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Histological description of digestive tract segments of commercially-produced pirarucu (*Arapaima gigas*)

Descrição histológica de segmentos do tubo digestório do pirarucu (*Arapaima gigas*) proveniente de produção comercial

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Highlights

Muscular layer of the mucosa is not present in the medial segment of the esophagus.
Gastric glands were observed in the mucosa of the stomach body.
Intestinal folds varied along the digestive tract.

Abstract

The aim of this study was to histologically characterize portions of the digestive tract (esophagus, stomach, pyloric caeca and intestine) of 10 juvenile pirarucu (*Arapaima gigas*), with an average weight of 3.45 kg ±0.67, captured at fish farms. Histological processing was performed in line with the methods proposed by the UFPI/CPCE Animal Anatomy Laboratory. The sections were analyzed under a trinocular biological microscope (Nova®). The structural composition of the organs was recorded via photomicrographs, using a five-megapixel digital camera coupled to a microscope, with Toupview® capture software. The muscular layer of the mucosa was not observed in the medial segment of the esophagus. Gastric glands were observed in the medial region of the stomach body, in the lamina propria of the mucosa and tunica muscularis, with considerable thickness. The histological characteristics of the pyloric caeca were similar to those of the first segments of the Pirarucu midgut, with apparent variation in the thickness of the mucosal folds, submucosa and tunica muscularis according to organ diameter. Intestinal fold organization and goblet cell distribution varied along the digestive tract. Histologically,

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the organs of the Pirarucu digestive tract exhibited characteristics similar to those of other carnivorous teleosts.
Key words: Microscopic anatomy. Fish farming. Teleost.

Resumo

O objetivo deste estudo foi caracterizar histologicamente porções do tubo digestório (esôfago, estômago, cecos pilóricos e intestino) de 10 juvenis de pirarucu (*Arapaima gigas*) com o massa média de 3,45 kg ±0,67 capturados em viveiros de piscicultura. O processamento histológico seguiu os métodos de rotina propostos pelo Laboratório de Anatomia Animal- UFPI/ CPCE. Os cortes foram analisados em microscópio biológico trinocular (Nova®). As composições estruturais dos órgãos foram registradas por fotomicrografias em câmera digital de cinco megapixels acoplada ao microscópio com auxílio do software de captura Toupview®. No segmento medial do esôfago não foi observada a camada muscular da mucosa. No estômago observou-se glândulas gástricas na região medial do corpo localizadas na lâmina própria da túnica mucosa e túnica muscular aparentemente com espessura considerável. Os cecos pilóricos apresentaram características histológicas semelhantes aos primeiros segmentos do intestino médio do Pirarucu com aparente variação na espessura das dobras da mucosa e das túnicas submucosa e muscular de acordo com o diâmento do órgão. Foram observadas variações quanto a organização das dobras intestinais e distribuição das células caliciformes ao longo do tubo intestinal. Histologicamente os órgãos do tubo digestório do Pirarucu, apresentaram características semelhantes a outros teleósteos com o hábito alimentar carnívoro.
Palavras-chave: Anatomia microscópica. Piscicultura. Teleósteo.

Introduction

Fish farming is one of the fastest growing sectors of the global animal-derived food industry (Ramalho, 2015). Brazil ranks 13th in fish farming and 8th in freshwater fish farming, while China still leads the ranking in both commercial fishing and aquaculture, with 57.9% of global production in 2018. Global aquaculture is expected to increase by 32% by 2030, especially in Africa and Latin America (Food and Agriculture Organization of the United Nations [FAO], 2020).

In native Brazilian species, data on the morphological and physiological traits of the digestive system are insufficient to support fish farming; however, several studies with different species of neotropical freshwater fish are currently underway in the country (Alves et al., 2021). Space constraints, high stocking density, artificial feeding and rapid growth rates are common characteristics of fish farming that differ drastically from those of natural environments. Little is known about the influence of artificial conditions on the digestive system of farmed fish (Lokka et al., 2013).

In teleosts, as in other vertebrate groups, the histological structure of the digestive system consists of four layers from the lumen outwards, namely the mucosa, submucosa, muscularis (or muscular layer) and serosa. However, there is some debate regarding which tissues belong to the mucosa and submucosa (Lokka et al., 2013).

Arapaima gigas, commonly known as pirarucu, is endemic to the Amazon region and can reach up 200 kg and 3 meters long in nature (Silva & Duncan, 2016). The species is found in Peru, Bolivia, Colombia, Guyana and Brazil. In Brazil, it inhabits the floodplains of the Amazon and Tocantins-Araguaia River basins (Amorim et al., 2020).

Pirarucu is a commercially appealing species because its traits favor fish farming, including rapid growth, reaching up to 10 kg within the first year of production, hardiness in terms of handling, adaptability to artificial feed, high carcass yield (51-57%) and nutritious mild-tasting fillets that can be sold dried or salted (Nunes et al., 2012; Lima et al., 2015; Santos et al., 2021).

According to Rodrigues and Cargnin-Ferreira (2017), one of the main technological obstacles in pirarucu production is the need for suitable feed that meets the nutritional requirements of the species in the different stages of production. Research on pirarucu is incipient and little is known about the morphology of its digestive system (Lima et al., 2015; Rodrigues & Cargnin-Ferreira, 2017).

In light of the above, this study aimed to histologically describe segments of the digestive tract organs of juvenile pirarucu obtained from commercial production facilities, thus providing support for subsequent nutrition research.

Material and Methods

The study was divided into two stages, conducted at different times. The first stage aimed to establish collection sites in the organs of the Pirarucu (*Arapaima gigas*) digestive tract to ensure that the structural composition of the tissue would be segment-specific and not exhibit the characteristics of transitional regions. In the second stage, tissue from the esophagus, stomach, pyloric ceaca and intestine were histologically processed to characterize its structural composition.

The viscera of juvenile pirarucu were donated by a fish farm in the municipality of Xique-Xique in Bahia state (BA), Brazil, in the so-called *Médio São Francisco* region. The study design was completely randomized (CRD), since the fish were randomly captured from the ponds with no interference from the donating facility.

Stage one was carried out in September 2019, using 3 sets of viscera from juvenile pirarucu, and stage two in June 2021 with 10 sets of viscera. The average weight of the fish in the two stages was 3.95 kg ± 0.56 and 3.45 kg ±0.67, respectively. In both stages, capture, measurement, slaughter and evisceration were performed by staff at the fish farm.

In the first stage, the esophagus and stomach were opened longitudinally with a scalpel for macroscopic observation of fold behavior and to determine the medial region of both organs. Histological sections of all the organs analyzed were prepared using disposable steel razor blades. Given the wide diameter of the two organs, a longitudinal section approximately 2 cm long was cut, in accordance with the shape and size of the histology cassettes. The medial region of the pyloric caeca was established by macroscopic observation, considering the length of these structures. Cross-sections measuring approximately 2 cm were cut. In order to observe possible transition regions of the fish intestine, longitudinal sections were cut along the entire digestive tract and re-segmented transversely (± 2 cm) to determine changes in the mucosa that identify these potential transitions.

In the second stage in March 2021, the previously established collection points (Figure 1), longitudinal sections of the esophagus and stomach, and cross-sections of the pyloric caeca were maintained, with the intestinal section modified to transverse.

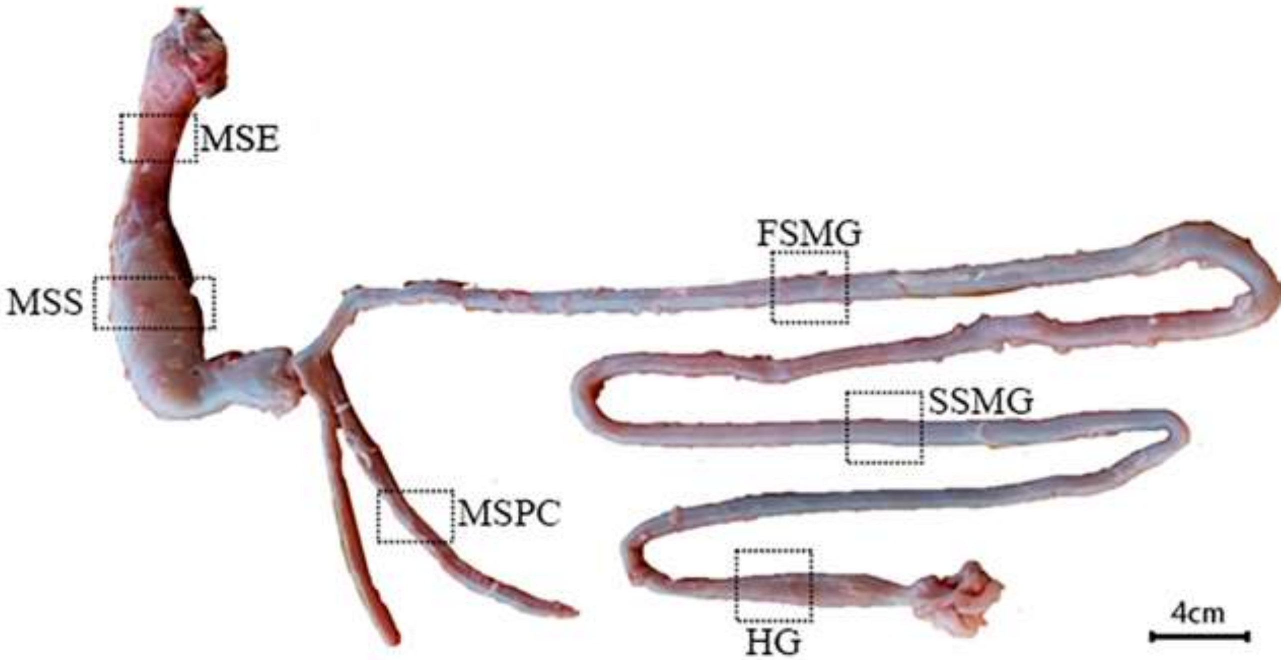

Figure 1. Macroscopic anatomy of the digestive tract of *Arapaima gigas*, highlighting the collection sites for histological processing: MSE – medial segment of the esophagus; MSS – medial segment of the stomach; MSPC – medial segment of the pyloric caeca; FSMG – first segment of the midgut; SSMG – second segment of the midgut and, HG - hindgut. Scale bar: 4 cm.

The transition sites of the organs (stage 1) and structural composition of the tissue (stage 2) were determined at that Animal Anatomy Laboratory on the "Prof.ª Cinobelina Elvas" *Campus* of the Federal University of Piauí (UFPI), in Bom Jesus, Piauí state (PI).

The sections of all the organs were washed with distilled water and individually embedded in duly identified histology cassettes. For cleaving, 2 cm fragments of each segment were placed in glass containers with 10% buffered formalin and fixed for 24 hours. The material was processed according to standard histology techniques via dehydration in increasing alcoholic solutions (50, 70, 80, 90 and 100%), followed by immersion in 100% absolute alcohol, and clarification in xylol, then soaked and embedded in paraffin, remaining in each solution for 60 minutes. The material embedded in paraffin blocks was cut into 3 to 4 μm histological sections in a semiautomatic rotary microtome (Leica ®). The sections obtained were submitted to distension in a water bath at 50° C and mounted on duly identified slides. The slides were stained with hematoxylin and eosin in accordance with Junqueira and Junqueira (1983).

After staining, the sections were analyzed under a trinocular biological microscope (Nova®). The structural composition of tissue from the esophagus, stomach, pyloric caeca and intestine were recorded via photomicrographs, using a five-megapixel digital camera coupled to a microscope, with Toupview® capture software.

Results and Discussion

Esophagus

Macroscopically, the esophagus was a tubular and seemingly short organ, with a considerably larger diameter than that of the intestine, and longitudinal folds along the lumen (Figure 1).

Histologically, irregular dense connective tissue was observed in the segment of the esophagus, making it difficult to distinguish between the lamina propria and submucosa (Figure 2). This was also reported by Rodrigues and Cargnin-Ferreira (2017) for the same species in different size classes, and by Lokka et al. (2013) in the esophagus of *Anablepsoides urophthalmus.* This difficulty in differentiation may be associated with the absence of a muscular layer in the esophageal mucosa. According to Cao and Wang (2009), histologically this muscular layer is responsible for creating a boundary between the mucosa and submucosa, but has been confirmed as absent in most fish species.

Figure 2. Photomicrograph of the medial segment of the esophagus of *Arapaima gigas.* Note the lumen (L) with the mucosa covered in nonkeratinized stratified squamous epithelium (*), dense connective tissue with no distinction between the lamina propria and submucosa (*), tunica muscularis (M) with distinction between the inner (Mi) and outer muscular layers (Mo) and the serosa (S). Scale bar: 200 µm.

The mucosa exhibited non-keratinized stratified squamous epithelium. The tunica muscularis showed apparent development in terms of thickness, with striated skeletal muscle tissue and an inner muscular layer composed of bundles of muscle fibers surrounded by dense connective tissue (Figure 3), and a circular outer layer. The serosa is situated externally to the circular outer layer and consists of loose connective tissue, lymphatic and blood vessels, and nerves. According to Moraes and Almeida (2014), the muscles of the circular outer layer of the esophagus are responsible for constricting the tube while the internal longitudinal layer expands the lumen, with the combined action of these two layers generating the peristalsis that moves food along the esophagus. Considering the carnivorous feeding habits of pirarucu, the striated fibers present in the esophageal muscles enable the voluntary regurgitation of large and rigid food items that might damage the wall of the tube (Moraes & Almeida, 2014).

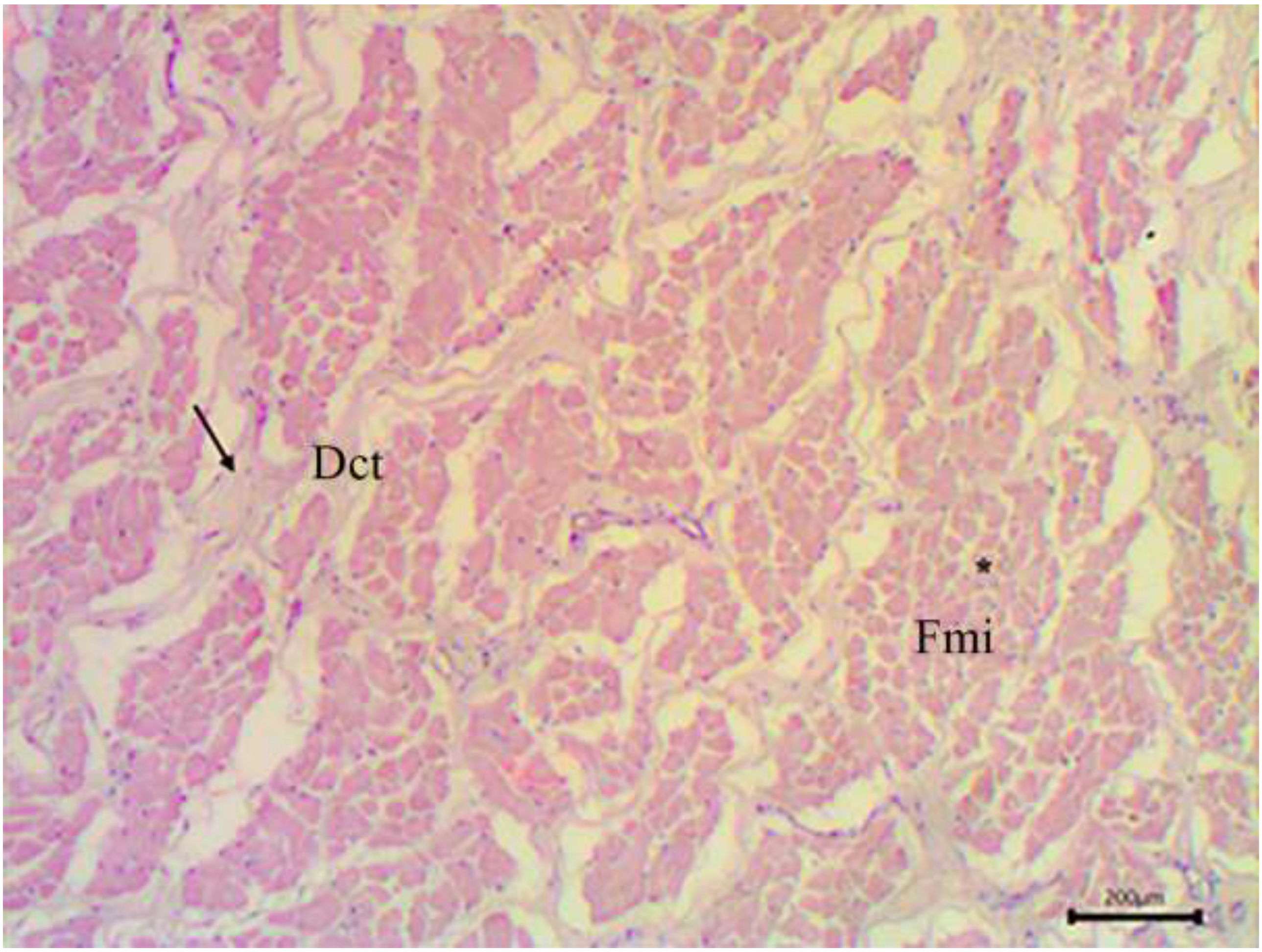

Figure 3. Photomicrograph of the inner muscular layer of the medial segment of the *Arapaima gigas* esophagus, showing bundles of muscle fibers (Fmi*) surrounded by dense connective tissue (Tcd). Scale bar: 200 µm.

Stomach

Macroscopically, the stomach is J-shaped (Figure 1), with a transition region from the esophagus to the stomach (cardia), and a proximal (body) and distal region (pylorus), both composed of longitudinal folds.

Histologically, the mucosa of the medial region of the body shows simple tubular gastric glands in the lamina propria, with simple columnar epithelial tissue (Figure 4). Ghosh and Chakrabarti (2015) found that the location and clustering of gastric glands may be directly related to the feeding habits of the species. For example, predatory species tend to have more glands in the cardiac region as a function of the rapid supply of digestive enzymes important in digesting prey swallowed live. This was evident in the species studied, considering the shape of the organ.

In *Hemisorubim platyrhynchos* and *Lates calcarifer,* fish with the same carnivorous feeding habit as pirarucu, gastric glands were observed in the cardia and fundus of the stomach, but not in the pylorus (Faccioli et al., 2014; Purushothaman et al., 2016). This was also observed by Germano et al. (2014) in an omnivorous fish species (*Pterodoras granulosus*). The authors attributed the larger concentration of these glands in the cardia and fundus to greater secretion demands due to the protein-rich foods consumed by the species.

In the submucosa, loose connective tissue with considerable thickness was identified in the smooth tunica muscularis. Subsequently, a muscular layer consisting of smooth internal oblique and circular external muscles was observed (Figure 4). According to Lokka et al. (2013), a thick gastric wall with strong muscles is important in mechanical food digestion, which is relevant given the carnivorous eating habit of pirarucu.

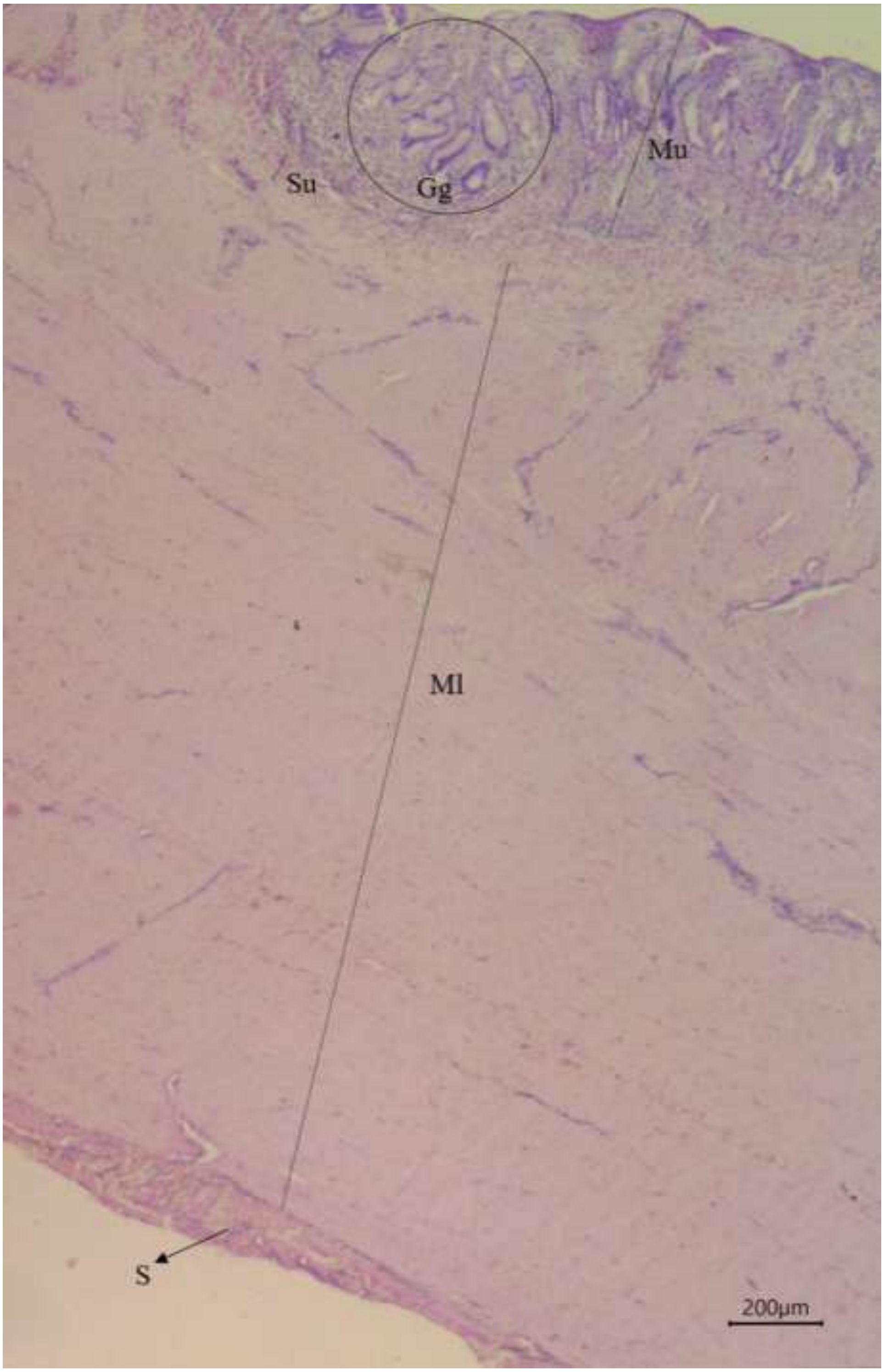

Figure 4. Photomicrograph of a longitudinal section of the medial region of the stomach body of *Arapaima gigas.* Mucosa (Mu) with the presence of tubular gastric glands (Gg) indicated by the dotted circle, submucosa (Su) formed by loose connective tissue, smooth muscular layer (Ml) and serosa (S). Scale bar: 200 μm.

Pyloric caeca and intestine

The intestine of the species studied is short, with seemingly the same diameter along the entire length of the intestinal tube (Figure 1). Two pyloric caeca with different diameters were observed in the region anterior to the midgut. The possible transition between the different regions of the intestine occurred through the behavior of the intestinal folds and goblet cell distribution on the simple cylindrical epithelium, forming absorptive cells known as enterocytes (Figure 5). There was no ileorectal valve marking the fore and hindgut regions in this species.

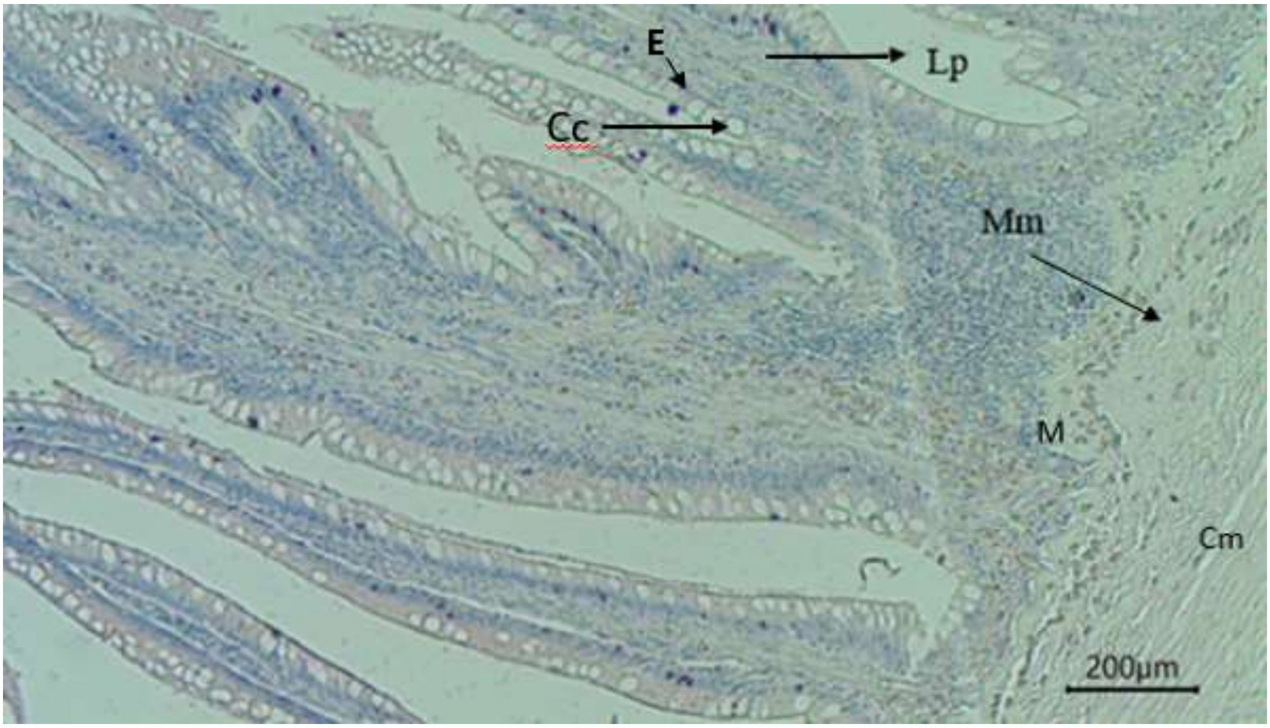

Figure 5. Photomicrograph of a cross-section of the medial segment of the midgut of *Arapaima gigas* showing the mucosa. Note the muscular layer of the mucosa (Mm), the lamina propria (Lp), goblet cells (Cc), enterocytes of the intestinal folds (E), muscles of the submucosa (M), and inner muscular layer (Cm). Scale bar: 200 µm.

The mucosa, submucosa, muscularis and serosa were observed in the cross-section of all the segments (first and second segments of the mid and hindgut), while the middle segment of the pyloric caeca exhibited similar characteristics to those of the first and second segments of the intestine.

Simple folds were noted in the middle portion of the pyloric caeca, which appear to be thinner than the folds in the midgut. In terms of thickness, this same characteristic was observed in the lamina propria of the mucosa, the submucosa and smooth muscular layer (Figure 6). Cao and Wang (2009) reported that, in some teleost species, the foregut is considered the main nutrient absorption site and as such, intestinal folds are important in expanding the absorption surface area. On the other hand, the smaller and less complex folds in the hindgut may be associated with the storage and concentration of the food bolus via water and ion absorption (Faccioli et al., 2014).

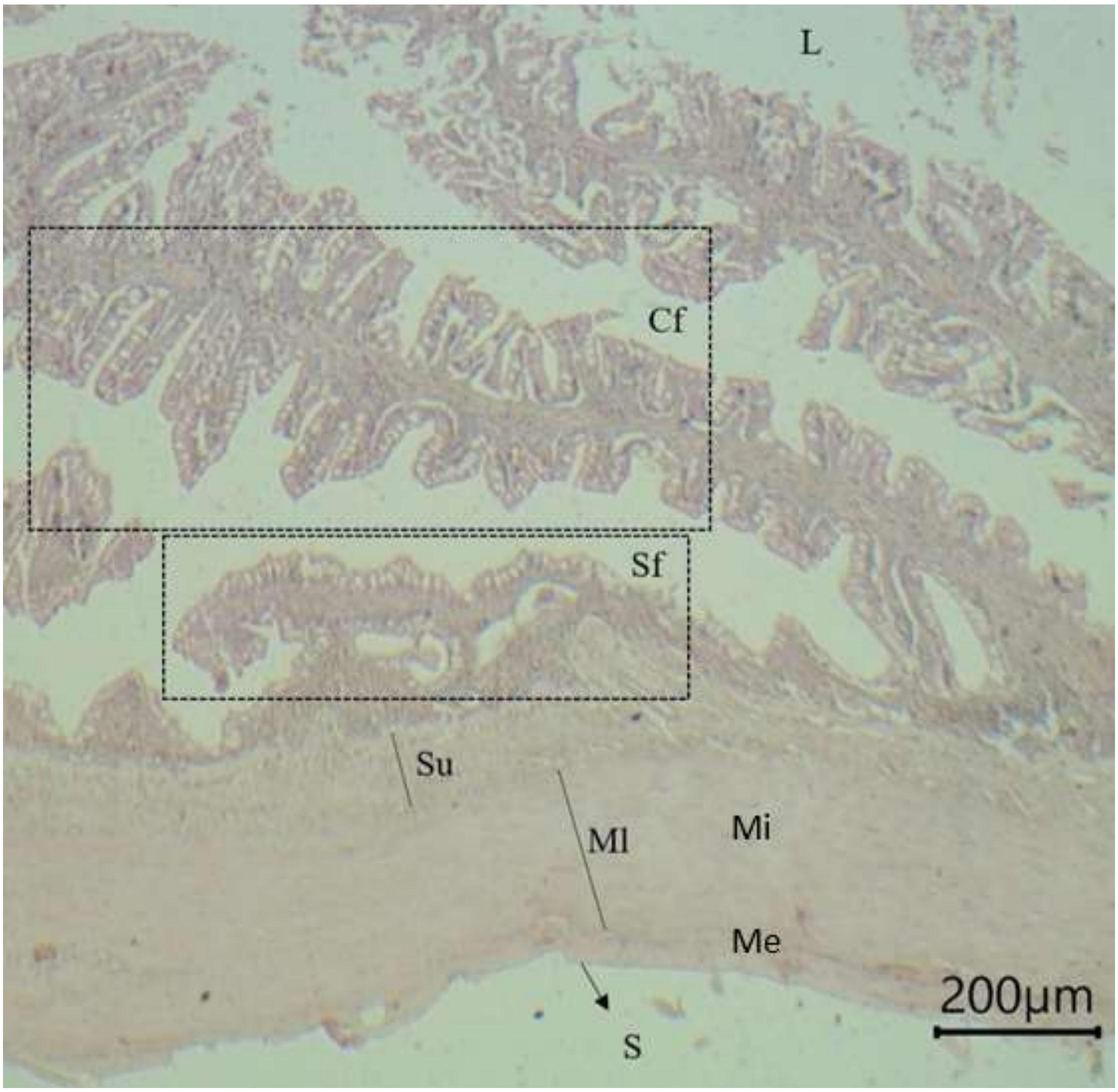

Figure 6. Photomicrograph of a cross-section of the medial segment of the midgut of *Arapaima gigas.* Lumen (L), mucosa (M) with simple (Sf) and complex folds (Cf); submucosa (Su); smooth muscular layer (Ml); inner circular (Mi) and external longitudinal layers (Me); and the serosa (S). Scale bar: 200 µm.

Conclusion

Histologically, the organs of the Pirarucu digestive tract exhibit characteristics similar to those of other carnivorous teleosts. Intestinal fold organization and goblet cell distribution made it possible to infer the likely different regions of the intestine in this species. This basic information can be used in future nutrition for the species aimed at achieving more efficient feeding management in commercial production.

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