 3 grams (g) of probiotics - Aquaplus® from Biomart and DBAqua® Imeve (Table 2), according to the manufacturer's procedures manual and Brito *et al*. (2019). In addition, 1.5g was added for every three phytogenic prebiotics, garlic (adapted from Abu Elala *et al*., 2016; Nyadjeu *et al*., 2020), rosemary (adapted from Dias *et al*., 2018; Naiel *et al*., 2020) and cinnamon (adapted from Abe *et al*., 2016). The jundiaras were fed *ad libitum* four times a day, during the 90 days of the experiment.

**Water Quality Parameters:** The water quality parameters in this experiment were maintained within the desirable standards for fish welfare (Wedemeyer, 1997; EMBRAPA, 2013). For this, a Closed RecirculatingWater System (CRWS) was used with ten boxes containing 80 L and a 1000 L renewal box, with a ½ CV propeller pump, with 6000 L / h renewal and a renewal rate of 4 at 5x / hour, with two filters, biological and sand, with aeration and replacement of approximately 1% of water per day or when necessary, disinfection by ultraviolet light, temperature control through thermostat and one to two siphoning/day.

**Table 1. Jundiaras experimental groups according to treatments (consisting of water, feed, vegetable oil, phytogenic prebiotics and/or commercial probiotics).G=Groups; W=water; R=Ratio; O=Oil; C=Challenge; $P_{HY}$=Phytobiotics; $PRO_1$=Probiotics one (AQUAPLUS); $PRO_2$=Probiotics two e (AQUAPLUS); (+) presence and (-) absence. Source: Adapted (Brito *et al*., 2019**

| G | W | R | O | C | $PRO_1$ | $PRO_2$ | $P_{HYTO}$ | $P_{HY}$ + $PRO_{1+2}$ |
|---|---|---|---|---|---|---|---|---|
| $C_1$ | + | + | + | - | - | - | - | - |
| $C_2$ | + | + | + | + | - | - | - | - |
| $T_1$ | + | + | + | - | + | - | - | - |
| $T_2$ | + | + | + | + | + | - | - | - |
| $T_3$ | + | + | + | - | - | + | - | - |
| $T_4$ | + | + | + | + | + | + | - | - |
| $T_5$ | + | + | + | - | - | - | + | - |
| $T_6$ | + | + | + | + | + | - | + | - |
| $T_7$ | + | + | + | - | - | - | - | + |
| $T_8$ | + | + | + | + | + | - | - | + |

**Table 2. Experimental diets**

| LEVELS OF ASSURANCE | (g) | LIVING ORGANISMS ANDADDED SUBSTANCE | |
|---|---|---|---|
| Ration Guabi | Aquaplus Probiotic | | DBAqua Probiotic |
| Humidity (max) | 100 | *Bacillus subtilis,* | *B. subtilis, B. bifidum,* |
| Total Crude Protein | 450 | *Bifidobacterium* | *L. acidophillus,* |
| Ether Extract | 80 | *bifidum, Enterococcus* | Mananoligosaccharide |
| Total Crude Fiber | 30 | *faecium,* | Dextrose Choline |
| Total Minerals | 160 | *Lactobacillus acidophillus,* | and Vitamins E and C |
| 5 to 20% biomass | 150 | *L. plantarum, L. cillus,* *L. casei, L. lactis, Pediococcus acidilactici,* Intensitive dry yeast of sugar cane, Iron Sulfate and cell wall | |

Source: In accordance with the manufacturer's data. gram (g), maximum (max).

**Table 3. Means and standard deviations of water quality parameters in 30 days**

| MEASUREMENTS | D/O | T | pH | EC | A | N | TA |
|---|---|---|---|---|---|---|---|
| Av | 7,2 | 27,6 | 7,38 | 0,22 | 0,24 | 145,67 | 0,03 |
| max. | 11,01 | 31,1 | 8,2 | 0,27 | 0,4 | 155 | 0,066 |
| min. | 3,1 | 24 | 6,95 | 0,11 | 0,01 | 132 | 0,00022 |
| PD | 7,2±1,58 | 27,6±1,31 | 7,38±0,23 | 0,22±0,04 | 0,24±0,12 | 155±2,23 | 0,03±0,0004 |

av=average, max=maximum, min=minimum, DO=Dissolved Oxygen (mg/L), T=Temperature=(oC), Hydrogenionic Potential=(pH), Standard Deviation (SD), EC=Electrical Conductivity (mS/cm), A=Ammonia (ppm), N=Nitrite (ppm), and TA=Toxic Ammonia (ppm). Source:Search data

The dissolved oxygen rate (DO mg/L) and the levels of total and non-ionized ammonia (toxic), nitrite (ppm), electrical conductivity (HANNA HI 9146 multifunctional device), pH (digital portable pH meter model WTW pH 330i) and temperature (bulb thermometer) were measured once a day (Table 3).

**Biometrics and productive performance:** At 90 days of feeding, the 120 fish (mean±sd - [n = 12]) were anesthetized in benzocaine solution for biometric measurements: TL (Total Length); SL (Standard Length); TL (Trunk Length); HL (Head Length); BH (Body Height); FW (Final Weight) and P-AD (Pre-Anal Distance). From these measurements, the productive performance was evaluated using two periods of the experiment, that is, with 90 total days, according to Zuanon *et al*. (2004); Yarmohammadi *et al*. (2012); Siddik *et al*. (2018); adapted from Brito *et al*. (2019) and Abdel-Aziz *et al*. (2020), for the following variables: Condition Factor (FC)=100xFinal Weigh/Final Length$^3$; Feed Efficiency Index (FEI)=Weight Gain/Total FeedConsumptionx100; and Feed Conversion Ratio (FCR)=Amount of Feed Offered/Weight Gain.

**Histological and histochemical analyses:** At the end of the experiment, (90 days) after 8 hours of fasting, the animals were euthanized by means of rapid cooling to 4° C in benzocaine solution, according to Ross & Ross (1999); Fujimoto (2015); Honorato *et al*. (2015); Brazil (2015)and eviscerated for anatomical observation of the intestinal tract. All organs were collected and fixed in 10% neutral buffered formalin.Fragments of the intestinal tract were cleaved and processed by histotechnology. Samples were included in paraffin blocks, cut 4 to 8 µm thick by microtome, and the slides were stained with Alcian blue (AB) associated with Periodic acid–Schiff (PAS) and Mallory's trichrome (MT) according to Caputo *et al*. (2010). Also, AB staining (pH2.5) combined with periodic acid-Schiff (PAS) (pH2.5) was used to identify and count goblet cells with acidic, neutral and mixed mucins, following Purushothaman *et al*. (2016) and Torrecillas *et al*. (2019). Histological analysis was performed with the aid of a light optical microscope (LOM) (Carl Zeiss Microscopy GmbH, model Axio Scope A1). Digital images (histomicrographs) were captured by an Axiocam 503 color camera coupled to the LOM, using the software ZEN lite for Windows. The free software ImageJ/Figi version IJ 1.46 was used for goblet cell count (available at https://imagej.nih.gov /ij/download.html).

600µm$^2$ in each group ($C_1$-$T_8$), with 601 villi and 6114 goblet cells differentiated by microscopy, through the enhancement of the (histochemical) staining performed with AB and PAS, adapted from Yarmohammadi *et al*. (2012); Mello *et al*. (2017); Schwarz *et al*. (2018); Abdel-Aziz *et al*. (2020) and Al-Deriny *et al*. (2020).The quantity of villi and goblet cells measured were compared for the same region.

# RESULTS

**Biometrics and productive performance:** The biometric results of Total Length (TL) and Final Weight (FW) showed significant statistically differences ($p<0.05$) when phytogenic prebiotics and/or probiotics were usedand were similar in the two control groups ($C_1$ and $C_2$) (Table 4). The biometric and productive performance results were not expressive in the treatments. The analysis of experimental data obtained from fish fed with commercial Aquaplus probiotics ($T_1$ and $T_2$) demonstrated that fish increased in length (TL) (19.35 and 18.57 cm) and presented a higher final weight (FW), when compared with other groups (Table 4). Final weight and growth were lower in the $C_2$ control, in treatments $T_4$ (DBAqua probiotics with challenge) and $T_8$ (3 phytogenic prebiotics and 2 probiotics with challenge) (32.80; 29.80 and 25.60 cm) and lower in $T_8$ and $T_5$ (3 phytogenic prebiotics without challenge) (15.75 and 16.35 cm), respectively (Table 4).

Fish that received food with commercial probiotics had better results in biometrics than those that received phytogenic prebiotics, but the isolated use of these additives demonstrated important responses, in which even whensanitation challenge was present, there were no deaths. Comparison between the biometric data measured in all groups was greater in the treatments without sanitation challenge, except in the $T_6$ treatment, in which, even with sanitation challenge, the biometric data were higher (Table 4). Despitesignificant growth, fish exhibited good behavioral, adaptive, physiological and nutritional characteristics to all diets offered in groups $C_1$-$T_8$, which can be seen by the analysis of the condition factor (CF). The treatment in which DBAqua was used, without sanitation challenge, ($T_3$) demonstrated better fish welfare and lower CF in the control group without the use of additives ($C_1$) (Table 4).

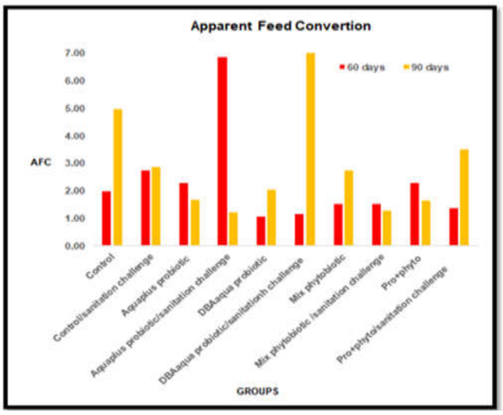
**Table 4. Averages of Biometrics and Performance of Jundiaras during 90 days of experiment**

| GROUPS | TL | FW | CF |
|---|---|---|---|
| Control | 18,50 a1 a2 | 39,00a1 a2 | 0,59a1 |
| Control/sanitation challenge | 17,5a1 a2 | 32,80a1 | 0,62a1 a2 |
| Aquaplus probiotic | 19,35 a2 | 52,80 a2 | 0,71a1 a2 a3 |
| Aquaplus probiotic/sanitation challenge | 18,57 a1a2 | 42,90a1 a2 | 0,66a1 a2 a3 |
| DBAaqua probiotic | 16,66 a1a2 | 36,60 a1a2 | 0,77 a3 |
| DBAaqua probiotic/sanitation challenge | 16,45 a1a2 | 29,80a1 a2 | 0,65a1 a2 a3 |
| Mix phytobiotic | 16,35 a2 | 36,40 a2 | 0,72a1 a2 |
| Mix phytobiotic /sanitation challenge | 17,90 a1a2 | 38,60 a1a2 | 0,60a1 a2 a3 |
| Pro+phyto | 17,85 a1a2 | 38,20 a1a2 | 0,67a1 a2 a3 |
| Pro+phyto/sanitation challenge | 15,75a1 | 25,60 a1 | 0,70 a1a2 a3 |
| CV | 11,71 | 32,55 | 13,4 |
| P | 0,0019 | 0,0005 | 0,0002 |

Means followed by different letters in the column were statistically different according to Tukey's test ($p<0.05$). TL=Total Length, FW=Final Weight, CF=Condition Factor, CV=Covariance and P=Probability (P=0.05). Isolated letters/numbers (a1, a2 or a3) represent significant statistical differences. Source: Research data.

**Statistical Analysis:** The biometric results and the productive performance (PP) were subjected to Analysis of Variance (ANOVA) to establish differences among the 10 groups.Subsequently, the Tukey test at 5% probability ($p<0.05$) was applied. The tables, calculations of mean and standard deviation of the Feed Conversion Ratio (FCR) and Feed Efficiency Index (FEI) were generated by Microsoft Excel 10. The ANOVA and Tukey's test were carried out on R a language and environment for statistical computing. The number of villi and goblet cells were counted through on-site observations by optical microscopy, using three intestinal regions: Proximal (PR), Medial (MR) and Distal (DR) as a sample parameter. Villi and goblet cells were counted in three intact fragments covering 200 µm$^2$, totaling

In the comparative analysis, it was possible to verify and affirm that phytogenic prebiotics have little influence on TL and FW.When compared with commercial additives, it was always less responsive to FW, whether in challenging conditions or not. It was noted that in the treatments in which commercial DBAqua probiotic was added, both without and with sanitation challenge ($T_3$ and $T_4$), the mean Feed Conversion Ratio (FCR) was 1.05 and 1.14 (60 days), and for Aquaplus probiotics with challenge ($T_2$) it was 6.85, presenting low response (Figure 1 and 2). Regarding FEI averages, more expressive responses were found for those with the addition of commercial DBAqua probiotics ($T_3$ and $T_4$) in 60 days, with 94.8 and 87.59% (Figs. 1 and 2).



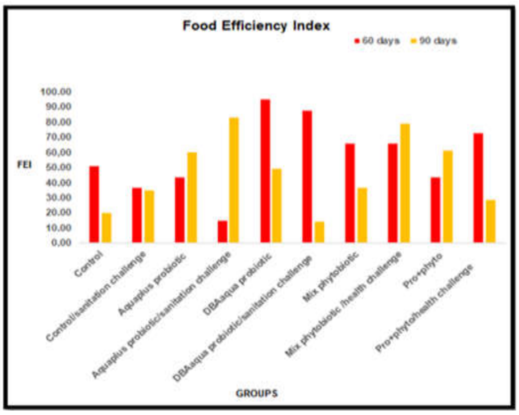




**Figure 1 and 2. Feed Conversion Ratio (FCR), Feed Efficiency Index (FEI -%) of Jundiaras during 60 and 90 days of experiments. Source: Research data. Feed Conversion Ratio (FCR), Feed Efficiency Index (FEI - %)**

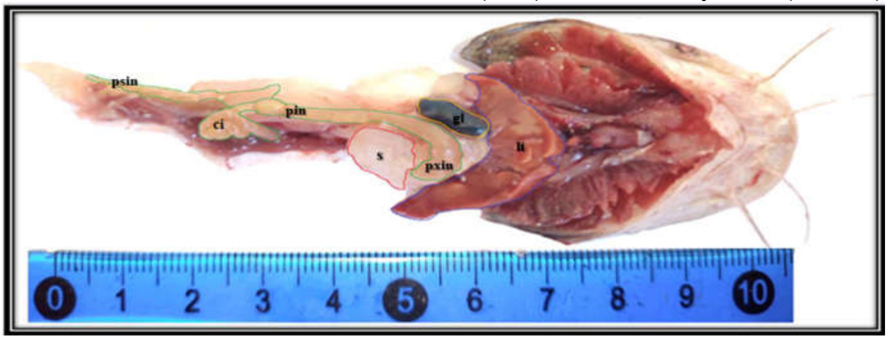



**Figure 3. Anatomy of the jundiaras' digestive tract: gallbladder (gall); liver (li), stomach (s), intestine proximal (pxin), medial (min) and distal (psin) and intestinal convolutions (ci). Source: Research data**

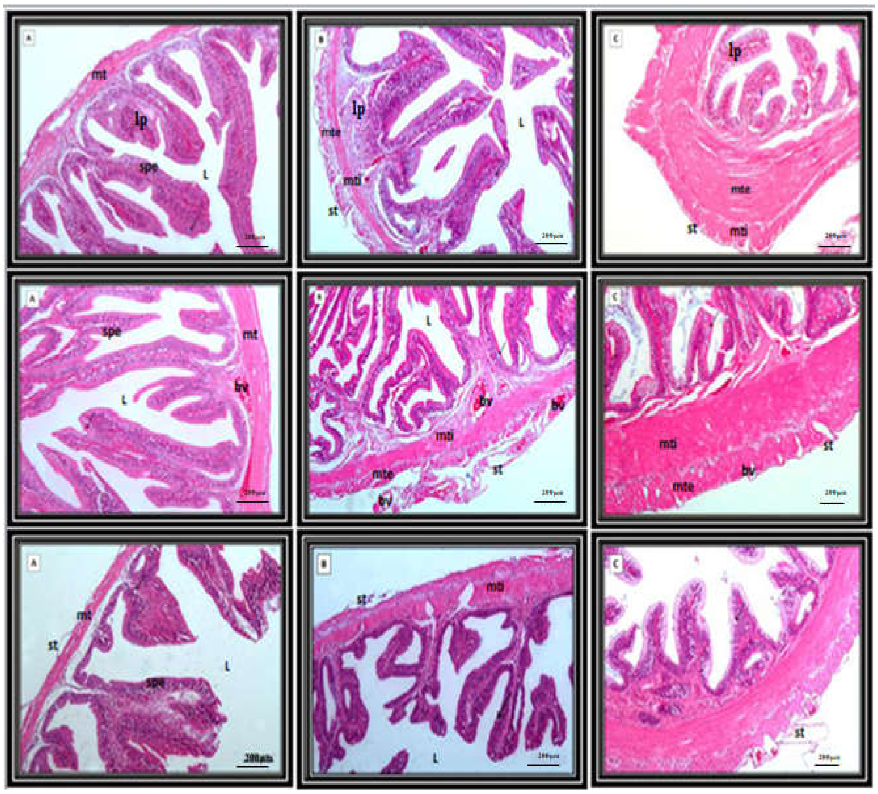



**Figure 4, 5 and 6 (A, B, C). Histomicrographs of the intestine of jundiaras, showing the differences in intestinal villi by regions in groups $C_2$, $T_4$ and $T_3$ (top to bottom). (A) proximal; (B) medial; (C) distal; simple prismatic epithelium (spe); goblet cells (arrow); lamina propria (lp); muscle tunics (mt); internal and external muscular tunics (mti and mte); serous tunic (st); Blood vessels (bv); lumen (L). Objective lens: 4x (A), (B), (C), HE/AB. Source: Research data**

At the end of the experiment (90 days), using commercial probiotic Aquaplus with sanitation challenge ($T_2$), the means of FCR (1.21) and FEI (82.83%) were better. Comparatively, the two treatments that contained phytogenic prebiotics, ($T_6$ and $T_8$), combined or not with the two commercial probiotics, revealed lower FCR values, and the worst responses were found where the 3 phytogenic prebiotics were used ($T_6$). It is important to highlight the effectiveness in $T_8$, because, even with the sanitation challenge, it had better result than $C_1$, which was not challenged. Regarding treatments $T_8$ (60 days) and $T_6$ (90 days), which received phytogenic prebiotics, both with sanitation challenge, the responses of FEI were effective with the same amount of feed offered. The lowest FEI was registered in the $T_4$ treatment at 90 days (14.29), with the use of commercial probiotic DBAqua and with sanitation challenge, while the FCR was lower in the $T_2$ treatment (1.21 at 90 days), to which the commercial probiotic Aquaplus was added, with sanitation challenge. In the treatments with sanitation challenge, at 90 days of experiment, the best productive performance (PP) responses were obtained with the use of the Aquaplus probiotic ($T_2$), followed by the ($T_6$) treatment in which 3 phytogenic prebiotics were used. These two treatments showed better responses in relation to $C_1$ (control),which did not include sanitation challenge; that is, the additives can be effective for improving the performance of jundiaras (Figure 1 and 2).

**Anatomical and histological analyses:** The elongated tubular intestine was visualized, curved anteriorly, with a "J" shape, denoting 3 distinct parts, anterior (proximal), middle (medial) and posterior (distal), with convolutions in the middle portion.The other anatomical characteristics are similar to other catfish (Figure 3).

**Histological comparison between different experimental groups:** In this study, biometric measurements, production performance and the effects of commercial additives DBAqua and Aquaplus stood out in groups $C_2$ (control with challenge), $T_4$ (DBAqua with challenge) and $T_3$ (DBAqua without challenge), presenting significant statistical differences ($P<0.05$). We compared the number of villi by regions, branches and morphology of the contours (apices) that occupied the intestinal lumen in all groups ($C_1$-$T_8$) (Figure4,5 and6 (A, B, C).The muscular tunic consisted of two layers, internal and external, consisting of smooth, circular, and longitudinal muscle fibers (Figure 4, 5 and 6 - C). It was noted that the internal muscular tunic was thicker than the external in all groups, gradually increasing in the cranio-caudal direction, reducing the intestinal lumen (Figure 4, 5 and 6- A,B,C). The serous tunic demonstrated no morphological and organizational differencesbetween groups, being thin and externally located, composed of dense vascularized connective tissue covered by a simple squamous epithelium (Figure 4, 5 and 6 - B).

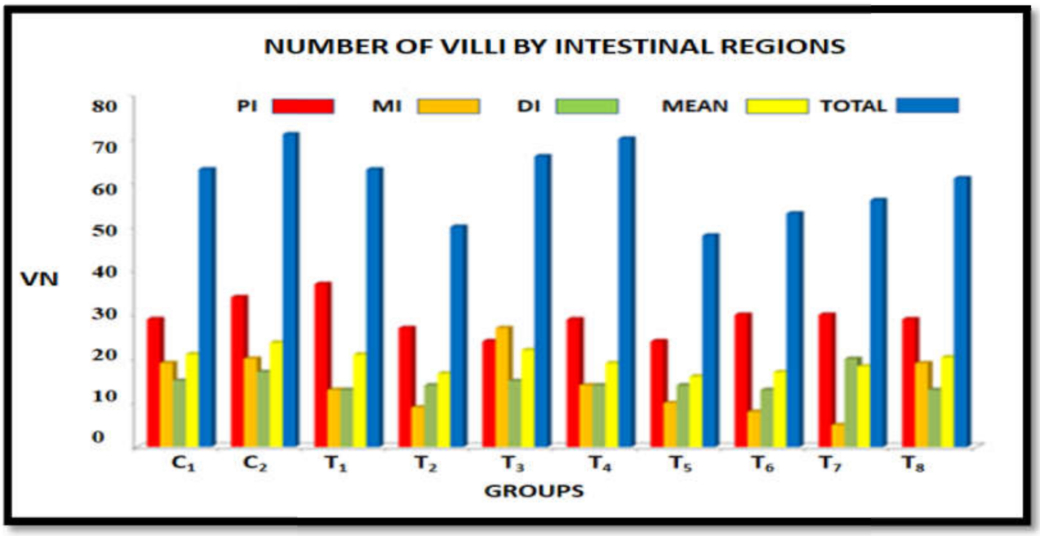



**Figure 7. Number of villi (NV) per group in each intestinal region of jundiaras. Proximal, Medial and Distal Intestines (PI, MI and DI), average and total. Source: Research Data**

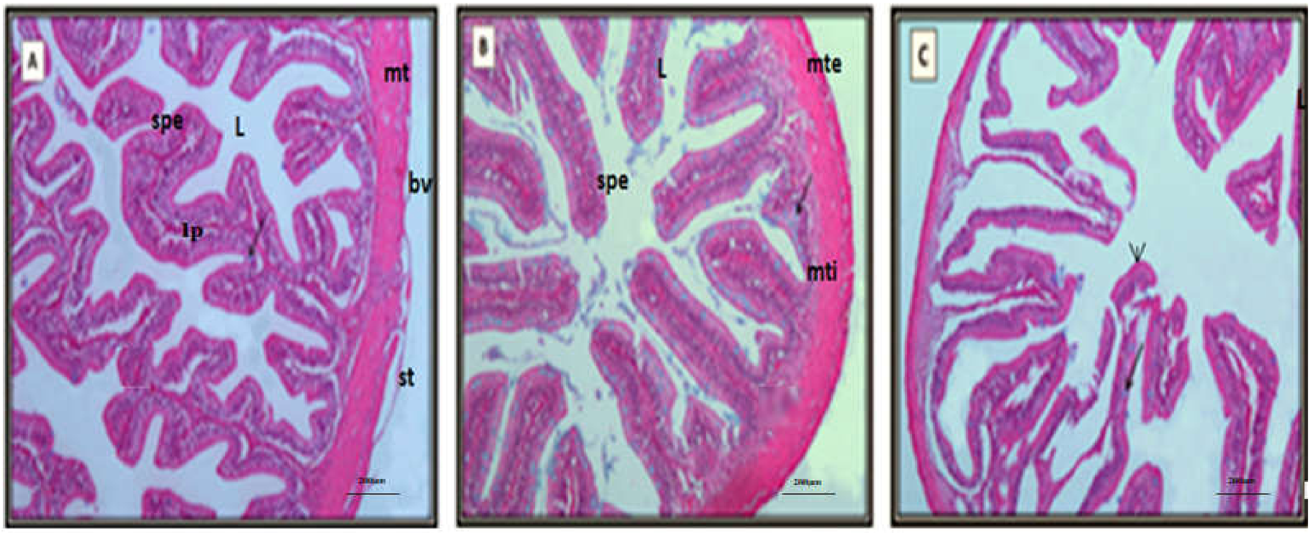



**Figure 8 (A, B, C). Histomicrographs of the jundiara intestines, showing the different regions of the previous $T_8$ treatment (A) proximal; (B) medial; (C) distal; simple prismatic epithelium (spe); goblet cells (arrow); brush border (arrowhead); lamina propria (lp); muscle tunics (mt); internal and external muscular tunics (mti and mte); serous tunic (st); blood vessel (bv); lumen (L). Objective lens: 4x (A), (B), (C), HE / AB. Source: Research Data**

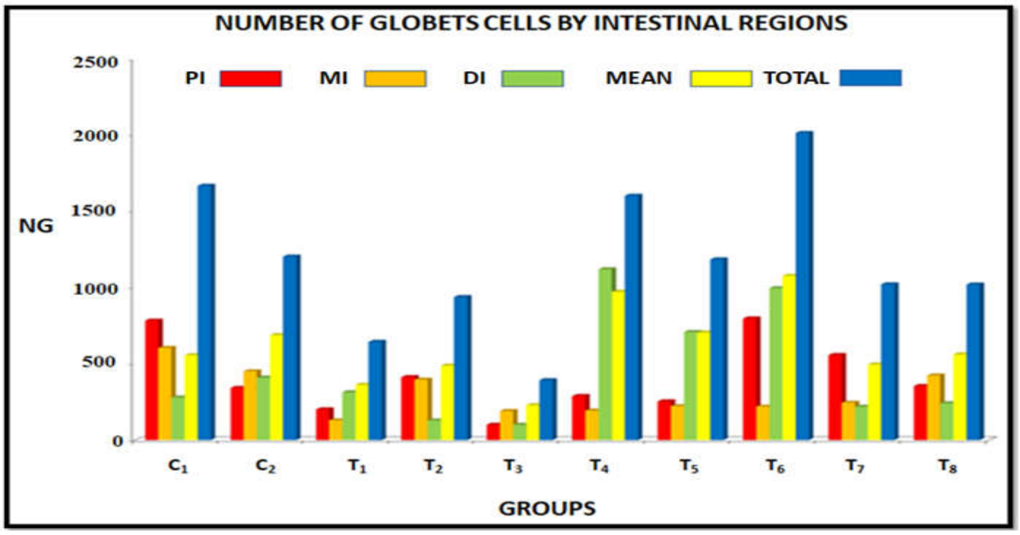



**Figure 9. Number of goblet cells in the proximal, medial and distal intestines (PI, MI, DI), average and total. Source: Research Data**

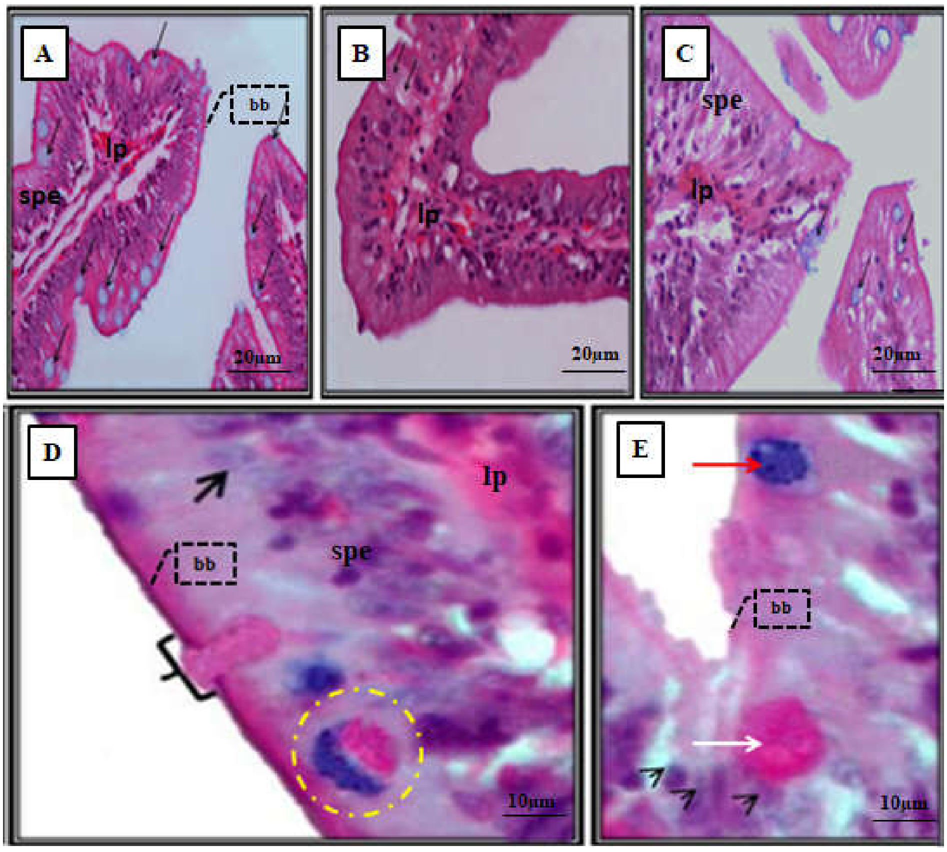



**Figure 10 (A, B, C, D and E). Histomicrographs of the enteric region of jundiaras, demonstrating different amounts of goblet cells in the epitheliumin the enteric mucosa of the previous $T_6$ treatment (A) distal; (B); $T_3$ medial; $C_2$ distal (C); simple prismatic epithelium (spe); goblet cells (arrow); brush border (bb); lamina propria (lp); (D and E) Details demonstrated different types of goblet cells.acidic (red arrow), neutral (white arrow) and mixed (yellow circle); lumen (L). Objective lens: 40x (A, B, and C) and 63 x (D and E), HE / AB. Source: Research Data**

In the three different portions of the intestine, a greater number of villi was observed in groups $C_2$ (control with challenge) and $T_4$ (DBAqua with challenge), and lower in $T_5$ (3 phytogenic prebiotics without challenge) (Figure 7). Regarding the distribution by regions, there was a predominance in the previous regions of all groups, with or without sanitation challenge, except for $T_3$ (DBAqua without challenge), which had more villi in the middle region. In the $T_4$ treatment (DBAqua with challenge), the villous were discontinuous, with irregular contours (apex) and edges, approaching filiform (Figs 7A, B) in the proximal and medial regions, in contrast to the posterior region, which presented less villi and little branching. Low values verified through biometrics and productive performance in every experiment (FCR and FEI), at 60 days, were satisfactory (Table 2 and Figure. 1 and 2).

Histologically, the villi showed long shape and complete morphology in the proximal and medial regions, with more regular contours, oval apexes, deeper crypts, and brush-bordered epithelium (Figure8 A, B, C). Comparatively, groups ($C_1$-$T_8$) showed a decreasing order of the total amount of goblet as follows $T_6 > T_4 > T_5 > C_2 > T_8 > C_1 > T_7 > T_2 > T_1 > T_3$, that is, 1073 > 969 > 703 > 686 > 560 > 554 > 494 > 486 > 361 > 228 measured units, respectively (Figure 9). The largest number of goblet cells was found in $T_6$ (with prebiotic additives and sanitation challenge), with prevalencein the distal region (Figure 10 A, B and C), identifying three different types of goblet cells, with a predominance of those containing acidic mucins (Figure 10 D and E). The $T_3$ treatment (with probiotic additives – DBAqua, and without challenge) had the smallest total number of goblet cells, with the highest number in the proximal and medial portions and the groups in which fishes were not subjected to sanitation challenge ($C_2$ control and $T_7$,combining all prebiotics and probiotics), had a greater number of goblet cells in the proximal region; in $T_3$ (DBAqua without challenge) and $T_5$ (3 prebiotics without challenge), in the medial region; and $T_1$ (Aquaplus without challenge), in the posterior region. The other variations and histological comparisons of jundiaras presented greater totality of goblet cells in the groups submitted to sanitation challenge: $T_2$ (Aquaplus) in thedistal portion; $C_2$ (control group with challenge) and $T_8$ (3 prebiotics and 2 probiotics with challenge) in the medial-enteric portion; and $T_4$ (DBAqua with challenge) in the distal portion(Figure9and10A,B,C, D and E).

It is important to address that, in the comparative analyses of biometrics, number of goblets and number of villi, greater growth and weight in $T_1$ and $T_2$ (Aquaplus with and without challenge)were noted but, on the other hand,these treatments did not showa greater number of villi and goblet cells regarding the productive performance evaluations (FEI), the highest occurred in $T_3$ (DBAqua without challenge) and in this specific treatment, the number of villi in the middle region and with a smaller number of goblet cells in all intestinal regions was even greater. A lower FCR was found in T4 (DBAqua with challenge), with better performance and higher number of cups in the intermediate region of all groups,and in villus count in all with sanitation challenge, only lower than $C_2$ (control group) with challenge.

# DISCUSSION

**Water quality:** In the present research, monitoring the experimental conditions of the water quality parameters was effective and avoided the emergence of pathologies in fish farming, as suggested by Assefa *et al*. (2018). The use of a Recirculating Aquaculture Systems (RAS )prevented toxicities and diseases, as mentioned by Toni *et al*. (2017) and Rajan *et al*. (2019), with the exception of 5 groups that were submitted to sanitation challenge. It is important to note that the RAS was kept stable for animal welfare, ensuring the quality and reproducibility of the results. Variations in any biotic and abiotic environmental parameter can potentially induce physiological responses, which can affect the experimental results (Toni *et al*., 2017). The environmental conditions measured in this study through experimental tests had a positive impact on water quality and tissue morphology modulations, aspreviously reported by Toni *et al*. (2017); Su *et al*. (2020), Mohapatra *et al*. (2012); Rehman *et al*. (2017); Toni *et al*. (2017) and Su *et al*. (2020).

**Biometric effects and productive performance of jundiaras under sanitation challenge conditions:** As an example of the importance of maintaining the health conditions of the captivity tank and in a comparative analysis with the studies by Faria *et al*. (2011) with other catfish known as cachadiá (*Pseudoplatystoma fasciatum* x *Leiarius marmoratus*), this study also found a high survival rate in seven months of cultivation with RAS. In this study, histomorphological modulations in jundiaras were demonstratedby theincrease in the number of intestinal villi and goblet cells in the enteric mucosa of fish supplemented with additives.The productive performance (PP) of jundiaras found in this experiment was inferior when compared to tilapia (*Oreochromis niloticus*), patinga hybrids (*Piaractus mesopotamicus* x *P. brachypomus*) and catfish hybrids (*Pseudoplatystoma* sp. x *L. marmoratus*), which had the greatest FCR, that is, the worst responses to the diet, evidenced by Vogel *et al.* (2019). Thejundiaras used in the present study result from the hybridization of native (non-hybrid) fish; thus,it is important to point out that dietary restrictions in captivity can be overcome and minimized by intergenus crossing, providing better hybrid vigor (Campos *et al*., 2010; Portalete *et al*., 2015; Fortes-Silva *et al*., 2016; Yabu *et al*. (2017)), as well as better productive performance, feed conversion ratio, development and meat quality (Yabu *et al*., 2017; Tavares *et al*., 2018). The positive results of the combination of phytogenic prebiotics and commercial probiotics used in treatments $T_7$ and $T_8$, showedimportant responses in morphology, biometrics and performance. The differences observed with the mixed use of phytogenic prebiotics and commercial probiotics with sanitation challenge ($T_8$) were similar to the results obtained by the studies shown inTable 3, mainly in relation to the CF and positive evaluations of the morphological modulations seen in the histological sections, such as: quantity, ramifications, integrity of villus contours, and high number of goblet cells. The present study is in agreement with Azevedo *et al*. (2015) and Amenyogbe *et al*. (2020) regarding the fact that the symbiotic theory can explain the effectiveness of combining prebiotics and probiotics, due to the synergy between them, as searched by Li and Gatln (2004); Rodrigues-Estrada *et al* (2009); Azari *et al*. (2013); Dehaghani *et al*. (2015); Abdulrahmar *et al*. (2015); Morshedi *et al* (2020); Azimirad *et al* (2016); Dowood *et al*. (2019); Sewaka *et al* (2019).

**Anatomy and histology:** The study of the functional morphology of the intestinal tract is complete, as mentioned by Rotta (2003); Ringø (2014); Fagundes *et al.* (2016); Burton & Burton (2018); Sado *et al.* (2020), and, more specifically Rodrigues *et al*. (2009), for parietals of the same genus as jundiara (*Pseudoplatystoma* ssp). The presence of convolutions, in the middle portion of the bowels, and of the rectilinear aspect of the intestinal tract of jundiaras described here, made possible to describe these fishes as carnivorous, with flexibility for plant diets, as reported by Albrecht *et al*. (2001); Seixas Filho *et al*. (2001); Rodrigues *et al*. (2009); Campos *et al*. (2010); Prieto-Guevara *et al*. (2015); Souza *et al*. (2017); Tavares *et al*. (2018); Yabu *et al*. (2017). Rodrigues *et al*. (2009) assessed natives of cacharas (*P. fasciatum*, Pimelodidae), noticing that fish establish relationships between size and shape of the gastrointestinal tract and eating habits. Other studies, such as the one by Petrinec *et al*. (2005), have foundthat the gut of the jundiaras is similar to that of large carnivores such as*Esox lucius* (Esocidae) and *Silurus glanis* (Siluridae).However, their findings differed from the ones described in the present study because they found fewer contortions in the intestines of the fish. In the histology of jundiaras intestine the number of villi in the proximal region was predominant,corroborating the findings by Alves *et al*. (2014) and Silva *et al*. (2015), who reported that this portion is responsible for the absorption of water, ions, and nutrients, and that themedial and distal regions of the gut are responsible for proteins and peptides. In addition to this quantitative inference, there was a greater amount of goblet cells in the distal region of the intestine ($T_4$ and $T_6$).

In the morphometric studies of the larger number of villi in the proximal region of the intestines, variations in the amount of goblet cells and the appearance of convolutions in the medial region of the jundiaras' gutcould be attributed to the nutrition used in the tests and also to the hybridization between their parental pimelodids, *Pseudoplatystoma reticulatum* and *Leiarius marmoratus*, since they are, in their native form, carnivores and omnivores, respectively. In this context Karachle &Stergiou (2010) justifiedthe assumption that morphological variations in the types and modes of fish intestines have been used to categorize fish in relation to food. Regarding the environmental conditions (Table 1) and different diets between the Groups ($C_1$ to $T_8$ – Table 2),this study observeddifferent quantitiesin intestinal villi and goblet cells, indicating histological modulations, as reported by Abdel-Tawwab *et al*. (2021). As assessed by Pirarat *et al*. (2010) in Nile tilapia, the jundiaras studied here presented different amounts of villi and goblet cells in all enteric regions, probably due to the influence of diet composition and supplementation with additives. In both studies, it was found that in the Control Group ($C_2$), which used probiotic additives, it was possible to visualize a greater number of villi in the proximal intestine, followed by the medial portion and the distal portion. The greater number of villi in the proximal portion of the jundiaras in this experiment was a response to the digestion and absorption in general, as also described forother Pimelodidae, like*cacharas* (*P. fasciatum*) supplemented with bovine colostrum studied by Rodrigues *et al*. (2010) and Nascimento Veiga *et al*. (2020) for hybrids *cachapintas* (*P. corruscans* x *P. reticulatum*).

In situations of sanitation challenge, that is, in conditions where the water had feces, there were still better responses of the tissues to these environmental stressors. Likewise, morphointestinal modulations can be explained by the generic ability of fish to quickly and reversibly adapt the characteristics of the gastro-intestinal tract to changes in functional demands that occur during their life history or more frequently to changes in their daily or seasonal diet or environmental conditions, depending on neuro-endocrine pathways (Merrifield *et al*., 2014) and the microbiota present in the intestinal tract (Egerton *et al*., 2018). The morphological modulations, from the histological study, were identified by comparing the control groups without additives, with and without challenge ($C_1$-$C_2$), presenting a greater number of villi in the proximalregion in all treatments. Comparatively with the lanceolate apex in proximaland medialregion, Nascimento Veiga *et al*. (2020) reported an increase in the area and size of intestinal villi in Brazilian hybrid catfish called *cachapinta* (*P. corruscans* x *P. reticulatum*), establishing a relationship with greater absorptive ease. Similarly,Dawood *et al*. (2019; 2020) showed improvement in performance, hematological parameters and antiparasitic activities in Nile tilapias (*Oreochromis niloticus*) supplemented with prebiotics and/or probiotics, confirming morphological modulations for this fish by the gradual growth of intestinal villi in the same direction as the jundiaras (cranio-caudal).

In this study, the intestinal mucosa of the jundiaras exposed clusters of goblet cells and enterocytes in the three regions ($C_1$-$T_8$), like the findings for other native and non-native teleost fish (hybrids), such as those mentioned by Pereira *et al*. (2019) for tambaqui (*C. macropomum*) and jundiaras (*P. reticulatum* × *L. marmoratus*), even without additive supplementation. The predominance of goblet cells in the proximalregion of the gut of the jundiaras differs from what was observed by Abdel-Aziz *et al*. (2020) in Nile tilapia (*O. niloticus*), as they found most of these cells in the proximal and distalregions in all groups. The histochemical detection of three types of mucus within goblet cells (acidic, neutral and mixed) in the intestinal mucosa of the jundiaras indicates histological diversity, suggesting possibilities for greater flexibility and functional versatility to protect environmental seasonality (with or without sanitation challenge), from the different combined and administered diets. The presence of adaptive characteristics such as variations in the number and size of goblet cells were decisive in promoting the final weight and final length of the studied animals, in agreement with the report by Siddik *et al*. (2018); Abdel-Aziz *et al*. (2020) in Nile tilapia (*O. niloticus*), as they found most of these cells in the proximaland distalregions in all groups. The histological characteristics revealed here are very relevant, considering the possibility of relating the higher number of goblets in $T_6$ (with challenge and three prebiotics), when compared to all other groups. These cellscan act as a defensive barrier against pathogens, since they produce mucins and are physiologically involved with defense mechanisms, as previously reported by Grau *et al*. (1992); Murray *et al*. (1996); Kalhoro *et al*. (2018). In this regard, after the production of acid and neutral mucins by the goblet cells, one more positive factor is perceived regarding supplements in the experiments, as these could trigger the protective effect of friction during swallowing, of lubrication, facilitating the transit of the bolus feed, and act as a barrier against pathogens, asreported by Kalhoro *et al*. (2018); Pereira *et al*. (2019); and Pontin *et al*. (2020).

The jundiaras' mucous membrane showed evident modulations due to the important number of goblet cells in the mucosal epithelium and intestinal villi of fish supplemented with prebiotics and probiotics, as reported in native cacharas (*Pseudoplatystoma fasciatum*) by Rodrigues *et al*. (2010), hybrid catfish (*Pseudoplatystoma corruscans* x *P. reticulatum*) by Nascimento Veiga *et al*. (2020), and Nile tilapia by Jesus *et al*. (2019). The jundiaras showed a significant number of goblet cells in the posterior enteric mucous layer, indicating better lubrication during the path covered by the food, as these unicellular glands produce chemically formed compounds mucin (glycoproteins) involved in the epithelial protection and absorption of nutrients in the intestine, as reported by Carrassón *et al*. (2006) for toothy fish (*Dentex dentex*), and by Rodiles *et al*. (2018). The finding of a large number of goblet cells was also reported by Tapia-Paniagua *et al*. (2014); Cámara-Ruiz *et al*., 2020;and Kalhoro *et al*. (2018) in the yellow croaker (*Larimichthys crocea*, Sciaenidae), and also by Pereira *et al*. (2019) in the jundiara hybrid, confirming the higher density of these cells in the middle and posterior region of the intestine. The hybrids studied here have a gradual increase in the concentration of goblet cells in the cranio-caudal direction, as described by Machado *et al*. (2013), which is attributed to the function of mucus to defend the intestinal lining epithelium and increase waste disposal insea bass (*Centropomus parallelus*, Centropomidae). The histochemical study of goblet cells in the gut mucosa of jundiaras, by regions, demonstrated different types of mucins located inside, which reacted positively to PAS, indicating the presence of neutral mucous substances in the enterocytes. This finding in jundiaras has already been reported inother teleost fish by Carrassón *et al*. (2006), validating several digestive functions. PAS and AB histochemical stainingin this research, showed that jundiaras presented the largest number of villi in the proximal region, with probable absorption function in the enteral mucosa, providing essential cofactors for the enzymatic degradation of nutrients.The positive reactions to AB for acid mucins are related to the protection, and inhibition of glycosidase on the mucous intestinal surface (Carrassón *et al*. 2006; Kalhoro *et al*. (2018). The great quantity of goblet cells found in the jundiaras identified by positive reactions to the AB/PAS staining, seems to be a response related to the supplemented diet in this experiment. In the medial and distal intestines of the jundiaras raised with sanitary challenge, it was noted that the presence of numbers of goblet cells indicates the presence of acidic mucopolysaccharides,which responded positively to the AB+ histochemical study.It is likely that the responses to the challenge are related to the inhibition mechanisms of protease for defense (protection) against invasion of pathogenic organisms and mechanical injuries, as previously reported by Campbell (1999) and Kalhoro *et al*. (2018; 2019).

In addition, the presence of goblet cells with neutral mucus (PAS +) in intestinal mucosa may be linked with digestive processes and nutrient emulsion, as initially described by Clarke &Witcomb (1980) and, later, by Kalhoro *et al*. (2019), for teleost fish. In line with the histological measurements of the number of goblet cells in the epithelial mucosa and intestinal villi of*jundiaras*, differences in the number of goblet cells were identified depending on the region, diet, and environment. It should also be noted that these fish were subjected to stress (sanitation challenge) in 5 groups, consequently manifesting responses, which represented effectiveness in the use of the additives, with promising results for health promotion, increases in growth, weight, improvements in productive performance and histological modulations in fish, as reported by Zahran *et al*. (2020), who verified the use of dietary supplementation with *Withania somnifera* and its influences on the histomorphologyof the intestine of healthy Nile tilapia (*O. niloticus*).

# CONCLUSION

The addition of phytogenic prebiotics and commercial probiotics combined or separated, in conditions of sanitation challenge or not, proved to be efficient for water quality, productive performance (Feed Efficiency Index, Feed Conversion Ratio and Condition Factor), length and final weight, maintaining the well-being of jundiaras in captivity. The use of food additives in the diet of jundiaras (commercial DBAqua and phytobiotics in sanitation challenge) showed to be more effective for maintaining anatomical and histological characteristics, providing tissue integrity and histological modulations, with significant differences in the quantity of goblet cells and intestinal villi. The findings of the present study indicates the use of supplementation with DBAqua at the beginning of captivity, for 60 days, and, subsequently,the use of Aquaplus and the 3 phytogenic prebiotics at the end of 90 days. It is noteworthy that the morphophysiological results of the intestinal tract and nutritional management found here with diets of high biological value and feed additives, improve the nutritional status of jundiaras and should be used as an alternative and innovative prophylactic supplement for morphophysiological modulation, health, and less impacting and more sustainable environmental conditions.

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