Original Article

Histomorphological and mucin histochemical study of the alimentary canal of pangas catfish, *Pangasius pangasius* (Hamilton 1822)

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Abstract: The present study describes the histological and mucin histochemical properties of the alimentary canal (AC) of the pangas catfish, *Pangasius pangasius*. The results revealed that the mucosa of the oesophagus was lined by a stratified epithelium containing chloride cells and taste buds which suggested mechanic, gustatory and physiologic roles of the oesophagus in this species. The stomach mucosa was lined by a simple columnar epithelium. The lamina propria-submucosa in cardiac and fundic stomach contained gastric glands. The pyloric stomach had the thickest muscularis layer among all the parts of the AC. The villi showed the maximum height and width in the middle intestine. The tunica muscularis and serosa showed the thinnest thickness among all parts of AC. The mucin histochemistry showed that the goblet cells of oesophagus and intestine contained both neutral and acidic with carboxylated and sulfated mucins and there was not acidic mucins in epithelial cells of the stomach.

Introduction

The alimentary canal (AC) in fishes, includes oesophagus, stomach and intestine which enable transportation, storage, digestion and absorption of the food (Wilson and Castro, 2011; Dos Santos et al., 2015). There are several reports available regarding histological study of the AC in numerous fish species (Albrecht et al., 2001; Diaz et al., 2003; Suicmez and Ulus, 2005; Chatchavalvanch et al., 2006; Cao and Wang, 2009; Hopperdietzel et al., 2014; Dos Santos et al., 2015) that show differences among species which are related to taxonomy, feeding habits, food, age, body shape and size. The mucin histochemistry of digestive tract has also been studied in different fish species which showed the diversity among species and along the fishes' AC (Pedini et al., 2001; Diaz et al., 2008; Faccioli et al., 2014). The presence of muco-substances in the fishes' AC is correlated with lubrication, protection against proteolytic degradation, inhibition of microorganisms and osmotic function (Loretz, 1995; Diaz et al., 2008).

Pangas catfish, *Pangasius pangasius* (Hamilton 1822) is a carnivorous species belong to the family Pangasiidae. It mostly feeds on mollusks, fishes, insects and crustaceans (Gupta, 2016). *Pangasius pangasius* is widely distributed in India, Bangladesh, Pakistan, Myanmar, Malaya-peninsula, Indonesia, Vietnam, Java and Thailand (Tripathi, 1996). However, over exploitation, habitat degradation, water pollution, destruction of the breeding grounds are major threats of this species in its natural habitats (Sarkar et al., 2006; Gupta, 2016). It is an important freshwater food due to its good taste and deliciousness, and also a popular game fish and recently is being kept as ornamental fish (Mohindra et al., 2015; Gupta, 2016).

There are no data is available about the morphology of the AC in *P. pangasius*, therefore, such a study can contribute to the digestive physiology, feeding habit, formulation of diet, diagnosis of disease and promote knowledge in zoological and phylogenetic field (Carrason et al., 2006; Chatchavalvanich et al., 2006;

Int. J. Aquat. Biol. (2017) 5(3): 208-217

209

Xiong et al., 2011). Considering that the literature is poor on digestive tract of aquarium fish species, the AC morphological investigation in this species is important (Hale, 1965; Caceci, 1984; Onal et al., 2010; Hopperdietzel et al., 2014). Hence, the present study was aimed to determine the detailed histological and mucin histochemical feature of the AC in *P. pangasius*.

Materials and Methods

Tissue sampling and histology: Seven healthy *P. pangasius* with total length of 50-55 cm were obtained from a fish ornamental shop. The fishes were anesthetized with 250 mg L^{-1} tricaine methane-sulfonate (MS-222, Argent Chemical Laboratories Redmond, WA, USA) based on the ethics and animal-care approvals (Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. N. 3867672). An abdominal wall incision was made and their AC were gently dissected. The entire AC was divided into the oesophagus, the cardiac, fundic and pyloric stomach and the anterior, middle and posterior intestine based on morphological differences. The body length, the length of AC and the lengths of each part of AC were measured in each specimen.

For histological investigation, the sections of each part of AC was cleaned of its contents using 0.01 mol l^{-1} phosphate buffer saline (PBS) and fixed in 10% neutral-buffered formalin. The histological sections were prepared based on Sadeghinezhad et al. (2015) and stained using hematoxylin and eosin (H&E) and Masson's trichrome for general histological examination. In addition, the following methods were used for the mucin histochemical study: Periodic acid-Schiff (PAS) staining to demonstrate neutral mucins (Schumacher et al., 2004), Alcian blue (AB) staining at pH 1.0 and 2.5 for acidic mucins (Bancroft and Cook, 1994), AB at pH 2.5 staining followed by PAS to detect neutral and acidic mucins (Mowry, 1956) and Aldehyde fuchsin (AF) with AB (pH 2.5) staining was also used to assess the nature of the acidic mucins (Spicer and Meyer 1960).

The mounted slides were observed under an Axioplan microscope equipped with Zeiss Axiocam MRm and the Axiovision software (Carl Zeiss, Oberkochen, Germany). The height and width of the intestinal villi, the thickness of the tunica muscularis, the thickness of tunica serosa and the number of goblet cells were measured in various parts of the AC. For each specimen, all measurements were made with ten replications in each section. The evaluation of the goblet cells was made on randomly selected 100 μm length of the mucosal epithelium.

Statistical analysis: Comparisons were performed using Kruskal-Wallis test. If it was significant, Mann-Whitney was used to determine the significant differences between the pairs of means. *P*<0.05 was considered statistically significant.

Results

Histomorphological characteristics: The AC of *P. pangasius* was 41±3 cm in length and consisted of the oesophagus, stomach (with cardiac, fundic and pyloric parts) and intestine (including anterior, middle and posterior parts). The wall of AC was divided into the mucosa, lamina propria-submucosa, muscularis and serosa. The results of the histomorphometrical study of various parts of the AC is summarized in Figure 1.

The oesophagus was a short tubular organ (2.7±0.5 mm in length) which connects the oropharynx to the stomach. Its mucosa was lined by a stratified epithelium containing surface epithelial, goblet and undifferentiated basal club cells (Fig. 2a). The chloride cells, contained the eosinophilic cytoplasm with a large central nucleus, were present in the epithelium. The taste buds were sparsely observed in the epithelium of the anterior part of the oesophagus (Fig. 2a). The muscularis mucosa was not observed and the lamina propria-submucosa was consisted of the dens connective tissue with abundant collagen fibers. The striated tunica muscularis was composed of an inner and outer muscle layers. The tunica serosa was the outermost layer and made up mesothelium with underlying connective tissue (Fig. 2b).

The stomach was a small blind sac consisting of three well-defined cardiac, fundic and pyloric parts. Their mucosa layers were lined by a simple columnar
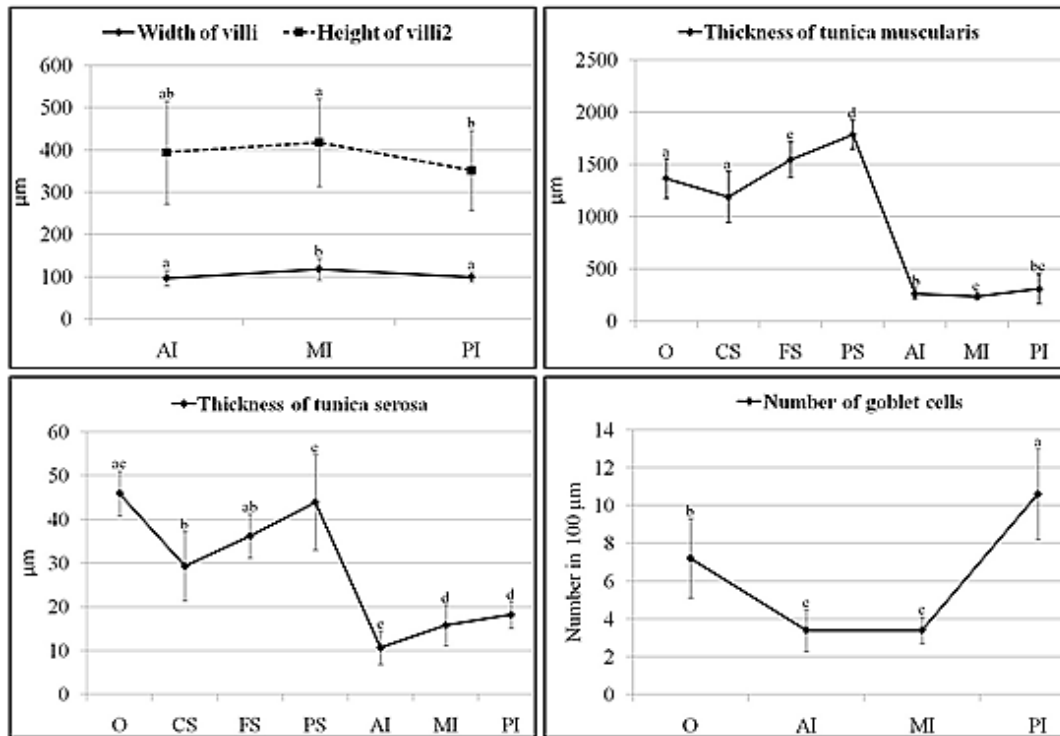
Figure 1. The histometric characteristics of the alimenray canal in *Pangasius pangasius* (Different superscript letters indicate a significant difference, *P*<0.05; O: oesophagus, CS: cardiac stomach, FS: fundic stomach, PS: pyloric stomach, AI: anterior intestine, MI: middle intestine and PI: posterior intestine).

Table 1. The histochemical characteristics of the mucous-secreting cells in the alimentary canal of *Pangasius pangasius*.

| Techniques employed | Mucous secreting cells | | | Remarks |
| | Goblet cells | | Epithelial cells of Stomach | |
	Oesophagus	Intestine		
PAS	+++	+	+++	R
AB (pH 1)	+++	++	-	B
AB (pH 2.5)	+++	++	-	B
PAS-AB (pH 2.5)	+++	+++	+++	RP, BP
AF-AB (pH 2.5)	+++	+++	-	B, P

R red, B blue; RP, reddish purple; BP, bluish purple; P, purple; PAS, periodic acid-Schiff; AB, alcian blue; AF, aldehyde fuchsin, - = negative staining; + = weak staining; ++ = moderate staining; +++ = intense staining.

epithelium. Many regular mucosal folds with secondary branches were seen in the pyloric stomach. The lamina propria-submucosa in the cardiac and fundic parts contained the gastric glands. These tubular glands were surrounded by a layer of connective tissue and opened into the bottom of the gastric pits. The limania propria-submucosa of the cardia and fundus contained collagen fibers with few thin strands of smooth muscles while in pyloric stomach the dense collagen fibers were predominant. The tunica muscularis is consisted of the smooth muscle which was formed an inner circular muscle layer (CML) and an outer longitudinal muscle layer (LML). The pyloric stomach had the thickest

muscularis (1783±139.6 μm) among all the parts of the AC (*P*<0.05). The histological features of serosa was similar to that of the oesophagus (Fig. 3a-c). The intestine was 33±2 mm in length and consisted of the anterior, middle and posterior parts showing similar histological structure. The pyloric caeca were not observed. The villi showed the maximum height (418±103.8 μm) and width (118.3±26 μm) in the middle intestine. The mucosa was lined by a simple epithelium, in which the enterocytes and goblet cells were present. The apical border of the enterocytes possessed many microvilli arranged as a striated border. The goblet cells were scattered between the enterocytes. The number of goblet cells significantly

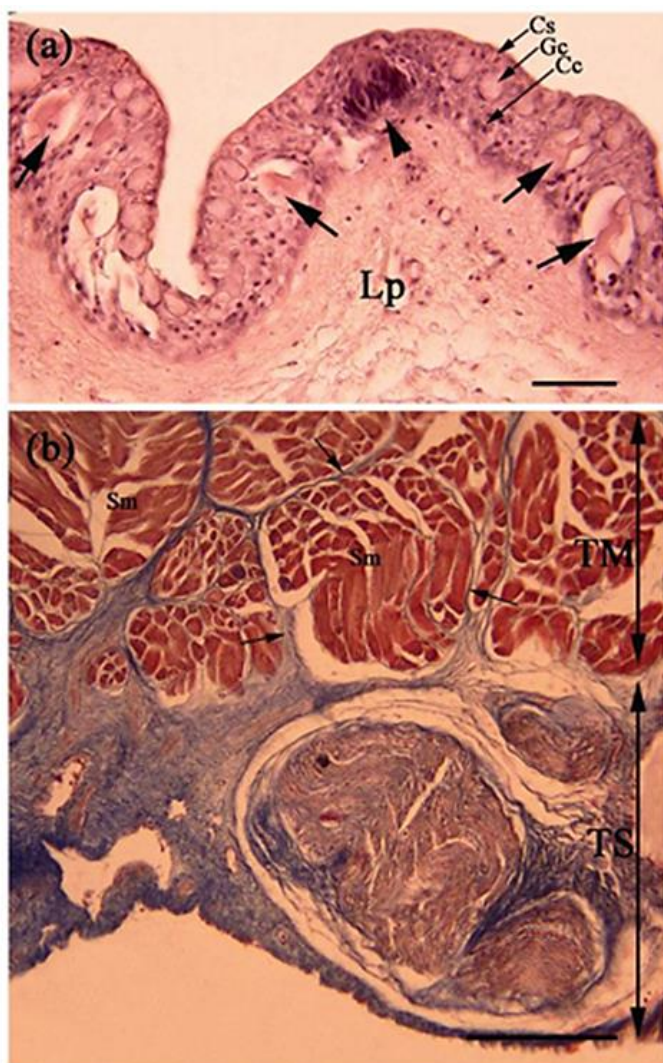Int. J. Aquat. Biol. (2017) 5(3): 208-217

211

Figure 2. The photomicrographs of *Pangasius pangasius* oesophagus. (a) The mucosa of the oesophagus. The epithelium was lined by a stratified epithelium containing surface epithelial cells (Sc), goblet cells (Gc) and undifferentiated basal club cells (Cc). Note the taste bud (arrowhead) and chloride cells (arrows) within the epithelium. The lamina propria (Lp) beneath the epithelium is labeled (H&E, scale bar=50 μm). (b) The tunica muscularis and tunica serosa of the oesophagus. Note the collagenous fibers (arrows) around striated muscle fibers (Sm) of the tunica muscularis (TM). The tunica serosa (TS) is the outermost layer (Masson's trichrome staining, scale bar=200 μm).

increased toward the end of the intestine (*P*<0.05) with the highest average number in the posterior intestine (106±2.4 cells in 100 μm). The muscularis was composed of the smooth CML and LML which showed the least thickness (267±51, 239.3±34.4 and 310.5±140.8 μm in anterior, middle and posterior intestine, respectively) part of the AC (*P*<0.05). The serosa of intestine had similar structure to other parts of the AC but without a distinct connective tissue and

thinnest serosa layer (10.7±3.8, 15.8±4.7 and 18.2±3.1 μm in anterior, middle and posterior intestine, respectively) (*P*<0.05) (Fig. 4a, b).

Mucin histochemical profile: The characteristics of the mucins secreted by the mucous secreting cells in the AC of *P. pangasius* are summarized in Table 1. Histochemical analysis showed that goblet cells in the oesophagus and intestine, and epithelial cells in the stomach as secretory unites. The neutral mucins were present in both mucous secreting unites that were PAS-positive. The apical cytoplasm of epithelial cell in the stomach showed a strong reaction. The intensity of the red color of the goblet cells was more in the oesophagus than intestine (Figs. 5a, 6a, 7a). The acidic sulfated and carboxylated mucins were observed in the goblet cells which were blue owing to the AB staining at pH 1.0 and 2.5, respectively. There was no acidic mucin in the epithelial cells of the stomach. The reaction in goblet cells of the oesophagus was stronger than those of intestine (Figs. 5b, 6b, 7b). The investigation of PAS-AB (pH 2.5) staining showed that the goblet cells stained bluish purple were more than the reddish purple stained cells, implying that the secreted mixed mucins were more acidic than neutral. However, the stomach epithelial cells were labeled red due to loss of acidic mucins (Figs. 5c, 6c, 7c). The results of the AF–AB (pH 2.5) staining indicated that both types of carbonic and sulfated acidic mucins were present in the goblet cells owing to blue and purple appearances, respectively (Figs. 5d, 6d, 7d). Regarding the histochemical reactions all observations mentioned above were seen in a similar way in different parts of stomach and also intestine.

Discussion

In the present study, the histomorphology and mucin histochemistry of the *P. pangasius* AC were examined for the first time. The results showed that the general morphological features were in accordance with those described for fishes, with some differences.

The oesophagus was lined with stratified squamous epithelium in this species as seen in most fishes. However, the stratified columnar epithelium was reported in some fishes like *Pelteobagrus fulvidraco*
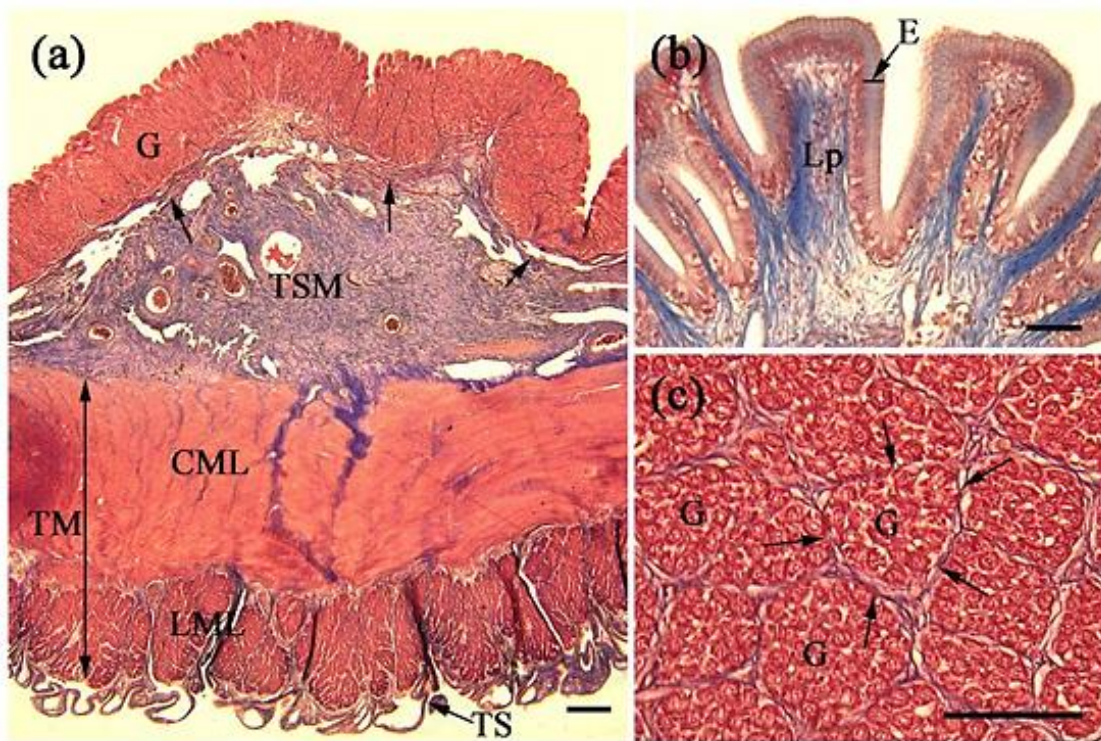
Figure 3. The photomicrographs of *Pangasius pangasius* stomach. (a) The cardiac stomach wall. Gastric glands (G) within the lamina propria are visible. The strands of the muscularis mucosae (arrows), tunica submucosa (TSM), tunica muscularis (TM) with the circular muscle layer (CML) and longitudinal muscle layer (LML), and tunica serosa (TS) are visible (Masson's trichrome staining, scale bar=200 μm). (b) The mucosal fold of the pyloric stomach. The secondary branches of a mucosal fold with simple columnar epithelium (E) and underlying lamina propria (Lp) are visible (Masson's trichrome staining, scale bar=50 μm). (c): The gastric glands of the fundic stomach. Note the layer of connective tissue (arrows) surrounded the glands (G) (Masson's trichrome staining, scale bar=100 μm).
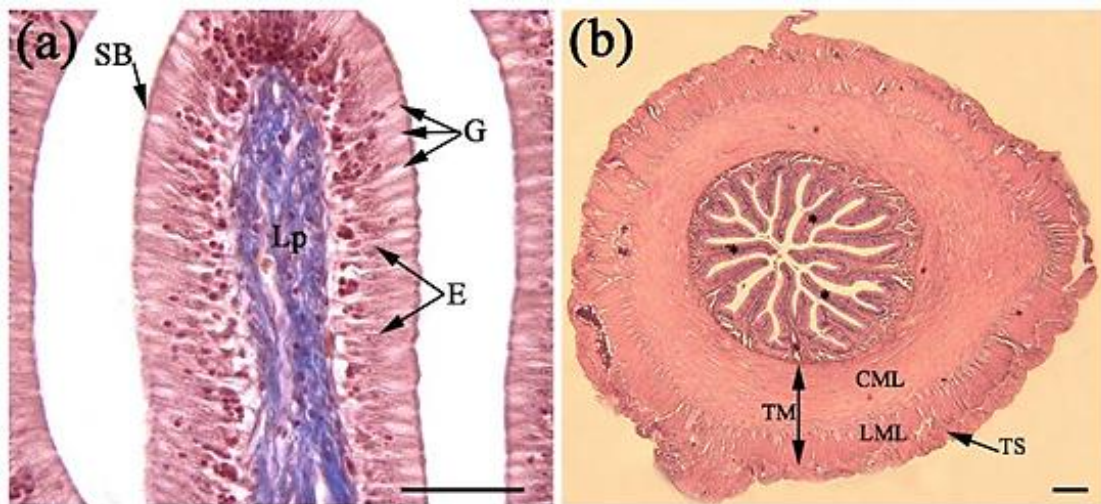

Figure 4. The photomicrographs of *Pangasius pangasius* intestine. (a) The intestine villus. The mucosa is lined with a simple columnar epithelium with enterocytes (E) and goblet cells (G). On the apical border of the enterocytes, microvilli are arranged as a striated border (SB). Note the presence of connective tissue fibers inside the lamina propria (Lp) of the villus (Masson's trichrome staining, scale bar=50 μm). (b) The intestinal wall. The intestinal villi (stars), tunica muscularis (TM) with the circular muscle layer (CML) and longitudinal muscle layer (LML), and tunica serosa (TS) are visible (H&E, scale bar=200 μm).

(Richardson 1846) (Cao and Wang, 2009), *Himantura signifer* (Compagno and Roberts, 1982) (Chatchav-alvanich et al., 2006) and *Raja clavata* (L. 1758) (Holmgren and Nilsson, 1999) in the oesophagus. The two different anterior and posterior regions in oesophagus mucosa with outer most layers of squamous and columnar cells were observed in some fishes e.g. *Engraulis anchoita* (Hubbs and Marini,
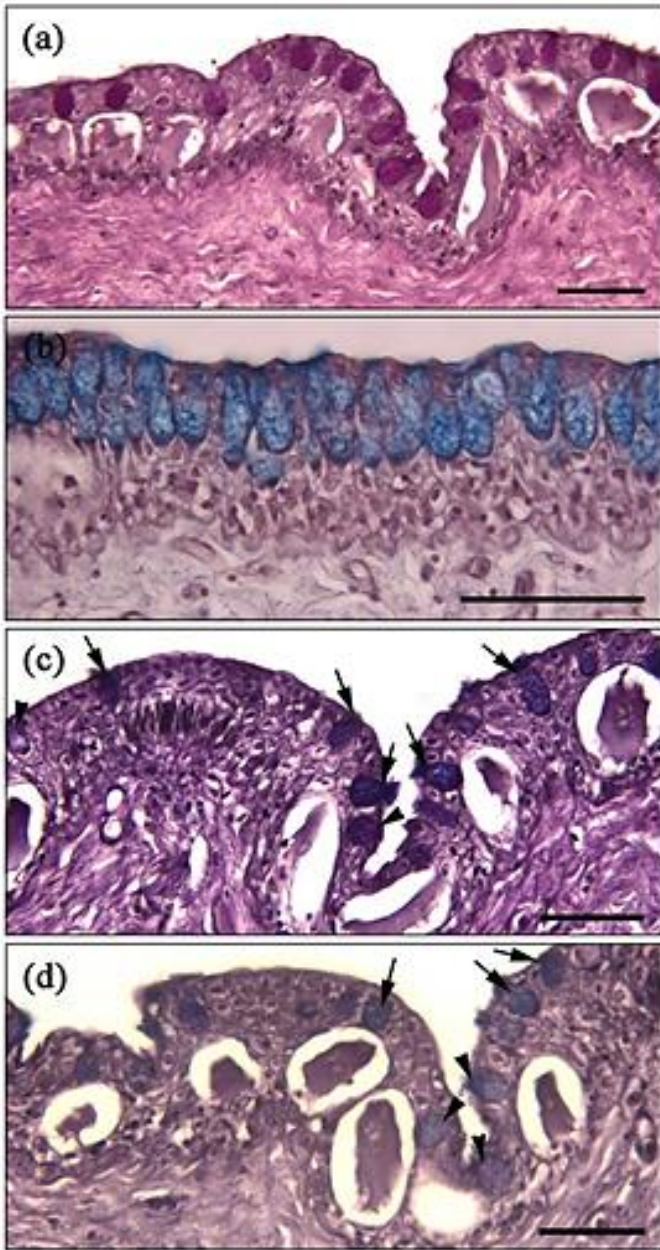
Figure 5. Histochemical characteristics of the goblet cells in the oesophagus of *Pangasius pangasius*. (a) The representative photomicrograph of PAS-stained goblet cells. (b) The representative photomicrograph of the alcian blue (AB) (pH 1.0) staining. (c) The representative photomicrograph of the PAS–AB (pH 2.5) stained goblet cells. Note that most goblet cells stained bluish purple and were secreting mixed mucins that were more acidic than neutral (arrows) and that the cells stained reddish purple secreted mixed mucins that were more neutral than acidic (arrowheads). (e) The representative photomicrograph of the AF–AB (pH 2.5) stained goblet cells. The sulfated mucin content (arrows) and the carbonic mucin content (arrowheads) are visible (scale bars=50 μm).
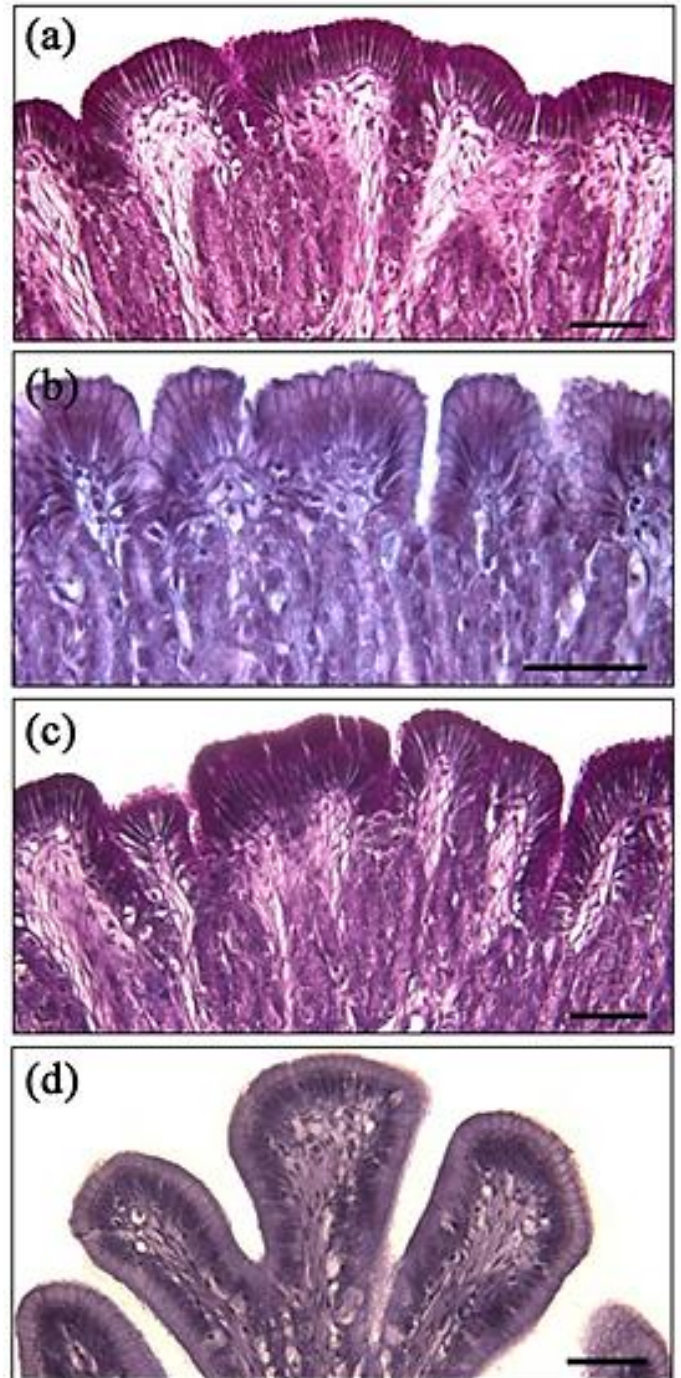

Figure 6. Histochemical characteristics of the mucous-secreting cells on the apical cytoplasm of epithelial cell of stomach in *Pangasius pangasius*. (a) The representative photomicrograph of the PAS staining. (b) The representative photomicrograph of the AB (pH 2.5) staining. Note that there is not any AB reaction within epithelial cells. (c) The representative photomicrograph of the PAS–AB (pH 2.5) staining. Note that all of the secreting units stained red, indicating only neutral mucin production. (d) The representative photomicrograph of the AF– AB (pH 2.5) staining indicating no reactions in epithelial cells (scale bars=50 μm).

1935) (Diaz et al., 2003), *Anguilla Anguilla* (L. 1758) (Abaurrea-Equisoain and Ostos-Garrido, 1996), *Scomberomorus maculatus* (Mitchill 1815), (Mota-

Alves, 1969) and *Seriola dumerili* (Risso 1810) (Grau et al., 1992). Albrecht et al. (2001) suggested the
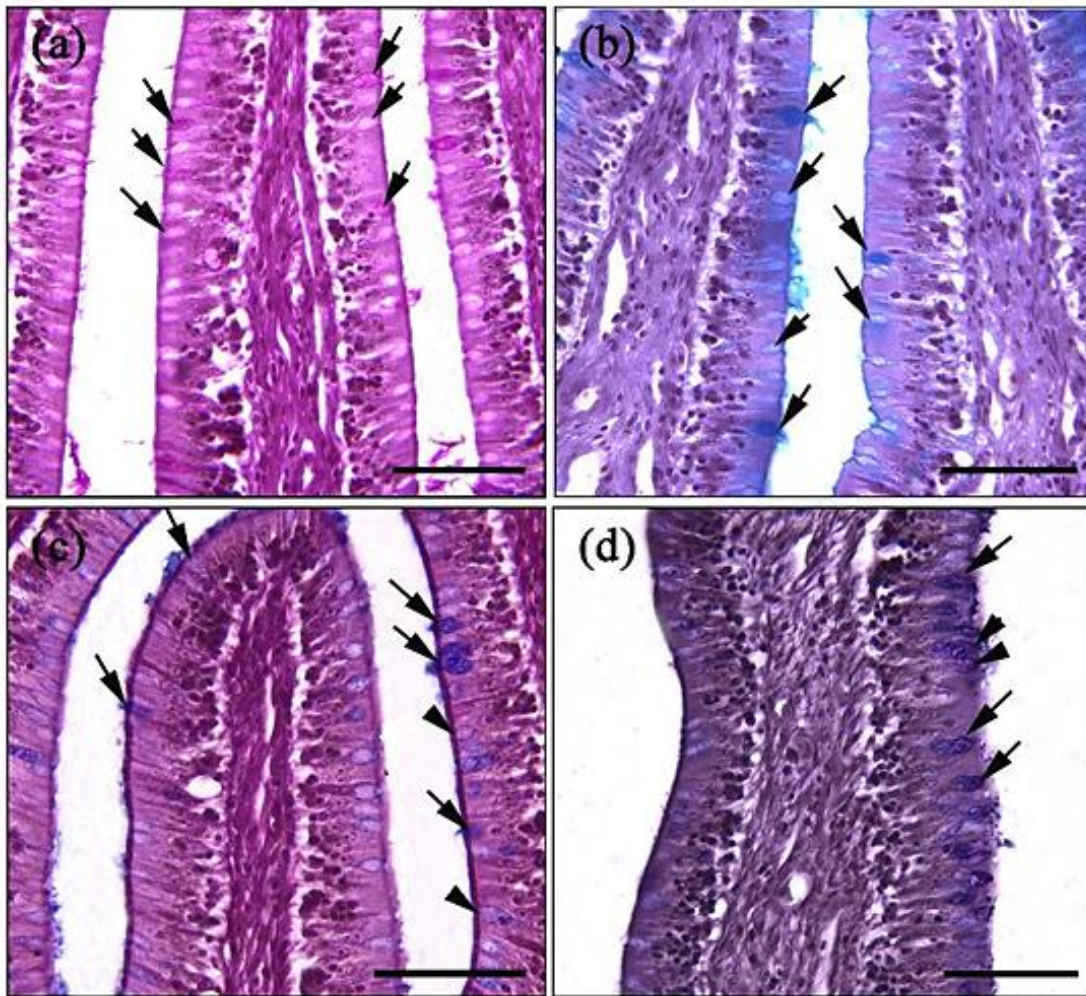
Figure 7. Histochemical characteristics of the goblet cells in the intestine of *Pangasius pangasius*. (a) The representative photomicrograph of the PAS staining indicating weak staining in intestinal goblet cells. (b) The representative photomicrograph of the AB (pH 1) staining. (c) The representative photomicrograph of the PAS–AB (pH 2.5) staining. The goblet cells secreting mixed mucins that were more acidic than neutral (arrows) and neutral than acidic (arrowhead) are visible. (d) Photomicrograph of the AF–AB (pH 2.5) stained goblet cells. The sulfated mucin content (arrows) and the carbonic mucin content (arrowheads) are visible (scale bars=50 μm).

protective role of the stratified epithelium against abrasion in oesophagus.

The longitudinal folds, which allow distention during swallowing, were present like other species (Holmgren and Nilsson, 1999). Goblet cells were scattered all over the esophageal epithelium of *P. pangasius*. The abundant goblet cells in the oesophagus of fishes may help the food transit to the stomach (Grau et al., 1992; Chatchavalvanich et al., 2006). The distribution of mucins secreted by these cells, detected by the series of histochemical tests, revealed that the goblet cells contained neutral, acidic carboxylated, and acidic sulfated mucins. The neutral and acidic mucosubstances in esophageal goblet cells were described previously with different intensity

(Pedini et al., 2001; Zdravko et al., 2005; Cao and Wang, 2009; Faccioli et al., 2014). Different amount of the acidic and neutral mucins might be related to their different functions. The secretions of acidic mucins are used to increase mucous viscosity, lubricate epithelium and protect against pathogens (Cao and Wang, 2009; Faccioli et al., 2014). The neutral mucins enable pre-gastric digestion in oesophagus with enzymatic digestion of food and its transformation into chyme (Cao and Wang, 2009). Chloride cells were observed in the esophageal epithelium of *P. pangasius*. These cells which have osmoregulatory function are reported in euryhaline species like *A. anguilla* (Genten et al., 2009). The presence of taste buds on the esophageal epithelium

indicates the gustatory role of the oesophagus in this species. It has been suggested that taste buds indicate final acceptance or rejection of food items passing in oesophagus towards the stomach (Oliveira-Ribeiro and Fanta, 2000). The presence of well-developed two layers of striated muscle in the oesophagus of *P. pangasius* facilitating antiperistalsis might suggest potential assessment of ingested food quality in this segment of AC. However, absence of taste buds in some species *Leporinus friderici* (Bloch 1794), *Leporinus taenifasciatus,* Britski 1997 (Albercht et al., 2001), *Orthrias angorae,* (Steindachner 1897) (Suicmez and Ulus, 2005), *S. dumerili* (Grau et al., 1992) and *E. anchoita* (Diaz et al., 2003) with different diets suggested that feeding habits was not related to the taste buds in the oesophagus.

The stomach of *P. pangasius* lined with single layer of the columnar epithelium was similar to that of other species. The gastric glands were absent in pyloric stomach that were similar with those of most species e.g. *L. friderici* and *L. taeniofasciatus* (Albercht et al., 2001), *H. signifier* (Chatchavalvanich et al., 2006), *P. fulvidraco* (Cao and Wang, 2009), *Hemisorubim platyrhynchos* (Valenciennes 1840) (Faccioli et al., 2014) and *Schizodon knerii* (Steindachner 1875) (Dos Santos et al., 2015). However, the tubular glands in pyloric stomach have been reported in *E. anchoita* (Diaz et al., 2003) and *Dentex dentex* (L. 1758) (Carrasson et al., 2006). The gastric glands were found in initial and terminal regions in *Oreochromis niloticus* (L. 1758) (Caceci et al., 1997) and only in the fundic stomach in *Oncorhynchus mykiss* (Walbaum 1792) (Arias and Garrido, 1994). The mucin histochemistry demonstrated extensive neutral mucins on the apical surface of the columnar cells in different regions of the *P. pangasius* stomach while the gastric glands were unreactive to histochemical tests. The neutral mucins in stomach with a role in absorption of easy digested substances (Murray et al., 1996) and buffering effects on acidic content of the stomach (Zdravko et al., 2005) have been also reported in *H. signifer* (Chatchavalvanich et al., 2006) and *P. fulvidraco* (Cao and Wang, 2009) on the gastric epithelial cells. The surface epithelium of the stomach

in *H. platyrhynchos* (Faccioli et al., 2014), *S. knerii* (Dos Santos et al, 2015), *D. dentex* (Carrasson et al., 2006), *Cynoscion guatucupa* (Cuvier 1830) (Diaz et al., 2008) and *E. anchoita* (Diaz et al., 2003) contained both neutral and acidic mucins. In *Umbrina cirrosa* (L. 1758) both epithelial surface and gastric glands show histochemical reaction with neutral and acidic profile (Pedini et al., 2001). In contrast, the secretory unites of the stomach in *Solea solea* (L. 1758) revealed no reaction with histochemical tests (Veggetti et al., 1999). These variations have been suggested to be related to different feeding habits or species specific (Gargiulo et al., 1997). The mucosa of cardiac and fundic stomach contained few smooth muscle fibers which were replaced by abundant collagen fibers in pyloric stomach. The same feature has been reported for descending and ascending stomach of *H. signifer* (Chatchavalvanich et al., 2006). The collagenous fibers in lamina propria-submucosa in some species may have a role in increase in elasticity for carrying food in stomach (Cao and Wang, 2009). The stratum compactum which protect the wall of AC has been reported in the stomach and intestine of some fishes (Diaz et al., 2003; Carrasson et al., 2006; Sadeghi-nezhad et al., 2015), however, it was not observed in *P. pangasius*. The muscular layers that cause motility in stomach and its thickening in pyloric stomach enhance the movement for passing food into the intestine (Dos Santos et al., 2015).

Histometrical results showed the significant dimension of the villi in the middle intestine. Similar results was reported in the initial parts of the intestine in *Esox lucius* (L. 1758) (Sadeghinezhad et al., 2015), *Amatitlania nigrofasciata* (Günther 1867) (Hopper-dietzel et al., 2014) and *P. fulvidraco* (Cao and Wang, 2009) and have introduced this part of the intestine as a major site for digestive processes. Goblet cells were scattered within epithelium of the intestine and their number were increased in the posterior part due to need for more mucosal lubrication and protection in this part of intestine (Pedini et al., 2001; Carrasson et al., 2006). Goblet cells were stained positive for both neutral and acidic mucins in *P. pangasius* intestine as found generally in the intestine of fishes with role in

lubrication of the mucosa and absorption of food (Pedini et al., 2001; Carrasson et al., 2006; Cao and Wang, 2009; Hopperdietzel et al., 2014; Dos Santos et al., 2015). Cao and Wang (2009) reported abundant neutral content with few acidic mucin in intestine of *P. fulvidraco* while contrariwise results in *P. pangasius* may be related to the different feeding habit. The thickness of tunica muscularis was consistent along the length of the intestine in *P. pangasius* similar to *A. nigrofasciata* (Hopperdiet-zel et al., 2014). In contrast, the thickness of tunica muscularis varies in the intestine of carnivorous fishes with irregular large food items to increase intestinal motility (Grau et al., 1992).

Based on the results, the histomorphological and mucin histochemical features described in *P. pangasius* can be considered as adaptations to feeding behavior of this species. Specific structures of the oesophagus in this species were indicative of mechanic, gustatory and physiologic roles in the oesophagus. The stomach and intestine presented the typical features of that of most fishes with some variations.

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Int. J. Aquat. Biol. (2017) 5(3): 208-217

217

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چکیده فارسی

مطالعه هیستومورفولوژی و هیستوشیمی موسین لوله گوارشی گربه ماهی پنگوسی (Pangasius Pangasius)

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چکیده:

مطالعه حاضر خصوصیات بافتی و هیستوشیمی لوله گوارش ماهی پنگوسی (Pangasius pangasius) را توصیف می‌نماید. نتایج نشان داد که مخاط مری بوسیله اپیتلیوم مطبق حاوی سلول‌های کلراید و جوانه‌های چشایی پوشیده شده بود که نقش‌های مکانیکی، چشایی و فیزیولوژیک مری را در این گونه نشان داد. مخاط معده بهوسیله اپیتلیوم استوانه‌ای ساده پوشیده شده بود. لایه پارین-زیر مخاط در کاردیا و فوندوس معده شامل غدد معدی بود. پیلور معده ضخیم‌ترین لایه عضلانی را در میان همه بخش‌های لوله گوارش داشت. بیشترین ارتفاع و عرض پرزها در روده میانی مشاهده شد. لایه عضلانی و سروز نازک‌ترین ضخامت را در میان همه بخش‌های لوله گوارش داشت. هیستوشیمی موسین نشان داد که سلول‌های جامی مری و روده دارای هر دو نوع موسین‌های خنثی و اسیدی کربوکسیلی و سولفاتی هستند و موسین‌های اسیدی در سلول‌های اپیتلیال معده وجود نداشت.

کلمات کلیدی: گربه ماهی پنگوسی، مری، معده، روده، ریخت‌شناسی.