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RESEARCH ARTICLE

Histology and histochemistry of the stomach of an Asian schilbeid, *Clupisoma garua* (Hamilton)

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Abstract. The cellular peculiarities and histochemical nature of the stomach in the freshwater catfish *Clupisoma garua* (Hamilton) were explored with light microscopic analysis. The pouch shaped muscular stomach was distinguished into the anterior cardiac and posterior pyloric regions. Histologically, the wall of stomach consisted of four distinct layers of the mucosa, submucosa, muscularis, and very thin serosa. The mucosa contained two kinds of epithelia: the superficial epithelium lined with columnar epithelial cells and the glandular epithelium crowded with numerous gastric glands that open through gastric pits. The vascularized submucosa was made up of loose connective tissues that extended into the mucosa forming lamina propria. The well-developed muscular layer was composed of an inner thick layer of circular muscle and an outer layer of longitudinal muscle bands. The detection and localization of mucopolysaccharides, glycogen, protein, and tryptophan in the various cells bounded in the stomach were described with the feeding behavior of the fish concerned.

Keywords: Garua bachcha, stomach, histomicroscopy, histochemistry

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Introduction

The structure of the digestive tract, particularly the stomach varies among fishes and is usually adapted to food and feeding habits. Morphological structures and the shape of the stomach are variable in the different species of fishes and are related to capacity in the body cavity (Pandey and Shukla 2019). The stomach is not differentiated externally from the esophagus but can be compared morphologically by dissimilarities in the mucosal lining, which is thin in the esophagus but sturdy and wavy in the stomach. The structural organization and function of the stomach in fishes has been reported upon earlier (Saadatfar et al. 2010, Naguib et al. 2011, Musa et al. 2013, Ghosh and Chakrabarti 2015, Moawad et al. 2017, De Felice et al. 2021). The gross anatomy and size of the stomach differs markedly among fishes in relation to the nature of the food they consume. The stomach is usually large and pouch-like with thick walls in predatory carnivorous fishes like *Wallago attu* (Bloch & Schneider), *Sperata aor* (Hamilton), and *Chitala chitala* (Hamilton) that feed on large prey (Khanna 2019). The stomachs of omnivorous and planktivorous fishes are shorter than those of carnivores. In *Tenualosa ilisha* (Hamilton), *Gudusia chapra* (Hamilton), and mullets the stomach is smaller in size but highly stiffened into a gizzard-like structure to triturate food items (Khayyami et al. 2015). Not all teleosts have a true stomach, and it is absent in several fishes. Among cyprinids, the anterior portion of the intestine just behind the esophagus is modified into a sac-like structure, and this intestinal bulb serves for the temporary storage of food (Hofer 2006). The stomach is lacking in *Hippocampus*, *Scomberesox* and *Xenentodon cancila* (Hamilton) (Fänge and Grove 1979). Lacunae still exist in some aspects of studies relating to structural adaptations of the stomach in Indian teleost fishes correlated with feeding habits. With this view, the important food fish *Clupisoma garua* (Hamilton) was selected for the present study.

C. garua (Siluriformes, Ailiidae) is a demersal, predaceous river catfish that feeds on mollusks, aquatic insects, crustaceans, small fishes, and decaying algal matter (Gupta and Banerjee 2016, Bhakta and Sonia 2020). An attempt was in the present investigation to describe the microstructure and function of the stomach of *C. garua* by applying histological and histochemical techniques. This microscopic survey of the stomach will provide information about the detailed composition and chemical nature of various cells in relation to digestion.

Materials and methods

Sample collection

Sexually mature specimens of *C. garua* (ranging from 10.56 to 21.42 cm standard length) were procured live from the Bhagirathi-Hooghly River near areas surrounding Kalyani in West Bengal using traditional fishing gear. They were identified following a key to the classification of fishes by Jayaram (2010). The specimens were anesthetized deeply with Benzocaine (4 mg l^{-1}), and the abdominal cavity of the fish was opened with scissors. The stomach part was removed, cut into small samples and processed with the relevant techniques.

Histological analysis

Small pieces of stomach were immediately fixed in aqueous Bouin's solution for 24 h. After fixation, the samples were washed well in 70% ethanol, dehydrated through graded series of (50–100%) ethanol, and cleared with xylene. The tissues were infiltrated with paraffin wax at 56–58°C for 1.5 h, and embedded in paraffin blocks. Serial sections were cut at 4 μm thickness using a rotary microtome (Weswox MT-1090A) and stained with Delafield's Haematoxylin-Phloxin (HP), Delafield's Haematoxylin-Eosin (HE) (Fischer et al. 2008), and Mallory's Triple (MT) stain (Mallory 1936) for light microscopic observation.

Histochemical analysis

Small fragments of stomach were kept in 10% neutral formalin for 18 h. After the routine procedure, the tissues were embedded in paraffin wax at 52–54°C and sectioned at an 8 μm thickness. The paraffin sections were subjected to the following histochemical investigations: periodic acid–Schiff's (PAS) reaction in combination with Alcian Blue (AB) (PAS-AB) for the detection of neutral and acid mucins (Yamabayashi 1987); Best's Carmine (BC) method for the detection of glycogen (Horobin and Murgatroyd 1971); the Mercuric Bromophenol Blue (MBB) method for detecting basic protein (Mazia et al. 1953); and the Dimethylaminobenzaldehyde (DMAB)-nitrite method for tryptophan (Adams 1957). The stained slides were examined and photographed using an Olympus-Tokyo PM-6 compound microscope at different magnifications.

Results

Histology

The stomach of *C. garua* was a pouch-shaped muscular structure with cardiac and pyloric regions. The wall of the stomach was comprised of four basic

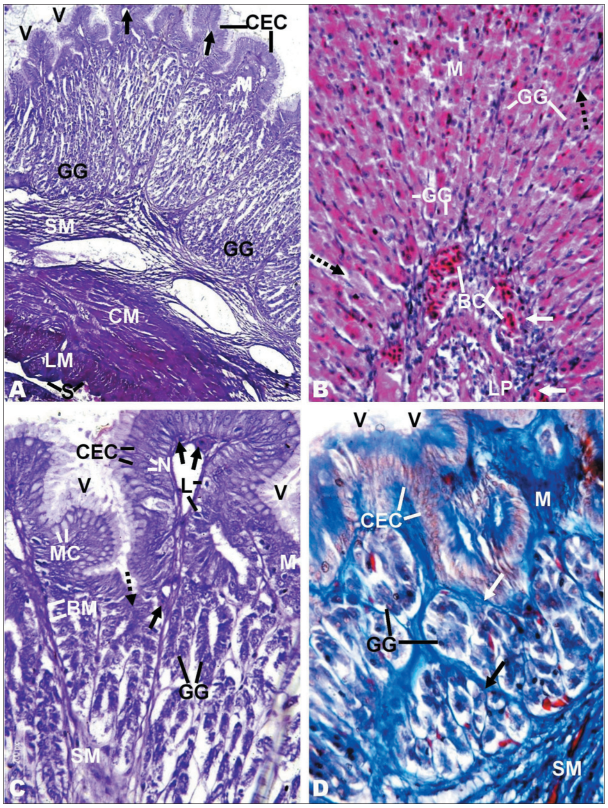

Figure 1. Microphotographs of the transverse sections through the stomach of *C. garua* showing cellular architecture stained with Delafield's Haematoxylin-Phloxin (HP), Delafield's Haematoxylin-Eosin (HE) and Mallory's triple (MT) stain. **(A)** The cardiac stomach shows mucosa (M) with numerous folds (solid arrows) provided with columnar epithelial cells (CEC) and gastric glands (GG), submucosa (SM), thicker circular muscle layer (CM), thinner longitudinal muscle layer (LM) and thin serosa (S). Solid arrows indicate thin top plate over CEC (HP × 4X). **(B)** Mucosa (M) shows clusters of tubular GG and submucosa with blood capillaries (BC) and eosinophilic granular cells (solid arrows) forming lamina propria (LP). Broken arrow marks the opening of GG to the gastric pit (HE × 15X). **(C)** Mucosa (M) containing CEC with prominent nuclei (N) and basal gastric glands (GG) distinguished from the submucosa (SM) by the basement membrane (BM). Note the presence of scattered mucous cells (MC) and lymphocytes (L) in mucosa and eosinophilic granular cells (solid arrows) in SM. Arrow heads specify mucus film over the apical surface of CEC and broken arrow marks the gastric pit (HP × 40X). **(D)** Magnified mucosa (M) exhibits compactly arranged CEC and GG bounded by connective tissue septa (solid arrows) arising from submucosa (SM). Arrow heads indicate mucin film over mucosal surface (MT × 45X).

histological layers: mucosa, submucosa, muscularis, and serosa (Fig. 1A). The mucosa was usually simpler than that in higher vertebrates and expanded by thick longitudinal folds, the rugae, the surface of which were carpeted with tiny depressions, the gastric pits. The cardiac stomach consisted of two types of epithelia: luminal epithelium with a monolayer of simple columnar epithelial cells and the glandular epithelium containing numerous gastric glands (Figs. 1C and D). The columnar epithelial cells were tall and elliptical in shape and tapered at the base. They had centrally or basally placed oval nuclei and their apical lining contained a prominent top plate of mucus. Mucous cells were scattered on the superficial epithelium (Fig. 1C). The glandular epithelium was packed with a cluster of gastric glands that were rounded or tubular in shape and present at the basal portion of the mucosa (Figs. 1B, C, and D). The gastric glands were composed of rhomboidal shaped granular cells with middling rounded nuclei distributed around a lumen. They usually opened into the lumen of the stomach trough the gastric pits (Figs. 1B and C). The gastric glands were firmly bordered by thin strips of connective tissues of lamina propria (Fig. 1D). Few lymphocytes were distinguished in the deeper layer of mucosa by their darkly staining conspicuous nuclei (Fig. 1C). There were no gastric glands in the pyloric stomach.

The submucosa was separated from mucosa by a basement membrane (Fig. 1C) made up of collagenous fibers, connective tissues, nerve fibers, and blood capillaries that protruded into the mucosal foldings forming lamina propria. (Fig. 1B). A few eosinophilic granular cells were also observed in the submucosa (Figs. 1B and C), which was thick in the pyloric stomach. The muscularis layer of the cardiac stomach was thinner than that in the pyloric stomach. It contained an interior circular and outward longitudinal muscular layer (Fig. 1A). The former layer was thicker than the latter. The outermost serosa layer was exceedingly thin and rarely impaled by blood vessels.

Histochemistry

Mucopolysaccharides

Combined periodic acid–Schiff's reaction and Alcian Blue (PAS-AB) showed a purple-bluish color of

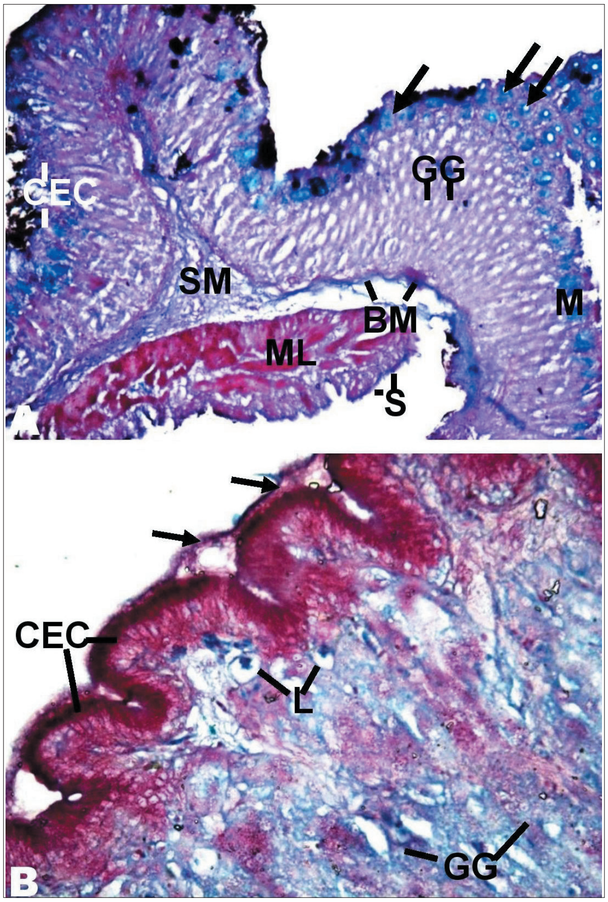

Figure 2. Photomicroscopy of the section of stomach displaying periodic acid–Schiff's (PAS) reaction conjointly with Alcian Blue (AB). **(A)** Localization of neutral mucopolysaccharide in the columnar epithelial cells (CEC) and both acid and neutral mucopolysaccharides in gastric glands (GG) of mucosa (M). Gastric pits (arrows) show bright blue color from the accumulation of acid mucopolysaccharides. The basement membrane (BM), submucosa (SM), muscularis (ML), and serosa (S) layers exhibit positive response (PAS-AB × 10X). **(B)** Intense PAS reaction in CEC along with luminal border of the mucosa (arrows). Note positive reaction in lymphocytes (L) and GG (PAS- AB × 40X).

varied magnitude conforming to the neutral and acid mucopolysaccharides of the different layers coating the stomach. The columnar epithelial cells of gastric mucosa were a bright purple color from PAS, while the gastric glands of glandular epithelium were a purple-bluish color because of the localization of both neutral and acid mucopolysaccharides (Figs. 2A and B). The gastric pits contained acid mucopolysaccharides. The secreted luminal mucin over the apical portion of the columnar epithelial cells were an intense purple color marking the presence of ample neutral mucin (Fig. 2B). The basement membrane between the mucosa and submucosa together with fibrous connective tissues exhibited a distinctive bluish-purple color. The lymphocytes of the mucosa and the muscularis and serosa layer expressed positive reactions with this combined test (Fig. 2A).

Glycogen

The results of Best's carmine test showed a deep red color in the columnar epithelial cells together with the gastric glands of the mucosa because of the marked amount of glycogen (Figs. 3A and B). The blood capillaries in the submucosa contained different shades of staining deposition of glycogen but the connective tissues exhibited a weak reaction. The subtle reaction of glycogen was discernible in the muscularis and serosa layer (Fig. 3A).

Protein

The mercuric bromophenol blue reaction revealed a dark blue color of diversified magnitudes in harmony with the localization of basic protein in various regions of the stomach. Mucous cells and columnar epithelial cells with coarse granules in the apical mucosa showed strong reactivity (Figs 4A and B). Gastric glands exhibited their protein inclusions as darkly stained fine grains dissipated homogeneously in the cytoplasm. The blood capillaries and connective tissues in the submucosa showed a positive reaction (Fig. 4A). The muscularis layer showed an intense reaction.

Tryptophan

The mucosa layer of stomach displayed a deep blue color considered a positive reaction for tryptophan (Fig. 5A). The zymogen granules of the gastric glands in the glandular epithelium showed the maximum intensity of the tryptophan reaction (Fig. 5B). The columnar epithelium cells of the superficial mucosa also furnished positive reactions for tryptophan though comparatively less than that of the gastric glands. The submucosa, the muscularis layer, and

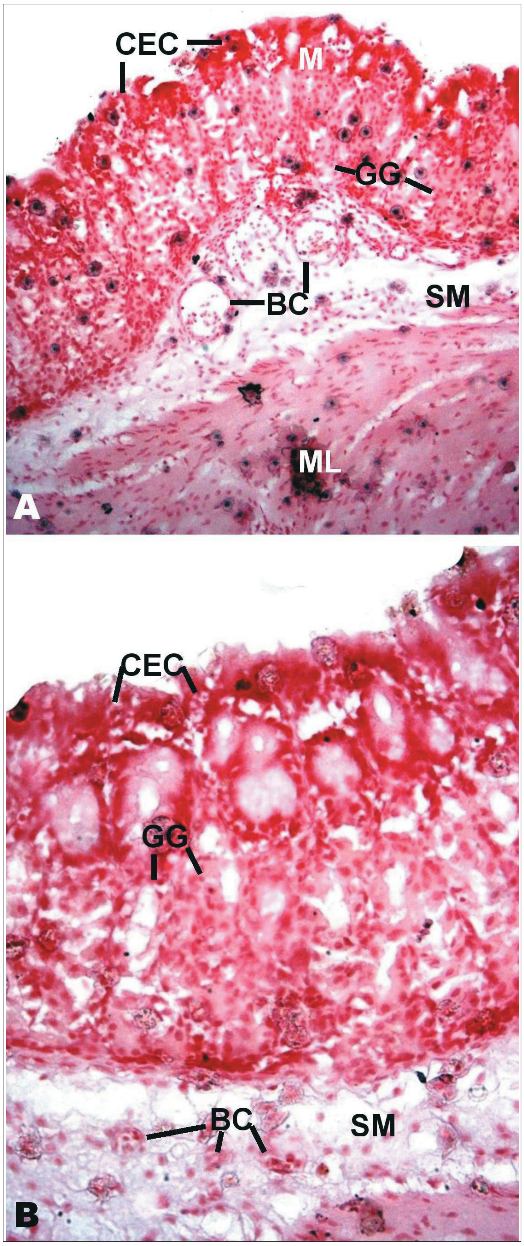

Figure 3. Microphotographs of the section of stomach exhibiting Best's Carmine (BC) reaction for the detection of glycogen. **(A)** Showing glycogen content in the columnar epithelial cells (CEC) and gastric glands (GG) of mucosa (M). Note moderate reaction in blood capillaries (BC) of submucosa (SM) and muscularis layer (ML) (BC × 10X). **(B)** Intense localization of glycogen in CEC and GG of mucosa. BC in SM display a moderate reaction (BC × 40X).

the serosa were colorless and exhibited negative responses to this test (Fig. 5A).

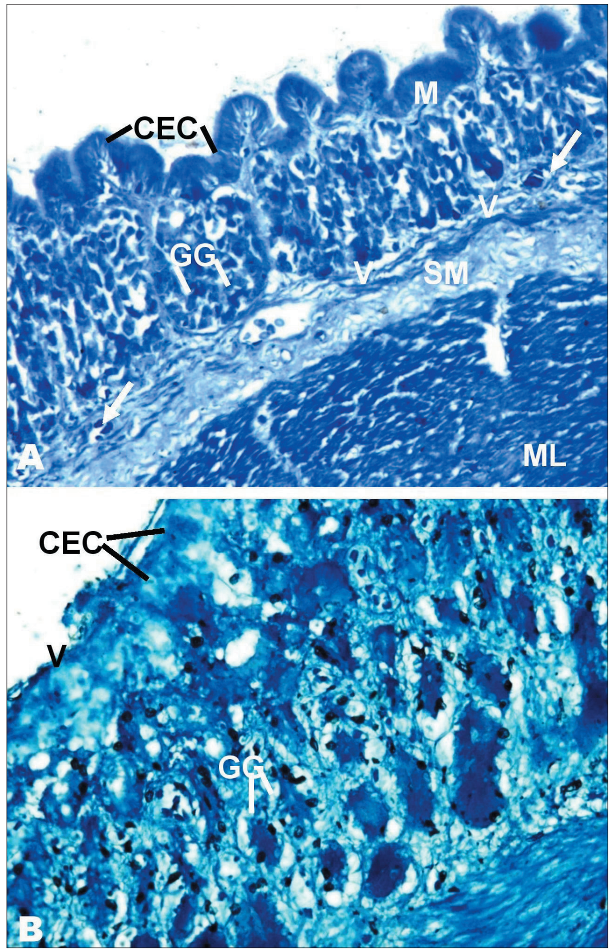

Figure 4. Transverse sections of stomach show Mercuric Bromophenol Blue (MBB) reaction. **(A)** Localization of protein in columnar epithelial cells (CEC) and gastric glands (GG) of mucosa (M). Note the positive reaction in the muscularis layer (ML), connective tissues (arrow heads), and blood capillaries (solid arrows) of the submucosa (SM) (MBB × 10X). **(B)** Inclusion of protein in CEC, GG, and mucous cell of the mucosa layer (MBB × 40X).

Discussion

The digestive system of teleosts exhibits much variation in structure and function and is adapted for particular foods. The anatomy of the stomach is distinctly variable in size and organization in relation to the quantity of food consumed by the species (Ostrander 2000). In *C. garua*, the stomach was pouch-like and thick walled since the fish is predatory in nature. The walls of the stomach were usually

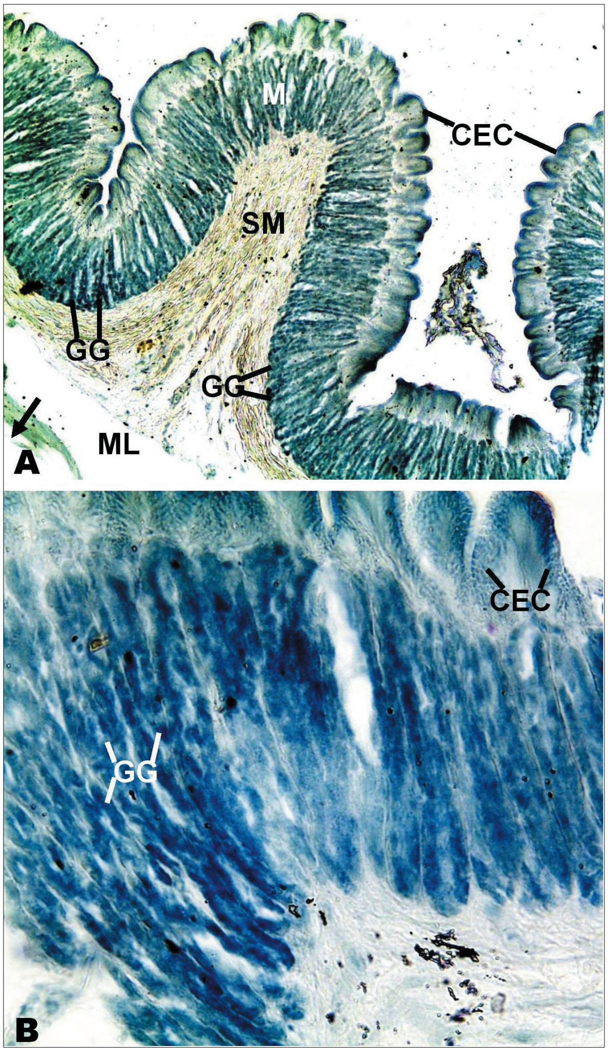

Figure 5. Microphotographs of the section of stomach in *C. garua* showing the localization of trypotophan (DMAB). **(A)** Mucosa (M) with columnar epithelial cells (CEC) and gastric glands (GG) show a positive tryptophan reaction. The submucosa (SM), mucularis (ML), and serosa (S) display negative reactions (DMAB × 10X). **(B)** Strong intensity of tryptophan reaction in GG and CEC. (DMAB × 40X).

folded and can expand. Histologically, the stomach of *C. garua* had four layers and is related to other teleost fishes (Arellano et al. 2001, Khayyami et al. 2015). The cardiac stomach was formed by two types of epithelia – surface epithelium and glandular epithelium. The surface epithelium was composed of columnar epithelial cells with a notable top plate of mucin and scanty mucous cells. Barrington (1957) reported that the columnar epithelial cells of the superficial epithelium secreted gastric mucin that protected the stomach surface from mechanical damage. The columnar epithelial cells of the superficial epithelium are described as absorptive in function by some researchers (Grau et al. 1992, Arellano et al. 2001). From the present study, it can be assumed that columnar epithelial cells of the mucosa served a dual purpose of the secretion of mucus and the absorption of digested food stuffs. Very few mucous cells were observed in the gastric mucosa of *C. garua*. Their lubricant secretions safeguard the gastric epithelium from the chemical and physical destruction that could occur (Mescher 2016). The lymphocytes in the mucosa layer of the stomach might have been engaged in protecting the fish against various pathogens (Park and Kim 2001, Moawad et al. 2017).

The gastric glands of the cardiac stomach were primitive in nature and not distinguished into oxyntic and peptic types. They opened into the lumen through the gastric pits and secreted pepsinogen and hydrochloric acid. The gastric histology of teleosts is usually simpler than higher vertebrates as the gastric glands bear a single type of cell that secretes both pepsinogen and hydrochloric acid (Rebolledo and Vial 1979, Albrecht et al. 2001). *C. garua, a* carnivorous catfish, needs the active secretion of digestive enzymes to adequately digest its proteinaceous foods, and, accordingly, profuse bunched gastric glands developed. The proteolytic enzyme pepsin originated in the gastric juice that was secreted by the granular cells of the gastric glands.

The presence of collagenous fibers and connective tissues in the lamina propria of the stomach might provide a protective, stiffened coat in addition to keeping the gastric gland in the perfect location (Albrecht et al. 2001). The muscular layer of the stomach was comparatively thick and formed by the usual inner wide circular layer and outer longitudinal layer of muscle fibers connected with the thin serosa. This organization of muscle layers probably helped to keep the enlargement of the wall of cardiac stomach within bounds. The greater development of the circular muscle layer presumably assisted in decisive contractions for comminuting semi-digested foods. Murray et al. (1994) and Khayyami et al. (2015) also report similar features.

PAS reactions in the columnar epithelial cells along with the apical border were indicated by the purple color from the presence of neutral mucin, whereas the bluish-purple color of the gastric glands illustrated the subsistence of both acid and neutral mucins. The mucoid nature of the gastric epithelium is reported by several researchers in many teleosts (Murray et al. 1994, Scocco et al. 1996, Raji and Norouzi 2010, Okuthe and Bhomela 2020). The apical border of the columnar epithelium produced a strong PAS reaction because of the presence of mucous cells. The surface epithelium possibly secreted mucus for the lubrication and propulsion of food stuffs toward the pyloric portion and also to neutralize the acid secreted by the gastric glands. The mucin secreted also shielded the gastric epithelium from mechanical abrasion and stoped autodigestion (Petrinec et al. 2005).

The Best's carmine response in the columnar epithelial cells of the stomach was conceivably associated with the synthesis of neutral mucin that needed energy, and the deposited glycogen provided it. The glycogen reaction in the gastric glands of *C. garua* was concomitant with the synthesis of zymogen granules and ergastic matters for the secretion of pepsinogen and HCl. Anwar and Mahmoud (1975) report the presence of ailing glycogen content in different layers of the intestines of Egyptian lizards used as an inception of energy but not accumulated.

The presence of basic protein in the mucosa, submucosa, and muscularis layers of the stomach is required for various metabolic and physiological activities. Arellano et al. (2001) report there are proteins rich in lysine, arginine, cysteine, and cystine in the columnar epithelial cells, gastric glands, lamina propria, and muscular layers of the stomach of *Solea senegalensis* Kaup. The gastric glands of the mucosa layer were firmly positive to mercury-bromophenol blue staining because of the existence of enzymatic precursors like pepsinogen or other gastrointestinal enzymes (Gutiérrez et al. 1986, Gisbert et al. 1999). The existence of a diversified proportion of protein and tryptophan in the columnar epithelial cells of *C. garua* might have been related to the metabolic processes of the cell. The presence of tryptophan residues in the gastric glands stemmed from the condensation of quantities of zymogen as a harbinger of pepsinogen. Medeiros et al. (1970) recorded the amino acids tryptophan, tyrosine, and arginine in the glandular cells of the stomach in *Pimelodus maculatus* Lacepčde for the synthesis of pepsinogen by the gastric glands.

In conclusion, the stomach of *C. garua* had a variety of cells that performed specific specialized functions. The glandular epithelium was characterized by hydrochloric acid and pepsin producing gastric glands, the principal site for gastric digestion. The present study provides information about the cellular features of the stomach that will help to further elucidate the nutritional physiology of this fish species.

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