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Histological variations and adaptability in some digestive organs of the thinlip grey mullet, *Chelon ramada* (Risso, 1827)

Diaa M. G. Farrag; Ahmad M. Azab* and Ahmed N. Alabssawy
Zoology Department, Faculty of Science, Al-Azhar University, Cairo
*Corresponding author: ahmadmosaad201@azhar.edu.eg

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ABSTRACT

The objective of the present study was to explain the histological variations in the pyloric caeca and anterior intestine of *Chelon ramada*.For this purpose, small pieces of the target organs were attained from specimens of *C. ramada*: for histological and histochemical procedures. The final sections were examined under a microscope, photographed and described.
Histologically, serosa, muscularis, submucosa, and mucosae are the main layers of the wall of pyloric caeca and anterior intestine in *C. ramada* indicating there are no remarkable differences in the basic structure. The mucosal folds of pyloric caeca are thin and deeply folded in a narrow lumen; in contrast, the mucosal folds of the anterior intestine are long protruding at a wide lumen. The mucosal layer of the anterior intestine is consisted of columnar and mucus cells as in the pyloric caeca, but mucous cells are numerous in number at the anterior intestine while it observed in fewer numbers at the mucosal layer of the pyloric caeca. In submucosal and mucosal layers, wandering cells and lymphocytes are plentiful especially among the columnar cells of the anterior intestine. These cells are more abundant in the anterior intestine than that observed in pyloric caeca; in contrast with the number of blood vessels. The present study concluded that the distribution and secretions of the mucosa are very clear with histochemical stains. This study is helpful for understanding the digestive physiology and the related functional mechanisms of fish digestion.

INTRODUCTION

Mugilidae (or mullets) are members of the Order Mugiliformes, that are widely distributed in all world's waters; tropical, temperate and also subtropical **(Khalaf-Allah, 2001; Durand *et al.*, 2012; Coad, 2015** and **El-Aiatt & Shalloof, 2018)**. This family is including about 75 teleostean species, which follow to about 20 genera **(Nelson *et al.*, 2016).** Mugilid species are commercially important as they are a major source of food for human in most countries of the world (**Durand *et al.*, 2012; Whitfield *et al.*, 2012**and **Farrag *et al.*, 2020).**

The Thinlip grey mullet, *Chelon ramada* (Risso, 1827) is a diadromous and euryhaline species **(Papa *et al.*, 2003)** which plays a vital roles in the economical-fisheries of Egypt and inhabits all waters: fresh; brackish and marine waters in temperate to tropical zones **(El- Aiatt & Shalloof, 2018).**

The digestive system in fishes, including two anatomical components: the digestive tract and its accessory glands. The histological investigations indicating the key function of the digestive tract in capturing and transformation of food items to small molecules which can be easily digest and absorb by the fish **(Nelson *et al.*, 2016).** The physiological and morphological adaptations of the digestive tract in fishes are mainly correlated to phylogeny and the different feeding habits amongst fish species **(Wilson & Castro, 2010; Faccioli *et al.*, 2014; Farrag, 2017; Kalhoro *et al.*, 2018; Alabssawy *et al.*, 2019; Gosavi *et al.*, 2019**; **Shalaby, 2020** and **Vidal *et al.*, 2020)**.

The wall structure of the digestive tract in fishes showing a strong matching to that of the higher vertebrates, as it composed of the upper layer of serosa; a part of the peritoneal tissue which enveloping muscularis. The latter is almost composed of the circular and longitudinal muscles. In addition to the internal mucosal epithelium that surrounded by submucosal connective tissue which supported by nerves and blood vessels **(Nelson *et al.*, 2016).**

Fish are the only vertebrates which have blind projections (pyloric caeca) at the gastro-intestinal junction: between the stomach and the anterior intestine (hence the name pyloric caeca) **(Kent, 1983; Ringø *et al.*, 2003; Wilson & Castro, 2010** and **Kalhoro *et al.*, 2018).**

The term “intestinal caeca” suggested by some authors for use instead of “pyloric caeca” **(Hossain & Dutta, 1996; Marchetti *et al.*, 2006** and **Khalaf-Allah, 2009).**

The pyloric caeca are finger-like pouches and show variety in their number, arrangement, shape and size at the various species in family Mugilidae. Number of the pyloric caeca has a significant key which used as a taxonomic character in identification of fish species **(Thomson, 1997; Roy & Gopalakrishnan, 2011** and **Sitar & Traynor, 2018**). The different species can be distinguish anatomically between family Mugilidae, as there are 2 pyloric caecae found in *Mugil cephalus*, while 6 – 8 (usually 7) pyloric caecae in *Liza aurata*, in umbrella-like shape covered the gizzard stomach **(Khalaf-Allah, 2001** and **Khayyami *et al.*, 2015).**

The histological structures of the intestinal caeca are similar to that of the anterior intestine **(Khalaf-Allah, 2009).** The intestine in fishes has a length variation due to the type of food. It is short in carnivorous fishes as in the perch (Perca). On the other hand; intestine often has many times the length of the body in herbivorous fishes such as in tilapias and mullets, while the intermediate lengths of intestine are found in omnivorous fishes **(Kumar & Tembhre, 1996; Khojasteh, 2012; Khalf-Allah, 2013, Farrag, 2017 Shalaby, 2017** and **Alabssawy *et al.*, 2019).** Histological and anatomical characters of the intestine in teleosts are correlated to its diet. It has been observed by many researchers **(Buddington *et al.*, 1997; Arellano *et al.*, 2002; Khojasteh *et al.*, 2009; El-Bakary** and **El-Gammal, 2010; Mokhtar *et al.*, 2015; Khadse** and **Gadhikar, 2017)**.

Histochemically, the mucosa is the only layer in contact with food and the external environment. Abundance and lack of the histological items as digestive glands, mucus-secreting cells, taste buds and the muscular coat is an evidence of the adaptation of the fish to their food and feeding habits. Several studies show that mucin of the mucosal folds perform essential functions like lubrication, protection against digestive enzymes, food digestion, osmoregulation and are also related to pathogen defence **(Humbert *et al.*, 1984; Petrinec *et al.*, 2005; Cao** and **Wang, 2009; Pérez- Sánchez *et al.*, 2013** and **Leknes, 2015).** Also, the mucosal layer may be prevention of infections **(Ringo *et al.*, 2003; Grove *et al.*, 2006** and **McGuckin *et al.*, 2011;)**. In addition to **Sveen *et al.* (2017**) and **Vidal *et al.* (2020)** who declared that mucin transcription is modified in response to diet, parasite infection and handling stress, suggesting that mucin may be used as markers for fish health.

Hence, the present study designed to provide a description on the histological adaptations in pyloric caeca and anterior intestine of *Chelon ramada* to understanding the role of pyloric caeca and anterior intestine in digestive physiology and the related functional mechanisms of fish digestion.

MATERIALS AND METHODS

1. Specimens collection:

A total of 25 specimens of *Chelon ramada* were collected from Dimru at Sidi Salem City, Kafr El-Shaikh governorate, Egypt **(Figure 1).** The specimens commonly caught by gill-net reaching the bottom where it usually lives or by trammel net **(Latif, 1974**). The specimens were examined fresh or after preservation in 10% Bowin's solution. Then they were transported to Laboratory of Marine Biology at Zoology Department, Faculty of Science, Al-azhar University, Cairo. In the laboratory, fish were confirmed according to **Boulenger (1907) and Sandon (1950)** then the following studies were carried out.

Fig. (1): Map of Nile Delta showing the location of Dimru fishing area (★).

2. Histological and histochemical studies:

For histological studies, small pieces (5 mm) of the pyloric caeca and anterior intestine of *M. cephalus* were removed from the dissected specimens, fixed in 10% formalin solution for at least 48 hours, dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene and embedded in wax (M.P.: 58°C). Transverse sections were cut at 4-6 µ in thickness, stained with Harris`s haematoxylin and eosin for routine histological examination **(Humason, 1979)**, Alcian blue and periodic acid Schiff (PAS) for acid and neutral of mucopolysaccharides **(Putt, 1971)**, Masson's trichrome for detection type of the connective tissue **(Masson, 1928)** and Bromophenol for detection of protein (**Mazia *et al.*, 1953**). Finally, the stained slides were microscopically examined with light microscope (XSZ-N107T) at different magnifications, and then photographed using digital camera (Toup Cam, Ver. 3.7) mounted on light microscope and described.

RESULTS

1. Anatomical structures:

In *Chelon ramada*, there are 6-8 cone-like shape of pyloric caeca present between the gizzard stomach and the anterior intestine. These caeca covering the gizzard stomach and relatively equal in size. Behind the pyloric caeca to the first convoluted part of the intestine: the anterior intestine extends and runs with the gizzard stomach at the right side of the abdominal cavity to get the form of U-shape **(Fig. 2)**.

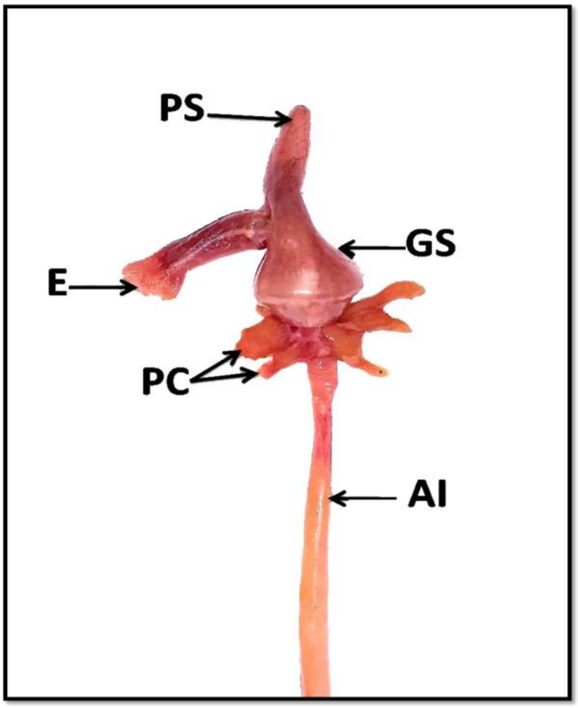

Fig. (2): A photograph of the digestive tract of *C. ramada* showing esophagus (E), proventriculus stomach (PS), gizzard stomach (GS), pyloric caeca (PC) and anterior intestine (AI).

2. Histological structures:

2.1. Pyloric caeca:

In *C. ramada*, wall of the pyloric caeca consists of the four main layers namely serosa, muscularis, submucosa and mucosa. The mucosa of the proximal portion in the pyloric caeca in *C. ramada* appeared in a several folds or caecal villi. These folds hanging inside a narrow lumen and give the shape of an irregular finger like shape, especially at the base, while it gets wide at middle of the fold **(PLATE I A&B)**.

The mucosal epithelia of the proximal portion are composed of goblet cells and ciliated columnar cells. Goblet cells are in less number at base of the mucosal folds but they are numerous and appears towards the inner surface of the epithelium **(PLATE II A&B)**.

The submucosa of caecal villi is relatively thin and cosists of connective tissue filled with blood vessels, lymphocytes and wandering cells**.** Blood vessels found in a large number and various sizes which extends inside the mucosal folds by a relatively wide lamina propria **(PLATE II B).**

The muscularis composed of inner circular fibers which is thicker than the outer thin longitudinal muscle fibers. Serosa is very thin of squamous epithelia, lining the muscularis **(PLATE I B)**.

The histological structure of distal region in pyloric caeca is significantly varied from that of described proximal region. The mucosal folds of the distal region are more folded. These folds are overlapped together and forming a reticular shape of very narrow lumen, as seen in transverse sections. The caecal villi hang in a very restricted lumen which reduced to several channels or lacunae **(PLATE I C&D)**.

Mucosa of the distal region in pyloric caeca resembles that in the proximal one, except the much number of goblet cells in addition to the less number of lymphocytes **(PLATE II C&D)**. Furthermore the other principle layers of the wall of distal caeca: include relatively reduced layers than in proximal ones: submucosa, muscularis and serosa **(PLATE I D).**

2.2. Anterior intestine:

Wall of the anterior intestine of *C. ramada* is composed of the usual four layers: the mucosa; submucosa; muscularis and serosa. The histological structure of anterior intestine is similar to that of pyloric caeca in except some differentiations which can be easily seen **(PLATE I E&F)**. The mucousal membrane of anterior intestine is greatly folded. Villi are diverse in length. Small folds are found in the bases between the relatively large folds; in addition to a wide lumen **(PLATE I E&F)**.

The epithelial mucosa of anterior intestine consists of two types of cells: mucus-secreting cells and columnar cells. The columnar cells are in the direction of the lumen. The mucus secreting cells have a goblet-like shape; concentrated on the sides of the folds, rarely on the crests. While in large areas along the bases, the mucus secreting cells are completely absent **(PLATE II E)**.

The submucosa is shown in the wall of anterior intestinal villi as a relatively thin layer. This connective tissue layer is filled with lymphocytes and wandering cells which tend to be more closely concenterated to the base of columnar epithelia and appear to be migrating towards the lumen **(PLATE II E)**.

Muscularis composed of two layers: inner circular muscle fibers which thicker than the outer thin longitudinal muscle fibers. Serosa is a very thin layer composed of simple squamous epithelia, lining the outer longitudinal muscle fibers **(PLATE I F & PLATE II F)**.

3. Histochemical structures:

3.1. Pyloric caeca:

The histochemical reaction in both proximal and distal regions of pyloric ceaca shows the aqueous content of the mucus secretions as a ngative reaction with Bromophenol blue **(PLATE III A&B)**. Furthermore, different concentrations of neutral mocopolysaccharides (MPS) at the sides of mucosal folds in addition to mixture of acid & neutral MPS resulted when reacted with Alcian blue and periodic acid Schiff (PAS) **(PLATE IV A&B)** for proximal and **(PLATE IV C&D)** for distal pyloric caeca.

A relatively thin layer of submucosa is shown in the wall of caecal villi. This layer contains an elastic fibers in connective tissue surrounding numerous blood vessels and extend inside the lamnia propria of the ceacal villi, lymphocytes and wandering. These elastic fibers are noticed as a reaction with Masson trichrome stain **(PLATE III B)**

3.2. Anterior intestine:

The histochemical reaction shows a watery content in the mucus secreting cells of the intestinal mucosa with Bromophenol blue stain **(PLATE III C)**. Moreover, when reacted with PAS stain, a mixture of acid & neutral MPS and different concentrations of neutral MPS are observed **(PLATE IV E&F)**. The comparatively thin submucosa of the wall of intestinal villi contains fibrous connective tissue which resulted by the histochemical reaction with Masson trichrome stain **(PLATE III F)**.

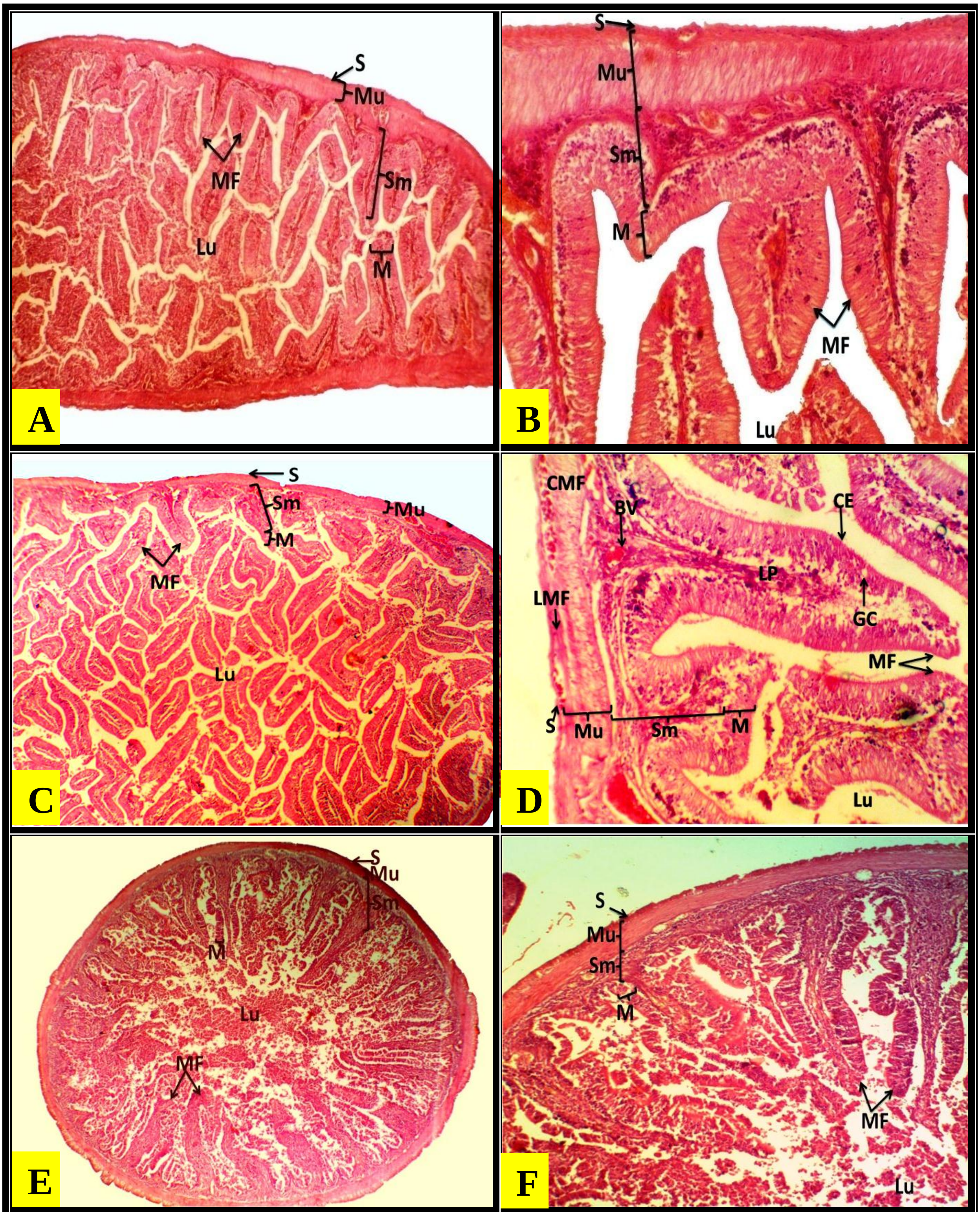

PLATE I

Photomicrographs of T.S. in the proximal pyloric caeca **(A&B)**, distal pyloric caeca **(C&D)** and anterior intestine **(E&F)** of *C. ramada* showing serosa (S), muscularis (Mu), submucosa (Sm), mucosa (M), mucosal folds (MF), lumen (Lu), longitudinal muscle fibers (LMF), circular muscle fibers (CMF), blood vessel (BV), lamina propria (LP) and columnar epithelium (CE) **(H&E; A&C&E: X40 and B&D&F: X200).**

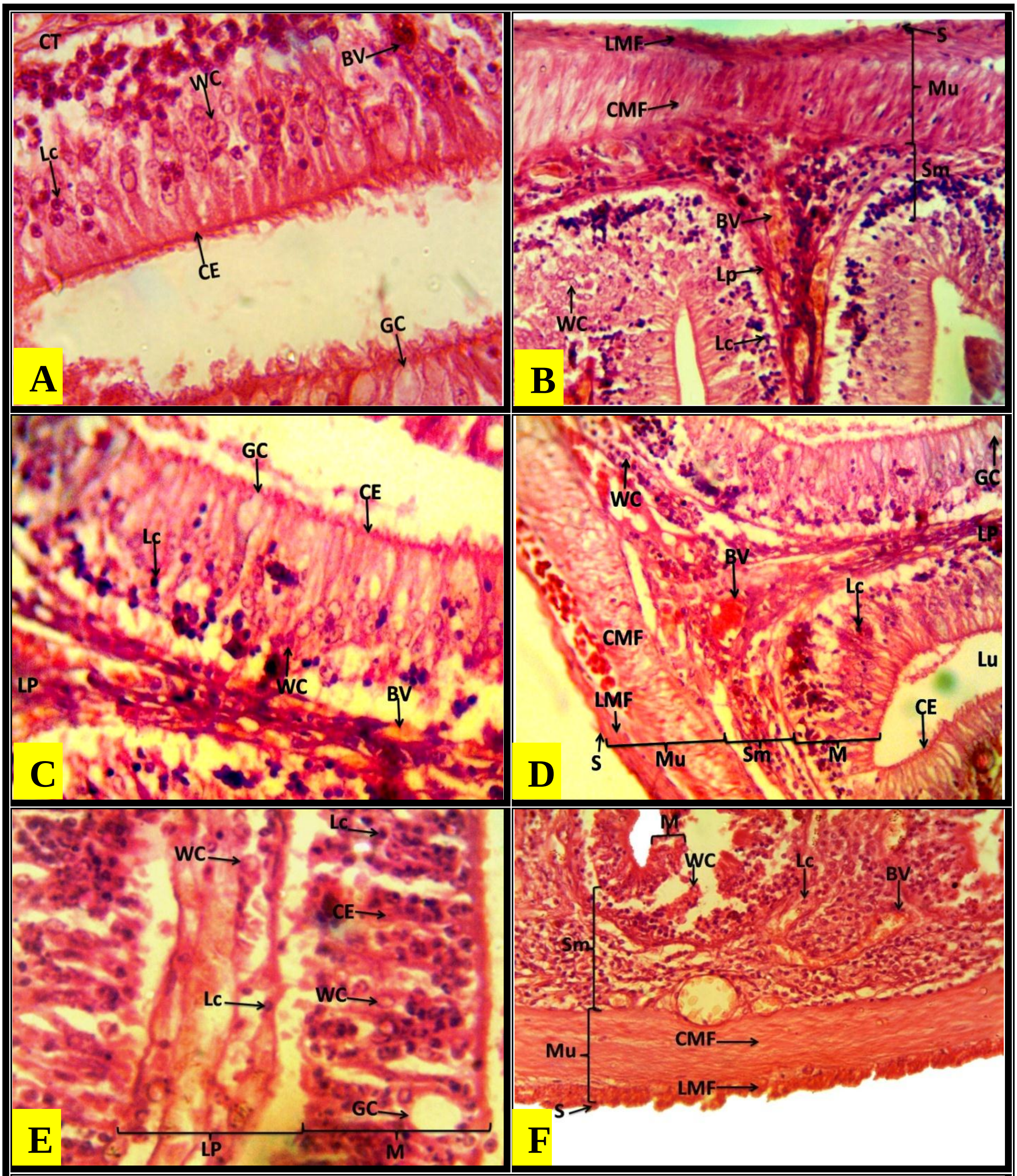

PLATE II

Photomicrographs of T.S. in the proximal pyloric caeca **(A&B)**, distal pyloric caeca **(C&D)** and anterior intestine **(E&F)** of *C. ramada* showing serosa (S), muscularis (Mu), submucosa (Sm), mucosa (M), mucosal folds (MF), lumen (Lu), longitudinal muscle fibers (LMF), circular muscle fibers (CMF), blood vessel (BV), lamina propria (LP), lymphocyte (LC), columnar epithelium (CE), goblet cell (GC) and wandering cell (WC) **(H&E; A&C&E: X1000 and B&D&F: X400).**

DISCUSSION

In the present study, the pyloric caeca of grey mullet, *Chelon ramada*, is consists of the four main layers namely serosa, muscularis, submucosa and mucosa. The mucosa appeared in several folds or caecal villi. These caecal villi are very long and hanging inside lumen to give the shape of an irregular finger indicates that the function is probably to increase the absorptive surface; as the fish, *C. ramada* is a herbivorous. These observations agree with the pyloric caeca of other mullet fish species, *Mugil cephalus* described by (**Farrag *et al.*, 2020).**

In our study, the pyloric caeca was close to the anterior intestine formation in their histological features. A similar indication was declared by **Mumford *et al.*, (2007).** Moreover, **Canan *et al.*, (2012)** and **Mitra *et al.*, (2015)** reported that the anterior intestine was histologically similar to the pyloric caeca. Our research showed that the pyloric caeca and intestine of *C. ramada* had the four basic layers; serosa, muscularis, submucosa and mucosa. This result is supported by results obtained from other species such as *Oncorhynchus mykiss* **(Khojasteh *et al.*, 2009)** and *Rastrelliger brachysoma* **(Senarat *et al.*, 2015)**, *Ctenopharyngodon idella* **(Mokhtar *et al.*, 2015)** and *Belone belone* **(Bocina *et al.*, 2017).**

In the current work, the mucosal folds in the anterior intestine are relatively long and closely set, due to the retention of food for longer period indicate that the function is probably to increase the digestive and absorptive surface; as the mullet fish, *C.ramada* is a herbivorous and need a long time and surface to digest the plant component. Similar observations were detected in *Mugil cephalus* by **(Farrag *et al.*, 2020)**.

Similar to our investigations of the study, **Murray *et al.*, (1996)** reported that the pyloric caeca had a thinner muscularis layer than the intestine, in Atlantic halibut, the winter flounder and the yellowtail flounder. The function of mixing the food with the digestive enzymes is contributing to the muscularis layer **(Mumford *et al.*, 2007).**

The present observations found that the submucosal layer of pyloric caeca in *C._ramada* is characterized by the presence of elastic fibers when stained by Masson Trichrome. These elastic fibers in connective tissues may play a role in its flexibility nature and peristaltic movement. Similar observations were detected in *Liza aurata* **(Khalaf–Allah, 2001)** and *Mugil cephalus* **(Farrag *et al.*, 2020).**

The submucosa of caecal villi is cosists of connective tissue filled with lymphocytes and wandering cells which may play a role in immuninity function**.** Moreover the blood veseels found in large numbers and sizes which extends inside the mucosal folds and may play a role in absorption function.

In addition the present results showed that the tunica mucosa of the pyloric caeca in the investigated fish is mainly composed of simple prismatic epithelia with mucus-secreting cells and longitudinal folded structure. The chief purpose of the tubular pouches is explained as "absorption" in contrast with mammals that function as fermentation. These are filled up as well as collapsed together by the anterior intestine, expanding the entire absorptive surface. Similar observations were detected in *Mugil cephalus* by **Farrag *et al.* (2020)**.

The present study observed that the intestinal mucosa is covered by columnar epithelia (enterocytes) and mucus secreting cells called the goblet cell, like in other teleosts. Histochemically, columnar cells contain neutral MPS content. The latter may help in its flexibility nature and its peristaltic movement. Goblet cells are relatively quite in size and number. It shows moderate reaction with Bromophenol stain while it shows more intensive positive reaction with PAS stain which indicates presence of mixture of acid & neutral MPS and different concentrations of neutral MPS. Similar observations were detected in *Liza* *aurata* **(Khalaf–Allah, 2001)**; *Siganus rivulatus* **(Abd Elwahab *et al*., 2017)** and *Mugil cephalus* **(Farrag *et al.*, 2020).**

The present study also observed that, the intestinal mucosa is covered by columnar epithelia (enterocytes) and dominant mucus cell type called the goblet cell, like in other teleosts. **Carrassón *et al.*, (2006**) stated that gel-forming mucin secretion produced by these goblet cells is involved in epithelial protection as well as in lubrication for nutrient pass way. The increased concentration of mucus-secreting cells appearing from the anterior to the posterior region followed by the rectum indicates that mucous production helps to defend the lining of the intestine and support waste exclusion **(Machado *et al.*, 2013).**

The mucosal layer is specialized and differentiated with distinct functions among the digestive tract regions include secretory cells that release large amounts of gel-forming mucin in the lumen **(Leknes, 2015)**. Thus, the mucosa has a vital function serving as a selective barrier and plays an essential role in digestive processes, ionic regulation and stress response **(Shephard, 1994; Cao & Wang, 2009** and **Sundh *et al.*, 2010)**. Moreover, the mucosal layer may be preventing of infections **(Ringo *et al.*, 2003; Grove *et al.*, 2006; McGuckin *et al.*, 2011;** and **Wang *et al.*, 2018)**.

The mucin histochemistry of digestive canal has been studied on different fish species that showed the variety among different species and along its alimentary canals **(Azab *et al.*, 1998; Khalaf–Allah, 2001; Diaz *et al.*, 2008; Faccioli *et al.*, 2014** and **Farrag, 2017)** Presence of mucus substances in the fishes' digestive tract is related to lubrication, inhibition of microorganisms, protection against degradation, beside osmotic function **(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).**

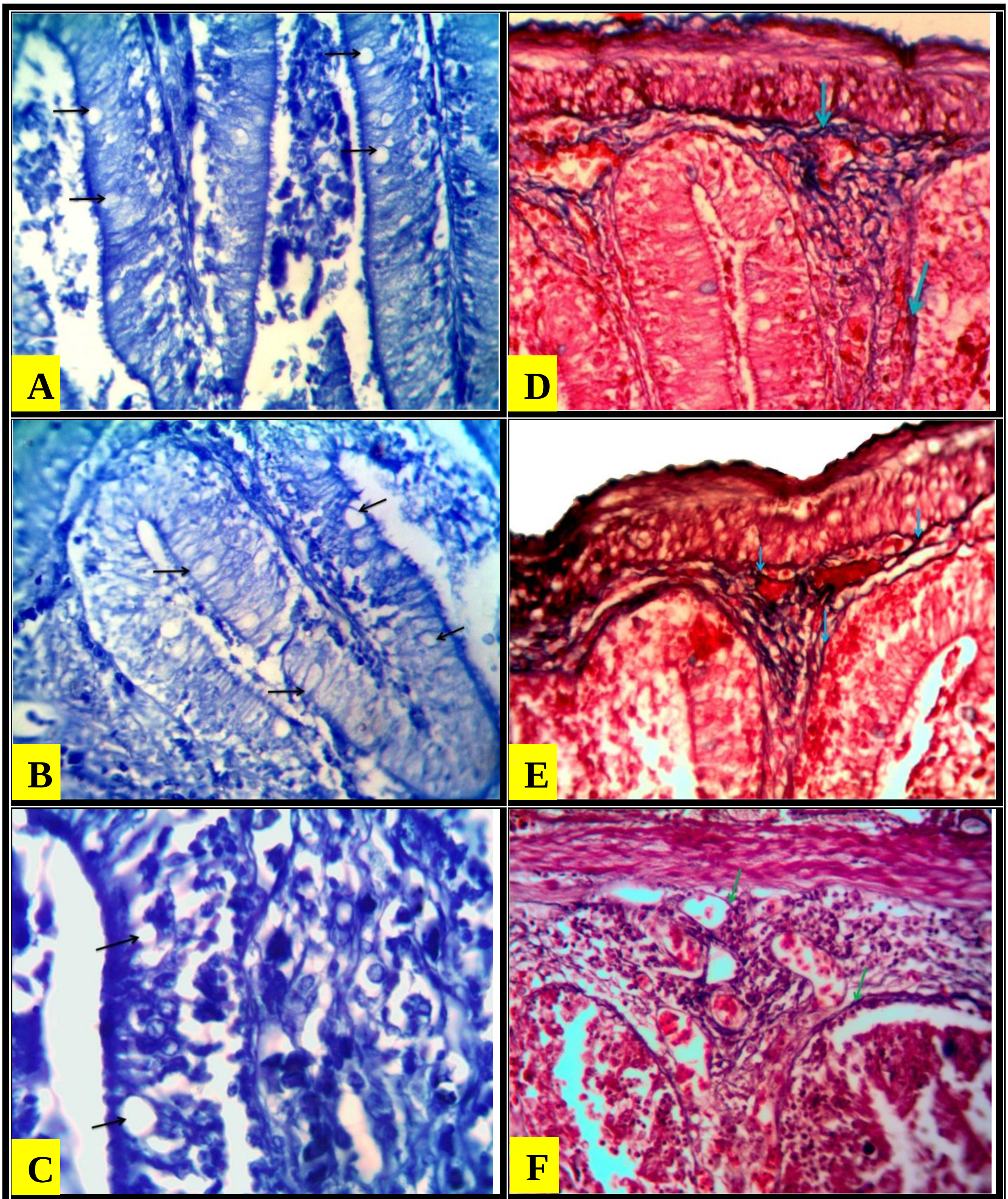

PLATE III

Photomicrographs of T. S. in the proximal pyloric caeca **(A&B)**, distal pyloric caeca **(C&D)** and anterior intestine **(E&F)** of *C. ramada* showing aqueous content (black arrows), elastic connective tissues (cyan arrows) and fibrous connective tissues (green arrows) **(Bromophenol; A&B: X400 & C; X1000 and Masson trichrome; D&E&F: X400).**

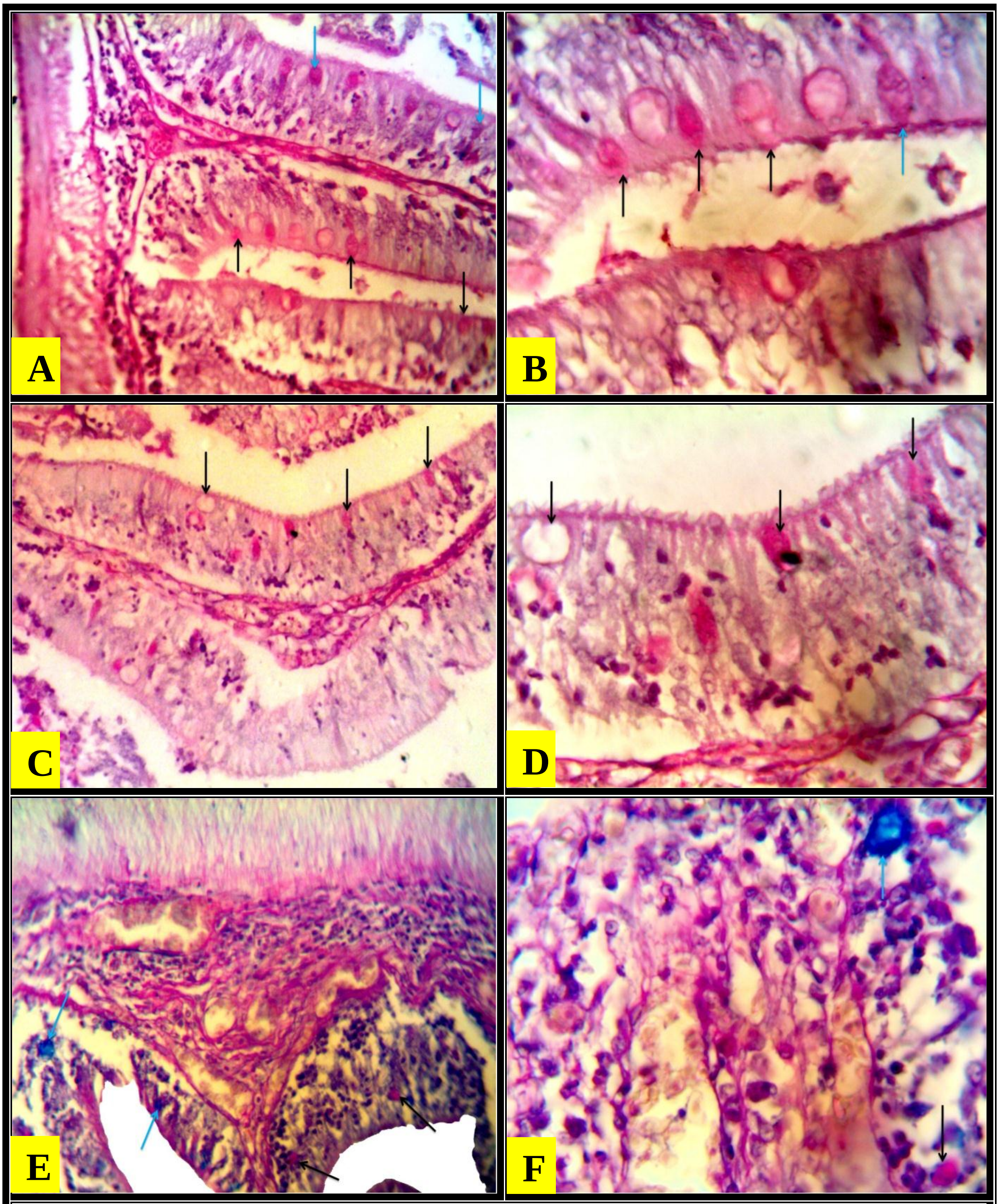

PLATE IV

Photomicrographs of T.S. in the proximal pyloric caeca **(A&B)**, distal pyloric caeca **(C&D)** and anterior intestine **(E&F)** of *C. ramada* showing mixture of acidic & neutral mocopolysaccharides (MPS) (cyan arrow) and different concentrations of neutral MPS (black arrows) **(Alcian PAS; A&C& E: X400 and B&D&F: X1000).**

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ARABIC SUMMARY

الإختلافات النسيجية والقدرة على التكيف في بعض أعضاء الجهاز الهضمي في سمكة الطوبار

ضياء مجدي جوده فراج، أحمد مسعد عزب، أحمد نصر محمد العبساوى
شعبة علوم البحار والأسماك - قسم علم الحيوان - كلية العلوم - جامعة الأزهر - القاهرة

تهدف الدراسة الحالية إلى دراسة التنوع فى التركيب الهستولوجي والهستوكيميائي لكلا من الأعاور البوابية والأمعاء الأمامية فى سمكة الطوبار. ولذلك ثلاثون عينة من أسماك الطوبار تم تجميعهم من دمرو – مدينة سيدى سالم – محافظة كفر الشيخ. وتم عمل قطاعات نسيجية من هذه الأعضاء وصباغتها بالطريقة المعتاده للدراسة النسيجية وكذلك استخدام بعض الصبغات الخاصة لتوضيح النشاط الكيميائي في الأنسجة، ثم فحص القطاعات وتصويرها ووصفها.

أوضحت نتائج الدراسة أن جدار الأعاور البوابية والأمعاء الأمامية في سمكة الطوبار لا يوجد بها إختلاف ملحوظ، حيث أنه يتكون من الطبقات الأساسية الأربعة (المصلية والعضلية وتحت المخاطية والمخاطية). وبينما تظهر ثنيات مخاطية عميقة في تجويف ضيق فى الأعاور البوابية ، وحيث أن سمكة البورى نباتية التغذية: فإن الثنيات المخاطية للأمعاء الأمامية تكون طويلة والتى هى متوائمة مع إبقاء الغذاء لكى يكتمل هضمه مع زيادة مساحة سطح الأمعاء للإمتصاص.
أوضحت النتائج أن الطبقة المخاطية لكلا من الأعاور البوابية والأمعاء الأمامية فى سمكة الطوبار تتكون من خلايا مخاطية وخلايا طلائية عمودية. بينت نتائج الدراسة أيضا أن الخلايا المخاطية تكون متوسطة الحجم والعدد في الأمعاء الأمامية ولكنها أقل في الأعاور البوابية. كما أن الخلايا الليمفاوية والخلايا المتجولة شائعة الإنتشار بين الخلايا العمودية للأمعاء الأمامية والأعاور البوابية بالإضافه لوجودها أيضا في الطبقة تحت المخاطية. وعلاوة على هذه الخلايا المناعيه فى الطبقة تحت المخاطية، توجد الأوعية الدموية بأعداد وأحجام كبيرة وتمتد داخل الثنيات المخاطية وقد تلعب دورًا في وظيفة الإمتصاص بالنسبة للأسماك.

خلصت هذة الدراسة الى أن التنوع فى التركيب الهستولوجي والهستوكيميائي لكلا من الأعاور البوابية والأمعاء الأمامية فى سمكة الطوبار أعطت تنوعا كبيراً مُلائما للوظيفة ومميزاً وفقاً لتوصيل وهضم وإمتصاص الغذاء، مما ساعد فى فهم ميكانيكية بعض الوظائف الفسيولوجية وأدى إلى معرفة فسيولوجيا الهضم فى هذا النوع من الأسماك.