World Applied Sciences Journal 8 (4): 477-485, 2010
ISSN 1818-4952

Comparative Histological, Histochemical and Ultrastructural Studies on the Proximal Intestine of Flathead Grey Mullet (*Mugil cephalus*) and Sea Bream (*Sparus aurata*)

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Abstract: The fish intestine plays the main role in the digestive and absorptive functions of the alimentary tract. The histology, histochemical characteristics and ultrastructure of the proximal intestine in *Mugil cephalus* (flathead grey mullet) and *Sparus aurata* (sea bream) were investigated to correlate the histological and cytological structure with the nature of diet in these fishes with different feeding habits. Samples of the proximal intestine from the investigated species were removed and processed for light and transmission electron microscopy studies. Histologically, the intestinal wall of the investigated species consisted of a mucosa, submucosa, muscularis and serosa. Histochemical analysis revealed that the proximal intestine of *Sparus aurata* differs from that of *Mugil cephalus*; the goblet cells in *Mugil cephalus* were numerous and concentrated at the base of mucosal fold and the apical parts of epithelial cells was highly reacted with PAS stain. Ultrastructurally, the epithelium of the intestine in both fishes consists of columnar epithelial absorptive cells as well as goblet cells. The apical part of the epithelial absorptive cells bears numerous microvilli. The epithelial cells in *Mugil cephalus* have lamellar structures. The histological and ultrastructural features of the proximal intestine in *Mugil cephalus* and *Sparus aurata* were different. This may related to the type of feeding. In conclusion, this study will help in understanding the digestive physiology of the investigated species and provides basis for diagnosing diseases that affect the digestive tract in two teleostean fishes (*Mugil cephalus* and *Sparus aurata*) with different feeding habits.

Key words: Histology · Histochemistry · Ultrastructure · Intestine · Teleosts

INTRODUCTION

The fish digestive tract shows a marked diversity of both morphology and function [1, 2]. In general, the intestinal morphology of fish can be influenced by feeding habits, frequency of food intake, as well as by body size and shape [3, 4]. Also, there are relationship between intestine length and body length [5]. Depending on diet, the fish intestine can vary morphologically from short straight to long coiled and complexly arranged [6, 7].

In fishes, as in many other taxa, intestine length is often an indicator of diet [5, 8]. The percentage of plant material in the diet is the major determining factor for intestinal length, species that eat only algae or higher plants ('herbivores') tend to have longer intestines than species that eat both plant and animal material ('omnivores') and these in turn tend to have longer intestines than species that eat only other animals ('carnivores') [3, 9, 10].

The fishes intestinal lumen is lined by a simple columnar epithelium (enterocytes) interspersed by goblet cells [11]. The epithelium, together with the underlying lamina propria constitutes the mucosa. The other layers of the intestine are: submucosa, muscularis (longitudinal and circular) and serosa, a connective tissue layer attached to the mesenteric tissue and they vary with species. The mucosal histology and the number and type of specific cell types vary according to the fish's diet [2, 12, 13].

Teleostean goblet cells are known to contain carbohydrates [11] without characterizing their specific nature. An abundance of mucous cells indicates that mucosubstances have some role in the digestive process [14] and different mucosubstances found in different regions of the gut are correlated with assorted digestive functions [15]. Additionally, the gut mucins lubricate and protect the mucosa against chemicals, parasites and acidity and form a diffusion barrier for various ions [16,17].

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Flathead mullet, *Mugil cephalus* is a catadromous fish with world - wide distribution [18]. Grey mullet feed on detritus, diatoms, algae and microscopic invertebrates, desmids, annelids, crustaceans, bivalves and fish parts, which they filter from mud and sand through their mouth and gills [19, 20].

Sea bream, *Sparus aurata*, is eurghali fish and is one of the most extensively cultured species in Mediterranean region [21]. Wild sea bream primarily feeds on molluscs and crustaceans, though occasionally it consumes algae [22]. These two fishes are economically important species for both aquaculture and commercial fisheries around the world [23, 24]. Previous data on the intestine of fingerling grey mullet [25]; larva and juveniles of thick lipped grey mullet [24] were reported. Also, the intestine of sea bream larva were studied [26, 27].

Scanty data could be found in the literature on the ultrastructure of adults *Mugil cephalus* and *Sparus aurata* intestine. Some morphometric measurements are necessary to define a model to forecast the absorptive surface area, combining metabolism and diet quality for fish [3].

The purpose of the present study was to describe the general histology and histochemical distribution of mucosubstances in fishes intestine, reports the existence of some histological peculiarities related to cell type distribution and ultrastructure and provides a morphological basis for the diagnosis of diseases that affect the digestive tract in two teleost fishes with different feeding habits.

MATERIALS AND METHODS

Sample Collection: Five live adult flathead grey mullet, *Mugil cephalus* (total body length ranging from 21 to 29 cm) and six adult sea bream, *Sparus aurata* (total body length ranging from 19 to 26.5 cm) were collected from Mediterranean Sea at Damietta region, Egypt.

The fishes were used without sexual distinction, after their identification and biometry, the body cavity was opened through a midventral incision and the proximal intestine was immediately fixed in Bouin's solution. The proximal intestine was defined in this study as the portion that extended three quarters the total length from the stomach.

Light Microscopy: After fixation, the samples of intestine were dehydrated in an ethanol series, cleared in xylene and embedded in paraffin wax and sectioned at 5μm. After dewaxing with xylene and hydration in ethanol series of descending concentration, sections were stained for general histological purposes with haematoxylin and eosin stain.

Histochemical techniques for the identification and differentiation of mucosubstances were applied using periodic acid schiff (PAS) and alcian blue AB (pH 2.5), for carboxylated and some sulfated glycoconjugates [28].

Transmission Electron Microscopy: Small fragments of intestine were placed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer at pH 7.2 for 3h at 4°C. After rinsing in buffer the specimens were post-fixed in 1% buffered osmium tetroxide at pH 7.2 for 1h at 4°C. They were then dehydrated and embedded in araldite. Thin sections were stained with uranyl acetate and lead citrate [29] and examined with JEOL transmission electron microscope and photographed.

Morphometric Measurements: Total body length was measured from cranial end of the lower lip to the caudal end of the caudal fin. Standard body length was measured from cranial end of the upper lip to the base of the caudal fin of the fish [30]. Intestine length was measured using calipers. Histological sections of intestine were submitted to measurements of: thickness of muscularis (thickness of circular and longitudinal smooth muscle layers), height and width of the mucosal fold. Five observations per fish were used for morphometric evaluation under X100 magnification. Measurements were performed with light microscope using an eye piece micrometer previously calibrated for the magnification used, with a stage micrometer. All data were expressed as mean ± SD and presented in Table 1.

Table 1: Morphometric characteristics of *Mugil cephalus* and *Sparus aurata*

	Fish total length (cm)	Fish standard length (cm)	Digestive tract length (cm)	Intestine length (cm)	Muscularis thickness (mm)	Longitudinal layer muscle thickness (mm)	Circular layer muscle thickness (mm)	Mucosal fold height (mm)	Mucosal fold width (mm)
Mugil cephalus	25 ± 3.53	23.2±3.65	85.26± 9.08	82.4 ± 9.09	5.75 ± 1.4	2 ± 1.2	3.57 ± 0.45	7.38 ± 2.18	2.18 ± 0.59
Sparus aurata	20.75± 2.86	18.58± 2.73	21.17± 3.86	18.5 ± 4.27	0.65 ±.06	0.25 ± 0.01	0.375 ± .01	1.9 ± 0.6	0.83 ± 0.39

RESULTS

The digestive tract of *Mugil cephalus* (85.26 cm) and *Sparus aurata* (21.17 cm) (Table 1) consisted of a pharynx, oesophagus, stomach and intestine that composed of proximal and distal intestine.

Light Microscopy: In the proximal intestine of *Mugil cephalus*, the histological findings showed that the basic organization of intestinal wall arrangement was similar to that in other vertebrates: an epithelial mucosal lining, a lamina propria/submucosa of loose connective tissue, tunica muscularis and a serosal layer external to the tunica muscularis (Fig. 1 a).

The tunica muscularis, 5.75 mm thick (Table 1) was organized in two layers, thick outer circular layer (3.07 mm) and inner longitudinal non continuous layer (2 mm) (Fig. 1 a). The mucosal surface of proximal intestine was thrown up in numerous elongated and deep folds, lined by microvilli lined by a simple columnar epithelium with mucus secreting cells which were characteristically goblet-shaped concentrated at the base of folds. The folds branched in parallel or united to form an extensive reticulate surface area. Height and width of the mucosal folds were 7.38 and 2.18 mm respectively. A thin connective tissue layer extended into the mucosal folds to form connective tissue cores (Fig. 1b).

In *Sparus aurata*, the intestinal mucosa is folded into short, branched and broad intestinal villi consisting of a single thinner layer of absorptive columnar epithelial cells (enterocytes), bearing a brush border (Fig. 1c). Small number of mucous goblet cells are interspersed among the epithelium and are found at all levels of the intestinal villi. The submucosa is thick and rich in blood vessels and the lamina propria invades the intestinal folds. Height and width of the folds were 1.9 and 0.8 mm respectively (Table 1). The muscularis externa is the thickest layer of smooth muscle of the proximal intestine. It consists of a thick inner circular layer (0.37 mm) and a thinner outer longitudinal layer (0.25 mm), (Table 1 and Fig. 1d). The serosa consists of mesothelial cells, small blood vessels, blood cells and loose connective tissue (Fig. 1c).

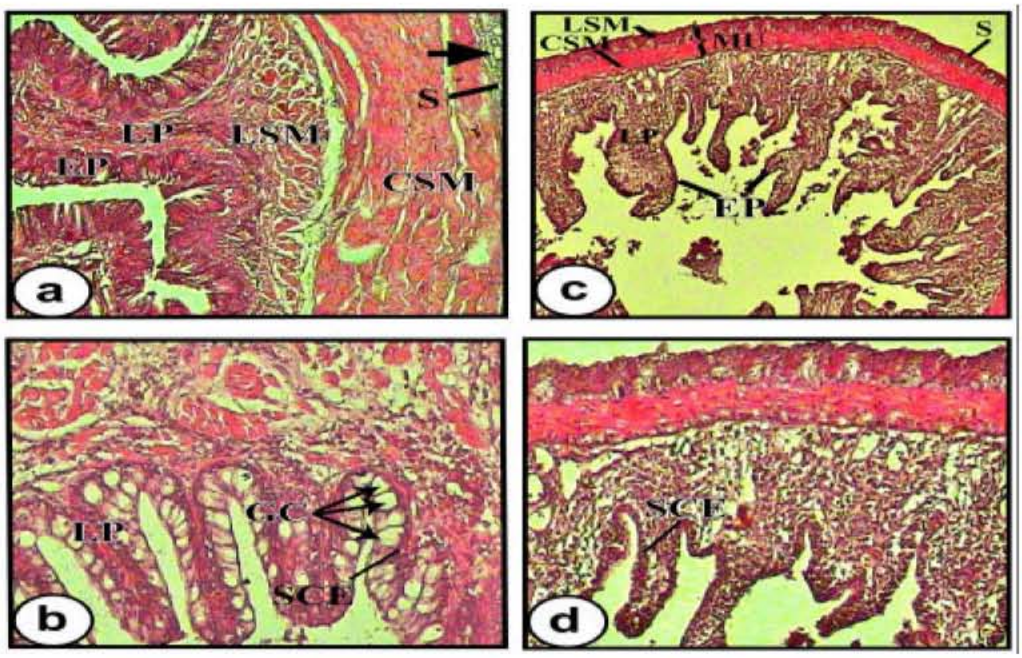

Fig. 1: (a) Intestinal wall of *Mugil cephalus* consisted of tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. (HE X100). (b) Mucosa of *Mugil cephalus* proximal intestinal wall containing simple columnar epithelial cells, many goblet cells and lamina propria.(HE X400). (c) Intestinal wall of *Sparus aurata* consisted of tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. (HE X100). (d) Mucosa of *Sparus aurata* proximal intestinal wall containing simple columnar epithelial cells and limit number of goblet cells. (HE X400). CSM, circular smooth muscle; EP, Epithelium; GC, goblet cell; LP, lamina propria; LSM, longitudinal smooth muscle; MU, muscularis; S, serosa; SCE, simple columnar epithelial cells.

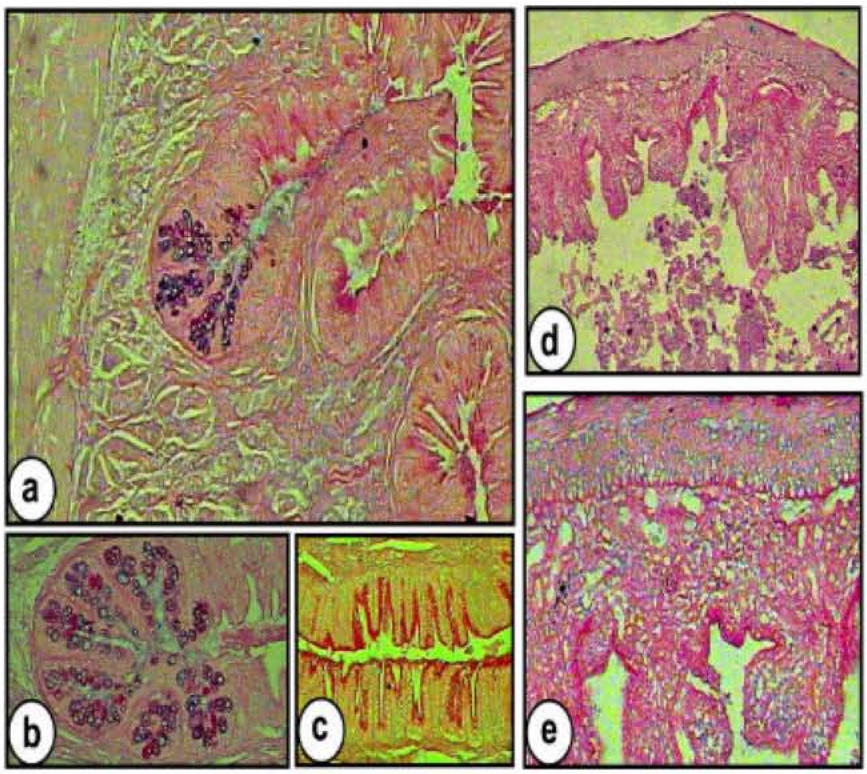

Fig. 2: (a) Proximal intestine of *Mugil cephalus*: Mucosal folds with numerous goblet cells. AB-PAS X100. (b) Mucosa of *Mugil cephalus* proximal intestine: Numerous goblet cells heavily stained with alcian blue within the base of mucosal fold. AB-PAS X400. (c)Mucosa of *Mugil cephalus* proximal intestine: Epithelial cells apical region heavily stained with PAS. AB-PAS X400. (d) Proximal intestine of *Sparus aurata*: The mucosal folds stained moderately with AB-PAS X100.(e) Enlarged portion of *Sparus aurata* proximal intestine. AB-PAS X400.

Histochemical analysis revealed that the proximal intestine of *Mugil cephalus* differs from that of *Sparus aurata* (Fig. 2d, e) in that the mucous cells in *Mugil cephalus* were more numerous and concentrated at the base of mucosal folds (Fig. 2a). All the goblet cells reacted positively to PAS/AB (Fig. 2b). The apical parts of mucosal folds were stained very strongly with PAS (Fig. 2c).

Transmission Electron Microscopy (TEM): Ultrastructuraly, long columnar epithelial absorptive cells with a well-marked striated border were observed in *Mugil cephalus* intestinal mucosa. The regular microvilli contained cores made up of fine filaments forming thick bundles which extended deeply into the terminal web of the apical cytoplasm (Fig. 3a). Invaginations of the apical plasma membrane were frequently seen among microvilli; vesicles and channel, which appeared to be continuous with these invaginations, were also present in the superficial cytoplasm. Ovoid mitochondria with a moderate number of cristae were scattered throughout the supranuclear cytoplasm and basal to the nucleus as well (Fig. 3b). A moderate amount of rough and smooth endoplasmic reticulum were evident especially near the nucleus. The large euchromatic oval or spherical nuclei with prominent nucleoli were situated either centrally or toward the base of the cells (Fig. 3b). The enterocytes were joined at the apical surface by junctional complexes including the zonula occludens and the desmosoma (Fig. 3c). Deep to the junctional complexes, the lateral plasma membranes were linear (Fig. 3c).

In the supranuclear region large electron-dense bodies, probably lysosomes, showing a granular and lamellar content could be detected Also, lamellar structures were observed in the columnar epithelial absorptive cells (Fig. 3c).

The large goblet cells were filled with numerous mucous droplets of low electron-density between surface epithelial cells (Fig. 3a). The intestinal mucosa also contained numerous endocrine cells. They were generally irregular shaped and were characterized by a clear cytoplasm, irregular shaped hyperchromatic nucleus and numerous electron-dense granules (Fig. 3d).

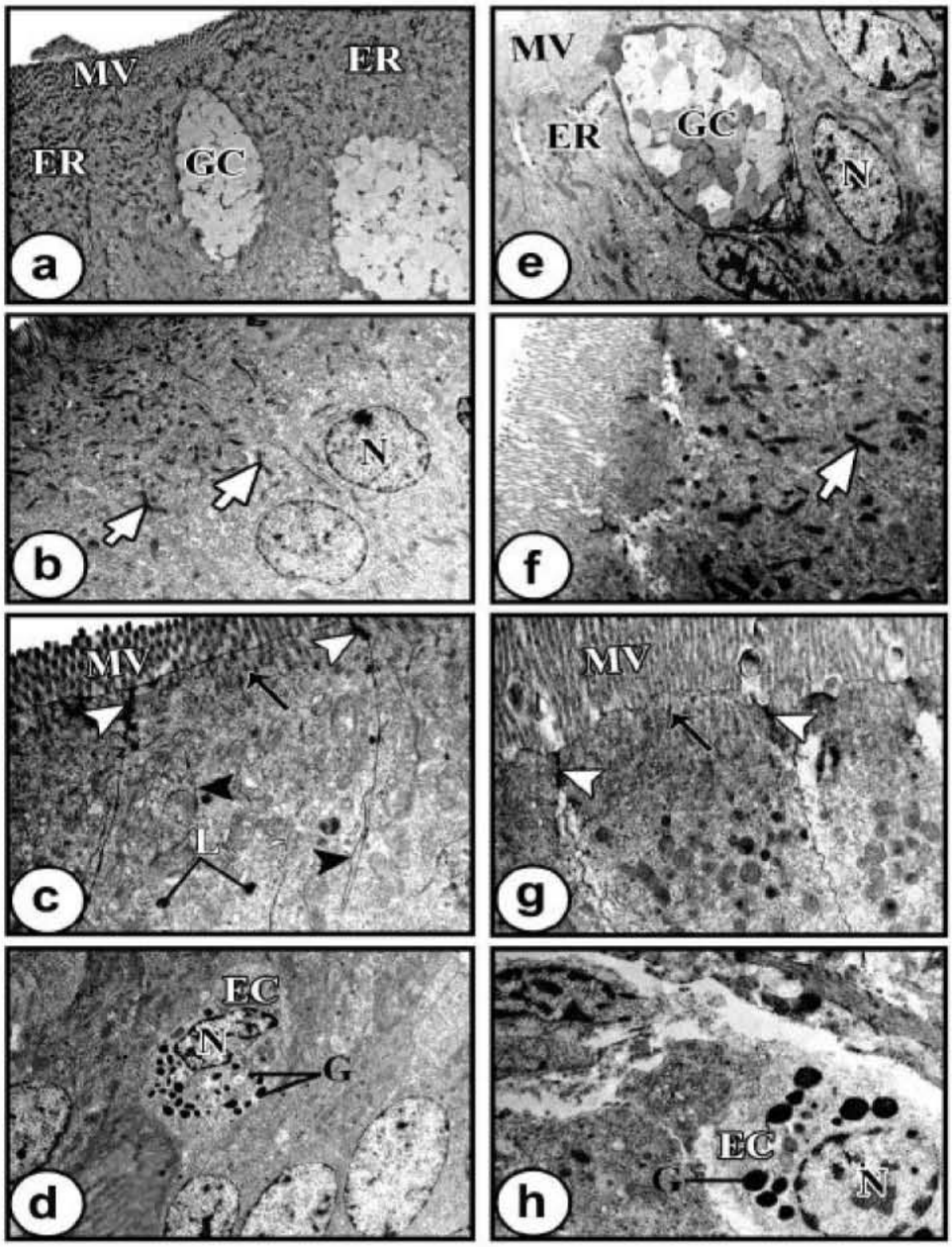

Fig. 3: Proximal intestine of *Mugil cephalus*. (a) Ultrastructure of the intestinal epithelium, columnar absorptive and goblet cells with many microvilli in their apexes and rough endoplasmic reticulum. X4000.(b) Numerous polymorphic mitochondria (white arrow) and basally located nuclei in columnar epithelial cells. X5000. (c) Apical junctional complexes (white arrow head) between columnar absorptive cells, many microfibrils (black arrow) in the apical cytoplasm below the microvilli, lamellar ucture and lysosomes. X10000 (d) Endocrine cell fine structure containing irregular nucleus and numerous electron dense granules. X5000. Proximal intestine of *Sparus aurata*. (e) Ultrastructure of the intestinal epithelium, columnar absorptive and goblet cells with many microvilli in their apexes and rough endoplasmic reticulum. X4000. (f) Numerous elongated and spherical mitochondria (white arrow) in the columnar epithelial cells. X5000. (g) Apical junctional complexes (white arrow head) between columnar absorptive cells, many microfibrils (black arrow) in the apical cytoplasm below the microvilli. X10000. (h) Endocrine cell fine structure containing regular nucleus and numerous electron dense granules. X5000. EC, endocrine cell; ER, endoplasmic reticulum; G, granules; GC, goblet cell; L, lysosome; MV, microvilli; N, nucleus.

The intestinal mucosal epithelial cells of *Sparus aurata* were similar to that of *Mugil cephalus* except in many differences. The most notable differences of the enterocytes was that they contained longer microvilli (Fig. 3e-g), elongated and spherical mitochondria (Fig. 2f), elongated or irregular shaped nuclei and the lamellar structures were rare in *Sparus aurata* (Fig. 3g).

In *Mugil cephalus*, Goblets cells in various stages of release were found interspersed at the base of mucosal folds. Mature goblet cells contained typically elongated cisternae of rough endoplasmic reticulum and a well-developed Golgi apparatus participating in the production of the secretory granules which filled the apical cytoplasm (Fig. 3e). The endocrine cells were characterized by a clear cytoplasm, spherical shaped hyperchromatic nucleus (Fig. 3h).

DISCUSSION

The histological studies of the alimentary channel across species of fish are becoming more valuable as the interest in fish culture expands and more information is required with regard to feeding and nutrition [31]. Mucosa is pivotal in digestion, absorption and metabolic processes [12]. It represents a selective barrier to nutrients and avoids several toxins and or pathogens. Moreover, it plays a part in the electrolytic balance, immune response and endocrine functions [3].

The present study revealed that the longer proximal intestine of *Mugil cephalus* and *Sparus aurata* have a mucosal folds lined by columnar epithelium, but the longest folds and cells were found in *Mugil cephalus* and, the mucous goblet cells are interspersed between the epithelial absorptive cells. The mucosal folds of intestine that are lined by a single layer of columnar cells with many mucous goblet cells was consistent with the results in other species [2, 23, 32, 33]. The columnar epithelium of the intestinal mucosa may have an absorptive function as reported in other fishes [8, 34, 35].

The pattern of muscularis layers followed equally and coherently the pattern of intestine mucosal folds. The muscularis externa consisting of an outer longitudinal and a thicker inner circular layer of smooth muscle showed in *Sparus aurata*, was described for *Ambassis sp.* [36], for Tilapia [37], for *Orthrias angorae* [38] and for *Rhamdia quelen* [33]. The thickness of the muscularis may be correlated with the temporary storage in and expulsion of faecal material from this area [39].

In *Mugil cephalus* a great amount of folds were observed in the proximal intestine, increasing the surface area and enhancing the absorptive activity [40]. In addition to the great amount of folds, thick outer layer of the smooth muscle cells as well as the inner non continuous longitudinal smooth muscle were found in *Mugil cephalus*. This may confirm the expectation that higher efficiency of mucosal folds occurs when the motility is increased. Therefore, this structural arrangement could be considered as a possible adaptation to omnivorous feeding habit. It has been reported that complex folding of the intestinal mucosa with the resultant increase in surface area aids the mixing of food with hepatic and pancreatic digestive juices as well as with mucus secreted by goblet cells [39].

Studying fishes with different types of feeding habits, suggested that the degree of increase in the digestive and transportive surface of the intestine to the microvilli may differ. Histochemically, proximal intestine of *Mugil cephalus* revealed numerous goblet cells concentrated at the base of mucosal folds and positively reacted with AB. The apical parts of mucosal folds, were positively reacted with PAS. Goblet cells are common components in the postgastric mucosa of fish [38, 41, 42]. Different mucosubstances have been correlated with assorted digestive function. The presence of mucosubstances, especially those sulfated in the intestine; possibly regulate the transfer of proteins, or a fragment of them, as well as of ions and fluids [15, 42, 44-47]. The possible role of mucosubstances in intestinal absorption processes is supported by the findings that confirmed starvation induced an increase in the number of intestinal goblet cells in carp [48]. From these points of view, the present histochemical investigation confirm the adaptation for feeding habits.

At the ultrastructural level, the epithelial absorptive cells of the proximal intestine display complement of the usual organelles and regular microvilli that increase surface area. An elaborate system of surface invaginations, cytoplasmic channels and pinocytotic vesicles as well as lysosomal system are typical features of the lining cells of the proximal intestine.

Similar results with findings of the present study regarding the presence of microvilli, junctional complexes, pinocytotic vesicles, lysosome and mitochondria in enterocytes, have been observed in the freshwater fishes [12], in *Solea solea* [49], in *Tilapia spp.* [37], in *Solea senegalensis* [4], in *Orthrias angorae* [38] and in *Oncorhynchus mykiss* [2].

The pinocytotic inclusions in the proximal enterocytes of the fishes provide evidence that an intracellular digestion of proteins in the proximal intestine may be important in the fish species [37].

Another feature of the mucosa of the proximal intestine of both *Mugil cephalus* and S*parus aurata* is the presence of lamellar structures. The lamellar structures in the cytoplasm were detected in the *Senegal sole* intestine [4]. However, they were abundant in the intestine of different larvae and adult fish species [50-52]. The lamellar structures increase the membrane surface that is in contact with extracellular spaces, probably facilitating lipoprotein transport. The involvement of these membrane infoldings in the transport of lipoprotein has been suggested [51]. In *Sparus aurata* larvae, they are associated with mitochondria in the basal cytoplasm [52] and their presence is probably due to an increased demand of energy for the osmoregulation processes, since the gut is the primary organ for absorbing water to maintain ion and water balance in marine fish species [53].

The endocrine cells are restricted to the proximal intestine confirming the immunohistochemical data that describe cells containing gastrin, cholecystokinin, glucagon and NPY in this portion of the intestine [37].

In conclusion, the histological, histochemical and ultrastructural features of the proximal intestine in *Mugil cephalus* and S*parus aurata* revealed an adaptation for the feeding habits. This adaptation occurred in order to protection and increasing of absorptive processes.

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