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Original Research

Histological and histochemical characterization of the digestive tract of European catfish (*Silurus glanis* Linnaeus, 1758)

S. Köprücü[1*], M. Yaman[2]

[1] Fisheries Faculty, Firat University, Elazig 23119, Turkey
[2] Veterinary Faculty, Firat University, Elazig 23119, Turkey

Abstract: In this study was to examined the morphology, histology and histochemically the digestive tract of European catfish, *Silurus glanis*. Two-four age *S. glanis* obtained from Çelik Lake (Gölbaşı-Adıyaman, Turkey). Six *S. glanis*, 59-71 cm length, 1.5-3.2 kg weight used for in the investigation. The body cavity was opened and samples of digestive tract (esophagus, stomach and intestine) were fixed in neutral buffered 10% formalin and embedded in paraffin. Dewaxed section (5-6 µm) were deparaffinized and stained with haematoxylin and eosin (H&E), Crossman triple, periodic acid-shiff (PAS), alcian blue (AB, pH 1.0 and 2.5) and PAS+AB pH 2.5 tecnique for different structures. The histological and histochemical structures of all specimens were viewed under a light microscope and microphotograph. The histological structure consists of four layer: tunica mucosa, submucosa, tunica muscularis and tunica serosa. The short esophagus of *S. glanis* have numerous deep longitudinal folds, squamous epithelium with numerous mucous cells which react positively to PAS and AB stains. The muscularis mucosa was organized in longitudinal and circular layers of striated muscular fibers. The stomach is sac-like shaped and its mucosa was formed by simple columnar epithelium with folds. Histologically, the stomach shows three different region: cardia, fundus and pylorus region. The surface epithelium of stomach reacted positively to PAS and AB (pH 1.0 and 2.5). The mucosal surface of intestine of *S. glanis* has numerous folds lined by simple tall columnar cells. There were large number goblet cells in the intestine. The goblet cells reacted to PAS and AB (pH 1.0 and 2.5).

Key words: *Silurus glanis*, histology, histochemical, digestive tract.

Introduction

Fish digestive tracts show remarkable differences in morphology and function. Differences observed at specific levels are related to the food, feeding habits, age, body weight, shape and sex (1-6).

The histology of fish digestive tract has been studied with light and electron microscope in numerous fish species. Generally, the basic histological structures are similar: wall of the digestive tract of many fish is composed of mucosa, submucosa, muscularis and serosa (7-17). The presence of mucosubstances in mucosa of the digestive tract has been observed in most teleosts (18-23).

The mucin layer of wall of digestive tract has various functions such as lubrication, digestion, absorption, protect the tunica mucosa against chemicals, parasites, control of the infectious diseases and colonization of the harmful or opportunistic microorganisms (24-28). Histochemical characterizations of mucins secreted by mucous cells in fish digestive tracts vary among different species, age and regions of the tract (29-32).

Silurus glanis is the largest bodied freshwater fish of Europe. Silurus is the only existing genus in Europe of the Siluridae family. It has a triangular-shaped head with small eyes and a large mouth, with two very long, slender. Pigmentation is generally darkalong its back with marbled sides, with a greyishwhite. The skin is scale-less, coated in mucus, contains sensory cells (33). *S. glanis* is an economically important species in commercial (34).

Presently, dozens of research projects have been focusing on the geographical distribution, environmental biology, parasitosis and nutriology of this species (33), feeding behavior (35), embryonic development (36), mucosubstances of the digestive tract (31). However, the information about histology features and mucin histochemistry of its digestive tract has been poorly understood.

The purpose of the present study was to describe histology and histochemically of the digestive tract on the light microscopic level of carnivorous fish species, *S. glanis*.

Materials and Methods

In this study, *S. glanis* obtained from Çelik Lake (Gölbaşı-Adıyaman, Turkey). Six adult *S. glanis* (Length: 59-71 cm, Weight: 1.5-3.2 kg, 2-4 age) were used for in the investigation. Six fish were killed after being anaesthetized in MS-222. The body cavity was opened and samples of digestive tract (esophagus, stomach and intestine) were fixed in neutral buffered 10% formalin and emmedded in paraffin. Dewaxed section (5-6 µm) were deparaffinized and stained for general morphological purpose with haematoxylin and eosin (H&E) (37), Crossman triple stains (38). Periodic acid-shiff (PAS) was used for demonstration of neutral mucosubatans. Alcian blue (AB) at pH 2.5 and pH 1.0 and were used for demonstration of various kinds of acid

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*** Corresponding author:** S. Köprücü, Fisheries Faculty, Firat University, Elazig 23119, Turkey. Email: skoprucu@firat.edu.tr

mucosubstans (37). Microphotography were taken with an light microscope.

Results

The digestive tract of *S. glanis* is composed of the esophagus, stomach and intestine. The short esophagus was followed by a sac-shaped stomach. The intestine had same thickness throughout and convoluted structure. Histologically, the wall of the digestive tract was made up of the mucosa, submucosa, tunica muscularis and tunica serosa.

The esophagus of *S. glanis* was found to have numerous deep longitudinal folds; it was lined by a few layers of stratified squamous epithelium with the numerous superficial mucous cells (Figure 1). All mucous cells show a moderate to high content of PAS-positive stained because of the neutral mucosubstances. But only some of them were positively stained by AB due to the acidic mucosubstance. The lamina propria was formed by the connective tissue but the lamina muscularis mucosa was not present. The muscularis was composed of two striated muscular layers, a thick inner circular and thinner outer longitudinal layer. The serosa consist of the mesothelial cells, small blood vessels, blood cells and loose connective tissue.

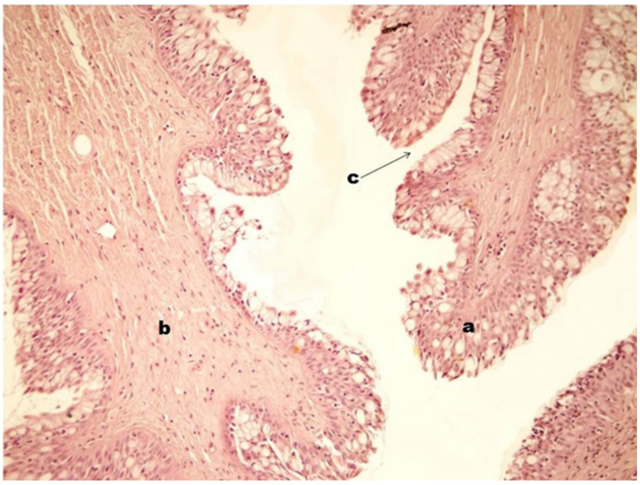

Figure 1. Esophagus; mucus cells (a), lamina propria (b), gastric pit (c) (H&E, x200).

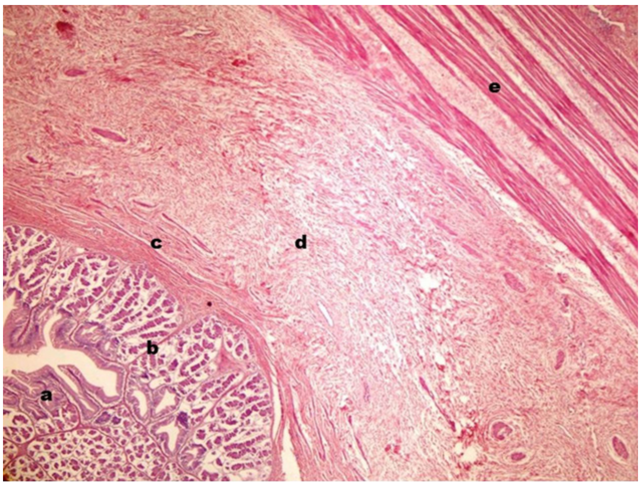

Figure 2. Cardiac stomach; tunica mucosa (a), gastric glands (b), lamina propria (c), submucosa (d), tunica muscularis (e), (H&E, x40).

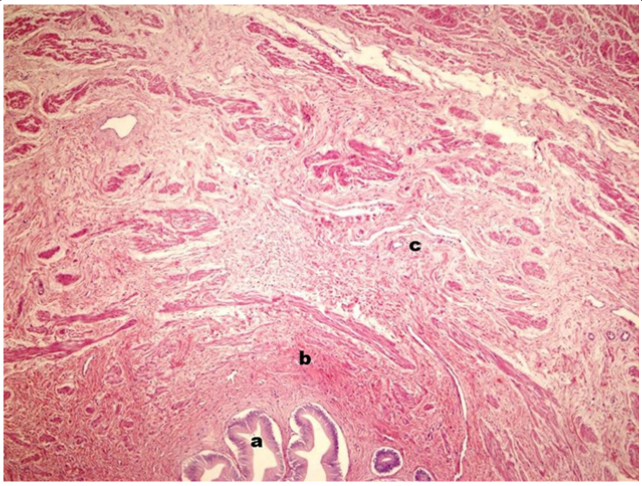

Figure 3. Piloric stomach; tunica mucosa (a), lamina propria (b), submucosa (c), (H&E, x100).

The stomach of *S. glanis* included the cardiac, fundus and pyloric region. The mucosa of the cardiac, fundus and pyloric were formed with the longitudinal folds that were shallow in the fundus. There were numerous gastric pits of the stomach formed by the invaginations of the mucosal layer into the lamina propria. The epithelium of mucosa was consisted of a single layer of the columnar cells and mucus cells. In the cardiac and fundic stomach, a plenty of gastric glands were observed between the epithelium and lamina propria (Figure 2), whereas in the pyloric stomach the epithelium connected the lamina propria directly and no glands existed (Figure 3). The gastric glands were branched tubular and surrounded by a layer of the connective tissue and opened into the bottom of gastric pits (Figure 4). The glands consisted of a single layer of cells. Mucus cells and the apical cytoplasm of the epithelial cells shows the presence of mucous substances stained strongly with PAS (Figure 5) and AB (pH 1.0 and 2.5). In the staining of PAS+AB pH 2.5 reacted positively to PAS in the apical portion of the cytoplasm and positively to AB in the mucus cells. A small amount of PAS-positively stained substances is observed in the lamina propria. The submucosa is a thin layer having a very vascularizated and dense connective tissue. The muscularis was made up of two layers, an inner circular and an outer longitudinal layer of the smooth muscle. Nerve plexus was present between these two muscular layers. The

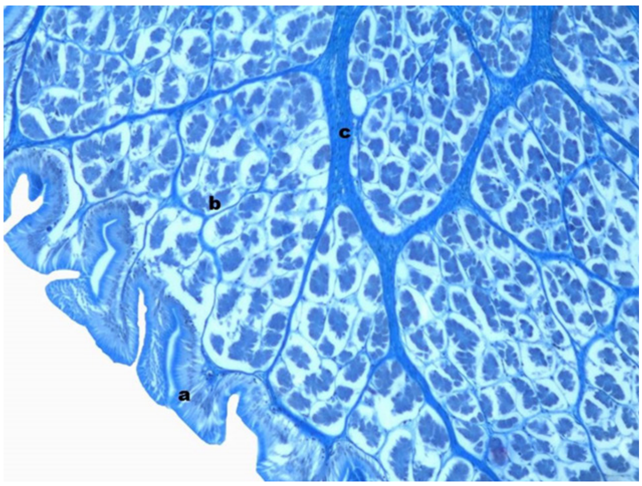

Figure 4. Stomach; columnar epitel cells (a), gastric glands (b), connective tissue (c), (Crossman, x200).

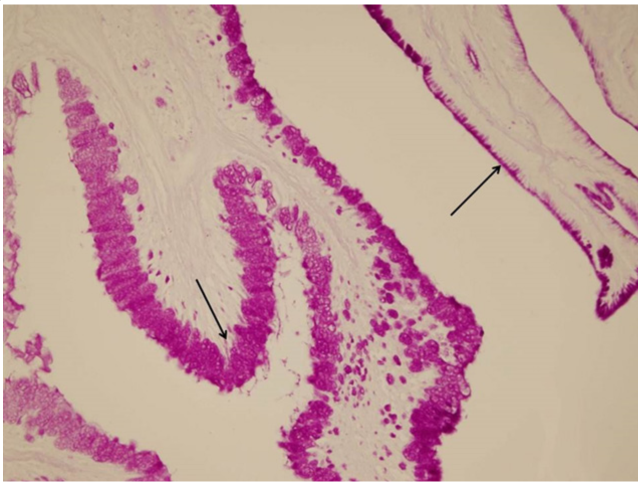

Figure 5. Stomach; PAS positive mucus cells and apical region (PAS, X200).

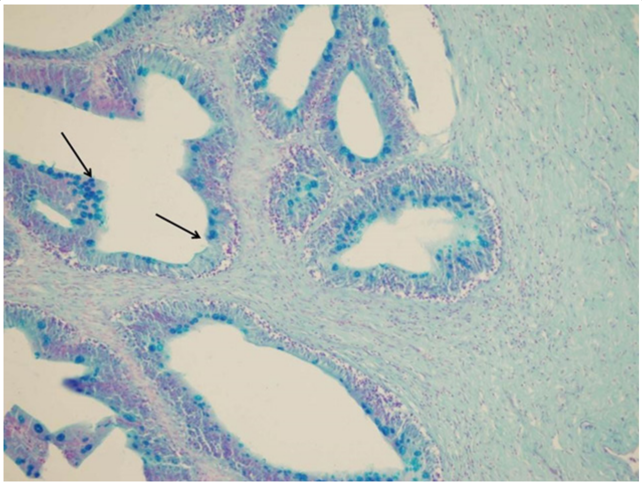

Figure 7. Intestine; AB positive goblet cells (ABX200).

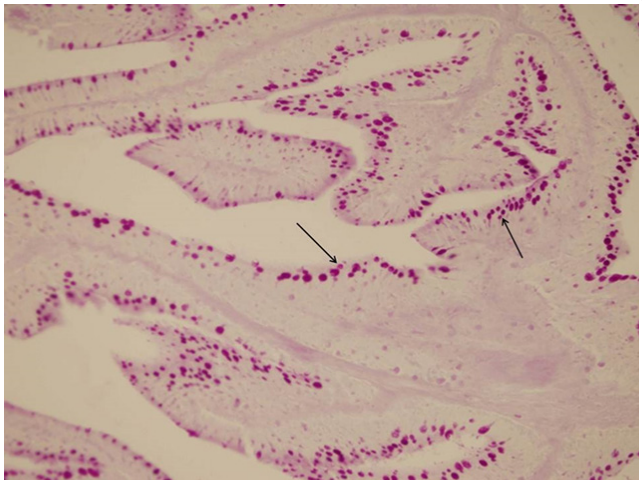

Figure 8. Intestine; PAS positive mucus cells (PASX200).

pyloric stomach had a thickest muscularis among all the parts of digestive tract. The serosa is composed by the connective tissue, mesothelium and blood vessels.

There was no pyloric caeca observed between the stomach and anterior intestine. The intestine is short and its mucosa has an almost uniform structure throughout its entire length. The mucosal surface in the intestine had numerous elongated and deep folds lined. This intestine is lined with simple columnar epithelium which is bordered by a layer of microvilli on their apical surface. The large numbers mucus-secreting goblet cells were found between the epithelial cells as well as occasional leucocytes and macrophages could be seen in the mucosa (Figure 6). The lamina propria-submucosa was formed by loose connective tissues with the many vessels and blood cells. The goblet cell contents stain intensively with AB (pH 1.0 and 2.5) (Figure 7) and they are also PAS-positive (Figure 8). The apical surface of the epithelial cells has a thin PAS-positive and AB (pH 1.0 and 2.5) brush border. The tunica muscularis was organized in two distinct layers of the smooth muscle; the inner circular and outer longitudinal layers. Between both layers can be observed neuronal mienteric plexus.

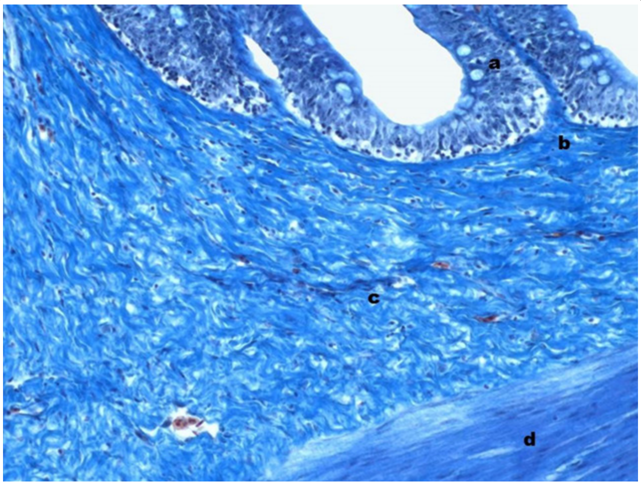

Figure 6. Intestine; tunica mucosa (a), lamina propria (b), submucosa (c), tunica muscularis (d), (Crossman, x200).

Discussion

As described in many other fishes (9, 10, 12-14, 16, 17, 39, 40) the wall of digestive tract in the *S. glanis* was divided into four layers.

The anatomy of the esophagus of *S. glanis* resembles that of the other teleosts (7, 9, 14, 17, 32, 40). The mucosal folds of the esophagus described by Cataldi et al. (7), Diaz et al. (16) and and Hernandez et al. (32) were similar to the esophagus of *S. glanis*. The present study revealed that the esophageal epithelium is stratified squamous with the mucous cells. The mucous cells were mainly PAS positive, indicating that their contents are of the neutral mucosubstans, although some contain AB positive, indicating their contents of acidic mocosubstans. This secretion is similar to the *Acipenser transmontanus* (20), *Rhamdia quelen* (32), *S. glanis* (31, 36), *Tilapia spilurus* and *Mylio cuvieri* (41). Lamina muscularis mucosa were not observed in the wall of the esophagus in the *S. glanis*, which is similar to the *Claris batrachus*, *Serrasalmus nattereri* (40) and *Polyodont spathula* (42). The muscle layers of *S. glanis* had the outer circular and inner longitudinal layers. It was the existence of a thick striated muscle fibril. These result were similar to that described by Simsek and Sarieyyupoglu (9), Suiçmez and Ulus (14), Diaz et al. (16), Hernandez et al. (32), Raji and Norouzi (40).

Generally, the stomach of fish is divided into categories depending on their shape; no stomach, straight with an enlarged lumen, U-shaped, Y-shaped and sac-shaped. The stomach is absent in many fish, including *Cryprinids*, where straight stomachs are rare, and seen in the carnivorous fishes, including in the pike, channel catfish and halibut, while *salmonide* are an example of fish with a U-shaped stomach (40). The present study revealed that the sac-shaped stomach consist of three regions; the cardiac, fundus and pylorus, which was similar to *Orthrias angorae* (14), *R. quelen* (32), *Pelteobagrus fulvidraco* (39) and *Oreochromis niloticus* (43). The primary and secondary folds of the stomach, described by Cataldi et al. (7), Simsek and Sarieyyupoglu (9) and Grau et al. (29) were similar to the stomach of *S. glanis*.

The gastric glands in *S. glanis* were well-developed in the cardiac and fundic stomach, and absent in the pyloric stomach. The gastric glands consisted of mucous cells are arranged as tubular branched glands surrounded by connective tissue. Mucus cells and the apical borders of the epithelial cells in *S. glanis* shows the presence of mucous substances stained strongly with PAS and AB (pH 1.0 and 2.5). In the staining of PAS+AB pH 2.5 reacted positively to PAS in the apical portion of the epithelial cell and positively to AB in the mucus cells. The acid mucosubstances are shown to be chiefly of the carboxylate and sialylated type. In fish, neutral mucosubstances secreted by the stomach epithelium have been related to the absorption of easily digested molecules, such as disaccharide and short-chain fatty acids (29). The mucosubstances facilitate the movement of large-sized food particles, as well as protecting the gastric mucosa from mechanical injury, chemicals, parasites, control infectious disease (24-28, 31). The presence of both neutral and acid mucosubstances was observed in the superficial gastric epithelium of the *A. transmontanus* (20), *R. quelen* (32), *P. fulvidraco* (39) and in developing larvae and adults of the *Sparus aurata* (19).

There was no pyloric caeca observed between the stomach and anterior intestine of *S. glanis* which is similar to the *P. fulvidraco* (39), *C. batrachus* (40).

In many fish as the *Oncorhynchus mykiss* (9, 17) and *Chanos chanos* (44) the submucosa presents an stratum compactum. That structure were not observed in the present study on the *S. glanis,* which agree with previous studies realized by Hernandez et al. (32) in the *R. quelen,* Sis et al. (45) in the *Ictalurus punctatus* and Pignalberi et al. (46) in the *Pimelodus albicans.* According to the results obtained from present study, the folds in the epithelium of intestine were abundant, the intestine of *S. glanis* contains numerous goblet cells in the mucosa which react positively to PAS and AB (pH 1.0 and 2.5). The presence of mucous-producing goblet cells in the intestinal mucosa has been reported in many fish studied earlier (9, 17-20, 32, 39, 47). The mucous secreted by goblet cells in the intestine has many functions. For example, it lubricates undigested materials for onward progression into the rectum. In addition, It has got a possible role in osmoregulation (31). The studies of Ribelles et al. (48) have shown that the quality of gut mucosubstances is directly related to environmental conditions, which in turn may directly affect the function of the alimentary tract. The presence of mucosubstances, especially those sulfated in the intestine, possibly regulate the transfer of proteins, or a fragment of them, as well as of ions and fluids (19, 24, 31). Like many other fishes (17, 32, 39, 47) a large number of microvilli and blood vessels were observed in the intestine of *S. glanis*.

This investigation, as well as previous investigations on the other fishes, demonstrates that the quality of gut mucosubstances varies in the different regions of alimentary canals of the *S. glanis*. It appears that histological structure and the quality of mucosubstances in particular parts of the digestive tract is similar to the investigated carnivorous fish. That results can offer a baseline for the future detailed immunohistochemical studies in the *S. glanis*.

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