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Ultrastructural and histological study of intestine of adult Nile Tilapia (*Oreochromis Niloticus*)

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ABSTRACT

Tilapia intestine is essential for both digestion and nutrient absorption, making its structural analysis crucial for understanding its functional adaptations. This study sought to examine the histological, ultrastructural, and histochemical features of the intestine in adult Nile Tilapia (*Oreochromis niloticus*) by employing histology and scanning electron microscopy (SEM). Histological analysis revealed that the mid-intestinal mucosa was composed of short, longitudinal folds. Intestinal villi were lined with simple columnar epithelium with numerous goblet cells relatively in a deeper position. Wandering cells appeared rounded with round nuclei basally located and migrated to the surface. The goblet cells displayed a positive reactivity to both Alcian blue (AB) and Periodic acid Schiff (PAS). Scanning electron microscopy (SEM) of the mid-intestinal mucosa showed the existence of irregular wavy folds. Intestinal mucosa showed honey bee-sided enterocytes with sac-like goblets. The striated borders between adjacent cells were clearly visible. These cells microvillus boundaries were clearly distinguishable as light bands which covered the cell apex. Enterocyte columnar cells were interspersed with goblet cells containing mucus. Prominent large pores on the villus surface lead to goblet cells. In conclusion, these findings provide fundamental information concerning the structure of tilapia small intestine to anatomists and nutritionists

1. INTRODUCTION

The Nile tilapia (Oreochromis niloticus) is a versatile member of the Cichlid family, known for its omnivorous feeding habits. It has gained popularity in aquaculture due to its fast growth rate and remarkable adaptability to various environmental conditions (Yan et al., 2013). The continuous demand for Nile tilapia, *Oreochromis niloticus*, in Egypt is rising due to its desirable characteristics for aquaculture such as rapid growth, tolerance of a wide range of water quality parameters, disease resistance, good taste, and high market value (Barcellos et al., 1999).

The intestine is divided into three parts according to thickness of the it's wall; anterior, middle (posterior) and rectum (Mokhtar et al., 2015). The mid-intestine plays an important role in food absorption and fish immunity (Ellis, 2001). Intestinal villi length and goblet cells number affect directly on fish digestion and nutrient absorption (Elsabagh et al., 2012) and also affecting the absorption area capacity so they are considered good healthy intestine indicators (Khojasteh et al., 2018).

The objective of this study is to examine the intestinal morphology of Nile Tilapia (*Oreochromis niloticus*) by utilizing both light microscopy and scanning electron microscopy.

2. MATERIAL AND METHODS

2.1. Ethical statement

The research was conducted at the Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Benha University, Egypt. All experimental procedures undertaken in this study received an approval from the Institutional Animal Care and Use Committee of Benha University and adhered to the guidelines established by the National Institute of Health (NIH) in Egypt (Ethical No. BUFVTM 07-12-23).

2.2. Sampling

For this study, a total of 20 adult Nile tilapia (*Oreochromis niloticus*) specimens, comprising both 10 males and 10 females, were randomly selected. The specimens were collected during the winter of 2022 from a fish farm located in Kafr El Sheikh. The average standard length of the specimens was measured to be 37.20±4.00 cm, while the average body weight was recorded as 421.60±8.70 g.

2.3. Histological examination

To prepare the samples for histological examination, the middle portion of the Nile tilapia intestine was dissected through a central incision in the abdominal cavity. Following dissection, all samples were preserved in 10% neutral buffered formalin for duration of 48-72 hours. Next, the samples underwent dehydration using a series of ascending ethanol concentrations and cleared in xylene. Samples embedded in paraffin were sectioned 5 to 8 µm in thickness. For general histological examination, these sections were stained with hematoxylin and eosin according to Bancroft and Gamble (2002).

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2.4. Histochemical analysis

Representative samples were stained with Alcian blue (AB) and Periodic Acid Schiff (PAS) to determine the acidic and neutral mucopolysaccharides content, respectively (Bancroft and Gamble, 2008).

2.5. Semi-thin section

To prepare the specimens for analysis, the mucosal surface of the intestine was carefully rinsed with normal saline to eliminate any food particles. Next, small sections of fresh specimens were obtained from the middle portion of the intestine and immersed in glutaraldehyde 2.5% solution at a pH of 7.4. The specimens were then fixed at a temperature of 4°C for three hours. Afterward, they underwent three washes with phosphate buffer saline, with each wash lasting for 10 minutes. Subsequently, the specimens were post-fixed in a 1% osmium tetroxide solution at room temperature for 30 minutes. Following this, a progressive series of ethyl alcohol concentrations (30%, 50%, 70%, 90% and 100%) was used for dehydration. Finally, the specimens were infiltrated with acetone.

2.6. Scanning electron microscopy

Each tissue sample was immersed in the respective solution for a duration of 30 minutes. Subsequently, the samples were subjected to drying using a Samdri-PVT-3B® critical point drying machine, employing liquid CO2. Once completely dried, the samples were mounted onto aluminum stubs and coated with a layer of gold, reaching a thickness of 0.04 µm, using a sputter-coating unit (JFC-1100 E). Finally, the coded specimens were observed using a Jeol-JSM-5300 LV scanning electron microscope (Japan) at an electron beam voltage of 20 KV. The observations were conducted at the electron microscopy unit within the Faculty of Science at Alexandria University in Egypt.

Samples preparations for semi-thin section and electron microscopy investigation were done according to procedures outlined by Bozzola and Russsell (1999).

3. RESULTS

3.1. Histological analysis

The Nile Tilapia's intestine was a tubular organ that consisted of several layers; tunica mucosa, sub-mucosa, muscularis, and serosa. Concerning the fine structure of the mid-intestine, the mucosa exhibited short longitudinal folds The tunica submucosa was made of a thin connective tissue layer containing delicate elastic fibers surrounding the blood vessels. Tunica muscularis was composed of two layers, inner circular and outer longitudinal smooth muscles. Finally, thin tunica serosa contained connective tissue and a layer of simple squamous epithelium (Fig.1A).

The lamina propria, which extended into the mucosal folds, was composed of loose connective tissue. It included smooth muscle fibers, fibroblasts, lymphocytes, and collagenous fibers (Fig.1B). Intestinal villi lined with simple columnar epithelium with numerous goblet cells relatively in deeper position. The columnar cells had a column-like shape with oval nuclei at the base and acidophilic cytoplasm. Goblet cells had a cup shape with basal nuclei and apical pale cytoplasm owing to presence of mucous granules. Wandering cell were one of connective tissue cells that migrated to the epithelial layer and acted as phagocytic cells. They appeared rounded with round nucleus. These cells were found at columnar cell bases and could migrate to columnar cells apical surface beside the goblet cells (Fig.1C). Neither multicellular intestinal gland nor crypts of Luberkhein were present in the intestine of Nile Tilapia. The brush border, which consisted of a continuous layer, was situated at the apical surface of the columnar epithelium. However, this continuous layer was interrupted by the presence of goblet cells (Fig.1D).

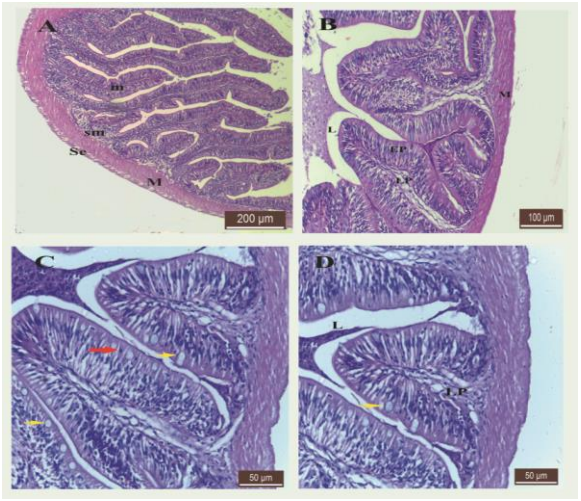

Figure 1 Histological characteristics of Nile tilapia's middle intestine. (A): The middle intestinal wall was composed of mucosal folds (m), submucosa (sm), muscularis (M), and serosa (Se). B: Several intestinal villi facing the luminal surface of the intestine (L) and lined by epithelium (EP) with underlying lamina propria (LP).C: The intestinal villi of Nile Tilapia showing oval- shaped Goblet cells (yellow arrow) dispersed between columnar cells and wandering cells (red arrow) D: The intestinal villi of Nile Tilapia showing lamina propria (LP) that consists of loose connective tissue extending into the folds of the mucosa and goblet cells (yellow arrow). H&E stain.

3.2. Histochemical analysis

The goblet cells were relatively numerous intermingled between columnar cells. They contained mucous granules which are mucopolysaccharides in nature either neutral or acidic. They exhibited intense positive reaction to Alcian blue (Fig.2A, B) and PAS stain (Fig.2C, D).

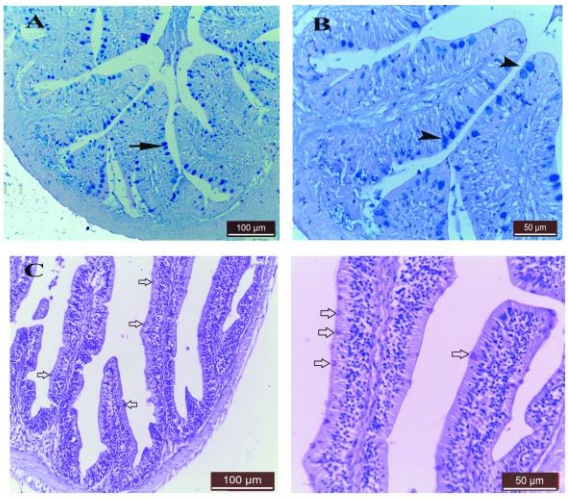

Figure 2 Histochemical characteristics of Nile tilapia's middle intestine. (A, B): Goblet cells exhibited a positive reaction to PAS (black arrow). (C, D): positive reaction of goblet cells to Alcian blue (white arrow).

3.3. Scanning electron microscopy

Our results gave a picture about the mucosa of the middle intestine of Nile Tilapia highlighting the existence of wavy irregular folds (Fig. 3A). The mucosa was lined with simple columnar epithelial cells which were densely packed clear polyhedral shape (honey bee-sided) enterocytes containing brush border (Fig. 3B). The striated borders between adjacent cells were clearly visible (Fig. 3C). These cells microvillus boundaries were distinguishable as bright bands covering the cell apex (Fig. 3D). The corrugated surface of

the villi showed discontinuity and disruption of the epithelial mucosa. Microvillus covered the luminal face of the middle intestine (Fig. 3E). Enterocyte columnar cells were interspersed with goblet cells containing mucus (Fig. 3F). Prominent large pores on the villus surface lead to goblet cells. Between the enterocytes, these characteristic goblet cells were visible (Fig. 3G).

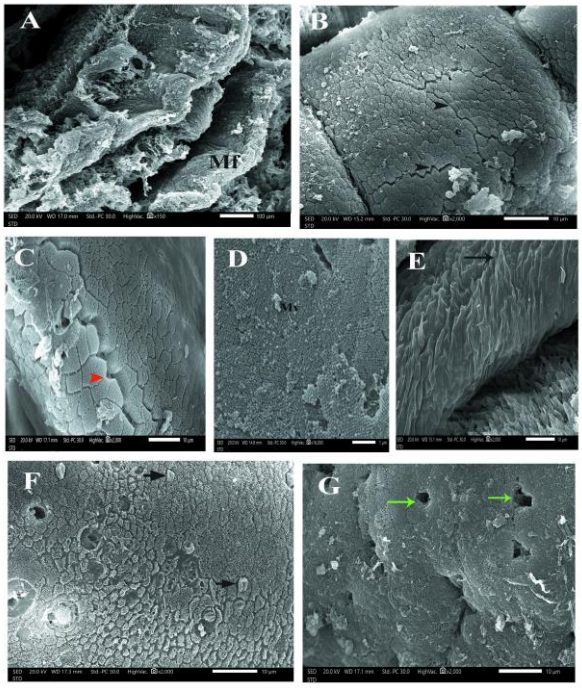

Figure 3 Scanning electron micrographs of the middle part of the Nile Tilapia's intestine. A: showing long mucosal folds arranged in an irregular pattern (Mf). B: showing the surface honey bee-shaped enterocytes (e); black arrowhead indicates striated borders between enterocytes C: showing epithelial polyhedral enterocytes of the Nile Tilapia's intestine as red arrowhead indicates striated borders. D: showing microvillus (Mv) covering enterocyte surface. E: showing typical brush border microvilli, the black arrow indicates the tip of microvilli covering the cell apex. F: showing sac-like goblet cell (black arrow) dispersed between enterocytes. G: showing large pores (green arrow) on the villus surface. The bar indicates magnification.

4. DISCUSSION

In this study, we focused on describing the Nile tilapia's mid-intestinal villi morphological structure and their distinct cellular distribution using scanning electron microscopy The current study showed that the mid-intestine of Nile Tilapia is a tubular organ that consists of several layers; tunica mucosa, sub-mucosa, muscularis, and serosa. There are numerous mucosal folds or villi that might increase the surface area of absorption. The mucosa had several short longitudinal folds which are consistent with Farrag et al. (2020), in *Mugil. Cephalus*, who stated that the comparatively long and closely set anterior intestine's mucosal folds allowed food to be retained for a longer amount of time, which increased exposed surface area and absorption time.

These features likely facilitate the partial retention of semi-digested food, promoting effective digestion and absorption processes. The presence of these undulating folds enables the fish to extend the exposure time of the semi-digested food to the surface area of the intestinal mucosa, potentially enhancing nutrient absorption as reported by Mokhtar et al. (2015). The same investigations were recorded by Hiroshi et al. *(*1984) in goldfish and by Mandal et al. (1996) in *Oreochromis mossambicus*.

In this study epithelium lining intestinal villi was composed of a simple columnar epithelium with a large number of goblet cells relatively in a deeper position. Similarly, Farrag et al. (2020) in *Mugil cephalus*, Farrag et al., (2021) in grey mullet, and Khalaf–Allah (2001) noticed that similar to other teleosts, the intestinal mucosa was lined by columnar epithelial cells known as enterocytes. Additionally, the mucosa contains goblet cells, specialized cells that secrete mucus. On the other hand, in Tilapia *sparrmanii*, Okuthe and Bhomela (2020) concluded that the epithelial cells that line the villi are primarily enterocytes, which possessed a brush border. These enterocytes were interspersed with goblet cells that contain mucus, with approximately one goblet cell found every 4 or 6 cells. The enterocytes, along with the presence of tight junctions, form a continuous barrier that regulates the diffusion of molecules through both transcellular and paracellular pathways. This barrier plays a crucial role in constituting the main component of the intestinal primary barrier, as highlighted by previous studies (Murray et al., 1994; Takashima and Hibiya, 1995; Beck and Peatman, 2015). Our study revealed that wandering cells are present around the bases of columnar cells. While Farrag et al. (2020) concluded that the submucosal layer and the spaces between columnar cells are full of wandering cells as these cells were one of connective tissue cells of submucosal.In histochemical analysis, goblet cells were relatively cup shape with pale cytoplasm. The presence of a mixture of acidic and neutral mucoploysaccharides is indicated by the more intense positive reaction with AB stain and PAS stain. Similar investigations were detected by Mokhtar et al. (2015) in grass carp and Khalaf Allah (2001). Also, numerous goblet cells in the mucosa of the intestines of walking catfish and piranha react positively to AB and PAS Rajii and Norouzi (2010). The goblet cells in the intestine are responsible for the secretion of gel-forming mucin. This mucin serves multiple functions, including providing protection to the epithelial layer and lubrication for the passage of nutrients. Additionally, the secreted mucin plays a role in inhibiting the growth of microorganisms, protecting against degradation, and contributing to the osmotic function of the intestine (Carrassón et al., 2006; Diaz et al., 2008; Khalaf–Allah, 2013; Hopperdietzel et al., 2014; Dos Santos et al., 2015; Farrag, 2017; Gosavi et al., 2019; Vidal et al., 2020 and Farrag et al., 2020).

In this research scanning electron investigation of intestinal mucosa showed honey bee-sided enterocytes with sac-like goblet cells. In the same line Gargiu et al. (2015) revealed that the enterocytes within the intestine displayed a columnar shape and were characterized by a distinct striated border. This border consisted of regular microvilli that contained cores composed of delicate filaments. These filaments formed dense bundles that extended deeply into the terminal web of the apical cytoplasm. However, Ringo et al. (2003) reported that the borders between adjacent enterocytes were clearly visible as are the microvilli which cover the cell apex in the midgut of Arctic charr. While in goldfish Caceci (1984) stated that individual enterocytes were distinguished as localized areas with polyhedral outlines.

5. CONCLUSIONS

Nile Tilapia's the mid-intestine was a tubular organ that had several layers. The mucosa had short several longitudinal folds. Epithelium lining of intestinal villi formed from simple columnar epithelium with numerous goblet cells relatively in a deeper position. Wandering cells presented around the bases of columnar cells. Intestinal mucosa showed honey bee sided enterocytes with sac like goblet cells. The striated borders between adjacent cells were clearly visible. Enterocyte columnar cells were interspersed

with goblet cells containing mucus. Prominent large pores on villus surface leading to goblet cells.

6. REFERENCES

1. Bancroft, J., D., & Gamble, M., 2008. Theory and practice of histological techniques.5th. Ed. Edinburgh. Churchill Livingstone Pub. pp 172-5, pp 593-620.
2. Barcellos, L.J.G., Nicolaiewsky, S., Souza, S.M.G. and Lulhier, F., 1999. The effects of stocking density and social interaction on acute stress response in Nile tilapia. Oreochromis niloticus (L.) fingerlings. Aquaculture Research 30: 887- 892.
3. Beck, B.H., Peatman, E., 2015. Mucosal health in aquaculture. Academic Press, Cambridge, pp.408 .
4. Bozzola, J.J. and Russell, L.D. 1999. Electron microscopy: principles and techniques for biologists. 2nd Ed. Jones and Bartlett Publishers
5. Caceci, T., 1984. Scanning electron microscopy of goldfish, Carassius auratus, intestinal mucosa J. Fish Biol. 25: 1-12
6. Carrassón, M., Grau, A., Dopazo, L.R., Crespo, S., 2006. A histological, histochemical and ultrastructural study of the digestive tract of Dentex dentex (Pisces, Sparidae). Histol Histopathol., 21: 579 - 593.
7. Diaz, A.O., Garcia, A.M., Goldenberg, A.L., 2008. Glycoconjugates in the mucosa of the digestive tract of Cynoscion guatucupa: a histochemical study. Acta. Histochemica., 110: 76 – 85.
8. Dos Santos, M.L., Arantes, F.P., Santiago, K. B., Dos Santos, J.E., 2015. Morphological characteristics of the digestive tract of schizodon knerii (Steindachner, 1875), (characiformes: Anostomidae): An anatomical, histological and histochemical study. Annals of the Brazilian Academy of Sciences, 87,2: 867 - 878.
9. Ellis, A.E., 2001. The immunology of teleosts. In: Roberts RJ (ed) Fish Pathology, London: WB Saunders. pp 133-150.
10. Elsabagh, M., Mohamed, R., Moustafa, E. M., Hamza, A., Eltholth, M., Farrag, F., Decamp, O. and Dawood, M.A.O., 2018. Assessing the impact of Bacillus strains mixture probiotic on water quality, growth performance, blood profile and intestinal morphology of Nile tilapia, Oreochromis niloticus. Aquaculture Nutrition, 24: 1613–1622
11. Farrag, D.M.G., 2017. Comparative studies on the skeletal elements and functional anatomy of the digestive tract and integument of some silurid fish species native to the River Nile, Egypt. M.Sc. Thesis, Zoology Department, Faculty of Science (Boys), AL-Azhar University, Pp: 329.
12. Farrag, D.M.G., Azab, A.M., Alabssawy, A.N., 2020. Comparative study on the histochemical structures of stomach, pyloric caeca and anterior intestine in grey mullet, Mugil cephalus (Linnaeus, 1758). Egypt. Egyptian Journal of Aquatic Biology and Fisheries, 24,7: 1055 - 1071.
13. Farrag, D.M.G., Azab, A.M., Alabssawy, A.N., 2021. Histological variations and adaptability in some digestive organs of the thinlip grey mullet, Chelon ramada (Risso, 1827) Egyptian Journal of Aquatic Biology & Fisheries 25,1: 241 – 256.
14. Gosavi, S.M., Verma, C.R., Kharat, S.S., Pise, M., Kumkar, P., 2019. Structural adequacy of the digestive tract supports dual feeding habit in catfish Pachypterus khavalchor (Siluriformes: Horabagridae). Acta Histochemica, 121,4: 437 - 449.
15. Okuthe, G.E., Bhomela, B., 2020. Morphology, histology and histochemistry of the digestive tract of the Banded tilapia, Tilapia sparrmanii (Perciformes: Cichlidae) ZOOLOGIA 37: e51043 https://doi.org/10.3897/zoologia.37.e51043
16. Hiroshi, I., Yamamoto, T., 1984. Morphological studies of the goldfish hindgut mucosa in organ culture. Cell Tissue Res 238: 523-528.
17. Hopperdietzel, C., Hirschberg, R.M., Hünigen, H., Wolter, J., Richardson, K. and Plendl J. 2014. Gross morphology and histology of the alimentary tract of the convict cichlid Amatitlania nigrofasciata. Journal of Fish Biology, 85: 1707-1725.
18. Khalaf–Allah, H.M.M., 2001. Ecological and biological studies on some fishes in Lake Qarun, Egypt. M.Sc. Thesis, Zool. Dep. Fac. Sci., Al–Azhar Univ., Egypt, pp.331
19. Khalaf–Allah, H.M.M., 2013. Morphological adaptations of digestive tract according to food and feeding habits of the broomtail wrasse, Cheilinus lunulatus. Egypt, J. Aquat. Biol. and Fish, 17,1: 123 - 141.
20. Khojasteh, S.M.B., 2012. The morphology of the post-gastric alimentary canal in teleost fishes: a brief review. International Journal of Aquatic Science, 3, 71-88
21. Mandal, DK., Chakrabarti, P., 1996. Architectural pattern of the mucosal epithelium of the alimentary canal of notopterus notopterus (pallas) and oreochromis mossambicus (peters) a comparative study. Acta Ichthyologica et Piscatoria 1: 15-22.
22. Mokhtar, DM., Abd-Elhafez, EA., Hassan, AH., 2015.Light and Scanning Electron Microscopic Studies on the Intestine of Grass Carp (Ctenopharyngodon idella): I-Anterior Intestine J Aquac Res Development 6:380 http://dx.doi.org/10.4172/2155-9546.1000374
23. Murray, HM., Wright, GM., Goff, GP., 1994. A comparative histological and histochemical study of the stomach from three species of pleuronectid, the Atlantic halibut, Hippoglossus, the yellowtail flounder, Pleuronectes ferruginea, and the winter flounder, Pleuronectes americanus. Canadian Journal of Zoology 72,7: 1199–1210.
24. Raji, A. R., Norouzi, E., 2010. Histological and histochemical study on the alimentary canal in Walking catfish (Claris batrachus) and piranha (Serrasalmus nattereri) Iranian Journal of Veterinary Research, 11,3:255-261
25. Ringo, E., Olsen, R.E., Mayhew, M., Myklebust, R., 2003. Electron microscopy of the intestinal microflora of fish Aquaculture 227: 395–415
26. Takashima, F., Hibiya T., 1995. An atlas of fish histology. Normal and pathological features. Gustav Fisher Verlag, Kodansha, Tokyo, pp.192
27. Vidal, M.R., Ruiz, T.F.R., Dos Santos, D.D., Gardinal, M.V.B., De Jesus, F.L., Faccioli, C.K., Vicentini, I.B.F., Vicentini, C.A., 2020. Morphological and histochemical characterisation of the mucosa of the digestive tract in matrinxã Brycon amazonicus (Teleostei: Characiformes). Journal of Fish Biology, 96,1: 251 - 260.
28. Wang, M., Lu, M., 2015. Tilapia polyculture: a global review. Aquacult. Res.
29. Yan, B., Wang, Z.H., Zhao, J.L., 2013. Mechanism of osmoregulatory adaptation in tilapia. Mol. Biol. Rep. 40, 925–931.