Macro, micromorphological and histological aspects of the intestine pirarucu *Arapaima gigas* (SCHINZ, 1822) (Osteoglossiformes: Arapaimidae)

Aspectos macro e micromorfológicos e histológicos do intestino do pirarucu Arapaima gigas (SCHINZ, 1822) (Osteoglossiformes: Arapaimidae)

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ABSTRACT: Currently, the fish farming with *Arapaima gigas* has suffered from technological obstacles in the fields of reproduction, health and nutrition, making it necessary to know the morphology of its structures, so that it can advance in more technified research in scope of production in Rondônia state, as well as in aquaculture nutrition and health. Therefore, the aimed is to characterize the macro and microscopic morphology of posterior digestive system of *A. gigas*. The intestine of six specimens *A. gigas* in ideal slaughter size was analyzed. The analyzes were performed using light-sheet microscopy (LM) and scanning electron (SEM) techniques. The intestine basically showes similar histological characteristics in three analyzed portions (proximal, middle and distal). Same type of simple columnar epithelium with goblet cells was evidenced, with subtle variations in pattern of villi in each segment, and in number of goblet cells. In the rectum, the amount of goblet cells and evident longitudinal villi was expressive. Macroscopic anatomy and histology of the intestine *A. gigas* analyzed showes characteristics of adaptation to cultivation, according to their diet and habitat. The intestinal mucosa can divided into three distinct portions: proximal, middle and final intestine, in addition to the rectum and anus. In the pyloric cecum, the folds are slightly higher and poorly branched. The rectum, compared to the midgut, showed a higher occurrence of goblet cells in the mucosa. This increase in goblet cells observed in the posterior portion may related to the assimilation of ions and fluids that occur at this location.

KEYWORDS: Animal Anatomy; Carnivorous Fish; Osteoglossiform; Scanning Electron Microscopy.

RESUMO: Atualmente, a piscicultura do *Arapaima gigas* têm sofrido com entraves tecnológicos nos campos de reprodução, saúde e nutrição, fazendo-se necessário conhecer a morfologia de suas estruturas, para que se possa avançar em pesquisas mais tecnificadas no âmbito da produção piscícola no estado de Rondônia, bem como na nutrição e na sanidade aquícola. Por isso, objetiva-se caracterizar a morfologia macro e microscópica do sistema digestivo posterior do *A. gigas*. Foi analisado o intestino de seis espécimes de *A. gigas* em porte de abate. As análises foram realizadas por meio de técnicas de microscopia de luz (ML) e eletrônica de varredura (MEV). O intestino apresenta basicamente características histológicas similares nas três porções analisadas (proximal, médio e distal). Evidenciou-se um mesmo tipo de epitélio colunar simples com células caliciformes, variando o padrão de vilos em cada segmento de forma sutil, e o número de células caliciformes. No reto foi expressiva a quantidade de células caliciformes e as vilos longitudinais evidentes. A anatomia macroscópica e a histologia do intestino de *A. gigas* analisadas apresentam características de adaptação ao cultivo, conforme sua alimentação e habitat. A mucosa intestinal pode ser dividida em três porções distintas: intestino proximal, médio e final, além de reto e ânus. No ceco pilórico, as dobras são ligeiramente mais altas e pouco ramificadas. O reto, comparado ao intestino médio, apresentou maior ocorrência de células caliciformes na mucosa. Esse aumento de células caliciformes observado na porção posterior pode estar relacionado com a assimilação de fons e fluidos que ocorrem neste local.

PALAVRAS-CHAVE: Anatomia Animal; Microscopia Eletrônica de Varredura; Osteoglossiforme; Peixe Carnívoro.

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INTRODUCTION

Pirarucu, *Arapaima gigas* (SCHINZ, 1822), is a carnivorous fish, known as Amazonian cod, because its cultivation is carried out with many zootechnical advantages, such as characteristics such as rustic management, high monthly growth rate, efficient feed conversion, good productive performance, among other advantages (ATENCIO et al., 2010; CAVALI et al., 2022). These characteristics make this fish a noble product in Amazonian aquaculture (REBAZA et al., 2010). The species *A. gigas* stands out in the aquaculture scenario, for having meat with worldwide acceptance, for containing remarkable organoleptic characteristics, with a unique flavor, being tender, and much appreciated for not having a spine, in addition to having great profitability (CALDAS et al., 2018; CAVALI et al., 2021; DANTAS FILHO et al., 2021; SILVA et al., 2022).

In the Amazon region, the species *A. gigas* is considered an important fishing resource, with market value, demonstrates demand both in local and international trade (AMARAL et al., 2011; SILVA et al., 2016; CAVALI et al., 2022). Due to the intense exploitation of natural stocks and the difficulty of monitoring fishing and trade throughout the Amazon region, worrying situations/issues for this species have arisen, such as the reduction of the natural population and almost disappearance in many environments where they were previously found, thus generating, the need to raise it in captivity (NUNES et al., 2012; CAVALI et al., 2021; CAVALI et al., 2022).

Rondônia state is the largest producer of native farmed fish in Brazil, corresponding to a total 59.6 thousand tons of fish produced in year 2021 (PEIXE BR, 2022), and A. gigas is one of the most cultivated fish. Together with tambaqui (Colossoma macropomum), they represent about 85% of the fish farmed in Rondônia state (MEANTE; DÓRIA, 2017). These results were achieved due to the soil and climate conditions, the proximity to a large consumer market, in addition to the high availability of water (GASPAROTTO et al., 2020). In addition, A. gigas meat is an important source of animal protein for traditional Amazonian populations and communities (SILVA et al., 2016; BARRO, 2017). Among the Amazonian species with aquaculture potential, in recent years, A. gigas has become probably the most promising species for the development of intensive fish farming in the Amazon region (LUXINGER et al., 2018). species of freshwater fish, with accelerated growth, reaching 10 to 12 kg in the first year. These productive characteristics and the decline of natural stocks, due to overfishing, make the species A. gigas very attractive for fish farming (REBAZA et al., 2010; CAVALI et al., 2020; GASPAROTTO et al., 2020; CAVALI et al., 2020; CAVALI et al., 2022).

In recent years, fish farming has shown the better results with feed for carnivorous fish in captivity (PEDROSA et al., 2018), with levels of 40 to 45% of crude protein, according to Sebrae (2013), preferably feed composed of at least 40% protein. However, Pedrosa et al. (2018) point out that, in fish nutrition, even after decades of investigations, indispensable information is still lacking to obtain satisfactory productive results. Furthermore, Ribeiro et al. (2012) and Dantas Filho et al. (2021) emphasize that the topic is very relevant in the nutrition of carnivorous fish, and that it meets the need to reduce the cost of production. However, technological obstacles in the fields of reproduction, health and nutrition are significant obstacles to the development of aquaculture (COSTA et al., 2020; CAVALI et al., 2022), making it necessary to know the macro and microscopic morphologies of the structures of the aquaculture. A. gigas, so that we can advance in development of more technified research in the scope of sustainable fish production in Rondônia state, as well as in its nutrition and in aquaculture health.

Given these assumptions, the aimed is to characterize the morphology of the posterior digestive system of the species *A. gigas*, using light-sheet microscopy (LM) and scanning electron microscopy (SEM) techniques.

MATERIAL AND METHODS

This article is derived from a Master's Thesis Research, at Programa de Pós-Graduação em Ciências Ambientais, Universidade Federal de Rondônia (UNIR) (PINTO, 2019). Due to fact that the study was carried out with animals slaughtered by those responsible for a fish processing unit, it was not necessary to submit the research to the Committee on Ethics in the Use of Animals (CEUA/UNIR). However, we attest that bioethical principles were respected.

The intestine of six specimens *A. gigas* in ideal slaughter size, on average of 12 kg, in the processing unit was analyzed. As mentioned above, the fish were donated and slaughtered by a fish processing unit - SIE/RO no. 093, located in the Ariquemes municipality, Rondônia state, Brazil. After collection, the viscera were washed in saline solution and the food content showes inside was removed. In which, it was filled with 10% buffered formalin solution. The collected fragments were processed and analyzed in collaboration with the Programa de Pós-Graduação em Anatomia dos Animais Domésticos e Silvestres, at Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo (FMVZ/USP).

It is important to emphasize, for study of anatomical structures of the intestines, rectum and anus *A. gigas*, the organs were dissected and the mucosa was exposed on a board (SCADENG et al., 2020).

Light-sheet Microscopy (LM) and Scanning Electron Microscopy (SEM)

Collected viscera were cut transversally, dehydrated in a series of ethanols in increasing concentrations (70 to 100%) and diaphanized in xylene, with subsequent inclusion in histological liquid paraffin. Then, microtomized at 4μ m thickness, being stained with Hematoxylin-Eosin (H/E). The images were obtained using the Nikon Eclipse E-800 light-sheet microscopy (LM). A complete description of LM methodology can be found in the research developed by Mangetti (2006) and Rodrigues et al. (2020).

Samples were fixed in 10% formaldehyde, dehydrated in increasing series of alcohols at concentrations of 50, 70, 90 and 100%, dried in a LEICA EM CPD300 critical point apparatus (FMVZ-USP), fixed with carbon glue on metallic bases of aluminum (stub) and metallized (sputting) with gold in the metallizing device EMITECH K550 (FMVZ-USP), being analyzed and photographed in a scanning electron microscopy (SEM) LEO 435VP (FMVZ-USP). The complete description of SEM methodology can be found in the research developed by Mangetti (2006) and Rodrigues et al. (2020).

RESULTS

The intestine *A. gigas* is formed by a muscular tube and, at the beginning of the proximal portion, it is marked by the presence of two pyloric cecum of different lengths. Macroscopically, the mucosal folds of proximal portion of the intestine are predominantly transverse, with simple, high, and numerous folds, compared to the medial portion of the intestine. In the final intestine, the same pattern of folds is observed, as seen in anterior portions of the intestine (Figure 1, I).

In the intestine *A. gigas*, it can observed that the pattern of folds in each segment varies in a subtle way. The mucosa of proximal intestine consists of a simple columnar epithelium with goblet cells, lamina unique, submucosa and two smooth muscle bundles, one in a circular configuration and the other longitudinal. This epithelium projects into the lumen of the intestine forming numerous and long leaf-shaped intestinal villi. From the lumen to serosa, after the mucosa, the mucosal lamina unique delimits the submucosa formed by dense connective tissue, followed by smooth muscle layers, with a circular layer and an outer longitudinal layer (Figure 1, II).

The midgut region *A. gigas* showes slightly larger and more complex villi compared to its anterior portion, the proximal intestine, with simple columnar epithelium and greater visualization of goblet cells (Figure 2, III). A low density of goblet cells was observed in the simple columnar epithelium, lamina unique delimiting the dense connective tissue in the submucosa and a thick smooth muscle layer, surrounded by the serous layer (Figure 2, IV).

Microscopically, it was possible to observe the villi formed by simple columnar epithelium, with a high density of goblet cells with invagination of lamina unique into the villi. This delimits the dense connective tissue in the submucosa and a thick muscular layer allowing great dilation of final portion of the intestine (Figure 2, V).

DISCUSSION

Based on visualization of the intestinal mucosa *A. gigas* (Figures 1 and 2), it is possible to divide the intestinal tract of this species into three distinct portions: proximal, middle and final intestine, in addition to the rectum and anus, as was observed in surubim (*Pseudoplatystoma corruscans*) by Cal (2006). According to Vernier (1990), the intestine of teleosts is divided into two distinct segments: anterior and posterior. This regional differentiation of the teleost intestine is directly linked to the absorption of some substances, in which lipids and proteins are absorbed according to conventional processes in the foregut and proteins are absorbed macromolecularly in the hindgut (CARDOSO, 2013).

In the pyloric cecum, the folds are slightly higher and less branched, corroborating other studies (MARTIN; BLABER, 1984; RODRIGUES; CARGNIN-FERREIRA, 2017; SCADENG et al., 2020). These studies emphasize that the architecture of the intestinal mucosal folds of teleosts becomes increasingly complex according to growth, with a significant increase in muscular thickness of the tunic being also observed (POZZER, 2015; RODRIGUES; CARGNIN-FERREIRA, 2017). Furthermore, according to Stoskopf (1993) and Farago et al. (2020), in teleosts the posterior portion of the intestine is difficult to identify. However, in some cases, the relief of the intestinal mucosa is a little simpler than that of mucosa of the anterior portion. Furthermore, the absence of an ileorectal valve makes it difficult to distinguish between the midgut and the rectum, the latter being distinguished from the former by its flattened shape (RODRIGUES; CARGNIN-FERREIRA, 2017). However, in species A. gigas, the differentiation was possible according to the rectal mucosa, which presents very evident and simple longitudinal folds (SCADENG et al., 2020).

In this study of A. gigas, the rectum, compared to the midgut, showed a higher occurrence of goblet cells in the mucosa. This increase in goblet cells observed in posterior portion may related to the assimilation of ions and fluids that occur at this location, as mentioned by Petrinec et al. (2005), as well as Khanna and Mehrotra (1971) and Alcântara et al. (2018) also suggest that the greater amount of goblet cells may facilitate the elimination of the food bolus. Furthermore, goblet cells secrete mucin (mucus). The greater presence of goblet cells in the rectum helps the elimination of fecal mass and reduces the abrasive damage that drier feces could cause to the epithelium (FARIAS, 2015). Alcantara et al. (2018) and Scadeng et al. (2020), when analyzing the morphology of A. gigas, through advanced analysis of 3D images, found two pyloric ceca in the intestine. While, the intestine passes posteriorly to the left of the esophagus and stomach rather than to the right, as is generally found in other teleosts, the intestine being in a back-and-forth cyclic format, circling four to five times within the coelom before end up in the rectum.

Gastrointestinal tract of species *A. gigas* is demonstrated as a tube that goes from the mouth to anus and through which the food passes. It can subdivided into the oral or oropharyngeal cavity, foregut (esophagus and stomach), midgut (intestine proper) and hindgut (rectum). The various tissues and organs related to it are involved with apprehension, chewing and swallowing, followed by digestion and absorption of nutrients, as well as excretion (ALCÂNTARA et al., 2018). Furthermore, the esophagus connects dorsally to the respiratory bladder through a muscular sphincter. According to Khojasteh (2012) and Watson et al. (2013), the intestine corresponds to 145% of the total length of *A. gigas*, that is, it is larger than would expected for a carnivorous fish (20% of total length) and shorter compared to what is found in herbivores (up to 2,000 % of total length).

It is worth emphasizing that, particularly in *A. gigas*, the intestines rotate back and forth (craniocaudally), but specifically within the coelom (SCADENG et al., 2020), in contrast to carnivorous fish trout (*Oncorhynchus mykiss*), where the intestine is a straight and short tube (KHOJASTEH, 2012). As mentioned above, the intestine *A. gigas* has two pyloric caeca, like most osteoglossid fish (WATSON et al., 2013). The intestine of osteoglossiformes is distinct from other teleosts, in that the intestine passes posteriorly to left



Figure 1. I) Photomacrography of the two pyloric cecum (2) *A. gigas* with unequal lengths and pleated appearance/folded, located between the stomach (1) and the intestine (3). Cross-sectional photomicrograph (H/E) showing a region of few goblet cells (*) of simple columnar epithelium (a), lamina unique (b) delimiting dense connective tissue in the submucosa (c) and a muscular layer (d). (A, B, C) H/E staining light-sheet microscopy – LM (D, E, F) and scanning electron microscopy – SEM; II) Photomacrography of the proximal intestine *A. gigas* with a pleated appearance. Cross-sectional photomicrograph (H/E) showing the mucosa of the simple columnar epithelium (a), goblet cells (*), lamina unique formed by loose connective tissue (b), delimiting the beginning of the submucosa (c) formed by dense connective tissue and circular smooth muscle (d). The epithelium projects into the lumen of the intestine forming numerous and long leaf-shaped intestinal villi (key), lumen of the intestine (L).



Figure 2. III) Photomacrography of the midgut *A. gigas.* It is observed that the pattern of folds in each segment varies in a subtle way, being more complex than the proximal portion of the midgut. Cross-sectional photomicrograph (H/E) showing low density of goblet cells (*) in simple columnar epithelium (a), lamina unique (b) delimiting dense connective tissue in the submucosa (c) and a thick smooth muscle layer (d); IV) Photomacrography of the final intestine *A. gigas* (1) ending in the rectum (2), where it is observed that there is a pattern in folds of anterior portions of the intestine. Cross-sectional photomicrograph (H/E) showing low density of goblet cells (*) in simple columnar epithelium (a), lamina unique (b) delimiting dense connective tissue in the submucosa (c) and a thick smooth muscle layer (d), surrounded by the serous layer (e); V) Photomacrography in V of the rectum *A. gigas* (1) positioned posteriorly to final portion of the intestine (2), where longitudinal folds are observed. Cross-sectional photomicrograph (H/E) of the rectum *A. gigas*, showing a high density of goblet cells (*) in the simple columnar epithelium (a), invagination of the lamina unique (b) into the villi, delimiting the dense connective tissue in the submucosa (c) and a thick smooth muscle layer (d), surrounded by the serous layer (e); V) Photomacrography in V of the rectum *A. gigas* (1) positioned posteriorly to final portion of the intestine (2), where longitudinal folds are observed. Cross-sectional photomicrograph (H/E) of the rectum *A. gigas*, showing a high density of goblet cells (*) in the simple columnar epithelium (a), invagination of the lamina unique (b) into the villi, delimiting the dense connective tissue in the submucosa (c) and a thick muscular layer (d) allowing great dilation.

of the esophagus and stomach (NELSON, 1972), instead of to the right (SCADENG et al., 2020).

CONCLUSION

Morphology of the intestine *A. gigas* analyzed, showes characteristics of adaptation to the cultive, according to its diet and habitat. The intestinal mucosa can divided into three distinct portions: proximal, middle and final intestine, in addition to the rectum and anus. In the pyloric cecum, the folds are slightly higher and poorly branched. The rectum, compared to the midgut, showed a higher occurrence of goblet cells in the mucosa. This increase in goblet cells observed in the posterior portion may related to the assimilation of ions and fluids that occur at this location.

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