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MORPHOLOGY OF THE AFRICAN CATFISH STOMACH

By

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SUMMARY

The purpose of the present study was to investigate the morphology, histochemistry and ultrastructure of the stomach of African Catfish *Clarias gariepinus*. Grossly, the stomach was J-shaped sac divided into cardiac, fundic and pyloric portions. Histologically, the wall of the stomach composed of four distinct tunics; mucosa, submucosa, muscularis externa and serosa. The mucosa of the three portions showed thick longitudinal folds lined with simple high columnar cells containing oval basally located nuclei. These columnar cells contained apically located mucosubstances that reacted positively with Periodic Acid Schiff (PAS) and negatively with Alcian-Blue (AB) stains. Many gastric pits were formed by invaginations of the mucosal layer. Both cardiac and fundic regions contained mucosal glands while the pyloric did not. The fundic glands lined with one cell type; oxynticopeptic cells. Enteroendocrine cells were distributed all over the gastric wall within the epithelial cells lining the gastric mucosa and glands. The lamina propria composed of extensive collagen fibers, many blood vessels and nerves. Strands of smooth muscle fibers separated the lamina propria from the submucosa forming lamina muscularis mucosa. Loose connective tissue was the main component of the tunica submucosa. The pyloric portion had the thickest musclosa that composed of three layers; thin inner and outer longitudinal and thick middle circular layers of SMF. A thin layer of loose connective tissue containing many blood vessels covered by a single layer of mesothelial cells constituting the serosal coat of the stomach. Based on the obtained results in the current study, we concluded that African Catfish *Clarias gariepinus* possessed a stomach resembles that of a carnivorous nutritious habits.

Keywords:

African Catfish; Morphology; Stomach; Immune Histochemistry; Ultrastructure.

INTRODUCTION

African Catfish *Clarias gariepinus* is widely distributed in Africa and parts of Asia (Syria and south of Turkey). Its main habitats are calm lakes, rivers and swamps in areas that flood on a seasonal basis **(De Graaf and Janssen, 1996**). The digestive system of fishes has a remarkable diversity in its morphological structures and functions, this diversity may be related to taxonomy and feeding habits (**AL-Abdulhadi, 2005**). According to the nature of food consumed by all fish species, the feeding activities are classified into; herbivores fish that eat plant material, carnivores one which consume animal material and omnivores fish which consume both plant and animal materials **(Evans and Claiborne, 2006)**. In addition, **Grau *et al.* (1992)** stated that, the main features of the carnivorous fish digestive tract are the presence of a large stomach and a short intestine. In this concern, the stomach of fishes is divided into many categories depending upon their shape; no stomach as in Cryprinids and Labrids, J-shape in African and American Catfish, straight with enlarged lumen in pike, channel Catfish and halibut, U-shaped in salmonid and Y-shaped in tilapia **(Smith, 1989)**. The approach to the histological structures of fish digestive tract resides in the application of this knowledge more in understanding the pathology of diseases, contributing in the development of fish farming and a rational use of the natural resources **(Pereyra *et al.*, 1999).** Moreover, knowledge about the structure of digestive system in various fish species is useful for the nutritional development researches and preparation of diets **(Rotta, 2003).** Even though investigations of most teleost are reported, but there is dearth of information about the immunohistochemistry and ultrastructure of the stomach in African Catfish from Nile River. In this context, the aim of the present work was to illustrate the morphological, histological, immunohistochemically and ultrastructure of the stomach of African Catfish. These observations will provide a basis for understanding the digestive physiology and help pathologists and nutritionists in the future studies on the diet and diseases affected the species under investigation to facilitate the histopathological diagnosis of these diseases.

MATERIAL AND METHODS

Freshwater African Catfish *Clarias gariepinus* (Class: Actinopterygii, Order: Siluriformes, Family: Clariidae) of both sexes were used in the current investigation Fig. (1a).

Collection of the samples:

A total number of forty adult, apparently healthy African catfish ranging from 20-30cm in length and 500-700g body weight were caught alive from the Nile River at different localities in Beni-Suef Governorate, Egypt. They were transported in plastic aquaria to the laboratory within two hours to allow the aerial respiration.

Gross examination:

Fifteen fishes were used to demonstrate the gross morphological features of their stomachs. The fishes were sacrificed and a ventral incision was made from the anal opening to the interbranchial membrane. The stomachs were grossly examined in situ and carefully dissected; lengths and diameters were measured using a caliber and photographed using a digital camera (Kodak, MP 12, 4x optical zoom).

For corrosion casting specimen:

The digestive tube was carefully removed outside the body then a cannula was fixed in the esophagus. A prepared epoxy (2 parts of polymer A and 1 part of hardener B, thoroughly mixed and colored red using rotting ink) was then injected. The obtained specimen was kept at room temperature for 24 hours to allow curing of the cast, then it was kept for 3 days in 3% KOH solution for corrosion.

Histological and ultrastructural studies:

Immediately after fish euthanasia, fresh specimens were carefully collected from the cardiac, fundic and pyloric regions of the stomach.

Light microscopy:

Fifteen fishes were used for the light microscopy, small pieces of 0.5 x 0.5cm from different portions of the stomach were fixed by immersion in aqueous Bouin's fluid for 24 hour, then dehydrated using ascending grades of ethanol (50-100%), cleared in xylene, embedded in Paraplast, sectioned at a thickness of 5 µm and mounted on a clean dry glass slides. The sections were stained with Harris' Haematoxylin and Eosin. Some sections were stained with periodic acid-Schiff reagent (PAS), Alcian blue (AB) and Crossman's trichrome stain. Grimelus silver method was used to demonstrate the, argyophilic cells, endocrine cells among the cells of the mucosal epithelium and gastric glands. The above-mentioned stains were applied as outlined by **Bancroft and Gamble (2008)**. *Transmission Electron Microscopy:* Five fishes were used for the transmission electron microscopy. Small pieces 2.0-3.0 mm from different portions of the stomach were fixed in 4% glutaraldehyde solution in phosphate buffer (pH 7.2-7.4) overnight at 4°C, then processed to prepare semi thin sections (1µm thick).The obtained sections were stained with Toluidine blue and examined with the light microscope to select the suitable areas representing the desired observations. Ultrathin sections (600A in thickness) were mounted on copper grids, stained with 5% uranyl acetate and lead citrate according to **Reynolds (1963).** Finally, these grids were examined using a JEOL 100S electron microscope at the Electron Microscope Unit at the National Cancer Institute, Cairo, Egypt. *Scanning Electron Microscopy:* The whole stomachs obtained from five fresh African Catfishes were incised longitudinally to expose their luminal surfaces. The tissues were then fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.3) and kept for 24 hour at 4°C. After fixation, the samples were rinsed in 0.2 M phosphate buffer, trimmed into 8-10 mm and post fixed for 2 hours in 1% osmium tetroxide in 0.2 M phosphate buffer (pH 7.3). The tissues were then dehydrated in ascending series of acetone, cleared in iso amyl acetate and critical point dried with carbon dioxide. The serosal surface of the cardiac, fundic and pyloric portions were mounted on metal stubs with mucosal surface uppermost and the specimens were coated with gold by using vacuum gold coater. The specimens were examined with a JEOL/EO-JSM-6510 LV scanning electron microscope at Faculty of Science, Beni-Suef University, Egypt.

RESULTS

Grossly:

The stomach of the African Catfish *Clarkias gariepinus* appeared as J-shaped muscular sac behind the liver on the left side of the abdominal cavity with average length from 3.3 - 4.5cm, while when the stomach is full it became. It extended from the esophagus to the proximal intestine. Externally Fig.(1b, c, e), it had a dorsal surface related to the kidney, and a ventral surface related to the proximal intestine and two curvatures started from the cardia on the right side to the pylorus on the left side. Internally Fig. (1d), the stomach was divided into three distinct regions; cardiac, fundic and pyloric. The cardiac region (right or descending limb) was the initial region of the stomach started from the esophagus in the form of a cone shape connected to the fundic region. It was ventral to the dorsal wall of the body cavity and dorsal to the proximal intestine and visceral surface of the liver. The inner surface of the cardiac region had longitudinal mucosal folds. The fundic region (middle part) constituted the majority of the stomach; it appeared as a pouch extended caudally and communicated with the other two regions. It was rostral to the spleen and ventral to the kidney and proximal intestine. Its internal surface was dark brown in color containing approximately twelve thick longitudinally corrugated mucosal folds. The pyloric region (left or ascending limb) represented the small terminal portion of the stomach that was related to the lateral and ventral wall of the body cavity. Externally, it showed a pyloric constriction with the initial part of the proximal intestine. Internally, it appeared paler in color, with no visible mucosal folds and connected with the proximal intestine by a narrow pyloric sphincter.

By light microscopic examination:

Three potions of the stomach; cardiac (anterior), fundic (middle and glandular) and pyloric (posterior and non-glandular) were observed.

Cardiac region:

The cardiac region of the stomach composed of four distinct concentric tunics arranged from inward to outward as follow; mucosa, submucosa, muscularis externa and serosa Fig. (2a), the tunica mucosa thrown up into several folds or rugae creating a narrow stellate lumen in empty stomach. These folds appeared as longitudinally oriented cup shaped folds of different sizes include the lining surface epithelium and the underlying lamina propria. It was lined with high columnar mucous secreting cells with homogenous faintly stained cytoplasm and basal oval nuclei Fig. (2b). the columnar cells contained apically concentrated secretory granules that reacted strong positively with PAS reaction Fig. (2c) and negatively with AB stain. Gastric pits (foveolae) were formed by invaginations of the mucosal layer into the underlying lamina propria and continuous with the openings of the gastric glands. These pits appeared short; shallow in depth lined with tall columnar cells represented a continuation of the surface epithelial cells Fig. (2d). numerous lymphocytes were observed under the epithelial cells lining the gastric mucosa in most examined sections of the cardiac region. The lamina propria extended deeply in the core of the mucosal folds contained loose connective tissue with extensive collagen fibers, many blood vessels and gastric glands Fig. (2e). those gastric glands appeared simple tubular parallel glands and lined with polyhedral shaped-cells and possessed darkly stained homogenous cytoplasm and basally situated oval nuclei Fig. (2d). these cells showed negative reaction to both PAS Fig. (2c) and

AB reactions. Enteroendocrinecells (argyophilic cells) were demonstrated within the epithelial cells lining the gastric mucosa Fig. (2f) and gastric glands Fig. (2g). Longitudinally arranged SMF situated in between the lamina propria and the tunica submucosa represented the lamina muscularis mucosae, in addition to diffuse strands of SMF were noticed in the tunica submucosa.The muscularis externa represented by inner circular and outer longitudinal SMF with inter muscular C.T contained blood vessels and nerves Fig. (2a). Areolar C.T, lamina subserosa, with abundant blood vessels covered with mesothelium, lamina epithelialis serosa, constituted the tunica serosa of the cardiac region Fig. (2a).

Fundic region:

The examined microscopical sections of the fundic region of the stomach showed four concentrically arranged tunics; mucosa, submucosa, muscularis externa and serosa. The tunica mucosa possessed several broad folds, which appeared lined with a single layer of columnar epithelial cells with oval basally located nuclei Fig. (3a) and apically located mucosubstances that reacted positively with PAS Fig. (3b) but not to AB. Threads of mucin covered the gastric mucosa secreted by the mucosal epithelial cells. Many depressions were noticed along the mucosal layer into the lamina propria continuous with underlying tubular glands forming the gastric pits. These pits appeared deeper than that observed in the cardiac stomach. The epithelial cells lined the pits appeared as a continuation of the columnar cells lining the gastric mucosa Fig. (3a, c). Numerous enteroendocrine cells were demonstrated within the epithelial cells lining the fundic glands (Fig. 3d). Many lymphocytes were seen in the tunica mucosa of the fundic region. The lamina propria appeared thicker than that of the cardiac stomach and showed extensive collagenic bundles in addition to many blood vessels. These bundles held the fundic glands and penetrated the mucosal folds Fig. (3e). The fundic glands arranged in straight parallel less coiled tubular manner perpendicular to the gastric surface mucosa occupying the entire mucosal layer beneath the surface epithelium and continuous with the gastric pits Fig. (3c). those glands were surrounded by a layer of C.T and lined with oxynticopeptic cells Fig. (3c) and enteroendocrine cells Fig. (3d). Two layers of SMF were situated between the lamina propria and the tunica submucosa forming lamina muscularis mucosa. Small muscular sheaves were intermingled with the connective tissues of the tunica submucosa Fig. (3e).Thick tunica submucosa appeared in between the lamina Muscular is mucosa and tunica muscularis externa composed mainly of highly vascularized loose C.T containing collagenic fibers and strands of SMF. The tunica muscularis externa was constructed by inner circular and outer longitudinal SMF. The serosa was thin loose C.T limited by a single layer of mesothelial cells.

Pyloric region:

The pyloric region of the stomach was easily distinguished microscopically by sudden disappearance of gastric glands, thick long mucosal folds; wide and deep gastric pits Fig. (3a).The epithelial cells lined the gastric mucosa were simple columnar cells with faintly stained homogenous cytoplasm and basally located oval nuclei. The apical portions of these cells were filled with intensely staining positive PAS Fig. (3b) and negative AB secretions. The lamina propria appeared thick and showed highly vascularized loose connective tissue rich in collagenic fibers that penetrate the mucosal folds Fig. (3a, c).The tunica submucosa was the thinnest layer when compared with those of the cardiac and fundic stomach and composed of vascularized loose C.T. The tunica musclosa of the pyloric region was the thickest among the other portions of the stomach forming the gastrointestinal sphincter. It composed of three layers; thin inner and outer longitudinal in addition to thick middle circular layers of SMF Fig. (3c). A thin serosal layer formed the outer lining of the pyloric stomach, constituted by a thin loose C.T lined with a single layer of squamous epithelium.

By scanning electron microscope:

The stomach showed primary longitudinal mucosal folds along the whole length of the cardiac and fundic parts, which left deep furrows in between Figs. (5a-6a). On the surface of these folds another secondary mucosal folds irregularly anastomosed with each other leaving discrete pockets in between Figs. (5b, c - 6b). The luminal surface of the columnar epithelial cells bearing microvilli and appeared as a honey comb polyhedral epithelial cells in the form of pentagonal or hexagonal elevations with the presence of gastric pits in between and extensive mucin droplets appeared as thin layer overlying the lining mucosa Figs. (5c,d-6c, d). Oval elevations were observed between the secondary mucosal folds represented the luminal surface of the goblet cells Figs. (5c - 6c). A transverse section of the fundic gland region showed primary folds with remarked gastric crypts of the gastric glands and deep gastric grooves Fig. (6e). These irregularly arranged mucosal folds with prominent gastric pits appeared as narrow concavities that were encircled by rosettes of columnar epithelial cells, oval shaped goblet cells and extensively scattered mucin droplets on the surface of the columnar cells. These columnar epithelial cells were provided with short and stubby microridges Fig. (6b, c, d). The transition area between the fundic and pyloric regions of the stomach, with a remarkable shallow groove could be seen Fig. (6f). There was a remarkable groove on the mucosa that indicated the position of the pyloric sphincter between the terminal part of the pyloric region and the initial part of the proximal intestine (Fig. 7a) and there were deep empty concavities formed by the anastomosis of the pyloric mucosal folds which possessed big and stubby micro-ridges Fig. (7b).

By transmission electron microscope:

The ultrastructural investigation of the three portions of the stomach of catfish showed that, the epithelial cells lining the gastric lumen and pits appeared tall columnar, electron-dense containing numerous apically located secretory granules that appeared homogeneous, elongated in shape with variable sizes. Some of these secretory granules were noticed fused with the cell membrane. The nuclei appeared large, oval basally located Fig. (8a, b) and surrounded by many cytoplasmic organelles; well-developed Golgi apparatus, rough endoplasmic reticulum and numerous mitochondria. The surface of the gastric epithelial cells exhibited many apically located microvilli. The cardiac glands showed only one type of cells, which appeared ultrastructurally polyhedral in shape containing rounded basal vesicular nucleus, many mitochondria and extensive network of rough endoplasmic reticulum. The fundic glands were lined with only one cell type; oxynticopeptic cells. The observed oxynticopeptic cells had polygonal shape and centrally situated nuclei. Numerous spherical shaped vesicles of different sizes were distributed in cytoplasm Fig. (8c). The supra nuclear part of their cytoplasm contained a tubulovesicular network, composed of numerous smooth small vesicles scattered throughout the cell cytoplasm especially at the apical region in between numerous tubules of rough and smooth types of endoplasmic reticulum and secretory granules Fig. (8d). numerous mitochondria, Well- developed Golgi apparatus and lysosomes were located all over the cytoplasm. The lateral cell membranes form smooth contacts with the neighboring cells and lack any interdigitating. Concerning the interior endocrine cells; they appeared ultrastructurally electron-lucent scattered throughout the gastric mucosal epithelium. They were rounded in shape with rounded euchomatic nucleus. Their cytoplasm contained rough endoplasmic reticulum, few rounded or elongated mitochondria and numerous scattered secretory granules exhibits variable shapes and sizes Fig. (8e).

DISCUSSION

The stomach of the African catfish *Clarias gariepinus* appeared grossly as J-shaped similar to that in South American Catfish **(Hernández *et al.,* 2009)**, channel Catfish **(Sis *et al.,* 1979)** and Rainbow trout **(Ezeasor and Stokoe, 1981)**, this shape may help in extending the time feed stays in the stomach, thereby ensuring greater degree of digestion by gastric juice. Moreover, different shapes of the stomach were reported in other fish species as Y-shaped stomach in *Oreochromis* niloticus **(Caceci *et al.,* 1997)** also Anguilla, the true cods, and ocean perch **(Smith, 1989),** U-shaped stomach in Salmo, Coregonus and Clupea and straight stomach in Esox **(Smith, 1989)**. The obtained corrosion cast of the stomach cavity, using epoxy polymer, and the observed extensive areas of folding ,using scanning electron microscopy, revealed that, the stomach is distensible and increased more than its normal size during food consumption. This would allow ingestion of large sized preys, extend the time of feed stay in the organ and increased the surface area for digestive enzymes activities permit efficient mixing of food with the digestive fluid **(Sis *et al.,* 1979; Osman and Caceci, 1991).** The histological examination of the stomach confirmed the anatomical observation as the stomach of African catfish composed of three distinct regions; cardiac, fundic and pyloric. These results were reported in many teleost's **(Raji and Norouzi, 2010; Xiong *et al.,* 2011; Ekele *et al.,* 2014)**. All examined sections from both cardiac and pyloric regions showed that the stomach of the African Catfish showed uniform histological features; superficial epithelial cells and simple straight tubular gastric glands in lamina propria surrounded by connective tissue. The presence of these gastric glands in both cardiac and fundic region and absence in pylorus has also been reported by **Chen *et al.* (2006)** in Kingfish, **Zacarias-Soto *et al.* (2006)** in *Paralichthy scaliforniaus*, **Yang *et al.* (2010)** in yellow Catfish, **Xiong *et al.* (2011)** in *Glyptosternum maculatum*. A similar arrangement of the gastric glands has been observed in other fish **(Domeneghini *et al.,* 1999** in white sturgeon; **Kozarić *et al.,* 2007** in blue fin tuna; **Chakrabarti and Ghosh, 2014** in Mystus vittatus). Our microscopical investigations of the three portions of the African Catfish's stomach showed that, the epithelial cells lining both the gastric mucosa and pits contain mucosubstances. These mucosubstances were intensely reacted with PAS and not to AB. This result indicates the presence of only neutral glycoproteins which might be related to conduction of food, provide efficient protection against proteolysis and mechanical injury **(Murray *et al.,* 1994; Domeneghini *et al.,* 1999;**

Petrinec ***et al.,*** **2005; Awaad** ***et al.,*** **2014)** and had buffering effects on the high concentration acid contents of the stomach **(Kozarić** ***et al.,*** **2008)**. Moreover, it may also involve in digestive activity by digestion and absorbing of easily digested molecules, such as disaccharides and short-chain fatty acids **(Grau** ***et al.,*** **1992).** According to **Osman and Caceci (1991),** these mucins also regulate the pH of the gastric fluid, explaining the variations in the PH of the gastric fluid in different species with different diets. On the other hand, the neutral mucous may serve to protect the stomach epithelium from auto-digestion processes caused by hydrochloric acid and the enzymes secreted by gastric gland by forming an adherent mucus gel **(Ferraris** ***et al.,*** **1987; Smith, 1989)** and provide complementation for the degradation **(Anderson, 1986).** Ultrastructurally, some secretory granules were frequently seen fused with the apical cell membranes, this may be correlated to empty their mucous contents into the gastric lumen via exocytosis process **(Ostos Garrido** ***et al.,*** **1993 ,** ***Oncorhynchus mykiss*****; Ostos Garrido** ***et al.,*** **1996 in** ***Anguilla Anguilla*****; Naguib** ***et al.,*** **2011 ,** ***Labeo niloticus*****)**. The apical borders of the gastric epithelial cells were observed carry microvilli that may be correlated to increase the absorptive activity **(Arellano** ***et al.,*** **2001).** Thus, the gastric epithelial cells lining the gastric mucosa and pits rich in neutral mucosubstances, mitochondria-rich cytoplasm and carrying microvilli implied that, the stomach of African Catfish had strong abilities for both digestion and absorption. The fundic glands show the presence of only one type of secretory cells, but no presence of any kind of glycoproteins. This result is accepted as there are general agreement, as pointed out by many authors, that only the gastric glands of mammals have a distinct acid-producing parietal cells (oxyntic cells) and zymogenic (chief) cells. In fish, amphibians and birds, both hydrochloric acid and pepsinogen are assumed to be secreted by only one cell type called oxynticopeptic cells **(Rebolledo and Vial, 1979; Ray and Moitra, 1982; Gisbert** ***et al.,*** **1999; Albrecht** ***et al.,*** **2001; Diaz** ***et al.,*** **2008)**.Ultrastructurally, features of both the oxyntic and peptic cells of the mammalian stomach were recorded in the oxynticopeptic cells of fundic region of Catfish's stomach the apical portion of the cytoplasm contains a well-developed vesiculotubular system many mitochondria which may be involved in secretion of hydrochloric acid secretion. Moreover, these cells posses well developed rough endoplasmic reticulum and numerous secretory granules that may be involved in production of pepsinogen. These results were in accordance with that obtained by **Ostos Garrido** ***et al.,*** **(1993) and Gallagher** ***et al.,*** **(2001)**. In this study, Enteroendocrine cells were noticed distributed all over the gastric wall between the epithelial cells lining the gastric mucosa and glands, these cells showed positive reaction to Grimelius stain. Ultrastructurally, it showed vesicular nuclei, rough endoplasmic reticulum and characteristic secretory granules. These cells may be involved in the secretion of various hormones that play an important role in digestive activities of the gut **(Podkowa and Goniakowska-Witaliñska, 2003; Lee *et al.,* 2004; Khidr, 2006)**. Moreover, **Ghattas (2004)** reported that, the gastric mucosa of catfish contain numerous enteroendocrine cells that secret many hormones; gastrin, somatostatin, and serotonin that might stimulate the glandular cells to increase the hydrochloric acid secretion. In agreement with several authors as **Osman and Caceci, (1991)** in tilapia Nilotica and **Park and Kim, (2001)** in mud loach), the lymphocytes that were observed in the mucosa of the stomach of the species under investigation may play an important role in protecting the fish from pathogenic microorganisms. Extensive collagenous fibers that were noticed in the lamina propria of three examined portions of the stomach might form a supporting, protective and strengthen layer as well as keep the gland in position. The powerful gastric musclosa of the carnivorous Catfish; the well-developed inner circular and outer longitudinal unstriated muscle fibers and collagenic fibers of the submucosa may involve in involuntary contraction for food digestion **(Albrecht *et al.,* 2001; Diaz *et al.,* 2008; Xiong *et al.,* 2011)**. So the retention time of ingested food in the stomach extended and the volumetric capacity of the stomach increased **(Albrecht *et al.,* 2001)**. Moreover, it may also be involved in trituration **(Ostos Garrido *et al.,* 1996 and Cao and Wang, 2009)** and for supporting and strengthening since stratum compactum is lacking in the species under investigation **(Burnstock, 1959).** In pyloric region, a Combination of a well-developed musculature, inner and outer longitudinal in addition to middle circular layers of smooth muscle fibers, and the absence of the pyloric glands may indicate that the primary function of this portion is the mixing of food and pushing it distally **(Reifel and Travill, 1978)**. In addition to, the absence of gastric glands may relate to adaptation of this species to reduce the quantity of hydrochloric acid entering the proximal intestine; hence, it might help the alkaline medium to maximize pancreatic enzyme actions in the proximal intestine **(Ribeiro *et al.,* 1999).** Presence of serosal coat consisting of mesothelial cells and loose connective tissue containing small blood vessels as seen in the present study has been reported in most other species of telosts **(Arellano *et al.,* 2001).**

CONCLUSIONS

The present study suggested that, the histological features of the stomach of African Catfish, *Clarias gariepinus* were consistent with the feeding habit of a carnivorous fish. (This (microanatomical structure) provides a baseline data for further investigative researches, help the fish clinicians in understanding the physiology and pathology of fish also aid nutritionists in feed management; preparation and handling of diets). However, further studies should be carried out for other explanations of the digestion process and nutrient absorption in those fish.

<u>Conflict of Interests:</u>

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

Al-Abdulhadi, H.A. (2005): Some comparative histological studies on alimentary tract of tilapia fish (*Tilapia spilurus*) and sea bream (*Mylio cuvieri*). Egypt. J. Aquatic. Res. 13, 387-397.

Albrecht, M.P., Ferreira, M.F.N. and Caramaschi, E.P. (2001): Anatomical features and histology of the digestive tract of two related neotropical omnivorous fishes (Characiformes; *Anostomidae*). J. Fish Biol. 58, 419- 430.

Anderson, T.A. (1986): Histological and cytological structure of the gastrointestinal tract of the luderick, *Girella tricuspidata* (Pisces, Kyphosidae) in relation to diet. J. Morphol. 190 (1), 109-119.

Arellano, J.M., Storch, V. and Sarasquete, C. (2001): Histological and histochemical observations in the stomach of the Senegal sole, *Solea senegalensis*. Histol. Histopathol. 16 (2), 511-521.

Awaad, A.S., Moawad, U.K. and Tawfiek, M.G. (2014): Comparative histomorphological and histochemical studies on the oesophagus of Nile Tilapia oreochromis niloticus and African Catfish Clarias gariepinus. J. Histology, Hindawi Publishing Corporation. 2014, Article ID 987041, 10 pages

Bancroft, J. and Gamble, A. (2008): Theory and practice of histological techniques, sixth edition. Churchill-Livingstone, Edinburgh, London, Melbourne, New York.

Burnstock, G. (1959): The morphology of the gut of the brown trout (*Salmo trutta*). Quart. J Micro. Sci. 100, 183-198.

Caceci, T., El-Habback, H.A., Smith, S.A. and Smith, B.J. (1997): The stomach of *Oreochromis niloticus* has three regions. J. Fish Biol. 50, 939-952

Cao, X.J. and Wang, W.M. (2009): Histology and mucin hstochemistry of the digestive tract of Yellow Catfish *Petteobagrus fulvidraco*. Anat. Histol. Embryol. 38, 254-261.

Chakrabarti, P. and Ghosh, S.K. (2014): A comparative study of the histology and microanatomy of the stomach in *Mystus vittatus* (Bloch), *Liza parsia* (Hamilton) and *Oreochromis mossambicus* (Peters). J. Microsc. Ultrastruct. 2, 245-250.

Chen, B.N., Qin, J.G., Kumar, M.S., Hutchinson W.G and Clarke, S.M (2006): Ontogenetic development of the digestive system in yellow tail Kingfish *Seriola lalandi* Larvae. Aquaculture. 256, 489 - 501.

De Graaf, G. and Janssen, H. (1996): Artificial reproduction and pond rearing of the African Catfish *Clarias gariepinus* in sub-saharan Africa. A Handbook, 362. FAO fisheries technical paper, Rome, 73pp.

Diaz, A.O., Garcia, A.M., Figueroa, D.E. and Goldemberg, A.L. (2008): The mucosa of the digestive tract in *Micropogonias furmieri*: A light and Electron microscope approach. Anat. Histol. Embryol. 37(4), 251-256.

Domeneghini, C., Arrighi, S. Radaelli, G., Bosi, G. and Mascarello, S. (1999): Morphological and histochemical peculiarities of the gut in white sturgeon, *Acipenser transmontanus*. Eur. J. Histochem. 43 (2), 135-145.

Ekele, I., Uchenna, N., Okechukwu, N. and Isaiah, A. (2014): The stomach of the adult African Catfish (*Clarias gariepinus*, *Siluriformes: Clariidae*) in farm conditions: A morphological and mucin histochemistry analysis. Rev. Fac. Cs. Vets. UCV. Histologia. 55 (1), 4 -10.

Evans, D.H. and Claiborne, J.B. (2006): The physiology of fishes, third edition. Taylor and Francis Group, Boca Raton, 54-57.

Ezeasor, D.N. and Stokoe, W.M. (1981): Light and electron microscopic studies on the absorptive cells of the intestine, caeca and rectum of the adult rainbow trout, *Salmo gairdneri*, Rich. J. Fish Biol. 18(5), 527-544.

Ferraris, R.P., Tan, J.D. and De La Cruz, M.C. (1987): Development of the digestive tract of milkfish *Chanos chanos* (Forskal): histology and histochemistry. Aquaculture, 61, 241-257.

Gallagher, M.L., Luczkovich, J.J. and Stellwag, E.J. (2001): Characterization of the ultrastructure of the gastrointestinal tract mucosa, stomach contents and liver enzyme activity of the pinfish during development. J. Fish Biol. 58 (6):1704 -1713.

Ghattas, S.M. (2004): A histological study on the digestive tract of eel (*Anguilla anguilla L.*) with special reference to ultrastructure of gastric glandular cells. J. Mansoura Vet. Med. anostomidae) Fish Biol. 58: 419 - 430.

Gisbert, E., Sarasquete, M.C., Williot, P. and Castello-Orvay, F. (1999): Histochemistry of the development of the digestive system of *Siberian sturgeon* during early ontogeny. J. Fish Biol. 55, 596-616.

Grau, A., Crespo, S., Sarasquete, M.C. and Gonzales de Canales, M.L. (1992): The digestive tract of the amberjack *Seriola dumerili*, Risso: a light and scanning electron microscope study. J. Fish Biol. 41(2), 287-303.

Hernández, D.R., Pérez Gianeselli, M. and Domitrovic, H.A., (2009): Morphology, Histology and Histochemistry of the Digestive System of South American Catfish (*Rhamdia quelen*). Int. J. Morphol. 27 (1), 105-111.

Khidr, B.M. (2006): Electron microscopic studies on the intestinal mucosa of the Nile catfish, *Chrysiehthys auratus* (Geoffroy) with special interest in its absorptive function. Egypt. J. Zool. 47, 143-165.

Kozaric, Z., Kuzir, S., Petrinec, Z., Gjurevic, E. and Baturina, N. (2007): Histochemistry of complex glycoproteins in the digestive tract mucosa of Atlantic bluefin tuna (*Thunnus thynnus L.*). J. Veterinarski arhiv. 77(5), 441- 452.

Kozarić, Z., Kužir, S., Petrinec, Z., Gjurčević, E. and Božić, M. (2008): The development of the digestive tract in larval European Catfish *(Silures glanis L.).* Anat. Histol. Embroyol. 37 (2), 141-146.

Lee, J.H., Ku, S.K., Park, K.D. and Lee, H.S. (2004): Immunohistochermical study of the gastro-intestinal endocrine cells in the Korean aucha perch. J. Fish Biol. 65 (1), 170-181.

Murray, H.M., Wright, G.M. and Goff, G.P. (1994): A comparative histological and histochemical study of the stomach from three species of pleuronectid, the Atlantic halibut, *Hippoglossus hippoglossus,* the yellowtail flounder, *Pleuronectes ferruginea,* and the winter flounder, *Pleuronectes americanus.* Canad. J. Zool. 72 (7), 1199-1210.

Naguib, S.A.A., EI-Shabaka, H.A. and Ashour, F. (2011): Comparative Histological and Ultrastructural Studies on the Stomach of *Schilbe mystus* and the Intestinal Swelling of *Labeo niloticus.* J. American Sci. 7(8), 251-263.

Osman, A.H.K. and Caceci, T. (1991): Histology of the stomach of *Tilapia nilotica* (Linnaeus, 1758) from the River Nile. J. Fish Biol. 38, 211-223.

Ostos Garrido, M.V., Nuñez Torres, M.I. and Abaurrea Equisoain, M.A. (1993): Histological, histochemical and ultrastructural analysis of the gastric mucosa in *Oncorhynchus mykiss*. Aquaculture. 115 (1-2), 121-132.

Ostos Garridoa, M.V., González Ollera, C. and Abaurrea Equisoaina, M.A. (1996): Effect of diet on gastric mucosa cells in the European eel (*Anguilla anguilla* L.). Histochemical and ultrastructural study. Micron, 27 (1), 25-34.

Park, J.Y. and Kim, I.S. (2001): Histology and mucin histochemistry of the gastrointestinal tract of the mud loach, in relation to respiration. J. Fish Biol. 58 (3), 861-872.

Pereyra, L.A., Domitrovic, H.A. and Sampietro, J.C. (1999): Microscopíaelectrónica de barrido del tubo digestive del surubí (Pseudoplatysto macoruscansy P. fasciaium) (Pisces, Siluriformes). Actas Reunión de Comunicaciones Científicas y Tecnológicas-UNNE. 10, 1- 4.

Petrinec, Z., Nejedli, S., Kuzir, S. and Opacak, A. (2005): Mucosubstances of the digestive tract mucosa in northern pike (Esox lucius L.) and European Catfish (*Silurus glanis* L.) Vet. Arhiv. 75(4), 317-327.

Podkowa, D. and Goniakowska-Witaliñska, L. (2003): Morphology of the air-breathing stomach of the catfish *Hypostomus plecostomus*. J. Morphol. 257 (2), 147- 163.

Raji, A. R. and Norouzi, E. (2010): Histological and histochemical study on the alimentary canal in Walking catfish (*Claris batrachus*) and piranha (*Serrasalm usnattereri*). Iranian J. Vet. Res. Shiraz Univ. 11(3), 255-261.

Ray, A.K. and Moitra, S.K. (1982): On the morpho-histology of the alimentary tract in the Indian climbing Perch, *Anabas tastudineus* (Bloch), in relation to food and feeding habits. Gegenbaurs Morpholoisches Jahrbuch.128, 778-798.

Rebolledo, I.M. and Vial, J.D. (1979): Fine structure of the oxynticopeptic cell in the gastric glands of Elasmobranch species (*Halaeluruschilensis*). J. Anat. Rec. 193, 805-822.

Reifel, C.W. and Travill, A.A. (1978): Structure and carbohydrate histochemistry of the stomach in eight species of teleosts. J. Morphol. 158, 155 - 168.

Reynolds, E.S. (1963): The use of lead citrate at high pH as an eIectron-opaque stain in electron microscopy. J. Cell Biol. 17, 208-2 12.

Ribeiro, L., Sarazquete, C. and Dinis, M.T. (1999): Histological and histochemical development of the digestive system of *Solea senegalensis* (Kaup, 1858) larve. Aquaculture. 171, 293-308.

Rotta, M.A. (2003): Aspectosgerais da fisiologia e estrutura do sistemadigestivo dos peixesrelacionados à piscicultura Documentos / Embrapa Pantanal. 53, 1517-973.

Sis, R.F., Ives, P.J., Jones, D.M., Lewis, D.H. and Haensly, W.E. (1979): The Microscopic anatomy of the oesophagus, stomach and intestine of the channel catfish, *Ichtalurus punctatus*. J. Fish Biol. 14, 179-186.

Smith, L.S. (1989): Digestive functions in teleost fishes. In: Fish nutrition, second edition. Academic Press London, 331- 421.

Xiong, D., Zhang, L., Yu, H., Xie, C., Kong, Y., Zeng, Y., Huo, B. and Liu, Z. (2011): A study of morphological and histology of the alimentary tract of *Glyptosternum maculatum (Sisoridae, silurifores*). Acta Zool. 96,161-169.

Yang, R., Xie, C., Fan, Q., GAO, C. and Fang, L. (2010): Ontogeny of the digestive tract in yellow Catfish *Pelteobagrus fulvidraco* larvae. Aquaculture. 302, 112-123.

Zacarias-Soto, M., Muguet, J.B., and Lzao, J.P. (2006): Proteolytic activity in California habitat larvae (*Paralichthy scaliforniaus*). J. World Aquacul. Soc. 37,175-185.

FIGURES LEGENDS

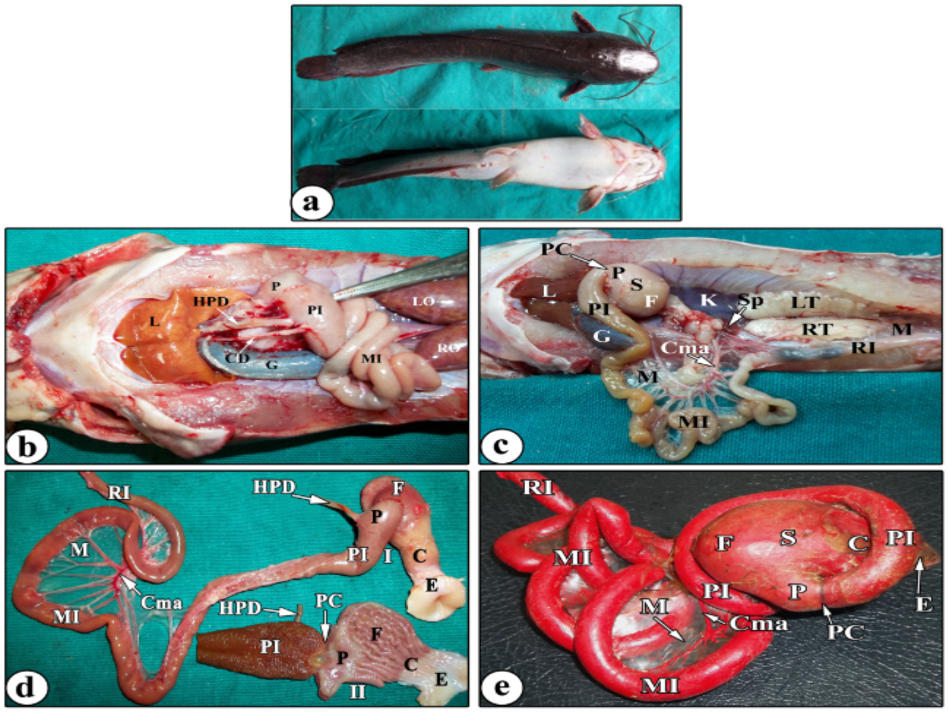

Fig. (1): Gross dissection of the African Catfish *C. gariepinus.* (a)- Dorsal and ventral views of the external features of the fish used in the present investigation. (b, c). The contents of the body cavity and divisions of the digestive tract, (d) - Divisions of the digestive tract outside the body cavity showing the outer structures (I) and the internal structures (II) of the stomach. (e)-Epoxy corrosion casting of the ventral aspect of the gastrointestinal tract. C= Cardiac part of the stomach, Cma= Cranial mesenteric artery, E= Esophagus, F= Fundic part of the stomach, G= Gall bladder, HPD= Hepato-pancreatic duct, K= Kidney, L= Liver, LO= Left ovary, LT= Left testicle, M= Mesentery of the intestine, MI= Middle intestine, P= Pylorus, PC= Pyloric constriction, PI= Pyloric intestine, RI= Rectal intestine, RO= Right ovary, RT= Right testicle, Sp= Spleen, S= Stomach.

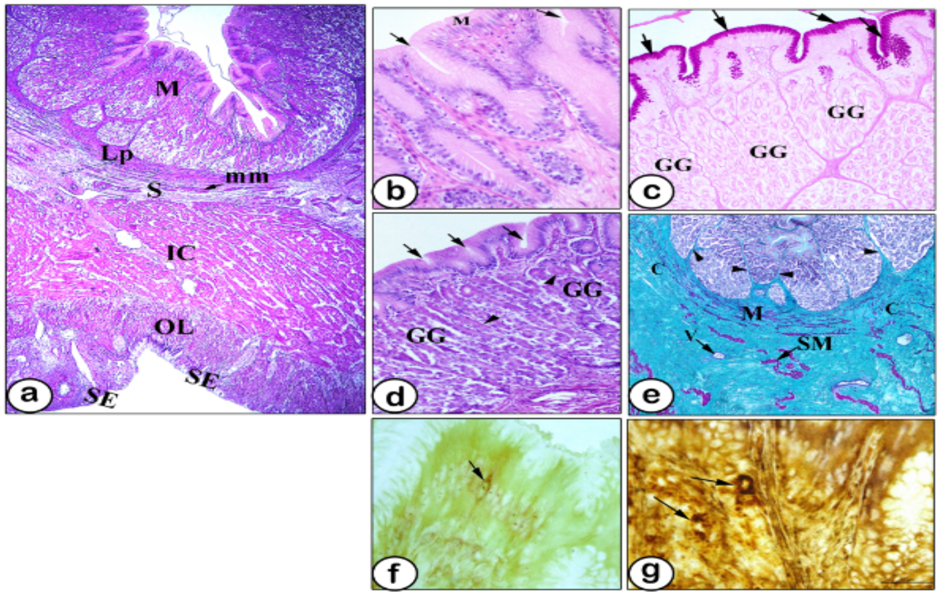

Fig. (2): Light photomicrographs of the cardiac region of the African catfish *C. gariepinus* showing: (a)- Four distict layers lining the cardiac region; mucosa (M), submucosa (S), Muscularis externa (composed of inner circular IC and outer longitudinal OL layers of smooth muscle fibers) and serosa (SE). Note, the lamina muscularis mucosa (mm) is situated between lamina propria (Lp) and tunica submucosa. H&E, X40. (b)- Cross section of the cardiac region showing cup-shaped mucosal folds (M) lined with simple columnar with oval basal nuclei. Note, shallow and narrow gastric pits (arrows) between the mucosal folds. H&E, X400. (c) - PAS-positive mucosubstance present in apical part of mucosal epithelial cells (arrows). Note, weak PAS reaction of the epithelial cells linning the gastric glands (GG). PAS, X100. (d)- Shallow gastric pits (arrows) lined with high columnar cells with oval basal nuclei. Note, plenty of parallel tubular gastric glands (GG) formed by cuboidal cells and oval basal nuclei (arrow heads). H&E, X200. (e)- Collagen bundles (C) run in between the gastric glands and extend to the mucosal folds (arrow heads), longitudinal arranged smooth muscles bundles (M) lie under the bases of the glands forming the muscularis mucosa. Note, the submucosa composed of extensive collagen bundles containing bundles of smooth muscles (SM) and many blood vessels (V). Crossmon's trichrom stain, X100. (f)- Oval shaped enterondocrine cell (arrow) lies between the gastric mucosal cells. Grimelius stain, X1000. (g)-Enteroendocrine cell (arrows) lies between the cells of the cardiac glands. Grimelius stain, X1000.

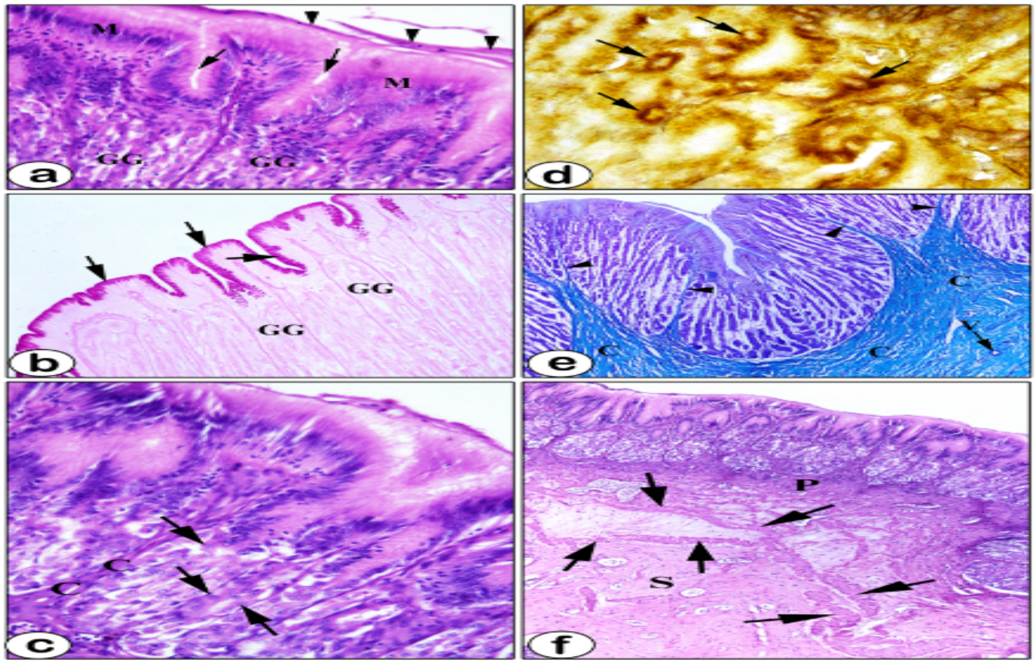

Fig. (3): Light photomicrographs of the fundic region of the African catfish *C. gariepinus* showing :(a) - Broad mucosal folds (M) lined with single layer of columnar cells with oval basal nuclei. Note, wide and deep gastric pits (arrows), gastric glands (GG) and mucous threads adhered to the surface epithelial cells (head arrows). H&E, X400. (b) - Apically concentrated PAS positive granules (arrows) of the epithelial cells line the mucosal surface epithelial cells and pits. Note, the parallel arranged straight tubular glands (GG). PAS X200. (c)- Tubular gastric glands formed by a single cell type (oxintopeptic) with central nuclei (arrows). Note, connective tissues (C) derived from lamina propria penetrate between the glands. H&E, X200. (d)-A transverse section showing numerous enters chromaffin cells (arrows) within the epithelial cells lining the fundic glands. Grimelius stain, X1000. (e)-Extensive collagenic bundles (C) containing blood vessel (V) located under the bases of the glands. Note, the tubular glands permeated by many collagen fibers (arrow heads). Crossman's trichrome stain, X100. (f)- A cross section showing double-layered of smooth muscle fibers (arrows) forming the lamina muscularis mucosa and separating the lamina propria (P) from the tunica submucosa (S). H&E, X100.

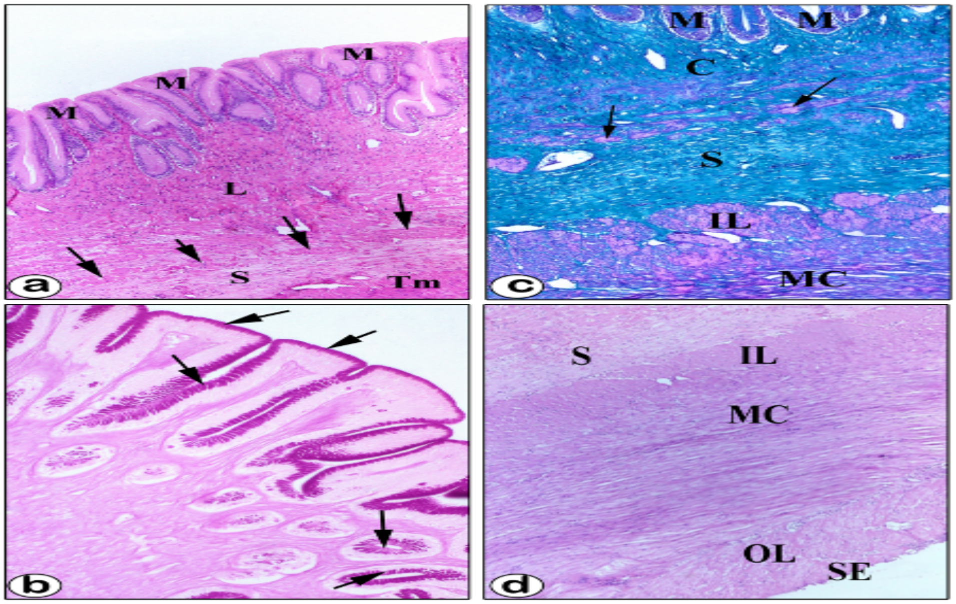

Fig. (4): Light photomicrographs of the pyloric region of the African Catfish *C. gariepinus* showing:(a)- Long folded mucosal folds (M) lined by columnar cells, devoid of gastric gland in a thick lamina propria (L). Note a layer of smooth muscle fibers (arrows) separated the lamia propria from the thin tunica submucosa (S) and tunica musclosa (Tm). H&E, X100. (b)- A cross section showing intense PAS positive reactions (arrows) at the apical portion of the epithelial cells lining the pyloric mucosa. PAS X200. (c)- Extensive collagen bundles (C) lie in the lamina propria and extend to the core of the mucosal folds (M). Note, lamina muscularis mucosa (arrows), submucosa (S), inner longitudinal (IL) and middle circular (MC) layer of tunica musculosa.Crossmon's trichrom stain, X100. (d)- Thick tunica musclosa composed of inner longitudinal (IL), middle circular (MC) and outer longitudinal (OL) layer of SMFNote, submucosa (S) and thin serosal coat (SE). H&E, X400.

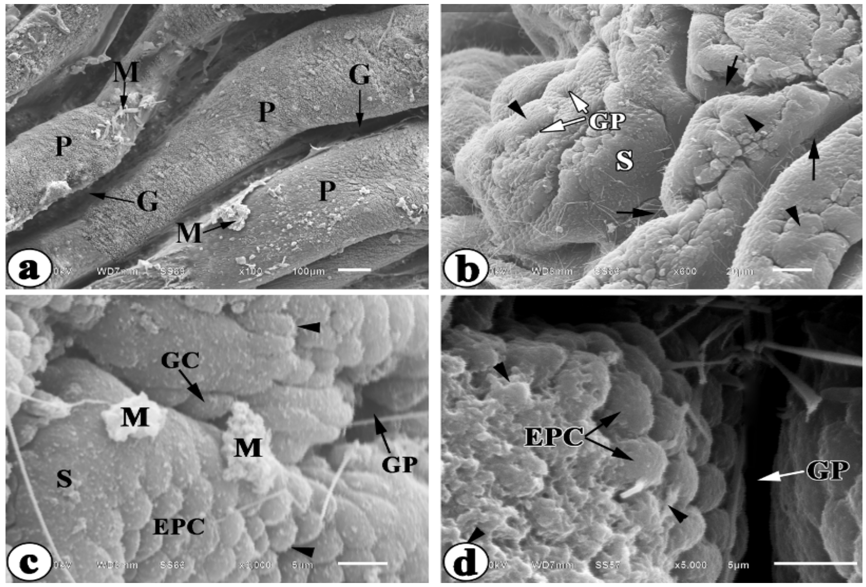

Fig. (5): Scanning electron micrographs of the cardiac region in the African Catfish *C. gariepinus*. (a)- A low magnification of the mucosal lining showing numerous primary mucosal folds (P) longitudinally arranged parallel to each other with the presence of grooves in between (G) and scatterd mucous secretion on its surface (M), (x100). (b)- Irregular anastomosis of the secondary mucosal ridges (S) leading to the formation of irregular discrete pockets (black arrows). Note the presence of microridges (arrow heads) on the secondary mucosal folds and lodging the gastric pits (GP) in between, (x600). (c)- Apical surface of the epithelial cells (EPC) bearing microvilli (arrow heads), oval elevations appeared between the secondary mucosal folds (S) represented the luminal surface of the goblet cells (GC). Note the accumulated mucin secretion (M) and the presence of the gastric pits (GP) between the columnar epithelial cells, (x3000). (d)- Luminal surface of the columnar epithelial cells (EPC) appeared as pentagonal and hexagonal elevations with the presence of gastric pits (GP) in between and extensive mucin droplets (arrow heads) appeared as thin layer overlying the lining mucosa, (x5000). Scale bars; (a- 100µm, b- 20µm, c and d- 5µm).

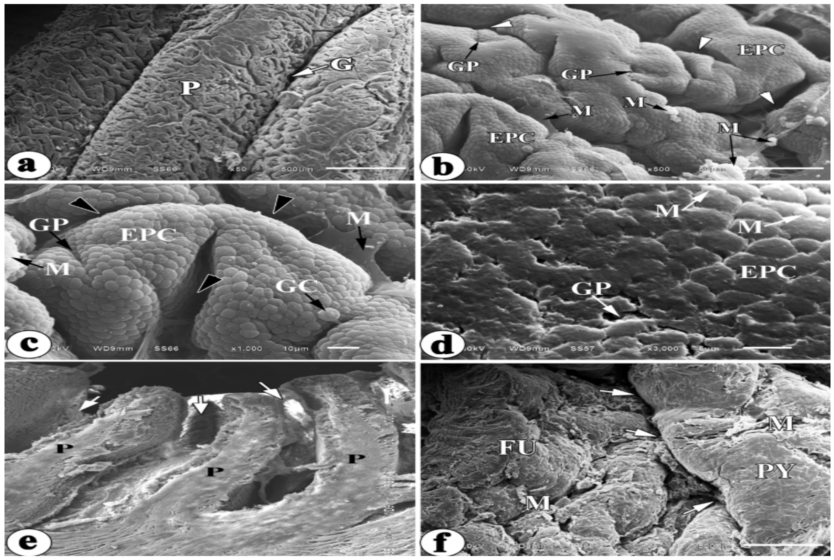

Fig. (6): Scanning electron micrographs of the fundic region in the African catfish *C. gariepinus.* (a)- A low magnification of the mucosal lining showing numerous primary mucosal folds (P) longitudinally arranged with deep furrows (G) in between, (x50). (b)- Irregular arranged secondary mucosal folds and narrow concavities in between them (arrow heads). Note prominent gastric pits (GP) encircled by rosettes of columnar epithelial cells (EPC), with scattered mucin droplets on their surface (M), (x500). (c)- Showing polyhedral (pentagonal or hexagonal) shaped columnar epithelial cells (EPC) provided with short and stubby microridges. Black arrow heads indicate concavities and prominent gastric pits (GP), mucin droplets (M) and oval shaped goblet cells (GC), (x1000). (d)- A higher magnification of the luminal surface of the polyhedral columnar epithelial cells (EPC) and mucin droplets (M). Note the gastric pits (GP) surrounded by the EPC, (x3000). (e)- A transverse section through the damaged surface of the fundic gland region showing primary folds (P), with remarked gastric crypts of the gastric glands (arrows), (x100). (f)- A low magnification showing the transition area between the fundic (FU) and pyloric (PY) regions of the stomach, with a remarkable shallow groove (solid arrows) could be seen, (x50). Scale bars; (a- 500µm, b- 50µm, c-10µm, d- 5µm, e- 100µm and f- 500µm).

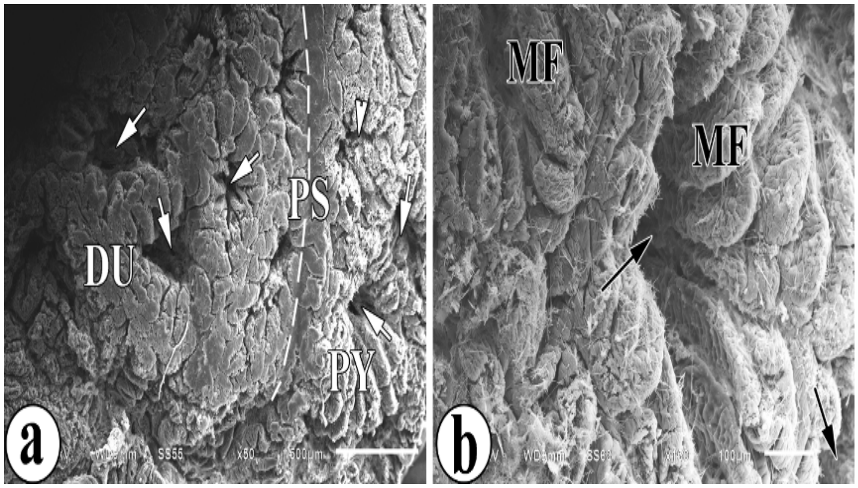

Fig. (7): Scanning electron micrographs of the pyloric region in the African Catfish *C. gariepinus.* (a)- A low magnification showing the terminal part of the pyloric region (PY), and the initial part of the proximal intestine; duodenum (DU), and there is a groove (white dotted line) indicates the position of the pyloric sphincter (PS) and there are deep empty concavities (solid arrows) formed by the anastomosis of the mucosal folds, (x50). (b)- The mucosal folds (MF) with big and stubby micro-ridges and deep empty concavities (solid arrows), (x100). Scale bars; (a- 500µm, b-150µm).

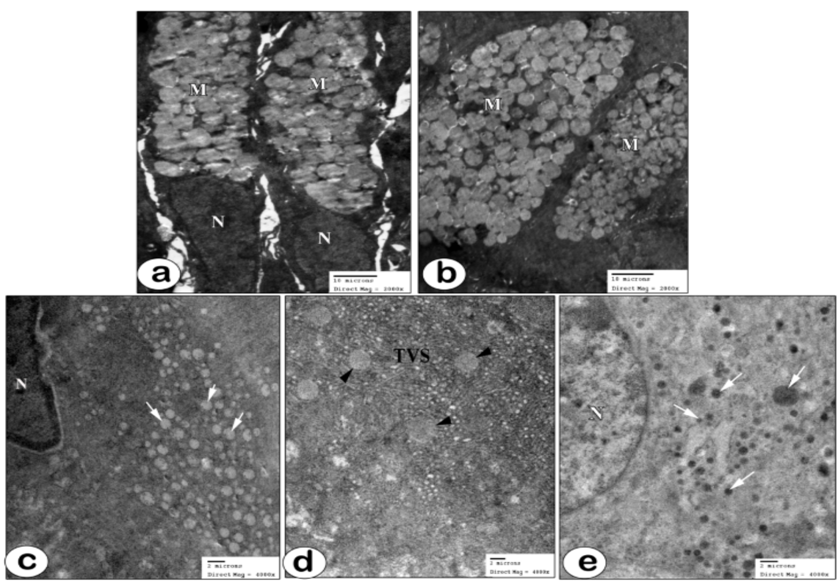

Fig. (8): Electron photomicrographs of the African Catfish *C. gariepinus* stomach.(a)-Cardiac mucosa showing luminal cells contain apically located secretory vesicles (M) occupy most of the cytoplasm and basally situated oval nucleus (N). Uranyl acetate and lead citrate, X2000. (b) -Gastric mucosal cells of fundic region containing excessive number of apically located secretory vesicles (M) occupy most of the cytoplasm. Note, some of these vesicles fused with the cell membrane. Uranyl acetate and lead citrate, X2000. (c) - Oxynticopeptic cell containing nucleus (N) and numerous spherical shaped vesicles (arrows) of different sizes. Uranyl acetate and lead citrate, X4000. (d)- Apical portion of oxynticopeptic cell lines fundic gland showing tubulo-vesicular system (TVS) and secretory granules (arrow heads) extensive network of rough endoplasmic reticulu (arrows). Uranyl acetate and lead citrate, X2000. (e): Electron micrograph of an endocrine cell lining the fundic gland showing euchromatic nucleus (N) and numerous electron dense secretory granules (arrows) of different sizes.Uranyl acetate and lead citrate, X4000.