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HISTOLOGICAL ASPECTS OF THE INTESTINAL TRACT OF LAMBARIS (*ASTYANAX ALTIPARANAE*: CHARACIDAE) PRODUCED IN CAPTIVITY

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ABSTRACT

Studies that address the morphology of the intestinal tract of fish have expanded the knowledge about the biology and nutritional management of native fish. The objective of this work was to describe some histological and histochemicalaspects of the intestinal tract of the yellow-tailed lambari (*Astiyanax altiparanae* - Charicidae) raised in captivity. The specimens obtained from the Grupo Acorci were immediately necropsied and the intestines were fixedin 10% formol. The fragments were subjected to automated processing, cut in a microtome at 4-µm thickness, and stained by Mallory's Trichrome, Hematoxylin and Eosin, Alcian Blue and Schiff's Periodic Acid (PAS). The histological study revealed three tunics: mucous, muscular and serous.The mucosa layer was consisted by a simple prismatic epithelium with basal nucleus and goblets cells PAS and AB positive, enterocytes and lamina propria with loose connective tissue and with nomucous muscle layer. The muscular tunic presented two muscular layers, internal and external, increasing the thickness in the cranio-caudal direction. Villi were morphologically differentin terms of height, thickness of branches, cell types and the presence of mucosal-associated intraepithelial lymphocytes.This study generated information on some histological particularities in the intestine of Astyanax, contributing to the improvement of nutritional management in captive.

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INTRODUCTION

The aquaculture sectorin Brazil has demonstrated great expansion with total production of 758.006 tons in 2019, representing an increase of 4.67% compared to 2018 production (PEIXE BR, 2020). According to FAO data (2018) for 2025, fish production is expected to be 162 million tons, based on consumption of 25 kg per capita/year. The yellow-tailed lambari (*Astyanax altiparanae*: Charicidae) is a small native species that inhabits the upper Paraná river basin, reaching 60 grams of live weight and 10 to 15 cm in length (Garutti and Britski, 2000; 2003; Grace and Pavanelli, 2013; Rodrigues *et al*., 2013). This species has diversification to feed for artificial feeding, with potential for fish farming (Jatobá *et al*., 2018). Some aspects of reproduction and morphology of *A. asltiparanae* are already knownin the literature, also, there are some studies involving nutrition and management (Viana *et al*., 2014; Siqueira-Silva *et al*., 2015; Carvalho *et al*., 2019). It is a generalist-opportunistic fish, with good results in fecundity and high growth rate, as well as with wide physiological adaptation to thermal oscillation in nurseries, rusticity, resistance to cultivation systems and good feed conversion (Sabbag *et al*., 2011). The Brazilian production of lambaris has been around 300 million units per year, being the State of São Paulo the largest producer. approximately 230 million per year (Carvalho *et al*., 2019). Of the native species used in food and human use, the species *A. altiparanae* has been accepted with great commercial interest and it has international coverage (all of South America), contributing to minimize pollution by the introduction of exotic species that can be used as live bait, eaten fried as processed and canned appetizers (Adrian *et al*., 2001; Martinez *et al*., 2012; Ferreira *et al*., 2016; Carvalho *et al*., 2019). Studies on the nutrition of native species in relation to morphophysiological aspects, nutritional need, digestibility and food management have promoted efficient application of methods of cultivation resulting in low production cost (Cyrino and Fracalossi, 2013). The morphology of fish digestive system and behavioral strategies become relevant for its adaptation to confinement, since it allows the association of eating habit with the physiological processes of responses to stress, intake, digestion and absorption of adequate nutrients (Molnár *et al*. 2018; Palińska-Żarska*et al*. 2020). Studies on the morphology of the intestinal tract of fish can provide information on nutritional dynamics, aiming to meet the needs for good health, well-being and achievement of good productive performance of animals in the face of the most competitive fish farming (Nakandakare *et al*., 2013; Mello *et al*. 2017). Due to the scarcity of morphological and histochemical studies of the intestinal tract of native cultivated fish, the morphological characterization of the yellow-tailed lambari (*A. altiparanae*) gut is considered relevant. Thus, the objective of this research was to perform histological and histochemical analysis of the intestine of *A. altiparane*.

MATERIAL AND METHODS

The experiments were carried out at the Laboratory of Fish Production and Health and Laboratory of Pathological Anatomy of the Catholic University of Don Bosco, in Campo Grande (MS), Brazil. The precepts contained in Law No. 11.794 of October 2008, decree no. 6.899 of July 15, 2009, and the rules issued by the National Council for the Control of Experimentation (Brasil, 2019), were authorized and registered with the Animal Ethics and Research Committee of Catholic University of Don Bosco under protocol 001/2019. This experiment was carried out in 2018-2019 with yellow-tailed lambari (*A. altiparanae*) cultivated at the Acorci Group Fish Farming Station, located in the rural area of Mato Grosso do Sul (Figure 1).

The animals were fed with commercial diet of the brand Guabi® extruded 1mm, presenting 42% protein content and 4,200 Kcal of crude energy, 4 times a day until satiety (*ad libitum*), for 90 days. They were anesthetized, euthanized and necropsied for the removal of biological material (intestinal tract), in order to elaborate histological slides according to the procedures of Caputo *et al*. (2010), delimiting the minimum area of 200μm2, totalizing 1200 μm2 of intact villi. Tissue fragments from each region (n=12) were collected, fixed, dehydrated in increasing alcoholic solutions (70% alcohol, 80%, 90% and 100% I and II), diaphanized in xylol and included in paraffin. The tissues were cut into a rotation microtome at 4 μm thick and stained by hematoxylin-eosin (HE), periodic acid Schiff acid (PAS), alcyan blue (alcian blue - AB) and Mallory tricrômico (TM) for morphological description. The slides were observed in a Composite Optical Microscope (MOC) of light, Carl Zeiss Microscopy GmbH, model Axio Scope A1, with the aid of Zen software and the photographic records by axiocam 503 color camera, coupled to MOC.

RESULTS

The histological analysis of the intestine of *A. altiparnae* allowed to describe the pattern of intestinal folds, which were variable in size, branches, types and cell densities, characterizing two distinct regions: anterior and posterior (Figure 2 A, B), which was confirmed by Cardoso *et al*. (2015); Riddle *et al*. (2018; 2020) for *A. altiparanae* and *A. mexicanus* (Charicidae), respectively. Histologically, the intestine of *A. altiparanae*, described here, was structured by 3 distinct tunics (a, b and c):(a) tunic mucosa, formed by the simple prismatic epithelium, with predominant absorbive cells (enterocytes) and brush edge on the surface; goblets cells increasing in the distal direction (posterior intestine) and lamina itself, formed by well-vascularized loose connective tissue (Figures 3A, B, C, D), similar to the described by Wilson; Castro (2011) and found by Rodrigues *et al*. (2013) in a study with pirarucus (*Arapaima gigas*: Osteoglossidae) and similarly identified for A. altiparanae by Cardoso *et al*. (2015) and Riddle (2020). The muscle layer of the mucosa was not visualized in *A. altiparanae* (Figure 3E).(b) Muscular tunic, below the tunic mucosa, consisted of 1 or 2 layers (Figure 3A, E), internal and external, the external being thicker in the posterior part of the intestine (Figure 2A, B). These were formed by smooth muscle fibers (Figures 3 and 4), a description similar to that of Castro *et al*. (2002) and, in curimbatás (*Prochiloduslineatus*: Prochilodontidae), piaus-de-três-pintas (*Leporinusrenhardti*: Anostomidae),dourados (*Salminusbrasiliensis*: Characidae) and, specifically, for *A. altiparanae* by Ferreira *et al*. (2016). In the present study, *A. altiparanae* demonstrated variation in the thickness of the muscular tunic in the anterior and posterior sections of the entire intestinal tract, as similarly reported by Castro *et al*. (2002) in three species of fish with different feeding habits, by Borges *et al*. (2010) in the carnivorous species true grouper (*Epinephelus marginatus*: Serranidae), Cardoso *et al*. (2015) for *A. altiparanae* and Riddle *et al*. (2018; 2020) for *A. mexicanus*. Ferreira *et al*. (2016) and Riddle *et al*. (2018) described that the greater thickness of the muscle layer in *Astyanax* would contribute to intestinal motility and protection against endoparasites. (c) More externally, the serous tunic was detected, which was coated with simple squamous epithelium, associated with dense connective tissue and blood vessel (Figures 3E).

In histochemical techniques, the presence of mucus secreting goblet cells (calliform), in pink/red color (PAS+), indicated the presence of neutral and acidic mucins in blue (HE/AB+), respectively (Figures 4A, B and 3A, B, C). The staining allowed the identification of these cells in the two intestinal parts, anterior and posterior. Acid mucins predominated in the posterior intestine, positively reacting to AB, staining strongly in blue (Figures 3A, B, C) and neutral mucins, also abundant in the posterior intestine, stained in dark pink/red by PAS dye (Figures 2 B). Other studies have found reactions similar to PAS and AB for other fish species. Riddle *et al*. (2020) mentioned the presence of goblets cells with acidic and neutral mucins in the intestine of *A. mexicanus*. For other teleosts, Hernández *et al*. (2009) found these cells in jundiá (*Rhamdiaquelen*: Pimelodidae), Santos *et al*. (2007) in fish trails (*Pseudopeneus maculatus*: Mulidae), in dentudos carnivores (*Oligosarcushepsetus*: Charicidae) by Abdallah *et al*. (2004), Fagundes *et al*. (2016) for the pufferfish (*Sphoeroidestestudineus* – Tetraodontidae), Kalhoro *et al*. (2018; 2019) in marine fish of the corvinas family (*Larimichthyscrocea* – Sciaenidae) and (*Larimichthyscrocea*: Acanthopterygii). The study of the cell types of villi located at the base of the crypt of *A. altiparanae* revealed: cells similar to those of Paneth, lymphoid cells and enteroendocrine cells

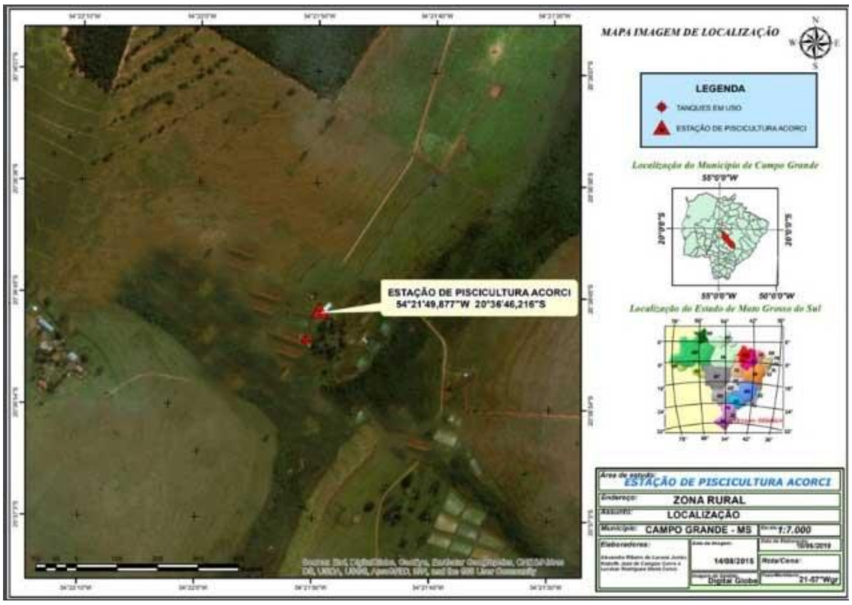

Figure 1. Location Map of the Acorci Group Fish Farming Station, Campo Grande (MS)

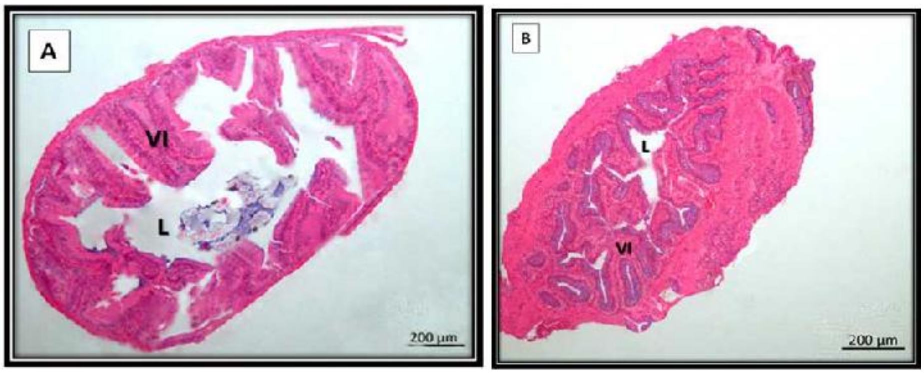

Figure 2 A, B. Histomicrography of the anterior (A) and posterior (B) intestines: intestinal villi (VI); lumen(L) of *A. altiparanae*. Objective 5x - AB+/HE

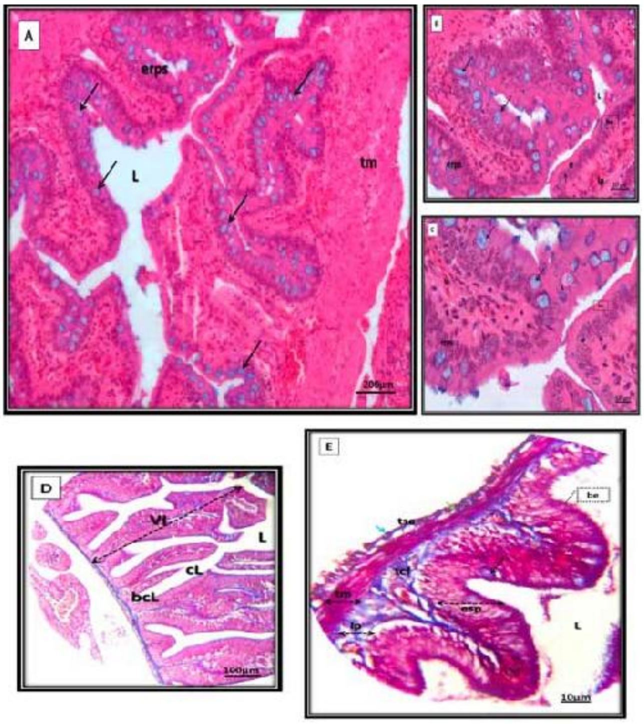

Figure 3 - Histomicrography of the intestine of *A. altiparanae*. (A) (B) tunic mucosa: simple prismatic coating epithelium (erps); HE-AB+ globets cells (black arrow). (C) brush edge (be). (D) Intestinal Villi (VI); lieberkühn crypt base (bcL); Lieberkühn crypt; L (light); serous tunic (tse); simple squamous epithelium (blue arrow); loose (tcf) and dense connective tissue (green arrow). (E) muscular tunic (tm); blood vessel (blue arrow). Lenses (A) 5x, (B) 40x, (C) 63x - HE/AB+, (D)10x, (E) 40x - TM.

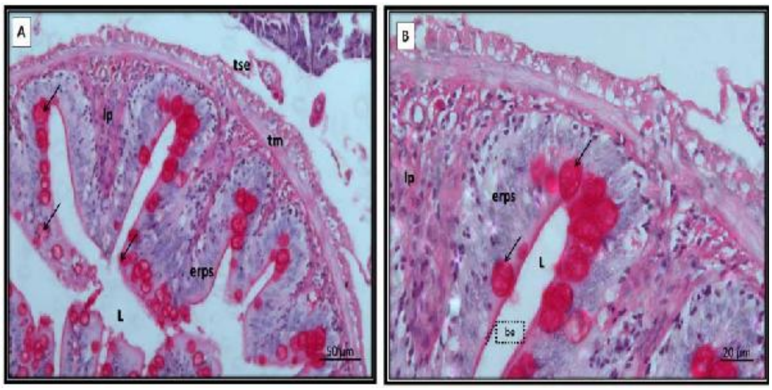

Figure 4 – Histomicrography of the intestine of *A. altiparanae*. (A) (B) tunic mucosa: simple prismatic coating epithelium (erps); PAS+ goblets cells (black arrow); muscle tunic (tm); mesoenteric plexus (pme); light (L). Lenses 20x, 40x - PAS+

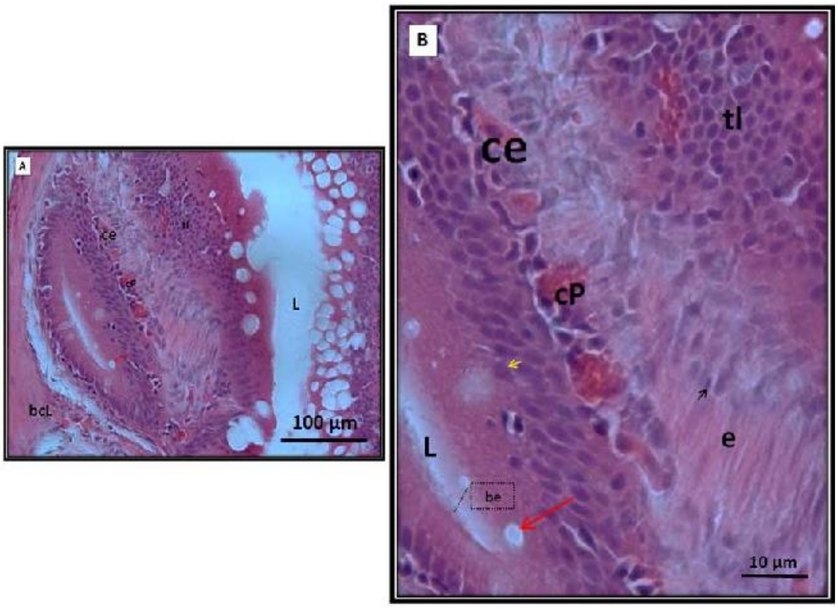

Figure 5 A and B - Cross-sectional photomicrography of the intestinal villi of *A. altiparanae* and details of the base of lieberkühn crypt (bcL) – (A and B) – goblet cell (red arrow); goblet cell nucleus (yellow arrow); absorbitive cell (enterocytes – e): basal nucleus of the absorbitive cell (black arrow); Paneth cells (cP), enteroendocrine (ce); lymphoid tissue (tl); light (L). Lenses: (A) 10x, (B) 40x - HE.

(Figures 5 A, B), already identified by Ourth *et al*. (1980); Min *et al*. (2009) in the American catfish (*Ictalurus punctatus*: Ictaluridae), in the medical fish (*Garrarufa*: Cyprinidae,) by Kuru *et al*. (2010), in jundiás (*Rhandiaquelen*: pimelodidae) in the work of Hernández *et al*. (2009) and more specifically for the yellow-tailed lambari (*A. bimaculatus*: Charicidae) (Cardoso *et al*. 2015).

DISCUSSION

The intestinal villi of the anterior intestine of *A. altiparana* were less numerous and branched and slightly more elongated compared to the posterior segment (Figure 2A, B). In the posterior segment, the villi were more numerous and more branched with gradual decrease of lumenin the distal direction (Figure 2B), as the reports of Ferreira *et al*. (2016); Riddle *et al*. (2020). Theoretically, it is agreed that differentiation into two different parts of the intestinal tract in teleost fish is related to the physiological processes of nutrient absorption: in the anterior intestine it predominates the lipids and, in the posterior, protein strain (Riddle *et al*., 2018; 2020). The origin and histological organization, concerning density and specific cell types (Baldisserotto *et al*., 2002; Baldisserotto, 2010; Becker *et al*., 2010; Mello *et al*., 2017; Riddle *et al*., 2018; 2020) are established by the characteristics (i) type of food ingested by fish, (ii) eating habits, whether carnivorous, herbivores, detritivorous or omnivorous (Ojeda, 1986; Sire; Vernier, 1992; Scocco *et al*., 1997; Moraes (Almeida, 2020), (iii) species or even nutritional physiological needs, sex and size (Santos *et al*., 2007; Santos *et al*., 2015; Ghosh; Chakrabarti, 2015; Moawad *et al*., 2017; Kalhoro *et al*., 2018; 2019).

The parts of the intestine, in the anterior to posterior sense, in the present study, presented different and specialized morph physiological characteristics, defined by the types and cell densities in the mucosa, thickness and organization of muscle layers, similar to that reported for a species of cave lambari in Mexico (*Astyanax mexicanus*: Charicidae) by Riddle *et al*. (2018; 2020). The greater amount of goblets cells in the posterior region of the intestine (Figures 3 A, B and C) has been related to the protection of the mucosa, its integrity and lubrication for the management and transit of the fecal bolus (Grau *et al*. (1992); Murray *et al*. (1996); Kalhoro *et al* (2918).

This finding was also reportedin pirarucus (*Arapaima gigas*: Osteoglossidae), by Rodrigues and Cargnin-Ferreira, (2017) who found gradual increase of goblets cells in the craniocaudal direction and higher density of goblets cells in the posterior intestine. Differently from this research, Hernández *et al*. (2009; 2012) in an immunohistochemical study of jundiás (*Rhamdiaquelen*: Pimelodidae) found 4 distinct tunics (mucosa, submucosa, muscular and serous). More specifically for *A. altiparanae*, the absence of the tunic submucosa in the cave lambari (*A. mexicanus*) (Riddle *et al*. 2018; 2020) was demonstrated. In this research, the first description of cells morphologically similar to paneth cells in *A. altiparanae* was reported, located at the base of lieberkühn crypts in the intestine. These cells were strongly stained by HE in bright red (Figure 5A), with rounded-aspect, and cytoplasmic vesicles with eosinophilic granular secretions inside (Figure 5B), previously reported by Sayyaf Dezfuli *et al*., 2018) in histochemical studies of the gastrointestinal tract of the marine stingray (*Raja clavata*: Rajiidae), that observed the presence of lysosis in the intestinal mucosa, relating them to Paneth cells in mammals and their role as a defense barrier. The specialized literature, since the last century, has been reporting many immunohistochemical studies of the presence of lysozymes in mucus, seum, organs, eggs, lymphocytes and macrophages in the mucosa of the intestinal tract of teleost fish (Fletcher *et al*., 1973; Ourth, 1980; Hikima *et al*., 1997; Berillis *et al*.,2017; Torcillas *et al*., 2019; evicán-Ansejo *et al*., 2019). It is supported by Almeida *et al*. (2016) the roles played by Paneth cells in the control of the intestinal microbiota, functioning as an important barrier in combating pathogenic organisms and foreign bodies. Previous studies mention the chemical composition of the granulosa vesicles inside Paneth cells as proteins, proteases, scaffolding molecules, cytokines and antimicrobial peptides, showing their physiological importance in integrity (defense), control of cellular signals that promote regeneration of the intestinal mucosa, and also secrete factors that sustain and modulate cells that promote the renewal of the epithelium (Clevers; Bevins, 2013; Sayyaf Dezfuli *et al* 2018; Holly, Holly. Smith, 2018).

More specific biomolecular studies clarify the nature of vesicles substances inside Paneth cells, identifying them as lysozymes (Garcia *et al*., 2009; Sayyaf Dezfuli *et al*., 2018), defensine, cryptine and murine (Ouellette, 2010; Bevins; Salzman, 2011; Andersson *et al*., 2012; Liu *et al*, 2012). Even with this evidence, Sayyaf Dezfuli *et al*. (2018), understand that there is still no confirmation about the presence of Paneth cells in fish. Most studies identify the presence of lysozyme, synthesized in Paneth cells in other vertebrates, but there is no certainty on the lysozyme production sites and the stimulus for its production in fish (Pausen *et al*., 2001). Another histological finding in the intestinal tract of *A. altiparanae* was the presence of lymphoid tissue at the base of lieberkühn's crypt (Figure 5A). Similarlyin this study, Gaines *et al*. (2012) visualized lymphoid tissue and the presence of defense cells in pirarucu (A. gigas), Venkatesh *et al*. (2014) in snakehead fish (*Channa punctatus*: Channidae), Srichaiyo *et al*. (2020a; 2020b) in Nile tilapia (*Oreochromis niloticus*), and more specifically Cardoso *et al*. (2015) and Moraes *et al*. (2020) for the genus *Astyanax*. In general, vertebrates' lymphoid tissues in the intestine functionas sites of cell production, phagocytic and immunological defense factors, forming defined tissues or clusters of cells in specific intestinal regions(Wang *et al*., 2017; Rebl; Goldammer, 2018). In fish, mucosal-associated lymphoid tissue (MALT) is subdivided into several anatomical factors of location and organization, observed here in *A. altiparanae* and the structure normally associated in small aggregates or diffuse, being named of lymphoid tissue associated with the intestine (GALT). These tissues are sites of protection and integrity of the intestinal mucosa since they are in contact with the environment through the lumen of the gastrointestinal tract, offering different responses to endogenous and exogenous factors (Campos, 2015, Srichaiyo*et al*., 2020a; 2020b). Studies of histology and histochemistry of the intestinal tract of fish is important for food management, as it reflects fishes eating habits, ethology and morphophysiology. intestinal morphology, once associated with nutrition, becomes an auxiliary mechanism to evaluate the adaptation of feeding animals in captivity in a more efficient way, which will favor animal welfare and increase production.

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Conclusion

The histological study of the intestine of *A. altiparanae* allowed the finding of morphological variations of structures and cells when compared to other fish species, highlighting the record of the presence of cells similar to paneth's. The comparative morphology of fish tissues becomes important for future research involving the increase in nutrient digestion and absorption processes, as well as defense to pathogens, providing better survival to captive-bred fish.

REFERENCES

Abdallah, V. D., Azevedo, R. K., Luque, J. L. 2004. Metazoários parasitos dos lambaris *Astyanax bimaculatus* Linnaeus, 1758, *A.parahybae*Eigenman, 1908 e Oligosarcushepsetus Cuvier, 1829 Osteichthyes: Characidae, do Rio Guandu, Estado do Rio de Janeiro, Brasil. Revista Brasileira de Parasitologia Veterinária, 32 pp. 57-63.

Adrian, I. F., Silva, H. B. R., &Peretti, D. 2001. Dieta de *Astyanax bimaculatus* Linnaeus, 1758 Characiformes, Characidae, da área de influência do reservatório de Corumbá, Estado de Goiás, Brasil. Acta Sci, 23, pp.435-440. https://doi.org/10.4025/actascibiolsci.v23i0.2735.

Almeida, W. M., Fraga, K. B., Aguiar Júnior, F. C. A., & Magalhães, C. P. 2016. Análise histológica do trato intestinal do*Caracaraplancus*Miller, 1777. Ciênc. anim. bras. Goiânia, 173, pp.425-434. https://doi.org/10.15.

Andersson, M. L., Karlsson-Sjoberg, J. M., Putsep, K. L. 2012. CRS-peptides: uniquedefensepeptides of mouse Paneth cells. Mucosal Immunol., 54, pp.367–376.https://doi.org/10.1038/mi.2012.22.

Baldisserotto, B. 2009. Fisiologia de peixes aplicada à piscicultura. Santa Maria: Ed. UFSM. 350p.

Baldisserotto, B., Val, A.L. 2002.Ion fluxes of metynnishypsauchen, a teleost from the Rio Negro, Amazon, exposed to an increaseof temperature. Brazilian Journal of Biology, 624B, pp.749-752.https://doi.org/10.1590/S1519-6984200200050 0003.

Becker, A. G., Gonçalves, J. F., Garcia, L. O. Behr, E. R., Graça, D. L., Kurtz Filho, M., Martins, T., Baldisserotto, B. 2010. Morphometric parameters 260comparisons of the digestive tract of four teleost with different feeding habits. Ciência Rural, Santa Maria, 40, pp.862-866.https://doi.org/10.1590/S0103-84782010005000049.

Berillis, P., Martin, S.,Mente, E. 2017. Histological methods to assess the effect of diet and a single meal on the liver and intestine of rainbow trout: fishmeal and fishoil replacement with plant protein and oil.In: Berillis P. Ed.. Trends in Fisheries and Aquatic Animal Health. Bentham Science publishers.

Bevins, C. L., Salzman, N. H. 2011. Panethcells, antimicrobialpeptidesandmaintenanceofintestinalhomeostasis. Nat. Rev. Microbiol., 9, pp.356–6, https://doi.org/10.1038/nrmicro2546.

Borges, J. C. S., Sanches, E. G., OliveirA, M. S. de; & Silva, J. R. M. C. da. 2010. Anatomia e histologia gastrintestinal da garoupa-verdadeira *Epinephelus marginatus* Lowe, 1834 Teleostei, Serranidae. Acta Scientiarum. Biological Sciences, 324,https://doi.org/10.4025/actascibiolsci.v32i4.4462.

Brasil 2019. Ministério da Ciência, Tecnologia e Inovação. Conselho Nacional de Controle de Experimentação Animal – CONCEA. Diretriz da Prática de Eutanásia. Anexo I. Brasília/DF - 2015. Available in: https://bit.ly/2X2qPX2.

Campos, S. M. N. 2015. Tecido linfoide associado ao trato gastrointestinal GALT, sua importância para a homeostasia orgânica e possibilidades de imunomodulação. Revista Brasileira de Nutrição Funcional, v.15, n. 62. Available in: https://bit.ly/2TEg3FE.

Caputo, L. F. G., Gitirana, L. B., Manso, P. P. A., Real, S. C. Técnicas histológicas. *In*: Guimarães, A. C. R., Souza, D. S., Alvez, E. A., Mota, E. M., BarbosA, H. S., MedradO, L. Orgs.,2010. Conceitos e Métodos para a Formação de Profissionais em Laboratórios de Saúde. Técnicas histológicas. v. 2, cap. 3. Rio de Janeiro: Fiocruz, Available in: https://bit.ly/2I2144h.

Cardoso, N. N., Firmiano, E. M. S., Gomes, I. D., nascimento, A. A., Sales, A., & Araújo, F. G. 2015.Histochemical and immunohistochemical study on endocrine cells 5HT, GAS, and SST of the gastrointestinal tract of a teleost, the characin *Astyanax bimaculatus*. Acta Histochemica, 1177, pp.595–604, https://doi.org/10.1016/j.acthis.2015.05.007.

Carvalho, J. G. de; Moura,M. de; Castellani, D., Gonçalves, G. S. Abimorad, E. G. 2019. Protocolos de coleta de fezes do lambari para estudo de digestibilidade. Capítulo 14. P. 101-109. Zuffo, A. M. Org. Aquicultura e Pesca: Adversidades e Resultados. Ponta Grossa PR: Atena Editora, 174 p. https://doi.org/10.22533/at.ed.272192903.

Castro, E. F., Fonseca, C. C., &Menin, E. 2002.Identificação de células endócrinas no aparelho digestivo de *Prochilodusmarggravii*Walbaum, 1792 Pisces, Teleostei, Characiformes, Prochilodontidae. ArqCiêncVetZool, UNIPAR 51: pp.71-78. https://doi.org/10.25110/arqvet.v5i1.2002.749.

Clevers, H. C., & Bevins, C. L. 2013.Paneth Cells: Maestros of the Small Intestinal Crypts. Annual Review of Physiology, 751, pp.289–311 https://doi:10.1146/annurev-physiol-030212-183744.

Cyrino, J. E. P., &Fracalossi, D. M. 2013 Eds.. Nutriaqua: nutrição e alimentação de espécies de interesse para aquicultura brasileira, 1[a] edição, Sociedade Brasileira de Aquicultura e Biologia Aquática, Florianópolis SC.

FAO. 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome.Available in: https://bit.ly/2uJVQpo.

FAGUNDES, K. R. C., ROTUNDO, M. M., & MARI, R.B. 2016. Caracterização morfológica e histoquímica do trato digestivo do baiacu *Sphoeroidestestudineu*s Linnaeus 1758 Tetraodontiformes: Tetraodontidae. *Anais da Academia Brasileira de Ciências*, 883, Supl, pp.1615-1624. https://doi.org/10.1590/0001-3765201620150167.

Ferreira, P. M.F., Caldas, D. W., Salaro, Ana L., SartorI, S. S.R., Oliveira, J. M., Cardoso, A. J.S., &Zuanon, J. A.S. 2016. Intestinal and liver morphometry of the Yellow Tail Tetra *Astyanax altiparanae* fed with oregano oil. An. Acad. Bras. Ciênc., Rio de Janeiro,882 pp. 911-922. https://doi.org/10.1590/0001-3765201620150202.

Fletcher, T. C. & White, A. 1973. Lysozyme activity in the plaice *Pleuronectes platessa* L.. Experientia, 29,pp.1283-1285.

Gaines, A.P.L., Sarmiento, L.E.E., Viana, G.M., Monteiro, P.C., Araújo, C.S.O. 2012. Tissue changes in the gut of *Arapaima gigas* Schinz, 1822, infected by the nematode *Spirocamallanusinopinatus* Travassos, 1929. Neotropical Helminthology, 62, pp.147 – 157,

Garcia, D. I. M., Aguilera, J. C., Becerril, I. V., & Montes, A. B. 2009. Cambios morfológicos producidosporelestrés sobre lapoblación de células de Paneth. Revista Médica Del Hospital General Del Mexico, Cidade do México, jul/set, 7230, pp.129-135.

Garutti, V. Piscicultura ecológica – Editora UNESP, São Paulo, 2003. Available in:https://bit.ly/2IdgPEh.

Garutti, V., Britski, H. A. 2000.Descrição de uma espécie nova de *Astyanax* Teleostei: Characidae da bacia do alto rio Paraná e considerações sobre as demais espécies do gênero na bacia. Comun. Mus. Ciênc. Tecnol.,13,pp.65-88.Available in: http://abre.ai/bwWK.

Ghosh, S. K., Chakrabarti, P. 2015. Histological and histochemical characterization on stomach of Mystuscavasius Hamilton, *Oreochromis niloticus* Linnaeus and *Gudusiachapra* Hamilton: Comparative study. The Journal of Basic & Applied Zoology, 70, pp. 16-24.https://doi.org/10.1016/j.jobaz.2015.04.002.

Grau, A., Crespo, S., Saraqueste, M. C., González Canales, M. L. 1992. The digestive tract of the amberjack *Seriola dumerili*, Risso: a light and scanning eléctronmicroscope study. J. Fish. Biol., London, 41, pp.287-303.https://doi.org/10.1016/j.ydbio.2018.01.006.

Hernández, D. R., Pérez Gianeselli, M., & Domitrovic, H. A. 2009. Morphology, Histology and Histochemistry of the Digestive System of South American Catfish *Rhamdiaquelen*. International Journal of Morphology, 271.https://doi:10.4067/s0717-9502200900 0100019.

Hernández, D. R., Vigliano, F. A., Sánchez, S., Bermúdez, R., Domitrovic, H. A., & QUIROGA, M. I. 2012. Neuroendocrine system of the digestive tract in *Rhamdiaquelen* juvenile: An immunohistochemical study. Tissue and Cell, v.44, n.4, p. 220–226. https://doi:10. 1016/j.tice.2012.03.005.

Hikima, J., Hirono, I., & Aoki, T. 1997. Characterization and expression of c-type lysozyme cDNA from Japanese flounder *Paralichthysolivaceus*. Molecular Marine Biology and Biotechnology, 6, pp. 339–344.

Holly, M., & Smith, J. 2018. Paneth Cells during Viral Infection and Pathogenesis. Viruses, 105, pp. 1-20. https://doi.org/10.3390/v10050225.

Jatobá, A., Moraes, K. N., Rodrigues, E. F., Vieira, L. M., & Pereira, M. O. 2018. Frequency in the supply of Lactobacillus influence its probiotic effect for yellow tail lambari. Cienc. Rural, SantaMaria, 4810. https://doi.org/10.1590/0103-8478cr20180042.

Kalhoro, H., Tong, S., Wang, L., Hua, Y., VOLATIANA, J. A., SHAO, Q. 2018. Morphological study of the gastrointestinal tract of *Larimichthyscrocea* Acanthopterygii: Perciformes. Zoologia, 351.https:// doi.org/ 10. 3897/zoologia.35.e25171.

Kalhoro, H., Tong, S., Wang, L., Hua,Y., Volatiana, J. A., Shao, Q. 2019.Gross anatomical and histomorphological features of the *Acanthopagrusschlegelii* digestive tract Bleeker 1854 Perciformes, SparidaeHameeda. Acta Zoologica, 100, pp.24–35. The Royal Swedish Academy of Sciences. Error! Hyperlink reference not valid..

Kuru, N., Çinar, K., Senal, N., Demirbag, E. &Diler, D. 2010. Endocrine cells in the gastrointestinal tract of *Garrarufa*. Kafkas Univ. Vet. Fak. Derg., 16supl, pp.35-41, Available in: https://bit.ly/32BhlFq.

Levicán-Asenjo, J., Soto-Rifo, R., Aguayo, F., Gaggero, A., &Leon, O. 2019. Salmon cells SHK-1 internalize infectious pancreatic necrosis virus by macropinocytosis.J Fish Dis, 427, pp.1035-1045. https://doi.org/1046.10.1111/jfd. 130 09.

Liu, J., Walker, N. M., Cook, M. T., Ootani, A. & Clarke, L. L. 2012. Functional Cftr in crypt epithelium of organotypic enteroid cultures from murine small intestine. American Journal of Physiology-Cell Physiology, 30210, pp.492–1503.https://doi.org/10.1152/ajpcell.00392.20 11.

Martinez, E. R. M., Alves, A. L., Silveira, S. M., Foresti, F., & Oliveira, C. 2012. Cytogenetic analysis in the incertaesedis species *Astyanax altipar* anae Garutti and Britzki, 2000 and *Hyphessobrycon eques*Steindachner, 1882 Characiformes, Characidae from the upper Paraná river basin. Comp Cytogen, 61, pp. 41-51. https://doi.org/10.3897/CompCytogen.v6i1.1873.

Mello, G. C. G., Santos, M. L., Arantes, F. P., Pessali, T. C., Brito, M. F. G., & Santos, J. E. 2017. Morphologicalcharacterisation of the digestive tract of the catfish *Lophiosilurusalexandri* Steindachner, 1876 Siluriformes, Pseudopimelodidae. Acta Zoologica, 1001.https://doi.org/10.1111/azo.12224.

Min, H.E., Kai-Yu, W. & Yu, Z. 2009. Imunocyto chemical identification and localization of Difuse Neuendocrine System DNES cells in gastrointestinal tract of channel catfish *Ictalurus punctatus*. Agric Sci China, 8, 2, pp.238-243. https://doi.org/10.1016/S1671-29270960032-8.

Moawad, U. K., Awaad, A. S., Tawfiek, M. G. 2017. Histomorphological, histochemical, and ultrastructural studies on the stomach of the adult African catfish *Clariasgariepinus*, Journal of Microscopy and Ultrastructure, 53, pp.155-166.https://doi.org/10.1016/j.jmau.2016.08.002.

Molnár, T., Csuvár, A., Benedek, I., Molnár M., KabaI, P.2018. Domestication affects exploratory behaviour of pikeperch *Sander lucioperca* L. during the transition to pelleted food. PlosOne 135.https://doi.org/10. 1371/journal.pone.0196118.

Moraes, G., &Almeida, L. C. de.2020. Nutrition and functional aspects of digestion in fish. Capitulo 11, p. 251-271, 2020. BALDISSEROTO, B., URBINATI, E. C., & CYRINO, J; E. P. Eds.. *In*: Baldisserotto, B. Urbinati, E. C., &Cyrino, J. E. P. Biology and Physiology of Freswater Neotropical fish. Academic Press ELSEVIER. 358 pp.https://doi.org/10.1016/B978-0-12-815872-2.00011-7.

Murray, H. M., Wright, G. M., Goff, G. P. 1996. Comparative histological and histochemical study of the post-gastric alimentary canal from three species of pleuronectid, the Atlantic halibut, the yellowtail flounder and the winter flounder. J. Fish Biol., London, 48949, pp.187-206.https://doi.org/10.1111/j.1095-8649.1996.tb011 12.x.

Nakandakare, I. B., Iwashita, M. K. P., Dias, D. de C., Tachibana, L., Ranzani-Paiva, M. J. T., & ROMAGOSA, E. 2013. Growth performance and intestinal histomorphology of Nile tilapia juveniles fed probiotics. Acta Sci., Anim. Sci., Maringá, 354, pp.365-370. https://doi.org/10.4025/actascianimsci.v35i4.18610.

Ojeda, F. P. 1986. Morphological characterization ofthe alimentary tract of Antarctic fishes and its relation to feeding habits. Polar biol., Berlin, 5, pp.125-128.https://doi.org/10.1007/BF00443 384.

Ouellette, A. J. 2010. Paneth cells and innate mucosal immunity. Curr. Opin. Gastroenterol, 26, pp. 547–53 12.

Ourth, D. D. 1980.Secretor IgM, lysozyme and lymphocytes in the skin mucus of the channel catfish, *Ictaluruspunctatus*. Immunology Developmental & Comparative, 4, pp.65-74. https://doi.org/10.1016/s0145-305x8080 009-7.

Palińska-Żarska, K., Woźny, M., Kamaszewski, M. *et al*. 2020. Domestication process modifies digestion ability in larvae of Eurasian perch *Percafluviatilis*, a freshwater Teleostei. Sci Rep, 102211.https://doi.org/10.1038/s41598-020-59145-6.

Peixe Br 2020. Associação Brasileira de piscicultura. Anuário da Piscicultura brasileira em 2020. Available in:https://bit.ly/38ZhSnZ.

Paulsen, S. M., Engstad, R. E., &Robertsen, B.2001. Enhanced lysozyme production in Atlantic salmon *Salmo salar* L. macrophages treated with yeast β-glucan and bacterial lipopolysaccharide. Fish & Shellfish Immunology, 111, pp.23–37. https://doi.org/ 10.1006/fsim.2000.0291.

Rebl, A., &Goldammer, T. 2018.Under control: The innate immunity of fish from the inhibitors' perspective.Fish & Shellfish Immunology, 77,pp.328-349. https://doi.org/10.1016/j.fsi.2018. 04.016.

Riddle, M. R., Damen, F., Aspiras, A., &Tabin, J. A., Mcgaugh, S. Tabin, C. J. 2020. Evolution of gastrointestinal tract morphology and plasticity in cave-adapted Mexican tetra, *Astyanax mexicanus*. BioRxiv. https://doi.org/ 10.1101/852814.

Riddle, M. R., Boesmans, W., Caballero, O., Kazwiny, Y., &Tabin, C. J. 2018. Morphogenesis and motility of the *Astyanax mexicanus* gastrointestinal tract. Developmental Biology, 44, 12, pp.285–296. https://doi.org/10.1016/ j.ydbio.2018.06.004.

RodrigueS, A.P.O., Lima, A.F., Alves, A.L., Rosa, K.D., Torati, S.L., Santos,V.R.V. 2013. Piscicultura de água doce multiplicando conhecimentos: Espécies de peixes para a 90 piscicultura. 1. ed, Empresa Brasileira de Pesquisa Agropecuária, p. 440.

Rodrigues, A. P. O., &Cargnin-Ferreira, E. 2017. Morphology and histology of the pirarucu *Arapaima gigas* digestive tract. Int. J. Morphol. 353, pp.950-957. https://doi.org/10. 4067/S07 17-95022017000300025.

Sabbag, O. J., Takahashi, L. S., Silveira, A. N., Aranha, A. S. 2011. Custos e viabilidade econômica da produção de lambari-do-rabo-amarelo em monte Castelo/SP: um estudo de caso. Bol. Inst. Pesca, São Paulo, 373, pp. 307 – 315.

Available in: https://bit.ly/3cowUos. Acesso em 03 de março de 2020.

Sayyaf-Dezfulia, B., Manerab, M., Bosic, G., Merellad, P. Depasqualee, J. A., Giaria, L. 2018. Intestinal granular cells of a cartilaginous fish, thornback ray *Raja clavata*: Morphological characterization and expression of different molecule. Fish and Shellfish Immunology, 75, pp.172-180. http://dx.doi.org/10.1016/j.fsi.2018 .02.019.

Santos, C. M., Duarte, S., Souza, T. G. L., Ribeiro, T. P., Sales, A., Araújo, F. G. 2007. Histologia e caracterização histoquímica do tubo gastrintestinal de *Pimelodusmaculatus* Pimelodidae, Siluriformes no reservatório de Funil, Rio de Janeiro, Brasil. Iheringia, Sér. Zool., Porto Alegre, 974, pp.411-417.http://dx.doi.org/ 10.1590/S0073-47212007 000400009.

Santos, M. L. dos; Arantes, F. P., Pessali, T. C., Santos. J. E. dos. 2015. Morphological, histological and histochemical analysis of the digestive tract of *Trachelyopterusstriatulus* Siluriformes: Auchenipteridae. Zoologia, 324, pp.296–305. http://dx.doi.org/10.1590 /S1984-46702015000400005.

Scocco, P., Mengui, G., &Ceccarelli, P. 1997.Histochemical differentiation of glicoconjugatesoccuring in the tilapine intestine. J. Fish Riol., 51, pp.848-857.https://doi.org/10. 1111/j.1095-8649.1997.tb02005.x.

Siqueira-Silva, D.H., Silva, A.P.S., Ninhaus-Silveira, A., &Veríssimo-Silveira, R.2015. Morphology of the urogenital papilla and its component ducts in *Astyanax altiparanae* Garutti & Britski, 2000 Characiformes: Characidae. Neotropical Ichthyology, 13, pp.309-316. https://doi.org/10.1590/1982-0224-20140102.

Sire, M. F., Vernier, 1. M. 1992.Intestinal absorption of protein in teleost fish. Comp. Biochem. Physiol., 103A4, pp.771-781, https://doi.org/10.1016/0300-96299290180-X.

Srichaiyo, N., Tongsiri, S., Hoseinifar, S. H., Dawood, M. A. O., Esteban, M. Á., Ringø, E., &Van Doan, H a. 2020. The effect of fishwort *Houttuynia cordata* on skin mucosal, serum immunities, and growth performance of Nile tilapia. Fish & Shellfish Immunology, 98, pp.193-200.https://doi.org/10.1016/j.fsi.2020.01.013.

Srichaiyo, N., Tongsiri, S., Hoseinifar, S. H., Dawood, M. A. O., Jaturasitha, S., Esteban, M. Á., Van Doan, H. b. 2020. The effects gotu kola *Centella asiatica* powder on growth performance, skin mucus, and serum immunity of Nile tilapia *Oreochromis niloticus* fingerlings. Aquaculture Reports, 16. https://doi.org/10.1016/j.aqrep.2019.100239.

VENKATESH, S. P., JEYAPRIYA, N. S., & VIVEKANANTHAN, T. 2014. Report on gut associated lymphoid tissue GALT in freshwater fish *Channa punctatus* BLOCH. International Journal of Pure and Applied Zoology, 22, pp.95-99. Available in: https://bit.ly/2vJmDCM.

Viana, L.F., Tondato, K.K., Súarez, Y.R., Lima-Junior, S.E. 2014. Influence of environmental integrity on the reproductive biology of Astyanax altiparanae Garutti & Britski, 2000 in the Ivinhema river basin. Acta Scientiarum Biological Sciences, 36, pp.165-17. https://doi.org/10.4025/actascibiolsci.v36i2.21052.

Wang, A. R., Ran, C., Ringø, E., & Zhou, Z. G. 2017. Progress in fish gastrointestinal microbiota research. Reviews in Aquaculture, 10, pp.1–15. https://doi.org/10.1111/raq .12191.

Wilson, J. M., Castro, L. F. C. 2011. Morphology diversity of the gastrointestinal tract in fishes. *In:*Crossel, M., Farrell, A. P., Braner, C. J. Eds.. Fish physiology: the multifunctional gut of fish. London: Elsevier, p.2-56,https://doi.org/10.1016/S1546-50981003001-3.

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