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Article:

Histological investigation of the anterior intestine of koi fish (*Cyprinus rubrofuscus*)

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Abstract

The fish's gastrointestinal system is considered the primary site of digestion and absorption of nutrients and has an important role in fish immunology. For that, the present investigation aimed to highlight the histology, histochemistry, and surface architecture of the anterior intestine of koi fish. In the current study, the morphological characteristics of the gut were observed in fifteen Koi fish specimens. The epithelium of the anterior intestine was composed of lymphocytes, goblet cells, enterocytes (simple columnar epithelium), and enteroendocrine cells. The enterocytes had a large number of large vesicles, which may be indicative of pinocytotic activity towards certain nutrients. Apart from their positive reaction to toluidine blue, the goblet cells also showed good reactions to PAS and alcian blue. Collagen and elastic fibers comprised the submucosa and lamina propria. In conclusion, the length and height of the mucosal folds of the anterior intestine serve as a substitute stomach, temporarily storing any food consumed.

Keywords: Anterior intestine, Koi, Histochemical analysis**,** Enteroendocrine cells.

Introduction

Koi fish is the ornamental type of domesticated carp known as Nishikigoi, often known as (*Cyprinus rubrofuscus*), is well-known around the world and a highly valuable member of the Cyprinidae family [1]. This kind of household is found in nature in North America, Asia, Europe, and Africa, but not in South America, Australia, or New Zealand [2]. With the exception of their scaleless heads, the entire body of koi carp is covered in enormous, colorful cycloid scales [3].Ion, water, and nutrient transportation and absorption occur in the fish gastrointestinal system, which serves as the primary site of digestion and absorption. Furthermore, the intestinal mucosal system appears to be a target for several illnesses in farmed fish and plays a crucial role in fish immunology [4]. Fish have the ability to quickly and reversibly modify the features of their gastrointestinal tract (GIT) to accommodate changes in their functional needs as they grow [5]**.** Many fish species' intestines are typically made up of mucosa, submucosa, muscularis, and serosa [6]**.** According to the findings of earlier research, age, body type, weight, and eating habits all have an impact on the histological structures of fish's intestines [7, 8]. The precise demarcation of the intestinal segments in fish aids in the advancement of research on a variety of intestinal activities. The current study aimed to investigate the histological structure of the anterior intestine of koi fish *(Cyprinus rubrofuscus*).

Materials and Methods

Ethical considerations

The current study was approved by the Veterinary Medical Research Ethics Committee, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt, according to the OIE standards for use of animals in research with number **Soh.un.vet /00065R**

Source of samples

The samples selected in this study were randomly obtained from 15 adult koi fish (Cyprinus rubrofuscus), that were

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were brought to the laboratory. Their average weight was about 607.3 ± 23.6gram, total length was 37.2 ± 0.5 cm, standard length was 28.7 ± 0.5 cm, forked length was 34.2 ± 0.6 cm and GIT length was 39.5 ± 0.9 cm.

Samples preparations

As soon as possible, the front section of the koi fish's intestine was dissected for histological analysis by a middle abdominal cavity incision. The thickness of the wall, the length of the mucosal folds, and the muscularis were used to divide the intestine of koi fish into three sections: anterior, middle, and posterior. Following a 1 × 1 × 0.05 cm dissection, every sample was instantly preserved for 22 hours in Bouin's solution. The fixed samples were washed by alcohol, then were dehydrated in a series of ethanol (Chem-Labs Supplies), followed by xylene (El Nasr Pharmaceutical chemicals) clearing and paraffin wax embedding. For a general histological investigation, transverse and longitudinal paraffin cuts with a thickness of 5-8 µm were cut using a Richert Leica RM 2125 Microtome, Germany, in the Histology Department, Faculty of Veterinary Medicine, Sohag University.

Histological and Histochemical analysis

Hematoxylin (Technopharma Pharmacy "Indian") and eosin were the staining methods used on paraffin sections for general structure [9]. In carbohydrate histochemistry, slices are stained with Alcian blue (SUVCHEM Laboratory Chemicals) (PH2.5) [10] for acidic mucin detection and Periodic Acid-Schiff (PAS) [11] for neutral mucins. Additionally, sections were stained for the distinction of muscle fibers and connective tissue using Crossmon's trichrome [12]. Crossmon's trichrome for fibrous collagen to distinguish smooth muscle cells from connective tissue and show that fibroblasts are present, utilize the Crossman's trichrome dye approach [13]. Sections were stained with bromophenol blue to show the protein contents [14]. To show the intestinal enteroendocrine cells and rodlet cells, sections were treated with silver nitrate stain [15]. Stained sections were examined using an OPTIKA B-293 microscope (OPTICA S.r.l., Ponteranica (BG), Italy), and digital images were acquired by OPTICA C-B10 camera and OPTICA PRO view software, in the histology department, Faculty of Veterinary Medicine, Sohag University.

Results

Histological analysis

Light microscopic analysis indicates that the tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa comprise the koi fish's anterior gut wall Figure 1.A. The tunica mucosa had a mean thickness of 3109.8 ± 53.9 µm. The average mucosal fold width measured was 261.1 ± 17.1 µm. It was made up of enterocytes, which are the basic columnar epithelium, and had numerous isolated goblet cells. Goblet cells were oval in shape, slightly deeper in location, and connected to the surface by a neck and microscopic aperture Figure 1.B. Thick inner circular and thin outside longitudinal smooth muscle fibers made up the tunica muscularis, which was followed by the thin tunica serosa Figure 1.A.

Histochemical analysis

Numerous goblet cells formed a continuous sheet and responded strongly and positively to both Alcian blue Figure 1.C and PAS Figure 1.D. Between the columnar cells were spindle-shaped enteroendocrine cells that responded positively to Grimelius stain. The majority of these cells extended to what may be an open-type lumen Figure 2.A. Telocytes and rodlet cells in the lamina propria and submucosa can be seen with the use of bromophenol blue Figure 2.B.

Discussion

Herbivorous animals had high relative gut length (RGL)values, omnivorous species had intermediate values, while carnivorous species had low values, according to Albrecht MP et al. [16]. Conveniently, our study revealed koi fish with a relatively high RGL. The lengthy, wavy mucosal folds were the most significant characteristics of the koi fish anterior intestine in the current study. Because of the lumen's large diameter, food may be able to be stored for longer periods of time to finish the process of digestion and absorption [17]. The type of food consumed affects how many mucosal folds an individual has [18, 19]. Our findings showed that the anterior intestine of koi fish has a high height of mucosal folds. These findings are consistent with those found by Unal and Mokhtar [20, 21] in Cyprinidae and may lead to an increase in the retention time of food and, consequently, an increase in the digestion of substances by pancreatic juices and muco-substances of goblet cells. Koi fish has neutral muco-substances and a large number of microvilli on their enterocyte surfaces, which may be connected to the absorptive function. The absorptive function of the surface epithelium is suggested by the increases in epithelium height. Fatty acid absorption occurs in the enterocytes of the koi fish's anterior section of the gut [22]. According to the current study, the anterior intestine contained oval-shaped enteroendocrine cells that stained positively for Grimelius; the majority of these cells entered the lumen.

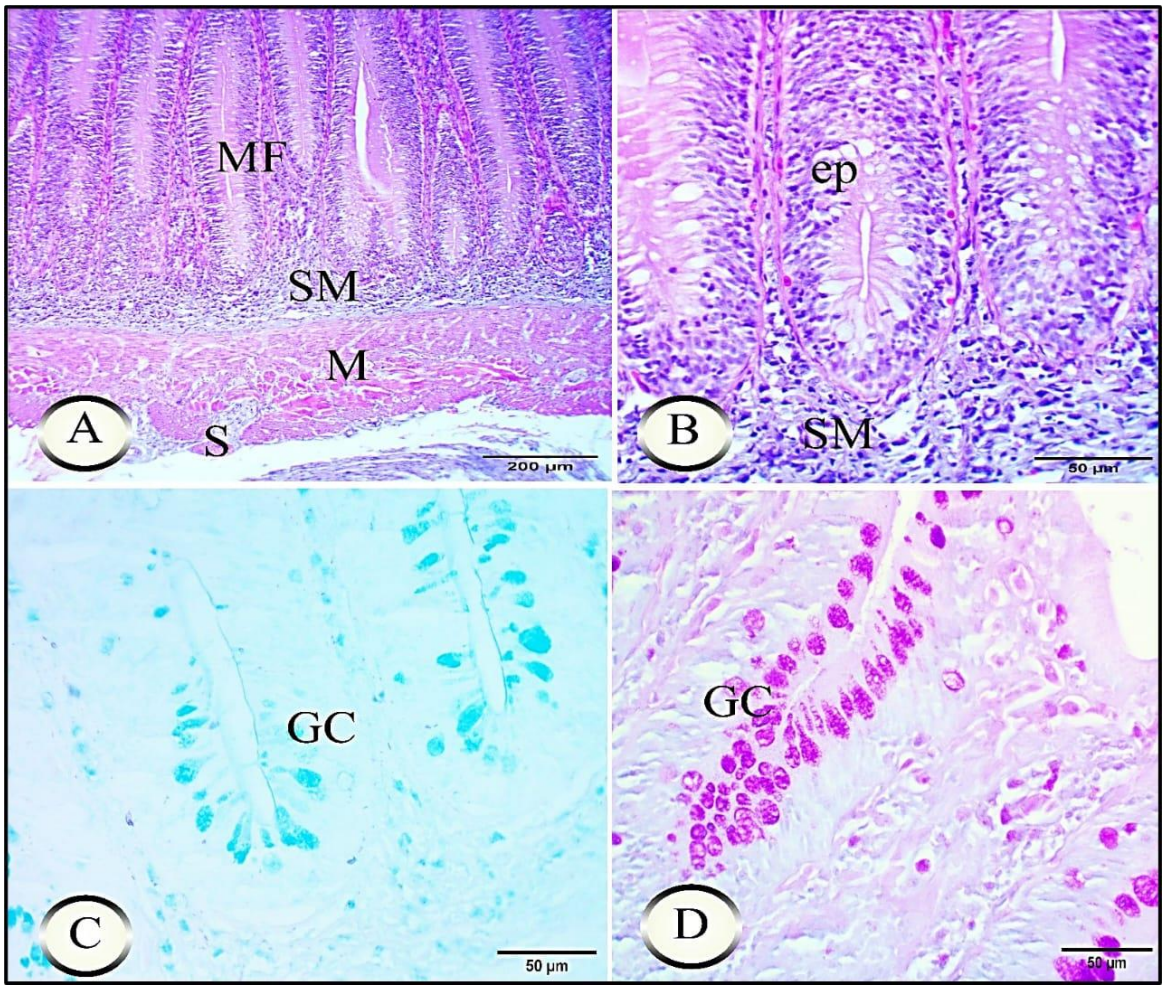

Figure 1: Histological structure of the anterior intestine of koi fish: **A.** Figure showing the wall of the anterior intestine consisted of folded mucosa which consisted of mucosal folds (MF) and lamina-propria filled the core of MFs, submucosa (SM), muscularis(M), and serosa (S). **B.** Figure showing paraffine section of epithelial cells (ep) of anterior intestine and submucosa layer (SM) stained with HX & E. **C.** Figure showing the positive reaction of goblet cell (GC) to alcian blue stain with different sizes and shapes. **D.** Figure showing the positive reaction of goblet cell (GC) to PAS stain.

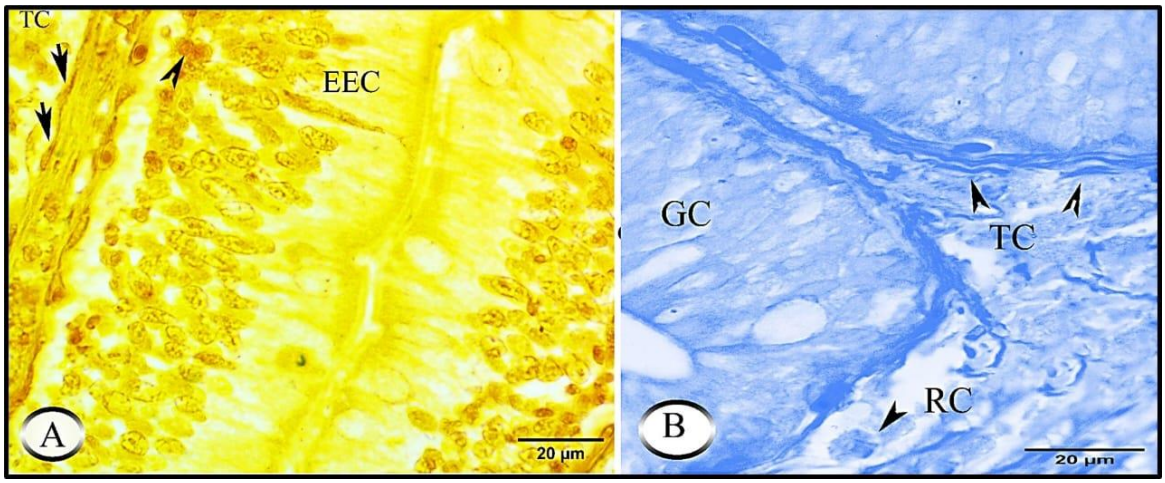

Figure 2: Histochemical analysis of anterior intestine: **A.** Paraffin sections stained by Grimelius silver stain showing positive reaction of open type enteroendocrine cell (EEC). Note the positive reaction of rodlet cell with its thick capsule (RC) and telocyte with spindle-shaped body (TC). **B.** Paraffin section stained with bromophenol blue showing positive reaction of rodlet cell (RC) and telocyte (TC) and negative reaction of goblet cell (GC).

A previous study showed that the elongated gut of koi fish contained endocrine cells that were responsible for the production of gastrin, gastric inhibitory peptide, glucagon, pancreatic polypeptide, substance P, VIP, and secretin [23, 24]. They also mentioned that the majority of the cells were open type, suggesting that the contents of the gut may operate on the apical portion of the cell to either stimulate or prevent the release of peptides from the basal section of the cell.

Conclusion

The current study concluded by describing the physical characteristics of the koi fish's anterior intestine and showing that it had a large luminal width as well as a large number and height of mucosal folds. Enterocytes covered in microvilli, intercalated with wandering lymphocytes, goblet cells, and enteroendocrine cells lined the anterior gut.

Authors' contribution

The work was equally distributed between authors. All authors have read and approved the final version of the manuscript.

Conflict of interest

There is no conflict of interest.

References

1. Domasevich MA, Hasegawa H, Yamazaki T. Quality evaluation of Kohaku Koi (Cyprinus rubrofuscus) using image analysis. Fishes. 2022 Jun 29;7(4):158.
2. McDowall RM. New Zealand freshwater fishes: an historical and ecological biogeography. Springer Science & Business Media; 2010 Jul 27.
3. Kohlmann K. The natural history of common carp and common carp genetics. Biology and Ecology of Carp. 2015 Jun 22:3-26.
4. Sun J, Wang Y, Lv A, Xian JA, Wang Q, Zhang S, Guo Y, Xing K. Histochemical distribution of four types of enzymes and mucous cells in the intestine of koi carp (Cyprinus carpio var. koi). Fish physiology and biochemistry. 2019 Aug 15; 45:1367-76.
5. Ray AK, Ringo E. The gastrointestinal tract of fish. Aquaculture nutrition: Gut health, probiotics and prebiotics. 2014 Oct 20:1-3.
6. Murray HM, Wright GM, Goff GP. A 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. Journal of Fish Biology. 1996 Feb;48(2):187-206.
7. Fugi R, Agostinho AA, Hahn NS. Trophic morphology of five benthic-feeding fish species of a tropical floodplain. Revista brasileira de biologia. 2001; 61:27-33.
8. Abdulhadi HA. Some comparative histological studies on alimentary tract of tilapia fish (Tilapia spilurus) and sea bream (Mylio cuvieri). (2005).
9. Harris HF. On the rapid conversion of haematoxylin into haematein in staining reactions. Journal of Applied Microscopic Laboratory Methods. 1900;3(3):777.
10. Steedman H. Alcian blue 8GS: a new stain for mucin. Journal of Cell Science. 1950;3(16):477-9.
11. McManus JF. Histological demonstration of mucin after periodic acid. Nature. Steedman HF. Alcian blue 8GS: a new stain for mucin. Journal of Cell Science. 1950 Dec 1;3(16):477-9.1946 Aug 10;158(4006):202-.
12. Crossmon G. A modification of Mallory's connective tissue stain with a discussion of the principals involved. The Anatomical Record. 1937 Aug;69(1):33-8.
13. Bancroft JD, Gamble M, editors. Theory and practice of histological techniques. Elsevier health sciences; 2008.
14. Pearse AG. Histochemical: Theoretical and applied. Churchill, London. 1985.
15. Grimelius L. A silver nitrate stain for alpha-2 cells in human pancreatic islets. Acta Societatis Medicorum Upsaliensis. 1968 Jan 1;73(5-6):243-70.
16. Albrecht MP, Ferreira MF, Caramaschi EP. Anatomical features and histology of the digestive tract of two related neotropical omnivorous fishes (Characiformes; Anostomidae). Journal of Fish Biology. 2001 Feb;58(2):419-30.
17. Mir IH, Channa A. A scanning electron microscopic examination of the intestinal tract of the snow trout, Schizothorax curvifrons Heckel. Journal of Fisheries and Aquatic Science. 2010 Jul 21;5(5):386-93.
18. Mercy TV, Pillai NK. The anatomy and histology of the alimentary tract of the blind catfish Horaglanis Krishnai Menon. International Journal of Speleology. 1985;14(1):8.
19. Monsefi M, Gholami Z, Esmaeili HR. Histological and morphological studies of digestive tube and liver of the Persian tooth-carp, Aphanius persicus (Actinopterygii: Cyprinodontidae). European Journal of Biology. 2010 Nov 11;69(1):57-64.
20. ÜNAL G, ÇETİNKAYA O, KANKAYA E, ELP M. Histological study of the organogenesis of the digestive system and swim bladder of the Chalcalburnus tarichi Pallas, 1811 (Cyprinidae). Turkish Journal of Zoology. 2001;25(3):217-28.

21. Mokhtar DM. Histological, histochemical and ultrastructural characterization of the pancreas of the grass carp (Ctenopharyngodon idella). European Journal of Anatomy. 2015 Apr 1;19(2):145-53.

22. Rombout JH, Lamers CH, Helfrich MH, Dekker A, Taverne-Thiele JJ. Uptake and transport of intact macromolecules in the intestinal epithelium of carp (Cyprinus carpio L.) and the possible immunological implications. Cell and Tissue Research. 1985 Mar; 239:519-30.

23. Noaillac-Depeyre J, Hollande E. Evidence for somatostatin, gastrin and pancreatic polypeptide-like substances in the mucosa cells of the gut in fishes with and without stomach. Cell and Tissue Research. 1981 Mar; 216:193-203.

24. Pan QS, Fang ZP, Zhao YX. Immunocytochemical identification and localization of APUD cells in the gut of seven stomachless teleost fishes. World Journal of Gastroenterology. 2000 Feb 2;6(1):96.