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Histomorphology and histochemistry of the digestive tract in meagre (*Argyrosomus regius*)

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ABSTRACT

This work presents a histomorphological and histochemical study of the digestive tract of meagre, *Argyrosomus regius*, a commercially-important species of Spanish indigenous fish with a great future in aquaculture. The interest of meagre culture in several countries due to the necessity of increase the diversification of fish species in aquaculture does very important to know the physiology of *A. regius* for improving culture aspects such us the feeding and the reproduction. The carbohydrates, proteins and phosphatase enzymatic activities distribution in the digestive tract of *A. regius* were studied in oesophagus, stomach, intestine and pyloric caeca samples providing useful information in the study of the digestive processes. Due to the histological and histochemical characteristics described in this work, we could suggest one secretor function for stomach and secretor-absorptive for the intestine and pyloric caeca.

KEYWORDS

Histochemistry;
Digestive tract;
Carbohydrate;
Phosphatase activity;
Argyrosomus regius;
Feeding;
Aquaculture.

INTRODUCTION

The aquaculture acquires special relevance in Spain for several reasons: the high consumption of fishes, the complexity to satisfy the consumer necessities by fishing and the existence of a tradition and infrastructure (industry and research centers) considerable to assure the initial steps of this activity. A recognized priority, at Europe and Spain, for a sustainable development of the aquaculture is the fish species diversification.

The interest in the culture of sciaenid fishes has grown in the last years. The meagre (*Argyrosomus regius*) is a commercially important fish species[1]. The meagre is a highly fecund species, widely distributed, with medium-high market prices and good acceptance on the part of the consumers. It presents the advantage of a eurihaline species which tolerate a wide range of salinity therefore allows its adaptation to diverse environments including the terrestrial aquaculture on brackish water. It resists the captivity perfectly (presence in great aquariums) and show high rates of growing and good food conversion efficiency.

Due to the interest of meagre culture in Spain ("Meagre Culture National Plant", also called PLANACOR), it's very important to know the physiology of *A. regius* for improving aspects of aquaculture like the feeding and the reproduction, therefore it's necessary the development of histological and histochemical studies of this species.

The morphology, histology and histochemistry of the teleosts digestive tract have been described for numerous species[2-10], but exist a few studies in the Argyrosomus genus[11-14], being practically nonexistent the histomorphological and histochemical studies in meagre, *A. regius*[15].

Concerning the histochemical aspect, many authors have focused on the importance of mucosubstances present in the fish digestive system and correlated these with the absorption and transportation of macromolecules[4], increase of digestive efficiency[16], buffering of intestinal fluid, prevention of proteolitic damage to the epithelium[17,18] and defences against bacteria[19].

To extend the histochemical studies in interest commercial teleost fish and considering the existence of larger interspecific and intraspecific histochemical variations in some digestive tract tissues, is necessary the study of the distribution of compound such us carbohydrates, proteins and enzymes in the meagre digestive tract in order to characterize the secretor cells/ tissues of those compounds and identify possible correlations with specific functional roles of the alimentary canal.

MATERIALS AND METHODS

Specimens of *A. regius* (mean body weight 80-90 g, total lengh ranging from 19-21 cm) were captured in Spanish southatlantic coast. Fish were maintaned in cilindric tanks of 1000 L in the Aquaculture Plant of Andalusian Superior Centre of Marine Studies until their utilization.

The specimens were anaesthetized and killed two hours after the capture by decapitation (the work described in this article has been carried out in accordance with "The code of ethics of the world medical association for animal experiments"). Oesophagus, stomach, intestine and pyloric caeca samples was then quickly removed. For light-microscopic studies of carbohydrates and proteins, the samples were fixed by perfussion with Bouin's Fluid for 24 h. After dehydratation in graded concentrations of ethanol, samples were embedded in paraffin wax. Dewaxed sections of 6-7 µm thickness obtained from samples fixed were stained with haematoxylin-eosin as well as haematoxylin-VOF trichromic[20] stains to show general morphology. The carbohydrate characterization was obtained using the following staining: Periodic acid-Schiff (PAS), Alcian Blue (AB) pH 2.5, Esterification-Alcian Blue (E-AB) pH 2.5, AB pH 1.0 and AB pH 0.5. The protein characterization was obtained using Bromofenol Blue (BB).

Enzymes, alkaline phosphatase (ALP) and acid phosphatase (ACP), were studied in sections (12µm) of unfixed samples embedded in Tissue Tek® from cryostat (Leica CM 1800) at -20ºC. Sections obtained from samples were treated with β-glycerolphosphate pH 9.4 (for demonstrate ALP) and β-glycerolphosphate pH 5.5 (for demonstrate ACP). References and compounds demonstrated by the different histochemical techniques are shown in TABLE 1.

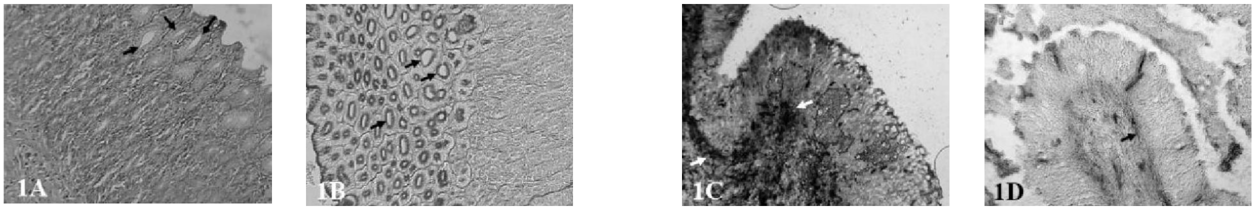
RESULTS

Oesophagus

The oesophagus was short with a wide lumen (at its connection with the stomach on one end and the pharynx on the other) and muscular walls.

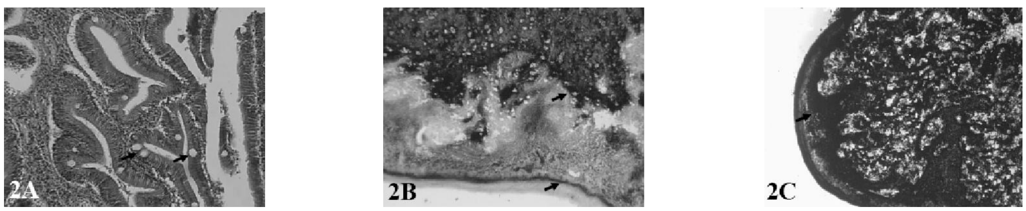
We observed a thick mucosa respect to wide submucosa layer. Oesophageal mucosa was organized in longitudinal deep folds of similar sizes. Taste buds were not present. The epithelium of mucosa was stratified at the anterior portion but becomes single-layered of columnar cells towards the transition to the stomach. The submucosa composed of a stratum compactum of connective tissue, presented a developed nervous plexus. Two layers of striated muscle occurred at the periphery of the connective tissue: an inner longitudinal layer and an outer circular layer. The serosa consisted of mesothelial cells and loose connective tissue.

Goblet cells were numerous in the mucosal epithelium of the end posterior oesophagus. Goblet cells were intensely PAS and AB pH 2.5 positive indicating the presence of glycoproteins and carboxylated mucines.



(A) (B) (C) (D)

Figure 1 : Histology and glycoconjugate histochemistry of the stomach in *A. regius*. (A) Morphological aspect of gastric glands (arrows). Haematoxylin-eosin×10. (B) PAS-positive material is present in the gastric glands (arrows). PAS×10. (C) Acid phosphatase activity in lamina propia and gastric glands (arrows). β-glicerophosphate x 25. (D) Alkaline phosphatase activity in lamina propia (arrow). β-glicerophosphate×25



(A) (B) (C)

Figure 2 : Histology and enzymatic activity of pyloric caeca in *A. regius*. (A) Morphological aspect of mucosa. Detail of globet cells (arrows). Haematoxylin-eosin x 10. (B) Alkaline phosphatase activity in submucosa and lamina propia (arrows). β-glicerophosphate×10. (C) Acid phosphatase activity in epithelium (arrow). β-glicerophosphate×4

Alcian Blue pH 0.5 showed the weak presence of ionised strongly sulphated glycoconjugates. Acid phosphatase activity is observed in the muscular layer. The histochemical results for oesophagus are summarized in TABLE 2.

Stomach

The stomach is a muscular appearance organ with a limited lumen that presented shape of sack. The final portion of the oesophagus and the anterior portion of the intestine are inserted proximally in the anterior portion of the stomach. The stomach posterior portion presented shape of blind-sac.

The transition from oesophageal to gastric mucosa was gradual, with a progressive replacement of stratified epithelium by secretory columnar cells, which were organized as a simple columnar epithelium. Like the oesophagus, the mucosa of the stomach exhibited characteristics folds, but the fine folds of the oesophagus became coarse and short folds in stomach. Presence of microvilli in the gastric epithelial cells was observed.

Gastric glands were nested in the lamina propria. These glands are observed in the almost totality of the stomach disappearing in the zone next to the intestine and open crypts of the mucosa folds. The secretory cells that compose the gastric glands were polygonal in shape.

The muscular layer consisted of two layers of smooth muscle: a circular internal and longitudinal external layer, this muscular structure is observed in both intestine and pyloric caeca. The gastric serosa was formed by connective tissue.

The epithelial columnar cells stained weakly with PAS and Bromophenol Blue whereas gastric glands stained strongly with PAS and showed moderate positivity to Bromophenol Blue. The stomach presented an intense acid phosphatase activity (gastric glands and lamina propria). The histochemical results for stomach are summarized in TABLE 3. The histology and glycoconjugate histochemistry of the stomach in *A. regius*. are showed in figure 1.

Pyloric caeca

Opened to the proximal zone of the intestine and next to the anterior portion of the stomach, we observed blind prolongations denominated pyloric caeca. The number of pyloric caeca found in the digestive system of *A. regius* was nine. The transversal section of pyloric caeca showed the same histological structure that proximal intestine. The surface of the caecal mucosa was covered with long and deep folds filling the lumen. The epithelium consisted of simple columnar cells which

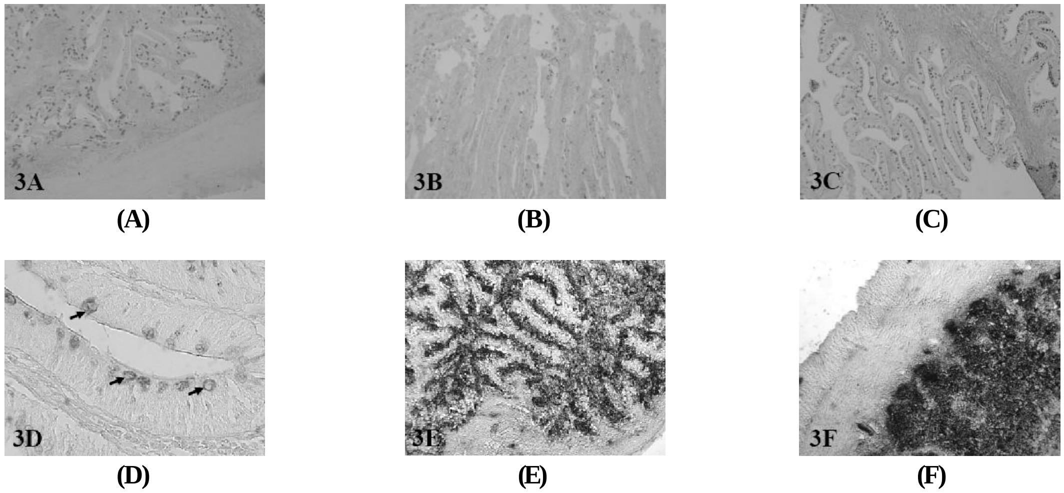


Figure 3 : Histology and glycoconjugate histochemistry of the proximal and distal intestine in *A. regius*. (A) Distribution of goblet cells in proximal intestine. AB 2.5×10. (B) Distribution of gobbet cells in distal intestine. AB 2.5×10. (C) Distribution of goblet cells in proximal intestine. PAS×10. (D) Detail of goblet cells in distal intestine (arrows). PAS×25. (E) Alkaline phosphatase activity in epithelium of proximal intestine. β-glicerophosphate×4. (F) Alkaline phosphatase activity in submucosa of distal intestine β-glicerophosphate×4

are interpersed with goblet cells. Goblet cells were intensely PAS and AB pH 2.5 positive. A discrete pisitivity is observed after AB pH 0.5 and AB pH 1. The columnar epithelium presented an intense alkaline and acide phosphatase activity. The histochemical results for pyloric caeca are summarized in TABLE 4. The histology and enzymatic activity of pyloric caeca in *A. regius* are showed in figure 2.

Instestine

The meagre intestine was relatively short with gross walls. It presented a greater diameter in the proximal zone that decreased to the medium zone and bring to enlarge again in the anal zone. The intestinal mucous showed long and deep primary and secondary folds lined by a simple columnar epithelium characterized by the presence of the enterocytes (abpsortive cells); numerous goblet cells are scattered among this columnar cells. Goblet cells were abundant in the proximal part of the intestine and decreased in number in the distal part.

The epithelium cells surface exhibited short microvilli-brush border-. The submucosa consisted of loose connective tissue and blood vessels. The serosa consisted of connective tissue containing capillaries.

The intestine portion next to stomach (so call "anterior" or "proximal" intestine) showed more primary and secondary folds than the intestine distal portion, the mucous cells were abundant and submucosa is thicker than the mucosa layer.

The intestine portion next to the anus (so call "posterior" or "distal" intestine) showed a decrease of the quantity and length of the folds. The quantity of secondary folds decreased since to disappear toward to rectal zone and the quantity of primary folds decreased and increased his diameter. The muscular layer diameter is greater in the rectal zone next to rectal zone. The quantity of mucous cells decreased respect to the proximal intestine.

The histochemical properties of proximal and distal intestine varied in some aspects. Most mucous cells of the proximal and distal intestine stained with PAS but the columnar epithelium in the distal portion stained intensely with AB pH 2.5 and in the proximal portion this reaction is weak. The mucous cells of the proximal intestine presented abundant carboxyl-rich glycoconjugates (sulphated or not) and a lesser proportion of sulphated glycoconjugates (weakly ionised). In the distal portion the quantity of carboxyl-rich glycoconjugates was greater in the columnar epithelium. The alkaline phosphatase activity was higher in the mucosal epithelium of proximal intestine compared to the distal intestine. On the other hand, acid phosphatase activity was stronger than the alkaline one in the distal intestine. The reaction of both enzymes at the epithelium was more significant than at the muscular or submucosal level. The histochemical results for

TABLE 1 : Histochemical reactions used to detect carbohydrates, proteins and enzymes

Reactions	References
Carbohydrates	
Periodic Acid Schiff (PAS) Glycogen, neutral mucosubstances and/or glycoconjugates	McManus[52]
Alcian Blue (AB) pH 2.5 Carboxyl-rich glycoconjugates (sulphated or not)	Steedman[53]
Esterification – Alcian Blue (E-AB) pH 2.5 Blockage of carboxylated and sulphated groups	Spicer &Lillie[54]
Alcian Blue pH 1.0, Sulphate glycoconjugates (weakly ionised)	Steedman[53]
Alcian Blue pH 0.5, Sulphate glycoconjugates (strongly ionised)	Steedman[53]
Proteins	
Bromophenol Blue (BB), General protein	Mazia et al.[55]
Enzymes	
β-glycerolphosphate pH 9.4, Alkaline phosphatase (ALP)	Gomori[56]
β-glycerolphosphate pH 5.5, Acid phosphatase (ACP)	Gomori[57]

proximal and distal intestine are summarized in TABLE 5 and 6. The histology and glycoconjugate histochemistry of the proximal and distal intestine in *A. regius* are showed in figure 3.

DISCUSSION

The fish digestive tract shows a marked diversity in its morphology and function influences by phylogeny, corporal size and diet and/or type of feeding[16,17,21-24], but it shows some basic structural similarities[9].

The meagre, is very voracious and usually is fed on mugilides, clupeids and some swimming crustaceans. This type of feeding (carnivorous) justifies the digestive tract morphology[25].

Oesophagus

In most teleost fish, oesophagus is short and thick walled; the muscularis is comprised of interweaving skeletal muscle fibres that may extend as far as the stomach. The stratified cubical or columnar epithelium may be ciliated and contains numerous goblet cells and occasionally taste buds. In addition, multicellular serous or cardiac glands may be found subsequently. The mucosa is thrown into longitudinal folds that end at the stomach. The serosa contains prominent nerve fivers of the vagus nerve[6,26].

The *A. regius* oesophagus presented an ample diameter and muscular walls and it was constituted fundamentally by neutral mucosubstances, being appraised slightly the presence of sulfomucines. The existence of neutral mucosubstances and sulfomucines in the oesophagi mucous cells of the fishes is constant[2,27]; the secretion of these compounds could protect of the abrasion that produce nutritional particles[28].

Stomach

A. regius presented a muscular appearance stomach of small light and sac shape. In most teleost fish, the gastric mucosa is composed by simple columnar epithelium that stains by "orange G" of the VOF[20]. The existence of microvilli in gastric epithelial cells with the presence of neutral mucosubstances could to imply certain absorptive function in the stomach as suggested Ezeasor and Stokoe[29] in other teleost fish. The lamina propria is composed by connective tissue supports gastric glands. Gastric glands can be observed in the oesophagus-stomach transition of the meagre digestive tract, these glands are also observed in *Seriola dumerlii*[25] and are not observed in some other teleost fish like *Solea senegalensis*[6]. Simple tubular gastric glands are formed by acidophilic granules and have a basophile basal nucleus. Gastric glands are present by almost totality of stomach like in gilthead sea bream, *Sparus aurata*[27], in meagre is observed a decreasing and disappearing of these glands in the portion next to intestine.

Stomach epithelium contains a high amount of neutral mucosubstances being observed a weak presence of acid mucosubstances, Murray et al.[30] indicated that the chemical composition of the gastric mucosa varies between the species and it is function of the diet. Neutral mucines have been described in stomach epithelium of *Sparus aurata*[31,32] and Senegal sole, *Solea senegalensis*[7] and have been related to the disaccharides absorption of easy digestion and fatty acids of short chain. The mucines presence in the gastric glands of teleost fish has been related with the type of feeding, suggesting that a piscivorous diet does not seem to demand any special carbohydrate, secreting indifferently neutral mucines or sulfomucines. According to Reifell and Travill[33], the sulfomucines secretion in the gastric crypts of different species increases when diminishes the prey size ingested, not observing sulfomucines in piscivorous species.

TABLE 2 : Histochemical distribution of carbohydrates, proteins and enzymes in oesophagus of *Argyrosomus regius*

	PAS	AB 2.5	E-AB 2.5	AB 1.0	AB 0.5	BB	ACP	ALP
Epithelium	+	- / +	-	-	-	+	+	-
Globet cells	+ + / + + +	+ / + +	-	-	- / +	+ / + +	-	-
Lamina Propia	+ / + +	+	-	-	- / +	+	+ / + +	-
Submucosa	+ / + +	+	-	-	- / +	+	+ / + +	-
Muscular	+ / + +	-	-	-	-	+ + / + + +	+ +	-

Results are reported considering the intensity of histochemical staining: - (unstained), + (weak staining), + + (moderate staining), + + + (intense staining)

TABLE 3 : Histochemical distribution of carbohydrates, proteins and enzymes in stomach of *Argyrosomus regius*

	PAS	AB 2.5	E/AB 2.5	AB 1.0	AB 0.5	BB	ACP	ALP
Epithelium	+	- / +	-	-	- / +	+	-	-
Gastric gland	+ + +	+	-	-	- / +	+ +	+ + / + + +	+
Lamina propia	+	+	-	-	- / +	+	+ + / + + +	+ / + +
Submucosa	+ / + +	+	-	-	- / +	+	+ / + +	+
Muscular	+ / + +	+	-	-	- / +	+ + / + + +	-	+

Results are reported considering the intensity of histochemical staining: / (unstained), + (weak staining), + + (moderate staining), + + + (intense staining)

TABLE 4 : Histochemical distribution of carbohydrates, proteins and enzymes in pyloric caeca of *Argyrosomus regius*

	PAS	AB 2.5	E-AB 2.5	AB 1.0	AB 0.5	BB	ACP	ALP
Epithelium	+	-	-	-	-	-	+ +	- / +
Globet cells	+ + +	+ +	-	+ / + +	+	+ / + +	-	-
Lamina Propia	+	-	-	-	-	+	+ / + +	+
Submucosa	+	- / +	-	-	- / +	+	+ / + +	+ / + +
Muscular	+	+	-	- / +	- / +	- / +	-	-

Results are reported considering the intensity of histochemical staining: / (unstained), + (weak staining), + + (moderate staining), + + + (intense staining)

TABLE 5 : Histochemical distribution of carbohydrates, proteins and enzymes in proximal intestine of *Argyrosomus regius*

	PAS	AB 2.5	E-AB 2.5	AB 1.0	AB 0.5	BB	ACP	ALP
Epithelium	+	+	-	-	-	-	+ / + +	+ / + +
Globet cells	+ + +	+ + +	-	+ / + +	+	+	-	-
Lamina Propia	+ / + +	+ / + +	-	- / +	- / +	- / +	+	- / +
Submucosa	+ / + +	+ / + +	-	- / +	- / +	+	+	+
Muscular	+	+	-	-	-	+ + / + + +	- / +	- / +

Results are reported considering the intensity of histochemical staining: / (unstained), + (weak staining), + + (moderate staining), + + + (intense staining)

Intestine and pyloric caeca

The *A. regius* intestine is relatively short with gross walls. It presents a greater diameter in his proximal zone that diminishing towards one middle portion from which it returns to be high towards rectal region. Opened to intestine proximal portion we found prolongations blind of a denominated pyloric caeca that in the case of the meagre are in a number of nine.

Although the intestine length is not correlated necessarily with the functional surface of the mucosa[29] is evident that the smaller length of the intestine of some carnivorous teleost fish is compensated with a complex relief of you fold mucous that increase the area or the surface that takes part in the digestive processes. The mucous folds present different morphologies in the different areas from the digestive tract[34]. These mucous folds increase the secretory-absortive surface of digestive tract and they even can retain the food. The functional surface of the mucosa adjusts to the variation of secretions and the absorptive demand[35]. The pattern of mucous folds observed in meagre extends from short folds of the oesophagus through to abundant and greater folds than we found in the stomach and the deep and numerous folds of the proximal intestine where we can observe primary and secondary ramifications. The number and the length of these folds diminish towards the rectal region.

The intestinal mucosa is composed by columnar cells (enterocytes) and goblet cells. The specific processes of absorption are produced at enterocytes level that presents prismatic columnar shape and a specialized free surface with brush border. In the absorption process participant the neutral mucosubstances and alkaline phosphatase presents in meagre intestinal epithelium of the meagre and other teleost fish[3,25].

In the meagre intestine, *A. regius*, emphasise the presence of an important production of rich glycoproteins with hydroxyls groups (PAS positive) as well as carboxyl-rich acid mucosubstances. The neutral mucines could cooperate in the enzymatic digestion of the food. These mucines and the detected alkaline phosphatase activity in the -brush border- could participate in absorptive functions as well as in the macro-molecule transport through the membranes[36].

In *Sparus aurata*[31], the goblet cells contain sulphated sialomucines partially acetylates because increases the positivity to the PAS after making an alkaline saponification and a small number of cells

TABLE 6 : Histochemical distribution of carbohydrates, proteins and enzymes in distal intestine of *Argyrosomus regius*

	PAS	AB 2.5	E-AB 2.5	AB 1.0	AB 0.5	BB	ACP	ALP
Epithelium	+	+ + +	-	+	+	-	+ / + +	- / +
Globet cells	+ + / + + +	+ / + +	-	- / +	-	+ / + +	-	-
Lamina Propia	+ +	+	-	- / +	- / +	+	+	- / +
Submucosa	+ +	+	-	- / +	- / +	+	+	+ +
Muscular	- / +	+	-	-	-	+ / + +	- / +	+

Results are reported considering the intensity of histochemical staining: / (unstained), + (weak staining), + + (moderate staining), + + + (intense staining)

contains neutral mucosubstances/glycoprotein. Reifel and Travill[37] suggested that the presence of two or more types of carbohydrates in the epithelium is indicative of different levels from maturation in the mucous secretion, synthesizing first neutral glycoprotein. In meagre, PAS reaction presents high positivity in the goblet cells of proximal intestine, presenting a smaller intensity in the distal intestine. These carbohydrates can provide a protection for the intestinal mucosa of the gastric material acidity[38] and preserve their hidratation degree of[39].

In higher vertebrates, the mucus secreted by the goblet cells (neutral and acid mucosubstances) takes part in the cells protection against the autodigestion at level of the proximal intestine, while in the distal portion it participates in the lubrication of the fecal material[40].

Enzymatic activities

The study of the hydrolytic enzymes in the teleost fish digestive tract has been treated by different authors[3,7,41-45]. The phosphatase activities distribution in the meagre digestive tract is similar to described in other teleost fish[7,31,42]. Acid and alkaline phosphatases are widely distributed in different organs and tissues from vertebrates. They are present in numerous biological functions: digestive absorption, secretion, bone formation, active transport through membranes, etc.[46]. Alkaline phosphatase participates in processes of bone formation and calcification, secretion, etc.[47]. On the other hand, Overnell[48] indicated the lisosomic origin and digestive function of phosphatase acid in the pyloric caeca and mesentery associated in *Gadus mohrua*.

In meagre, is observed a high acid phosphatase in the gastric glands and lamina propria of the stomach; being the alkaline phosphatase activity more patent at level of columnar epithelium of the intestine (apical portion and brush border of the enterocytes).

The no presence of the phosphatase activities in the stomach mucosa and its positivity in the glands of anterior region is also observed in the studies made by Vegas-Velez[49] that affirms that the stomach pyloric region of different teleost fish does not have greater digestive importance because the rate of enzymatic secretions is very low. In the different portions from intestine (distal and proximal) the phosphatase activities distribution is similar.

Comparing the phosphatase activities distribution in intestine and pyloric caeca of meagre we did not observe appreciable differences. This fact could be interpreted due to the similar histology of these two portions of the digestive tract in different teleost fish[50]. The absorption of lipids, aminoacids and carbohydrates is related with the location of alkaline phosphatase. The development of this enzymatic activity agrees with the appearance of the brush border in the enterocytes, increasing its presence with the consumption of these substances and proteins[36,51].

The distribution of acid phosphatase in meagre is similar to the alkaline phosphatase, although its intensity is from medium to strong. One distribution compares between acid and alkaline phosphatase was observed by Gonzalez de Canales[3].

Due to the histological and histochemical characteristics described in this work could suggest one secretory function for stomach and secretor-absorptive for the intestine and pyloric caeca.

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