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Histological and Histochemical Characteristics of the Digestive Tract of Tire Track Eel, *Mastacembelus Favus* (Hora, 1923) (Synbranchiformes: Mastacembelidae)

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Abstract

Mastacembelus favus (*M. favus*) is an important fish for local fisheries in Southeast Asia, however the corresponding microanatomy research has been scared. Therefore, this research was aimed to examine the histological and histochemical characteristics of the digestive tract of *M. favus*. The digestive tracts of ten adults *M. favus* from Phatthalung province, Thailand, were obtained using a paraffin technique process and stained with hematoxylin and eosin, periodic acid Schiff's, alcian blue pH 2.5 and AB pH 1.0. The findings explained that the digestive tract was consisted of pharynx, esophagus, stomach (cardiac, fundic and pyloric stomachs), pyloric caeca and intestine (anterior, middle and posterior intestines). The secreting-mucous and goblet cells of pharynx and esophagus was positively stained with PAS, AB pH 2.5 and pH 1.0, whereas the epithelium of stomach was only stained with PAS. The enterocytes of intestine and pyloric caeca were positively stained with PAS, whereas the goblet cells displayed positively stained with PAS and AB pH 2.5. All portions could secrete neutral glycoproteins, whereas only pharynx, esophagus and intestine could provide carboxylated glycoprotein. Meanwhile, sulfated glycoprotein was secreted by pharynx, esophagus and pyloric caeca. The gland was observed only in cardiac and fundic stomachs and its epithelium cell only secreted neutral glycoprotein. The wall of all portions surrounded by smooth muscle layers except for pharynx and esophagus were skeleton muscle. From overall results and intestinal coefficient (0.22) could indicate *M. favus* as a carnivorous fish. The present study may offer valuable profiles for investigating a comparative anatomy, ecology, pathology and future aquaculture.

Keywords: Carnivorous fish, Digestive system, Gastric gland, Glycoprotein, Histochemistry, Histology, Intestinal coefficient, *Mastacembelus favus*

Introduction

The digestive system is a crucial system for most living organisms because of providing the nutrients to produce adenosine triphosphate. However, the composition of digestive system in each species of vertebrate is different, including of fish, which has a diversity among species [1-3]. It's well known that the diversity of morphology and function depends on diet [2], eating behavior [4,5], body shape, evolution and environment factor. In aquaculture, the knowledge of the histological characteristics of a fish digestive system can be participated in assessing the pathological condition and promoted the nutrition and feed management [6]. The macroscopic characteristics of the fish digestive tract are generally similar among species,

but the secretion and other microscopic features are different [2]. Consequently, many studies have been conducted to clarify and identify the components of the digestive apparatus [2,7-10]. However, there are many specific differences in the digestive organs, particularly in anatomy, histology and histochemistry. For example, variations are observed in *Sphoeroides testudineus* [11], *Xiphophorus maculatus*, *X. helleri* [12], as well as in fish from the family Cyprinidae, Labridae, Gobiidae, Scaridae, Cyprinodontidae and some species in Poeciliidae that are stomachless fish [11,13]. These fish have an esophagus that directly connects to the intestine or is modified into an intestinal bulb [2]. In contrast, some fish possess glands in the esophagus, while others have taste buds in the pharynx and esophagus. In fact, each species has its own unique gastrointestinal tract morphology, histological structure and functional characteristic, particularly in stomach [14,15].

Based on the feeding habits and food consumption, fish can be categorized into three groups: Herbivorous, carnivorous, and omnivorous [16]. Herbivorous fish, consuming plants have a small stomach with less develop gastric glands. Compared to other fish, herbivorous species have the longest intestine [17]. Carnivorous fish, feeding animals have a large J- or U-shaped stomach with well-developed gastric glands and the shortest intestine [18]. Additionally, the gut is generally shorter than the body length [19]. Conversely, omnivorous fish consume both plant and animals, where the digestive tract morphology varies, depending on the primarily diet. Notably, the intestinal coefficient (IC) is used to assess the diet alongside anatomical, histological, stomach content and other methods. For example, the IC range for carnivorous fish is between 0.2 - 2.5, while the herbivorous and omnivorous fish are in the range from 0.8 - 15.0 and 0.6 - 8.0, respectively [20,21]. The fish digestive tract consists of the pharynx, esophagus, stomach and intestine [22]. Whereas the alimentary canal consists of all those portions except the pharynx [23]. Histological and histochemical profiles of the fish digestive system have been collected for improving the relation of anatomy, physiology [24], biology [25] and toxicology [26] among other fields. The specific sections of the digestive system have extensively focused, for example: The esophagus alone in *Heteropneustes fossilis* [27], both the esophagus and stomach in *Parupeneus forsskali*, *Popilloculiceps longiceps* and *Acanthurus sohal* [28]. As well as stomach alone in *Clarias gariepinus* [29], *Mystus cavasius, Oreochromis niloticus, Gudusia chapra* [30] and *Liza klunzingeri* [31]. Besides, other works have been investigated from the esophagus to the intestine, such as *Oncorhynchus mykiss* [2], *Trichomycterus bogotensis* [3], *Synodus variegatus* [5], *Larimichthys crocea* [6], *Alestes baremoze* [8], *Sarpa salpa* [17], *Lates calcarifer* [21], *Cichlasoma dimerus* [25], *Scorpaena porcus* [32], *Merluccius merluccius* [33] and *Sparus aurata* [34]. Furthermore, the entire digestive tract has been investigated, from the pharynx to the intestine [11], or the intestine alone such as in *X. helleri* [12], *Misgurnus anguillicaudatus*, *Ctenopharyngodon idellus*, *Pelteobagrus fulvidraco* [35]. The pharynx is located between mouth and esophagus, where its lateral sides are connected to the gills. The esophagus, a short muscular tube is responsible for transport food to the stomach or intestine in agastric fish [36]. The shape, portion and size of stomach can vary significantly among different species. This organ plays a key role in food storage, hydrochloric acid (HCl) production and protein digestion [17,21]. The intestine is the major site for the digestion and nutrient absorption, opening to external body by passing the anus.

The tire track eel, *Mastacembelus favus* (Hora, 1923), belongs to the family Mastacembelidae. The form of slender body is elongated, and delicately flattened on lateral side. The caudal, dorsal and anal fins are connected, while the pectoral fins are large. The body color varies from light gray to yellowish-brown with black bands of ring or net-like pattern covering the entire side of the body. The body size is large up to 70 cm in length and it feed on insect larvae, worms and various organic matter [37]. The *M. favus* has high economic value in local fisheries due to its delicious meat [38]. In many areas, the demand for its meat is very popular, leading to more farming. Recently, numerous studies have examined on various biological aspects of reproduction [39], genetic diversity [40] and dietary composition (additional ingredients in *M. favus* feed) [41] as well as in fingerlings *M. favus* feed [42]. As we known, the histology and histochemistry of the body system of *M. favus*, particularly the digestive system, have scarcely explored, which is crucial for survival. Therefore, for this work, the histological and

histochemical characteristics of the digestive tract of *M. favus* were investigated seriously. It may offer valuable knowledge and insights about the roles of *M. favus* on an ecosystem that additionally applied in physiology and histopathology, as well as future aquaculture practices.

Materials and methods

Sample collection

Ten adult tire track eels, *M. favus* (five males and five females) has mean total length 38.14 ± 2.45 cm (**Table 1**), and were collected from Phatthalung province, Thailand (7°15'05.3"N 100°26'24.0" E) during September, 2024 to January, 2025. The samples were carried to the laboratory at the Faculty of Science and Digital Innovation, Thaksin University. The *M. favus* was anesthetized using a dosage of 100 mg·L^{-1} of tricaine methanesulfonate (MS-222) solution [43]. After anesthesia, an abdominal incision was made from the anus to the mouth for observing the position of the organs and taking photographs. The digestive tract, including of the pharynx, esophagus, stomach and intestine, was removed. Each portion was measured by using a measuring tape. The intestinal length (IL) was used to calculate the intestinal coefficient (IC), as follows: [21,44].

$$\mathrm{IC} = \frac{\text{Intestinal length (IL)}}{\text{Standard length (SL)}} \quad (1)$$

The digestive tracts were placed in Bouin's fixative for 48 h and then preserved in 70% ethanol. This research received the approval from the Animal Ethics Screening Committee, Thaksin University (Permit number: COA TSU 2024-009 IACUC No. 0009).

Histological and histochemical study

The digestive organs were cut into small pieces (1×1 cm) and processed using a paraffin technique. This involved dehydration with a graded series of ethanol (70%, 80%, 95% and absolute ethanol), clearing with xylene, infiltration and embedding with Paraplast Plus® (Sigma-Aldrich, USA). The 5 µm serial sections were produced by rotary microtome. Subsequently, these sections were stained with 5 stains dyes, namely, hematoxylin and eosin (H&E) for charactering general tissue, periodic acid Schiff's (PAS) for describing neutral mucopolysaccharides/glycoproteins, alcian blue pH 2.5 (AB pH 2.5) and AB pH 1.0 for demonstrating carboxylated and sulfated acid mucopolysaccharides/glycoproteins, respectively and Masson's trichrome (MT) for discriminating muscle tissue form connective tissue (collagen fiber) [45]. Permanent slides were examined under a compound light microscope and photographed using a digital camera.

Histometric analysis

Histological metrics were used to describe and discriminate each portion of the digestive tract. Images of each organ were conducted using Image J software to measure various histological parameters. The data were presented as mean ± standard deviation.

Results and discussion

Gross anatomy

The digestive apparatus of the tire track eel, *M. favus,* with the mean tract length of 20.10 ± 0.53 cm (**Table 1**), was covered by the peritoneum, consisting of pharynx, esophagus, stomach and intestine (**Figures 1(A) - 1(C)**). The composition of its digestive tract is similar to those of other fish, which was different from stomachless fish [12]. In *S. testudineus*, the posterior esophagus could generate a sac-like structure called the abdominal pouch, which was directly connected to the intestine [11,12]. Observation at the ventral body and anatomical position, the pharynx linked with the mouth to the esophagus and gills. The pharynx wall exhibited thickened-longitudinal folds and surrounded by muscular layer. The esophagus is a short-straight and thick-wall muscular tube that located behind the liver, connecting the pharynx to the stomach (**Figure 1(A)**). For the esophagus, the mean length was 2.20 ± 0.26 cm, contained the longitudinal folds. The findings showed that the characteristics of pharynx and esophagus were in accordance with those reported in the previous researches [5,8,17,25,33]. The presence of longitudinal folds and distensibility could expand the pharynx and esophagus of *M. favus* while swallowing large prey. Similar findings have been reported in *Tilapia spilurus* [4], *S. variegatus* [5], *P. forsskali*, *P. longiceps A. sohal* [28] and *Tylosurus choram* [46]. At the gastroesophageal junction, the transition from the esophagus to stomach was observed by the constrict

portion or sphincter, where the stomach diameter exhibited a clearly larger than that of esophagus (**Figures 1(A)** and **1(C)**). The muscular sac stomach was white J-shaped, and in ventral side of the body, situated beneath the liver with a mean length of 4.90 ± 0.10 cm (**Table 1**). The stomach is divided into three portions, namely, cardiac, fundic and pyloric stomach (**Figures 1(A) - 1(C)**), according to the findings in *O. mykiss* [2], *A. baremoze* [8] and *L. calcarifer* [21]. However, some species such as *C. gariepinus* [29] and *L. klunzingeri* [31], possessed only two regions; cardiac and pyloric stomach. In *M. favus,* the fundic region occupied in the largest portion of the stomach. The pyloric stomach was the ascending terminal curve of the J-shaped stomach, creating a narrow section that connected to the intestine (**Figures 1(A) - 1(C)**). The J-shaped stomach was in accordance with those of *C. gariepinus* [9], *H. fossilis* [27] and *Rhamdia quelen* [47], however, such species as *O. mykiss* [2], *M. cavasius* [30] and *L. klunzingeri* [31], contained U-shaped stomach. Moawad *et al*. [9] stated that the J-shaped stomach might prolong the food storage time, and mix food with digestive enzymes [48]. In addition, the sac-like stomach of *M. favus* could be stretched, reflecting a feeding type of carnivorous fish. At the junction between the pyloric stomach and anterior intestine, two short finger-like straight pyloric caeca were observed. These structures were blind-ended extensions, branching from the stomach-intestinal junction, with a mean length of 0.97 ± 0.12 cm (**Table 1**). The observation of pyloric caeca was corresponding to those of *L. klunzingeri* [31] and *Mugil cephalus* [49], whereas, *C. dimerus* [25], lacked pyloric caeca entirely. The number of pyloric caeca is different based on fish species, for example: *S. salpa* contained four pyloric caeca [17], while the species in genus *Mugil* possessed 2 to 22 and the species in genus *Liza* presented two seventeen [49,50]. The intestine extended from the pyloric stomach, separated by a sphincter and occupied in the abdominal cavity, was the longest portion of the digestive organ with a mean length of 8.21 ± 0.15 cm (**Table 1**). From gross anatomy, the distinct intestinal regions could not be clearly differentiated, however, the diameter gradually decreased from the anterior to the posterior intestine (**Figures 1(A) - 1(C)**). These characteristics were consistent to those observed in *T. bogotensis* [3] and *Barbus altianalis* [51]. At the terminal end, the posterior intestine opened to the external body through the anus.

Table 1 Mean ($\bar{x}$) and standard deviation (S.D.) digestive tract length (cm) and intestinal coefficient (IC) in *M. favus* (n = 10).

	Standard length	Digestive tract length	Pharynx-esophagus length	Stomach length	Pyloric caeca length	Intestinal length	IC
$\bar{x}$ ± S.D.	38.14 ± 2.45	20.10 ± 0.53	2.93 ± 0.15	4.90 ± 0.10	0.97 ± 0.12	8.21 ± 0.15	0.22

Histological and histochemical characters

The digestive tract wall of *M. favus* comprised of four tunics arranged from the inner to outer layers, namely, mucosa, submucosa, muscularis and serosa. The observed structure was consistent with those found in other fish [2,11,25]. The mucosa could be further divided into an epithelium (packed-cell situating on basement membrane), a lamina propria, a layer of connective tissue, nerves and blood vessels as well as muscularis mucosae and a thin band of muscle cells, however, some portions lacked the muscularis mucosae. The submucosa was composed of the connective tissue, without glands. The muscular tunic was consisted of two layers; an inner circular layer and an outer longitudinal layer, where both contained nerve plexuses. The outermost layer was the serosa, which contained thin connective tissue, blood vessels and mesothelium.

The pharynx

The mucosa consisted of the epithelium and lamina propria, but lacked a muscularis mucosae (**Figure 2(A)**). The mean fold width was 249.75 ± 14.94 μm (**Table 2**). The mucosal epithelium was lined by non-keratinized stratified squamous epithelium with the number of layers of epithelial cell ranging from 4 - 7 layers (**Figures 2(B) - 2(F)**). The mean epithelial layer thickness was 31.72 ± 4.49 μm (**Table 2**). The cells were divided into four types; globular unicellular mucous cell, goblet cell, non-mucous cell and basal cell. The nuclei of globular unicellular mucous and goblet cells

located at the basal domain, while the cytoplasm was filled with mucin substances (**Figures 2(B) - 2(F)**).

The shape of globular unicellular mucous cells was round, while the goblet cells were goblet-shaped. The non-mucous cells found an oval nucleus that located centrally within the cell (**Figures 2(A) - 2(B)**). The mucins were appeared in the cytoplasm of the globular unicellular mucous cells and goblet cells that strongly stained with PAS (**Figure 2(D)**) and AB pH 2.5 (**Figure 2(E)**) and weakly stained with AB pH 1.0 (**Figure 2(F)**). These results were indicatable that both cell types secreted the neutral mucins, carboxylated and sulfated acid mucins, respectively. Whereas the cytoplasm of non-mucous cells showed negatively staining with PAS (**Figure 2(D)**), AB pH 2.5 (**Figure 2(E)**) and AB pH 1.0 (**Figure 2(F)**). These staining profiles in *M. favus* were consistent to that found in *B. altianalis* [51]. The neutral mucins in the pharynx assisted on transporting the food to esophagus [27], while the carboxylated acid mucins might aid in the lubrication and facilitated the food movement. In addition, the sulfated mucins might serve as a defense mechanism, protecting against a microbial invasion or harmful pathogen [51].

The stratified squamous epithelium found in *M. favus*, was according to those of *X. maculatus*, *X. helleri* [12], *B. altianalis* [51] and *Anguilla anguilla* [52] but the taste bud was not observed for *M. favus*. In contrast, taste buds were found in those fish [12,51], suggesting that *M. favus* was unable to perceive taste. The stratified epithelium of the pharynx likely played a protective role, shielding the underlying tissue from mechanical injury, bacterial invasion and chemical damage. Due to lacking a muscularis mucosae, it could not discriminate the boundary between the lamina propria and submucosa. The muscular layer was composed of a thick skeleton muscle, where muscle fibers arranged in longitudinal direction (**Figure 2(A)**). These characteristics were similar to that observed in *B. altianalis* [51]. The skeletal muscle of pharynx aided in swallowing under voluntary control and helped to expel unpleasant food. The mean muscular layer thickness was 245.00 ± 17.59 µm (**Table 2**).

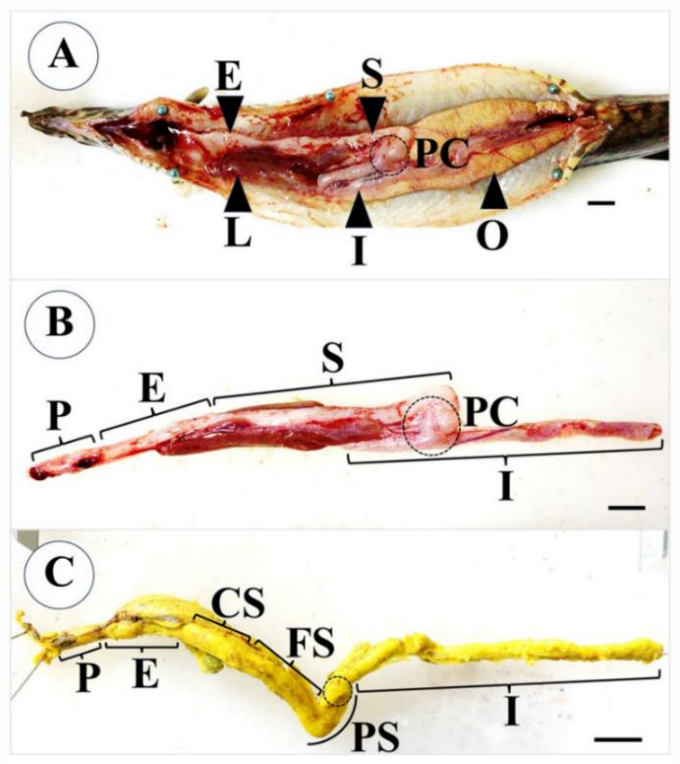

Figure 1 (1A) - (1C) The organs of digestive tract, consisting of pharynx (P), esophagus (E), stomach (S) and intestine (I), where the stomach containing three regions; cardiac stomach (CS), fundic stomach (FS) and pyloric stomach (PS). The dotted lone circle representing two pyloric caeca (PC). Liver (L) and ovary (O), with scale bar = 1 cm.

Table 2 Mean ($\bar{x}$) and standard deviation (S.D.) of the measurements (μm) of mucosal fold width (MFW), epithelial layer thickness (ELT), muscular layer thickness (MLT) of the pharynx and mucosal fold width, mucosal fold height (MFH), epithelial layer thickness, and muscular layer thickness of the esophagus in the *M. favus* (n = 10).

	Pharynx			**Esophagus**			
	MFW	**ELT**	**MLT**	**MFW**	**MFH**	**ELT**	**MLT**
$\bar{x}$ ± S.D.	249.75 ± 14.94	31.72 ± 4.49	245.00 ± 17.59	232.85 ± 21.65	993.00 ± 21.51	40.84 ± 4.31	302.91 ± 49.53

The esophagus

The mucosa of esophagus showed long-thick longitudinal folds, which was round blunt tips. The mucosal fold was consisted of three layers (epithelium, lamina propria and muscularis mucosae) (**Figure 2(G)**). The mean fold width and height were 232.85 ± 21.65 and 993.00 ± 21.51 μm, respectively (**Table 2**). The epithelium was lined by the stratified squamous/cuboidal epithelium, which contained of goblet cells that located between the globular unicellular mucous cells. Furthermore, non-mucous cell and basal cell were observed (**Figures 2(H) - 2(J)**). The number of epithelial cell layers was approximately 6 - 9 layers and the mean epithelial layer thickness was 40.84 ± 4.31 μm (**Table 2**). At the base of the epithelium, the basal cells were cuboidal in shape (**Figures 2(I) - 2(J)**). Furthermore, the lymphocyte was found near the base of epithelium with the nucleus oval and darkly stained with H&E (**Figures 2(H)**).

The stratified squamous/cuboidal epithelium of the esophagus in *M. favus* (**Figures 2(H) - 2(I)**) was similar to those observed in many fish but differed from some fish showing various apical cells. For example, *A. baremoze* [8], *C. dimerus* [25], *M. merluccius* [33] and *A. anguilla* [52] presented the stratified squamous epithelium and *S. variegatus* [5] contained simple columnar epithelium, as well as *Anablepsoides urophthalmus* possessed the stratified cuboidal epithelium [53] or pseudostratified ciliated columnar epithelium in *A. bicolor bicolor* [54]. Although, the apical cells of the stratified epithelium varied in shape, the basal and intermediate cells were typically cuboidal or polygonal in many fish [27,25,33].

The difference between the pharynx and esophagus lied in the transition from the stratified squamous epithelium of pharynx to the stratified squamous/cuboidal epithelium of the esophagus, furthermore, the number of epithelial cell layers was also different. The globular unicellular mucous cells and goblet cells were positively stained with PAS (**Figure 2(J)**), AB pH 2.5 (**Figure 2(K)**) and pH 1.0 (**Figure 2(L)**), whereas the basal cells were negatively stained. The findings of positive-staining with PAS and AB pH 2.5 in *M. favus* was in accordance with to those observed in many fish [21,25,27,34,51], however, the cells were positively stained with AB pH 1.0, which was different from *Odontesthes bonariensis* [55] that were negatively stained with AB pH 1.0. For positively staining with AB pH 1.0 of cell of *M. favus* could demonstrate the presence of sulfated mucin, which contributed to high viscosity of the mucus that aided in trapping small particles.

The esophagus exhibited a greater thickness than that of pharynx due to multiple cell layers. Therefore, it was assumed that the esophagus served as a connection between the oro-pharynx and gill, both of which were exposed to the environment. Thus, the epithelium thickness of esophagus might play a key role in the protection against the abrasion, facilitating the transport of rough solid food [27], and preventing other injuries. Correspondingly, the positive-staining of globular unicellular mucous and goblet cells with AB pH 2.5 and AB pH 1.0 could be indicatable that both pharynx and esophagus protected the mucosa from microbial invasion, various pathogens and chemical factors [27,28]. Furthermore, PAS-positive mucus in globular unicellular mucous and goblet cells also contributed to the lubrication and mucosal protection [11,27]. However, several authors suggested that the abundance of goblet cells observed in both pharynx and esophagus represented an adaptation to compensate for the absence of salivary glands. Besides, carnivorous fish tend to have more mucous and goblet cells than those of herbivorous fish [51]. Meanwhile, the taste bud was absent in the esophagus, corresponding to other fish species [27,32] and carnivorous species i.e. *A. bicolor*

bicolor [54]. Based on the observation, it was thought that the esophagus functioned solely in conducting and swallowing food rather than perceiving taste.

The lamina propria and submucosa were comprised of dense connective tissue (**Figures 2(G) - 2(I)**), which were richly supplied with blood vessels and fibrocytes. The muscularis mucosae was not observed in *M. favus,* similar to other fish species [25,27,32,33]. It was found that *M. favus* lacked esophageal glands (**Figure 2(G)**), according to other fish species [25,53], but differed from *S. testudineus* [11]. However, the esophageal gland in *S. testudineus* was the compensatory structure because *S. testudineus* was no stomach [11]. Fagundes *et al*. [11] suggested that the esophageal gland secreted the variety of glycoconjugate mucins, depending on the feeding habits and compensated for the absence of salivary glands in fish [21]. Although *M. favus* was no esophageal gland, its epithelium contained numerous PAS and AB pH 2.5 and pH 1.0 positively globular unicellular mucous and goblet cells. Therefore, the mucins secreted by these cells might functionally compensate for the absence of salivary glands.

Underneath the submucosa, the inner longitudinal and outer circular skeleton muscle layers were observed (**Figure 2(G)**), corresponding to the findings reported by other fish species [3,6,25,32] including of *A. bicolor bicolor* [54] and *Macrognathus siamensis* [56]. However, another work [57] displayed a longitudinal direction of skeleton muscle in both layers of *Belone belone* or inner circular and outer longitudinal layers in *A. baremoze* [8], *L. calcarifer* [21], *A. urophthalmus* [53] and *Hydrocynus forskahlii* [58]. The presence of skeleton muscle in esophageal wall of *M. favus* could reject the undesirable food and control the swallowing by the peristalsis, as well as exert the voluntary force for food movement. Therefore, these findings were indicatable that *M. favus* might have a greatly efficient mechanism for controlling the swallowing process. Moreover, the carnivorous fish have a thicker muscular layer than that of herbivorous fish, which helped to contract and protect the mucosa from the damage during prey swallowing [54]. The mean muscular layer thickness was 302.91 ± 49.53 µm (**Table 2**).

The stomach

Histologically, the J-shaped stomach of *M. favus* was consisted of four basic layers; mucosa, submucosa, muscularis and serosa. The stomach was divided into three regions, namely, cardiac, fundic and pyloric stomachs. These three portions were similar to other fish such as *H. forskahlii* [58] and *M. siamensis* with the same family [56]. The mucosal folds in all regions fabricated longitudinal ridges or rugae and the muscularis mucosae was observed. The mucosa was discriminated from the submucosa layer by a thin muscularis mucosae. The muscular layer in all three regions contained an inner circular and an outer longitudinal smooth muscle layer, although at the end of pyloric stomach or pyloric sphincter, three layers of smooth muscle could be observed in some regions.

Gastric glands were present in the lamina propria of cardiac and fundic regions, but are absent in the pyloric region. The submucosa in all three regions was consisted of dense connective tissue, blood vessels, smooth muscle that mixed with collagen fiber and lymph vessels, functioning to limit excessive expansion of the stomach [2]. The serosa was represented by lining of mesothelial cells, blood vessels, thin band of smooth muscle and loose connective tissue, where the structure was consistent with that observed in other fish species [2,3,8,25,28].

The cardiac stomach was connected to the esophagus and at the junction transition of esophagus to cardiac stomach was observed by abruptly changing of epithelium, gastric gland and muscular sphincter. The epithelium changed from the stratified cuboidal epithelium of the esophagus to a simple columnar epithelium in the stomach, where the gastric gland was observed. Additionally, the striated muscle of the esophagus was replaced by the smooth muscle in stomach. These observations were consistent with the findings in other fish species [30,59,60]. The mucosa of cardiac stomach of *M. favus* was consisted of epithelium, lamina propria and muscularis mucosae (**Figures 3(A) - 3(B)**). The mucosa and submucosa layers generated wide-large and tall longitudinal folds with round tips for supporting the expansion of the stomach to allow for food storage [2]. The mean mucosal fold width and height were 702.73 ± 119.89 and 1,465.82 ± 239.19 µm, respectively (**Table 3**). The cardiac epithelium was lined by a simple tall columnar

mucous-secreting epithelium and goblet cell was absent (**Figures 3(A) - 3(F)**). These results were in accordance with those observed in *L. crocea* [6], *C. dimerus* [25], *L. klunzingeri* [31], *A. bicolor bicolor* [54] and *M. siamensis* [56]. Meanwhile, the nuclei of the epithelial cells located in the basal region, where the mean epithelial cell height was 18.53 ± 2.44 µm (**Table 3**). Histochemical analysis revealed that the epithelial cells showed strong PAS-positive staining (**Figure 3(D)**) but were negatively stained with AB pH 2.5 (**Figure 3(E)**) and AB pH 1.0 (**Figure 3(F)**). The presence of PAS-positive epithelium in the cardiac region was consistent with those observed in many fish [6,9,21,25] including of *A. bicolor bicolor* [54].

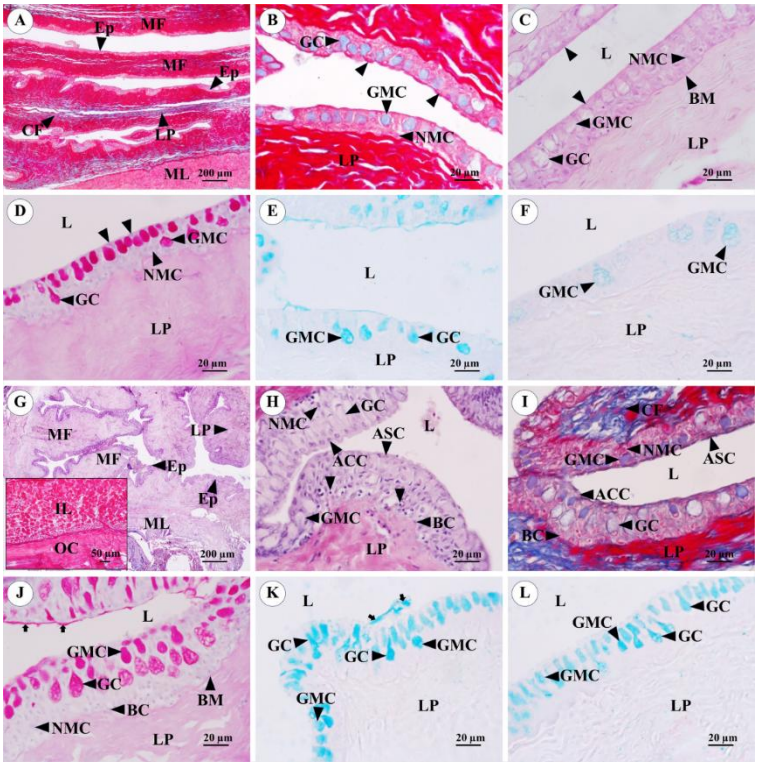

Figure 2 (2A - 2F) Pharynx of *M. favus*. (2A): Distinct layers, including epithelium (Ep) of mucosal fold, lamina propria (LP) and muscularis layer (ML). (2B - 2C): An enlarged picture of mucosa of pharynx, depicting goblet cell (GC), globular unicellular mucous cell (GMC) and non-mucous cell (NMC). (2D - 2F): Goblet cell and globular unicellular mucous cell of pharynx, containing neutral mucins, carboxylated and sulfated acid mucins, respectively. (2G): An enlarged view of esophagus, consisting of epithelium and lamina propria of mucosal folds. The box highlights the skeleton muscle of esophagus, consisting of inner longitudinal (IL) and outer circular (OC) layers. (2H - 2I): An enlarged picture of mucosa of esophagus, depicting goblet cell, globular unicellular mucous cell, non-mucous cell, basal cell (BC) and representing apical squamous-shaped cell (ASC) and apical cuboidal-shaped cell (ACC). (2J - 2L): Goblet cell and globular unicellular mucous cell, containing neutral mucins, carboxylated and sulfated acid mucins, respectively. Basement membrane (BM), collagen fiber (CF), lumen (L), muscular layer (ML), arrow head in (2B - 2D) showing apical squamous cells, arrow head in (2H) showing lymphocyte, arrow in (2J - 2K) showing PAS-positive mucin and AB pH 2.5-positive mucin, respectively. (2A - 2B, 2I and the box in 2G: MT), (2C, 2G - 2H: H&E), (2D, 2J: PAS), (2E, 2K: AB pH 2.5), (2F, 2L: AB pH 1.0).

The gastric gland in the cardiac stomach, also known as the cardiac gland, was a simple tubular gland that lined by cuboidal cells or polygonal shape (**Figures 3(C) - 3(F)**). The cardiac gland opened into the lumen through gastric pits, which was arisen from the invagination of the epithelium. These characteristics are comparable to those observed in many fish [2,8,9,25,28]. Vieira-Lopes *et al*. [61] described that

these features were common in carnivorous fish such as *A. bicolor bicolor* [54]. The mean cardiac gland width and height were 71.26 ± 6.38 and 58.70 ± 4.85 µm, respectively (**Table 3**). The epithelial cells of the gland exhibited weak PAS staining (**Figure 3(D)**) but were negatively stained with AB pH 2.5 (**Figure 3(E)**) and AB pH 1.0 (**Figure 3(F)**) that were consistent with the observations in *C. gariepinus* [9] and *C. dimerus* [25]. The cardiac gland was surrounded by thin collagen fibers (**Figures 3(A) and 3(C) - 3(D)**). Beneath the gland, the muscularis mucosae was present (**Figures 3(A) - 3(B)**) that also observed in many species, but was absent in *O. mykiss* [2]. The submucosa of the cardiac stomach possessed the dense connective tissue containing numerous blood vessels, lymph vessel and fibrocytes. Additionally, smooth muscle fibers occupied within the submucosa. The muscularis layer of the cardiac stomach was comprised of inner circular and outer longitudinal layers (**Figures 3(A) - 3(B)**) with a mean thickness of 314.24 ± 14.63 µm (**Table 3**). The smooth muscle was to increase the digestion efficiency by contracting to facilitate the food movement and churning.

Table 3 Mean ($\bar{x}$) and standard deviation (S.D.) of the measurements (µm) of epithelial cell height (ECH), mucosal fold width (MFW), mucosal fold height (MFH), muscular layer thickness (MLT) of cardiac and fundic stomachs, cardiac gland width (CGW), cardiac gland height (CGH) of cardiac stomach, fundic gland width (FGW), fundic gland height (FGH) of fundic stomach in the *M. favus* (n = 10).

	Cardiac stomach						Fundic stomach					
	ECH	**MFW**	**MFH**	**MLT**	**CGW**	**CGH**	**ECH**	**MFW**	**MFH**	**MLT**	**FGW**	**FGH**
$\bar{x}$ ± S.D.	18.53 ± 2.44	702.73 ± 119.89	1,465.82 ± 239.19	314.24 ± 14.63	71.26 ± 6.38	58.70 ± 4.85	24.43 ± 3.54	865.87 ± 73.55	1,556.07 ± 60.38	393.47 ± 61.97	29.90 ± 3.89	199.51 ± 22.69

The mucosa of the fundic stomach was lined by simple columnar epithelium (**Figures 3(G) - 3(L)**), where the epithelial cells stained positively with PAS (**Figure 3(J)**) and provided the staining negatively with AB pH 2.5 (**Figure 3(K)**) and pH 1.0 (**Figure 3(L)**), indicating the presence of neutral mucins. The mean epithelial cell height was 24.43 ± 3.54 µm (**Table 3**). The mucosal folds were resembled to those of the cardiac stomach, but were wider and taller. The mean mucosal fold width and height were 865.87 ± 73.55 and 1,556.07 ± 60.38 µm, respectively (**Table 3**).

The gastric or fundic gland was a simple tubular gland, occasionally exhibiting a slight coiling (**Figures 3(G) - 3(L)**). The mean gland width was 29.90 ± 3.89 µm and the mean gland height was 199.51 ± 22.69 µm (**Table 3**), which was taller than that of the cardiac gland. The fundic gland opened into the lumen through gastric pits**.** However, the epithelium of gland was lined by simple cuboidal-columnar and polygonal cell (**Figures 3(H) - 3(L)**), which was weak-stained positively with PAS (**Figure 3(J)**) and negatively with AB pH 2.5 (**Figures 3(K)**) and AB pH 1.0 (**Figures 3(L)**). The fundic glands were surrounded by a thin collagen fiber (**Figures 3(H) - 3(J)**), at the beneath, the muscularis mucosae was present. The submucosa was consisted of dense connective tissue and the muscular layer was composed of two sub-layers; an inner circular and an outer longitudinal layer (**Figure 3(G)**). These profiles were corresponding to the observation in *C. gariepinus* [9]. The mean muscular layer thickness was 393.47 ± 61.97 µm (**Table 3**).

The mucosa of pyloric stomach in *M. favus* generated the longitudinal folds that was corresponding to those observed in the cardiac and fundic stomach, but were the shortest folds among the three stomach regions. The shape of fold was blunted in the terminal end (**Figure 4(A)**), meanwhile the mean mucosal fold width and height were 727.80 ± 104.54 and 1,150.49 ± 240.87 µm, respectively (**Table 4**). The epithelium of the pyloric stomach was lined by simple tall columnar epithelium, which formed the pits but lacked gastric glands (**Figures 4(B) - 4(C)**). The mean epithelial cell height was 26.88 ± 4.29 µm (**Table 4**). This epithelium stained positively with PAS (**Figures 4(D)**) but negatively stained with AB pH 2.5 (**Figure 4(E)**) and AB pH 1.0 (**Figure 4(F)**). The absence of gastric glands

made a wider lumen, where the phenomena were consistent with those observed in many fish [25,29,30], which offered the difference of the pyloric stomach from other two stomach regions.

The submucosa of pyloric stomach was consisted of thin dense connective tissue, whereas a very thick muscular layer was observed, composing of two distinct layers, namely, an inner circular and an outer longitudinal layer (**Figure 4(A)**). These thick muscular layers played a crucial role in regulating the passage of the digested food into the intestinal portion. Furthermore, the thick muscularis externa of pyloric stomach acted as a pyloric sphincter. The observed structure in of *M. favus* aligned with the findings in other studies [4,62]. The mean muscular layer thickness was 1,126.67 ± 80.67 µm (**Table 4**).

The mucous-secreting cell with apical mucus, occupied throughout all regions of the stomach in *M. favus*, was according to those observed in other fish species [29,30]. The epithelium stained positively with PAS but negatively stained with AB pH 2.5 and AB pH 1.0, indicating that mucous-secreting cell synthesized and secreted only neutral mucopolysaccharides. These phenomena were according to the previous studies on *C. gariepinus* [9], *C. dimerus* [25], *M. cavasius, O. niloticus, G. chapra* [30], *Astyanax bimaculatus* [63] and *A. bicolor bicolor* [54]. The neutral mucopolysaccharides might play the role in the food conduction and the protection of the epithelium from high acidity, digestive enzymes, autodigestion [21,64,65] and mechanical injury [9,30,66]. Additionally, Mokhtar *et al*. [67] suggested that the neutral mucins contributed to the absorption of disaccharides and short-chain fatty acids. Moreover, the glycosaminoglycans secreted by epithelial cells, exhibited hydrophilic properties, attracting water to create a viscoelastic barrier, which ensured the mucosal hydration and protection [2,68,69]. In *M. favus*, the gastric glands, specifically the cardiac and fundic glands, were observed in the cardiac and fundic stomach, respectively. This classification allowed the use of the term glandular stomach for both cardiac and fundic regions. In contrast, the pyloric stomach lacked a gastric gland that was therefore referred to as the non-glandular stomach, corresponding to the previous studies on *Mylio cuvieri* [4], *C. gariepinus* [9], *Mulloidichthys flavolineatus* [62] and *L. crocea* [6].

The gastric glands in both cardiac and fundic stomach of *M. favus* exhibited a positive PAS reaction, according to the observation in *M. cavasius, O. niloticus* and *G. chapra* [30]. Moawad *et al*. [9] explained that the epithelial cells lining these glands were oxynticopeptic cells. Many researchers agreed that in fish, amphibians and birds, the oxynticopeptic cells were the sole cell type secreting both of pepsinogen and hydrochloric acid (HCl). Contrasting with mammals, the gastric glands contained two distinct cell types; chief cells-secreting the pepsinogen and parietal cells-secreting HCl [9,70]. Therefore, it was assumed that the gastric gland cells in *M. favus* were oxynticopeptic cell, as observed in most fish species. However, next studies using advanced techniques could confirm and characterize these cells. Because the major foods of *M. favus* is microinvertebrates such as crustaceans and insect larvae, therefore the histological structure and histochemistry of stomach reflected the carnivorous feeding habits of the *M. favus*. Additionally, the absence of gastric glands in the pyloric stomach of *M. favus* might serve to reduce the acid content, entering the anterior intestine, thereby optimizing the function of pancreatic enzymes in an alkaline environment.

A comparative observation of the muscular layer thickness of the cardiac stomach, fundic stomach and pyloric stomach revealed that the muscle layer of pyloric stomach was the thickest, corresponding to the findings in other fish species [9,31,48] including of *H. forskahlii* [58], *A. bicolor bicolor* [54] and *M. siamensis* [56]. In addition, the gland was not observed in the pyloric stomach, indicating that the pyloric primarily functioned in the food storage [21] and in the contraction to facilitate the influx of food or chyme into the cardiac portion, where food was churned and mixed with HCl and enzymes. Importantly, the thickest muscular layer in the pyloric stomach functioned as the pyloric sphincter. The pyloric sphincter also played a critical role in preventing food from being transported to the anterior intestine without undergoing chemical digestion [59] and in regulating the passage of food into the intestine [28].

Table 4 Mean ($\bar{x}$) and standard deviation (S.D.) of the measurements (μm) of epithelial cell height (ECH), mucosal fold width (MFW), mucosal fold height (MFH) and muscular layer thickness (MLT) of pyloric stomach and pyloric caeca in the *M. favus* (n = 10).

	Pyloric stomach				Pyloric caeca			
	ECH	**MFW**	**MFH**	**MLT**	**ECH**	**MFW**	**MFH**	**MLT**
$\bar{x}$ ± S.D.	26.88 ± 4.29	727.80 ± 104.54	1,150.49 ± 240.87	1,126.67 ± 80.67	41.96 ± 4.90	85.87 ± 5.85	1,077.24 ± 39.46	158.90 ± 26.72

The pyloric caeca

At gastrointestinal junction, two pyloric caeca were observed, which were similar to the fish in the same family, *M. siamensis* [56], where the wall contained four distinct layers, according to other parts of the digestive tract. The mucosa of pyloric caeca presented numerous thin folds that branched tall-deeply in the form of a mesh-work (**Figure 4(G)**), corresponding to that observed in *M. cephalus* [49]. The mean mucosal fold width and height were 85.87 ± 5.85 and 1,077.24 ± 39.46 μm, respectively (**Table 4**). The epithelium was lined by simple columnar epithelium with microvilli or brush border. The goblet cell was found among the columnar cells (**Figures 4(H) - 4(J)**) in accordance with those observed in other fish species [6,17,21,31,49]. The mean epithelial cell height was 41.96 ± 4.90 μm (**Table 4**). The microvilli functioned in the absorption, which could confirm that the pyloric caeca in fish played the role in the absorption or by increasing the surface area, according to that reported by Purushothaman *et al*. [21]. In contrast, the pyloric caeca in mammals served a different function, specifically a fermentation [49]. The columnar epithelium cell was positively stained with PAS (**Figure 4(J)**) and negatively stained with AB pH 2.5 (**Figure 4(K)**) and AB pH 1.0 (**Figure 4(L)**). Whereas the goblet cell was positively stained with PAS (**Figure 4(J)**), AB pH 2.5 (**Figure 4(K)**) and AB pH 1.0 (**Figure 4(L)**). Besides, the epithelium stained positively for PAS, demonstrating that the epithelial cell secreted neutral glycoprotein. Based on PAS-positive staining profiles, Purushothaman *et al*. [21] suggested that the pyloric caeca served as the structures that fish used to increase the absorption and aided in the digestion. However, in *M. favus*, the numerous microvilli were observed at the apical of epithelium cell (**Figures 4(H) - 4(J)**). Therefore, the pyloric caeca showed a primarily role in the absorption. Beneath the epithelium, the lamina propria was consisted of loose connective tissue with numerous blood vessels. The muscularis mucosae was not observed (**Figures 4(H) - 4(J)**), while the thin submucosa contained dense connective tissue (**Figure 4(G)**). Meanwhile, the muscular layer presented three layers; inner oblique, middle circular and outer longitudinal smooth muscles (**Figure 4(G)**). The mean thickness of muscle layer was 158.90 ± 26.72 μm (**Table 4**). Generally, carnivorous fish tended to have pyloric caeca with the different amounts based on species [21] such as sixteen pyloric caeca in *L. crocea* [6], nine in *A. baremoze* [8], five in *L. calcarifer* [21], seven in *S. porcus* [32], four in *S. aurata* [34] and twenty to twenty-two in *H. forskahlii* [58].

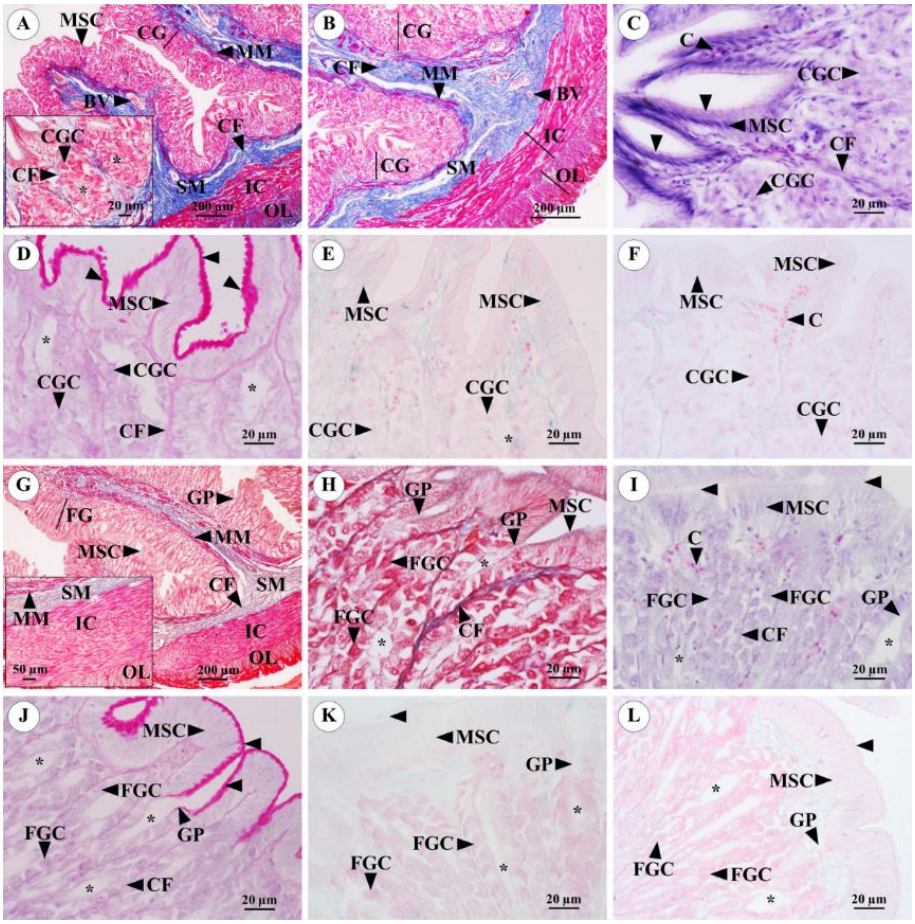

Figure 3 (3A) - (3F): Cardiac stomach of *M. favus*. (3A) - (3B): Distinct layers and cardiac glands (CG) in the lamina propria. Underneath the submucosa (SM), showing inner circular (IC) and outer longitudinal (OL) smooth muscle. The box in (3A): Cardiac glands and cardiac gland cells (CGC) surrounded by collagen fiber (CF). (3C): Mucous-secreting cells (MSC) with a clear apical cytoplasm. (3D): An enlarged view of PAS-positive mucous-secreting cells and a weak PAS-positive cardiac gland cell. (3E) - (3F): Negatively-staining with AB pH 2.5 and AB pH 1.0 of mucous-secreting cells and cardiac gland cells. (3G) - (3L): Fundic stomach. (3G): Distinct layers and fundic glands (FG), where the box representing muscularis mucosae (MM), submucosa and two distinct muscularis layer layers. (3H) - (3I): An enlarged photograph of mucosa, showing mucous-secreting cells, fundic gland cells (FGC) and gastric pits (GP). (3J): PAS-positive mucous-secreting cells, fundic gland cells and gastric pit. (3K) - (3L): AB pH 2.5 and AB pH 1.0 negative mucous-secreting cells, fundic epithelial cells and gastric pit, respectively. Blood vessel (BV), capillary (C), collagen fiber (CF), lumen (L), asterisk representing lumen, arrow head showing apical cytoplasm of mucous-secreting cell. (3A - 3B, 3G - 3H: MT), (3C, 3I: H&E), (3D, 3J: PAS), (3E, 3K: AB pH 2.5), (3F, 3L: AB pH 1.0).

The intestine

The histological features of the intestine in *M. favus* were consistent to those observed in other fish species, where the wall was comprised of four layers; mucosa, submucosa, muscularis and serosa (**Figures 5(A), 5(E)** and **5(I)**). Based on the histological characteristics and histometric measurements, the intestine of *M. Favus* could be divided into three parts, namely, anterior, middle and posterior intestine in accordance with the finding described in other fish species [5,27,71].

The mucosa fabricated villi that protruded into the lumen with the lamina propria located centrally. The epithelium in all portions of the intestine was lined by simple columnar cells, consisting of enterocytes and goblet cells (**Figures 5(B), 5(F)** and **5(J)**), according to those observed in other fish species [17,25,33,35,49,51]. In addition, the intraepithelial lymphocyte and capillary

that filled with erythrocyte were observed. Meanwhile the lamina propria of all intestine portions was richly supplied with blood vessels and lymphatic vessels (**Figures 5(B), 5(F)** and **5(J)**), particularly in the anterior intestine. Whereas gland was not observed in any portion of the intestine. All sections of the intestine were composed of two layers of muscularis tunic; an inner circular and an outer longitudinal tunic (**Figures 5(A), 5(E)** and **5(I)**), corresponding to other fish species [33,57,63]. The serosa lined by mesothelium and loose connective tissue with several blood vessels (**Figures 5(A), 5(E)** and **5(I)**).

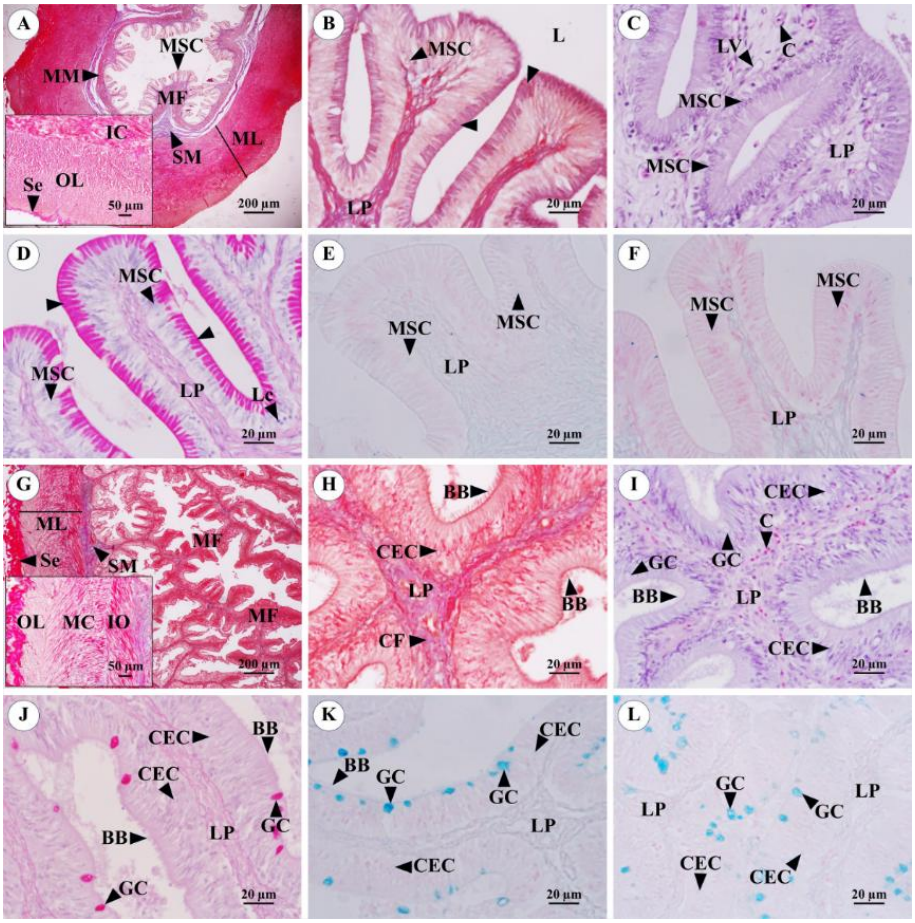

Figure 4 (4A) - (4F): Pyloric stomach of *M. favus*. (4A): Distinct layers and the box representing an enlarged view of muscular layer (ML), consisting of inner circular (IC) and outer longitudinal (OL) layers. (4B) - (4C): An enlarge view of mucosa, depicting mucous-secreting cell (MSC), lymph vessel (LV) and capillary in lamina propria (LP). (4D): PAS positive mucous-secreting cell (arrowheads). (4E) - (4F): AB pH 2.5 and AB pH 1.0 negative epithelial cells, respectively. (4G) - (4L): Pyloric caeca of *M. favus*. (4G): Distinct layers and the box representing an enlarged view of muscular layer, consisting of inner oblique (IO), middle circular (MC) and outer longitudinal (OL) layers. (4H) - (4I): An enlarged view of mucosa, consisting of goblet cell (GC) and columnar epithelial cell (CEC) with brush border (BB). Underneath epithelium, the lamina propria containing collagen fiber (CF) and capillary (C). (4J): PAS-positive goblet and columnar epithelial cells. (4K) - (4L): AB pH 2.5 and AB pH 1.0 positive goblet cells, respectively. Lumen (L), leukocyte (Lc), mucosal fold (MF), muscularis mucosae (MM), serosa (Se), submucosa (SM), arrow head showing apical cytoplasm of mucous-secreting cell. (3A - 3B, 3G - 3H: MT, (3C, 3I: H&E), (3D, 3J: PAS), (3E, 3K: AB pH 2.5), (3F, 3L: AB pH 1.0).

The anterior intestine showed numerous long, thin and round tips of villi (**Figure 5(A)**) and the mean mucosal fold height and width were 1,130.55 ± 24.85 and 116.76 ± 12.57 µm, respectively (**Table 5**). These

characteristics of the mucosal fold or villi created the spaces between them, which helped to increase the surface area for the food storage, digestion and nutrients absorption [12]. The enterocyte showed microvilli or brush border at the apical domain as the nucleus located near the basal domain of the cells. The mean epithelial cell height was 45.37 ± 4.17 µm (**Table 5**)**.** Between the enterocyte, some goblet cells were observed, meanwhile the lamina propria, were consisted of loose connective tissue containing blood vessels and lymphatic vessels. In addition, the lymphocytes observed in this layer with the nucleus that stained with hematoxylin (**Figure 5(B)**). These features were in accordance with to those observed in *S. testudineus* [11] and *M. cephalus* [49]. The mean muscular layer thickness of anterior intestine was 96.11 ± 6.74 µm (**Table 5**).

The middle intestine contained four tunics (**Figure 5(E)**) in accordance with the anterior intestine, while the mucosa was lined by enterocyte and goblet cells. However, the goblet cells tended to increase with larger amount than that of anterior intestine (**Figure 5(F)**). The mean epithelial cell height was 37.77 ± 2.04 µm (**Table 5**). The lamina propria formed villi with the mean mucosal fold height and width of 696.13 ± 77.65 and 105.68 ± 9.30 µm, respectively (**Table 5**). The muscular layer was divided into two layer (**Figure 5(E)**) that similarly mention in the anterior intestine but presented a thicker muscular layer than that of anterior portion (the mean muscular layer thickness was 114.91 ± 10.30 µm (**Table 5**).

The posterior intestine was the last part of the intestine that connected to the anus and external environment. The mucosa and epithelium similarly explained to those of the anterior and middle portions, but this portion contained larger amounts of goblet cells than the previous portions (**Figures 5(I) - 5(J)**). These characteristics were comparable to those observed in many fish species, such as [5,11,25]. The mean mucosal fold height and width were 445.64 ± 59.10 and 100.36 ± 9.82 µm, respectively. The mean epithelial cell height was 35.28 ± 4.05 µm and the mean thickness of muscular layer was 142.16 ± 14.45 µm (**Table 5**).

Table 5 Mean ($\bar{x}$) and standard deviation (S.D.) of the measurements (µm) of epithelial cell height (ECH), mucosal fold height (MFH), mucosal fold width (MFW) and muscular layer thickness (MLT) of anterior, middle and posterior intestines in the *M. favus* (n = 10).

	Anterior intestine				**Middle intestine**				**Posterior intestine**			
	ECH	**MFH**	**MFW**	**MLT**	**ECH**	**MFH**	**MFW**	**MLT**	**ECH**	**MFH**	**MFW**	**MLT**
$\bar{x}$ ± S.D.	45.37 ± 4.17	1,130.55 ± 24.85	116.76 ± 12.57	96.11 ± 6.74	37.77 ± 2.04	696.13 ± 77.65	105.68 ± 9.30	114.91 ± 10.30	35.28 ± 4.05	445.64 ± 59.10	100.36 ± 9.82	142.16 ± 14.45

The simple columnar epithelium of all three intestinal parts in *M. favus* was consistent with the findings in most fish studied [11,21]. The enterocytes in all portions showed positive PAS staining in the apical cytoplasm and at the base of the microvilli (**Figures 5(B), 5(F)** and **5(J)**), while those were negatively stained with AB pH 2.5 (**Figures 5(C), 5(G)** and **5(K)**) and AB pH 1.0 (**Figures 5(D), 5(H)** and **5(L)**). These results suggested that the enterocyte exhibited a function in the nutrients absorption. Moreover, the enzyme from brush border at the apical domain of enterocyte played a key role in food digestion, therefore the major function of fish's intestine was the site for the completion digestion and absorption [54]. Additionally, Infante and Cahu [72] stated that the brush border of teleost represented peptidase and disaccharidase enzymes. Whereas in *A. anguilla* showed esterase, alkaline and acid phosphatase and aminopeptidase, which acted importantly for the digestion and absorption [73]. However, the goblet cells showed positive-staining with PAS and AB pH 2.5, corresponding to the findings in *A. bimaculatus* [63]*, L. calcarifer* [21] and *M. cephalus* [49]. The goblet cell was relatively scared in the anterior intestine but became more quantity in the posterior intestine. This observation was in accordance with findings in many fish species [8,12,21,32,63]. The neutral mucins secreted by goblet cells in the anterior and middle intestine, might function to lubricate the lumen for food transport, while the mucins secreted by goblet cells in the posterior intestine, likely played the role in fecal transportation and luminal protection [11,25,46,71]. Moreover, the carboxylated acid mucin

secreted by goblet cells might have a crucial function for preventing the bacterial or other pathogenic bacterial invasion, and aiding the protein digestion [12]. Pereira *et al*. [74] stated that the goblet cell was generally observed in both carnivorous and omnivorous species. However, the presence of little amount of goblet cell at posterior intestine of herbivorous fish, was reported [54].

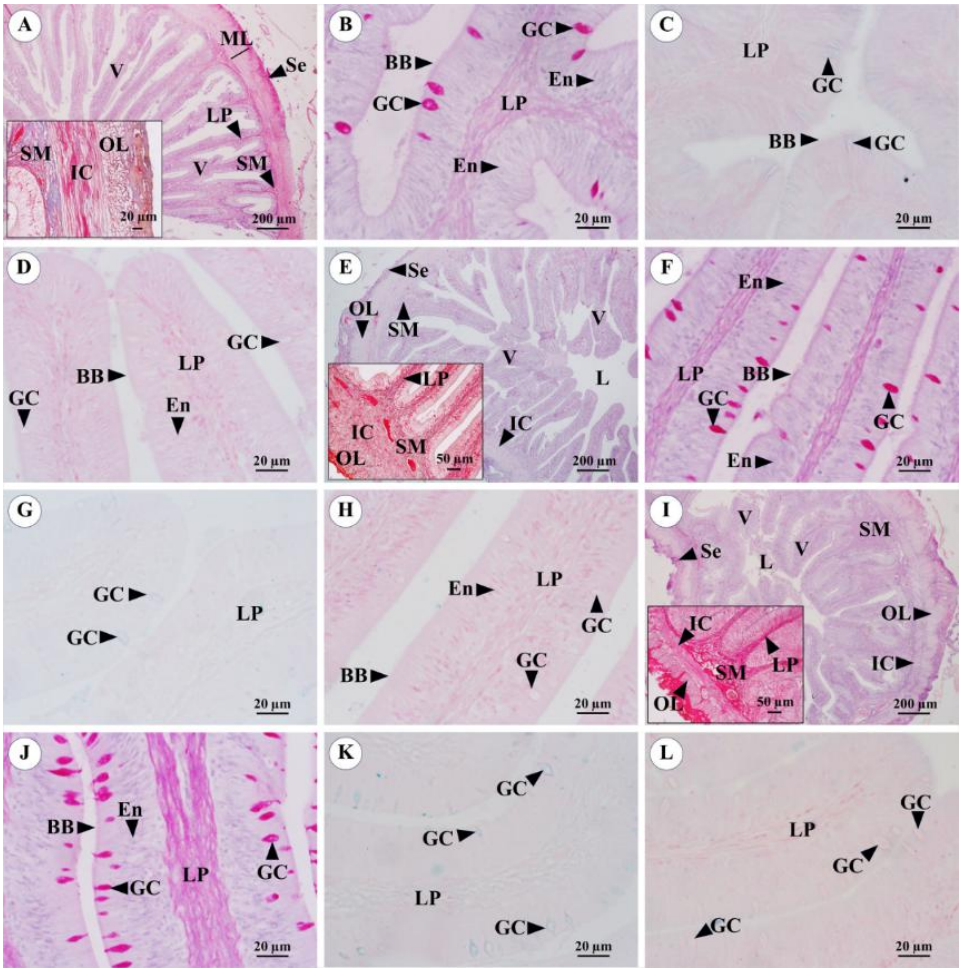

Figure 5 (5A) - (5D): Anterior intestine of *M. favus*. (5E) - (5H): Middle intestine of *M. favus*. (5I) - (5L): Posterior intestine of *M. favus*. (5A): Distinct layers and villi (V), consisting mucosa, submucosa (SM), muscular layer (ML) and serosa (Se). The box representing an enlarged view of submucosa and muscular layer (inner circular (IC) and outer longitudinal (OL) layers). (5B), (5C) and (5D): Villi of anterior intestine, with strongly PAS-positive, weakly AB pH 2.5-positive and AB pH 1.0-negative goblet cells (GC), respectively. (5E): Distinct layers and villi of middle intestine and the box illustrating an enlarged view of submucosa and muscular layer. (5F) - (5H): Strongly PAS-positive, weakly AB pH 2.5-positive and AB pH 1.0-negative goblet cells, respectively. (5I): Distinct layers and villi of posterior intestine and the box illustrating an enlarged view of submucosa and muscular layer. (5J) - (5L): Strongly PAS-positive, weakly AB pH 2.5-positive and AB pH 1.0-negative goblet cells, respectively. Brush border (BB), enterocyte (En), lamina propria (LP), lumen (L). (5A, 5E, 5I: H&E), (5B, 5F, 5J: PAS), (5C, 5G, 5K: AB pH 2.5), (5D, 5H, 5L: AB pH 1.0), (the box in (5A), (5E), (5I): MT).

From histometric observations, the mean epithelial cell height and mucosal fold height and width decreased from the anterior to the posterior intestine, which was similar to *A. bicolor bicolor* [54]. Therefore, this study could confirm and demonstrate that the anterior intestine was the most suitable site for the absorption and digestion, as stated by Ikpegbu *et al*. [75] and Nascimento *et al*. [53]. The tallest mucosal folds in the

anterior intestine enhanced the digestion and absorption efficiency by increasing the surface area [51,63], which were consistent with the intestine of other predator fish species such as *Esox lucius*, *Lota lota* [76] and *H. forskahlii* [58].

The posterior intestine was the smallest, according to the findings reported in *S. porcus* [32] and *A. bicolor bicolor* [54]. The muscular layer of posterior intestine was the thickest compared to other portions of intestine. This suggested that the posterior intestines showed the capacity for sphincter. Furthermore, the carnivorous fish feed the food from many kinds of protein sources and needed the force of muscular contraction for the digestion, absorption and propulsion of food. On the contrary, the herbivorous fish consumed the plant with high fibrous components, which might encourage the peristalsis, therefore the contraction of muscle was less needed [54].

The IC of *M. favus* was 0.22, which was in the typical range for the carnivorous fish. This IC was like to other carnivorous fish such as *A. bicolor bicolor* [54], *A. anguilla* [73] and *M. armatus* (0.27-0.34) with the same genus [77]. In considering the histological and histochemical characters, it could be concluded and confirmed that *M. favus* was a carnivorous fish. Generally, carnivorous fish possessed shorter intestine compared to herbivorous and omnivorous fish [59] because the diets possessed lower percentage of plants. Consistently, the structure and morphology of the intestine were closely related to the food consumption, digestion, absorption [17] and feeding behavior.

Conclusions

In conclusion, the study on the gross anatomy, microanatomy and histochemistry of the digestive tract of *M. favus* represents the first report on this species. The findings reveal similarities to carnivorous and some omnivorous fish, while differing from herbivorous fish in certain aspects. However, the basic structures of the digestive tract were consistent with those observed in Teleostei. The features of the digestive organs-namely, the pharynx, esophagus, stomach (cardiac, fundic, and pyloric stomach) and intestine (anterior, middle, and posterior portions) were observed to be adapted to food and feeding habits. Based on results, it can be suggested that the digestive tract of *M. favus* is closely relate to its carnivorous feeding habits. First, the presence of longitudinal folds and skeleton muscle in the pharynx and esophagus allows *M. favus* to swallow large microinvertebrates and extend the lumen when needed. Second, the cardiac and fundic stomach regions possess well-developed gastric glands, which are characteristic of carnivorous fish. Third, the presence of pyloric caeca, commonly found in carnivorous species, supports this classification. Fourth, the intestinal was relatively short in length compared to the standard body length, another feature typical of carnivorous fish. Furthermore, the IC value, a measure used to indicate the feeding habits of fish, confirmed that *M. favus* is a carnivorous fish. This study provides valuable insights into the relationship between diet, microanatomical structure and the function of each digestive organ in *M. favus.* These findings serve as a foundational reference for future studies in areas such as fish physiology, pathology and aquaculture.

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Declaration of Generative AI in Scientific Writing

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