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Original Research Article

Comprehensive Study of the Digestive Tract in the European hake (*Merluccius merluccius*)

Key words

European hake;
Merluccius merluccius;
histology;
histochemistry;
digestion;
enzymes

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Abstract: The European hake (*Merluccius merluccius*) is an important commercial fish that is widespread in the Adriatic Sea. It is a carnivorous fish whose diet consists mainly of fish. The aim of this study was to perform a detailed analysis of the digestive tract in the European hake. Therefore, the anatomy and histology of the posterior part of the digestive tract (from the esophagus to the rectum) were described. The fish were caught by longline fishing in the middle part of the Adriatic Sea along the Croatian coast. A total of 33 adult specimens were analyzed. Tissue components were visualized using hematoxylin-eosin, Mallory's trichrome, Verhoeff-Van Gieson, Alcian Blue-PAS kit and a reticular fiber staining kit. To investigate cellular digestion, the localization and activity of alkaline phosphatase, acid phosphatase, non-specific esterase and aminopeptidase were measured. All parts of the digestive tract were composed of mucosa, submucosa, muscularis and serosa. The type of epithelium varied from part to part. The muscular layer of the mucosa was not identified in the esophagus, the intestine proper and the rectum. The muscularis consisted of smooth muscle cells, except in the esophagus, where it consisted of striated muscle fibers. All parts of the digestive tract are involved in the digestion and absorption of nutrients. Results observed from optical density of enzymes highlighted that although the intestine was the main site for lipid and protein digestion, the stomach and rectum were also important locations for protein digestion. Although the anatomy and histology of the digestive tract in European hake have been partially described, there is no data on the optical density of the enzymes in the available literature. To achieve objective results that allow for precise data comparison, the optical density of the enzymes was measured in this study. This research provide comprehensive findings and introduced new knowledge that significantly expands and partly differs from what is known so far, highlighting the necessity for further studies in this area.

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Introduction

In fish, the digestive system consists of the digestive tract and associated glands (liver, pancreas or hepatopancreas). In the digestive tract the anterior and posterior parts are distinct from each other (1, 2, 3). While the structures of the anterior part are involved in food intake and its mechanical processing, the posterior part of the digestive tract is responsible for the intensive chemical digestion of food.

According to the Eschmeyer's Catalog of Fishes, 36893 fish species are recorded worldwide (4). Some of these species have similar habitats and feeding habits, while others have entirely different ecological niches, leading to multiple adaptations and variations in their digestive tract. Anatomical features and dietary habits strongly influence the distribution and intensity of enzymes in the digestive

tract (5). Alkaline phosphatase (ALP) comprises of a group of enzymes that catalyze the hydrolysis of monophosphate esters of phosphoric acid at a pH of 9.0 to 10.5 (6, 7, 8). In the digestive tract of fish, ALP has a variety of roles in the digestion and absorption of nutrients (9). Some of these roles include the hydrolysis of phosphate molecules from carbohydrates, fats and proteins (10). ALP is also involved in the metabolism of calcium, phosphorus and fatty acids (11). It serves as a barrier by regulating enterocyte pH, controlling tight junctions, detoxifying inflammatory microbial components and modulating the gut microbiota (11, 12). Acid phosphatase (AP) comprises of a group of enzymes that catalyze the hydrolysis of monophosphate esters of phosphoric acid at a pH of 4.0 to 6.0 (6, 7, 8). In fish, AP contributes to metabolic processes within the cell through pinocytosis. It is also involved in protein metabolism (5, 10, 13) and secretory processes in gastric glands (13). The non-specific esterase (NSE) comprises of the group of enzymes whose optimal activity is in a pH range of 5 to 8. The enzyme is involved in the digestion of glycerides and lower fatty acids. Aminopeptidases (A) are exopeptidases that hydrolyze peptide bonds at the N-terminus of a peptide or protein substrate. The role of this enzyme is associated with protein synthesis and degradation.

The European hake (*Merluccius merluccius*) is an important commercial fish widely distributed in the Adriatic Sea (14). Adult hake eat mainly fish and, to a lesser extent, mollusks and crustaceans (14, 15, 16). The aim of this study was to provide a comprehensive analysis of the digestive tract in the European hake. Therefore, it included the identification and description of the anatomy and histology of the posterior part, as well as the determination of the localization and activity of enzymes involved in cellular digestion.

Material and methods

The research is performed in accordance with Animal Protection Act by the Ministry of Agriculture and approved by The Committee for Ethics in Veterinary Medicine, Faculty of Veterinary Medicine (No. 251-61-01/139-20-32). The fish were caught by longline fishing in the middle part of the Adriatic Sea along the Croatian coast. A total number of 33 healthy adult European hakes (*Merluccius merluccius*) with a body mass of 417.50 ± 502.13 g and a total length (TL) of 35.56 ± 9.45 cm were investigated. After dissection, the macroscopic characteristics of the digestive tract were determined. The length of the intestine (IL) was measured from the caudal part of the pyloric sphincter to the anal opening and the relative intestinal length (RIL) was calculated (17). Tissue samples were taken from parts of the digestive tract based on the previously defined boundaries (Figure 1). To observe the general structure, organs samples were fixed in 10% neutral buffered formalin, embedded in paraffin and cut into 6 µm sections using a microtome (Slee Mainz, CUT 5062, GmbH, Germany). The following histological and histochemical staining methods were used: hematoxylin and eosin (18), Mallory trichrome (19, 20), Verhoeff-Van Gieson (20), Alcian Blue- PAS kit (Biognost, Zagreb, Croatia) and the seven-reagent kit for the determination of reticulin fibers (Biognost, Zagreb, Croatia). A total of 450 slides were analyzed to determine the microscopic structure of different parts of the digestive tract. To determine the localization and measure the intensity of the enzymes, the tissue samples were fixed in 10% formal calcium (4°C). After 24 hours they were transferred to a 30% gum sucrose solution (21), embedded in Cryofix gel (Biognost, Zagreb, Croatia) and cut into 8 µm sections using a cryostat (Thermo Shandon, Tamiko Instruments). The following azo-coupling methods were used to visualize the enzymatic reactions: ALP (6), AP (22), NSE (22) and A (20). Positive reactions were observed by different intensities of blue (ALP and NSE), pink (AP) and red (A). Negative controls without substrate were also prepared.

Slides were analyzed with a Digicyte DX50 microscope (Digicyte, Zagreb, Croatia) and photographed with a Digicyte BigEye camera and processed with Digicyte Capture software. ImageJ software (USA National Institutes of Health, MD, USA, www.imagej.net) was used to measure the optical density (OD) of the positive enzyme reactions on five randomly selected fields of the slide. The OD of the enzymes was measured on images obtained using a 10x magnification objective. Due to the field size (10.11 px × 3.96 px), rare reactions were only described descriptively. A total of 1963 measurements were taken in the digestive tract. The Mann-Whitney U-test was used for statistical analysis. The significance of differences in mean optical densities (MOD) of ALP, AP, NSE and A between the same layers in different parts of the digestive tract was tested. In addition, the significance of differences in MOD in the same parts of the digestive tract between different layers was tested. All differences were considered statistically significant if $P < 0.05$.

Figure 1: Sampling scheme of the digestive tract of the European hake. Black rectangles mark the sampling sites for histological and histochemical examination. Red rectangles mark the sampling sites for the examination of localization and enzymatic activity of the digestive tract. The following areas are shown: the anterior (1) and posterior (2) part of the esophagus, the anterior (3) and posterior (4) part of the stomach, the pyloric sphincter (5), the anterior (6), middle (7) and posterior (8) part of the intestine and the rectum (9)

Results

Macroscopic structure of the digestive tract

The digestive tract of the European hake was shown in Figure 2. The esophagus was short and straight and its mucosa formed longitudinal folds towards the stomach. The stomach was Y-shaped. Its anterior part had a wide lumen and extended into a long, blind sac. Unlike the anterior part, the posterior part of the stomach was short with a narrow lumen and ended with a muscular pyloric sphincter. The intestine of the European hake was long and formed several coils. Based on the appearance of the intestinal wall, it could be divided into an anterior, middle and posterior part. The anterior part of the intestine merged into the middle part, which had a significantly thinner wall and a larger lumen compared to the anterior part. The middle part of the intestine was the longest segment and formed several coils in the body cavity. A reduction in the lumen and thickening of the wall characterized the transition from the middle to the posterior part of the intestine. The RIL of the intestine proper was 0.41 ± 0.13. The posterior part of the intestine continued into the rectum, the wall of which was pigmented.

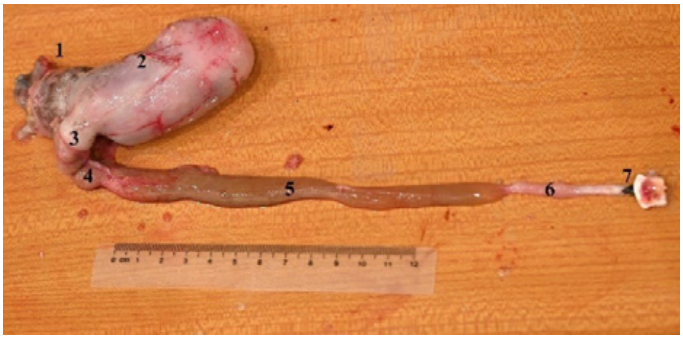

Figure 2: Digestive tract of the European hake: esophagus (1), anterior (2) and posterior (3) part of the stomach, anterior (4), middle (5) and posterior (6) part of the intestine and rectum (7)

Microscopic structure of the digestive tract

Esophagus

The layers of the esophagus were indicated in Figure 3A. The mucosa of the esophagus was lined with a stratified squamous non-keratinized epithelium. Among the surface epithelial cells were numerous unicellular mucous glands with AB+PAS- (Alcian Blue – Periodic Acid-Schiff) secretion. The epithelium was separated from the lamina propria by a PAS+ basement membrane. The lamina propria consisted of dense connective tissue with a predominance of collagen fibers and a smaller number of elastic fibers. However, the muscular layer of the mucosa was not differentiated. The lamina propria and the submucosa differed in the type of connective tissue of which they were composed. The submucosa of the esophagus consisted of loose connective tissue and was well vascularized. The muscularis of the esophagus consisted of an inner and an outer layer of striated muscle fibers. The outer surface of the esophagus was covered with a serosa layer consisting of connective tissue and mesothelium.

Stomach

The layers of the stomach were shown in Figure 3B and 3C. The mucosa of the stomach was lined with a simple columnar epithelium with microvilli. In some specimens AB-PAS+ secretion was observed in the apical part of the epithelial cells. The lamina propria consisted of collagen fibers with additionally differentiated reticular fibers between the glands. The gastric glands in the anterior part of the stomach consisted of a single type of cell (Figure 3B). The nuclei of these cells were light-colored, round and located closer to the basal part of the cell. In the posterior part, epithelium extended into tubular non-specific mucous glands (Figure 3C). The muscular layer of the mucosa was well differentiated and consisted of smooth muscle cells. The submucosa of the stomach consisted of collagen fibers and contained elements of the vascular system. The muscularis consisted of an inner circular and an outer longitudinal layer of smooth muscle cells. The outer surface of the stomach was covered with serosa. In the area of the pyloric sphincter, the lamina propria and the muscular layer of the mucosa were not visible. This area was dominated by connective tissue interspersed with smooth muscle cells, while the muscularis was notably thickened.

Intestine

The structure of the intestine proper was shown in Figure 3D – 3F. The mucosa of the intestine was lined with a simple columnar epithelium with microvilli. Among the epithelial cells unicellular mucous glands containing AB+PAS- secretion were present. In the anterior and middle part, the muscular layer of the mucosa was absent, so that the dense connective tissue of the submucosa lay directly on the loose connective tissue of the lamina propria (Figure 3D and 3E). In the posterior part, a muscular layer of the mucosae was identified in some specimens (Figure 3F). The folds of the intestine were made of mucosae and submucosa. The anterior part of the intestine had a narrow lumen with wide and low folds (Figure 3D). The middle part of the intestine had a thicker lumen with folds that were considerably higher than those in the anterior and posterior parts. The posterior part of the intestine had a similar lumen diameter to the anterior part, but the folds were higher than those in the anterior part and shorter than those in the middle part (Figure 3F). The connective tissue layers were more developed in the anterior part than other parts of the intestine. The muscularis of the anterior and posterior part was well developed and consisted of an inner circular and an outer longitudinal layer of smooth muscle cells. In the middle part, the structure of the muscularis was similar except that the inner layer was thinner than the

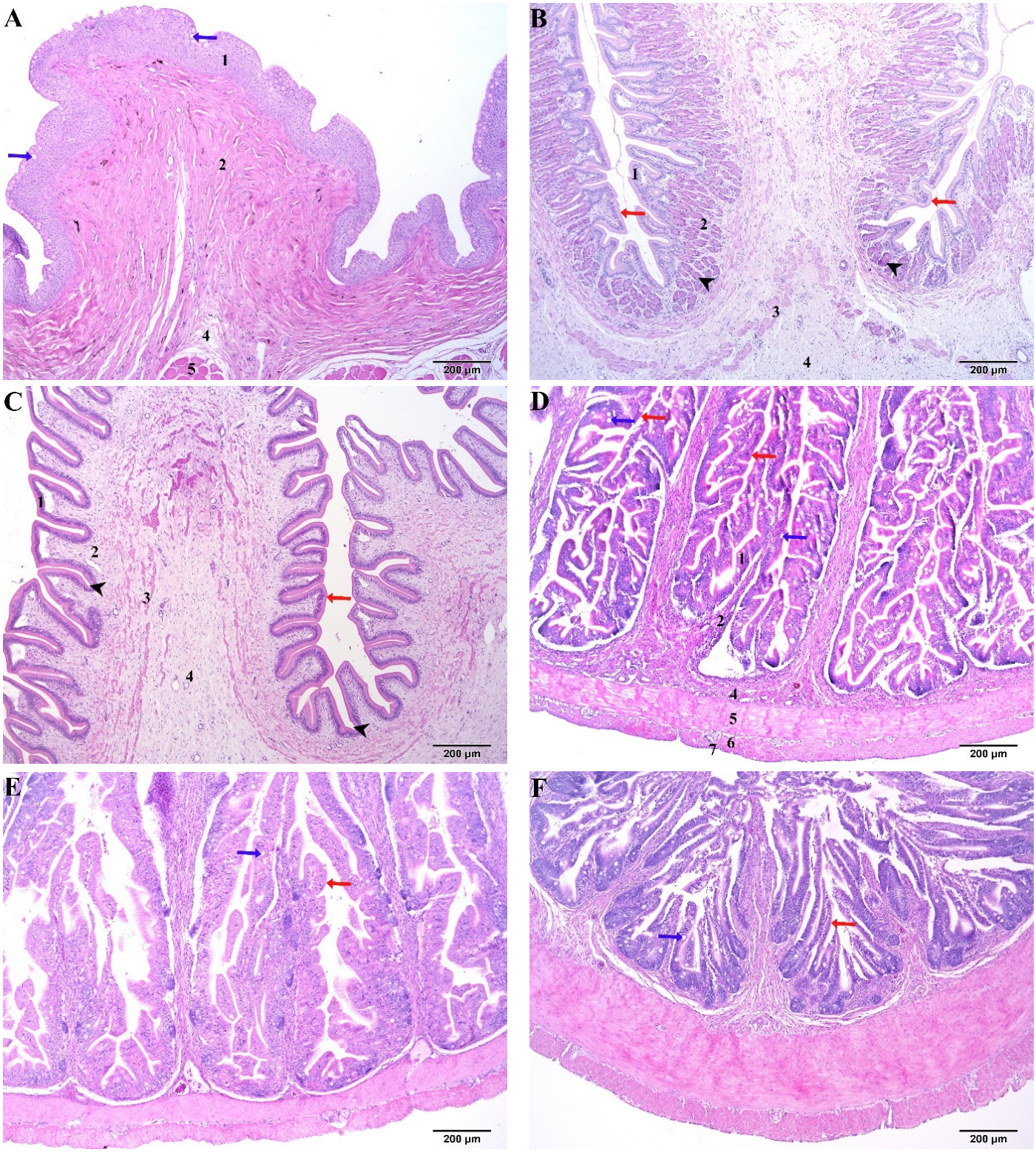

Figure 3: Different parts of the digestive tract of the European hake. Hematoxylin-eosin staining method. The figure shows esophagus (A), anterior (B) and posterior (C) part of the stomach, anterior (D), middle (E) and posterior (F) part of the intestine and rectum (G). The layers of the digestive tract are labeled as it follows: epithelium (1), lamina propria (2), muscular layer of the mucosa (3), submucosa (4), inner (5) and outer (6) layer of muscularis and serosa (7). Figures E and F show the ratio of the different layers and the height and width of folds compared to the anterior part of the intestine. The blue arrows indicate unicellular mucous glands, while the red arrows show microvilli. The arrowheads indicate the glands in the lamina propria

same layer in the other parts of the intestine (Figure 3E). The outer surface of the intestine was covered with serosa.

Rectum

The structure of the rectum was shown in Figure 3G. The mucosa of the rectum was lined with a special form of

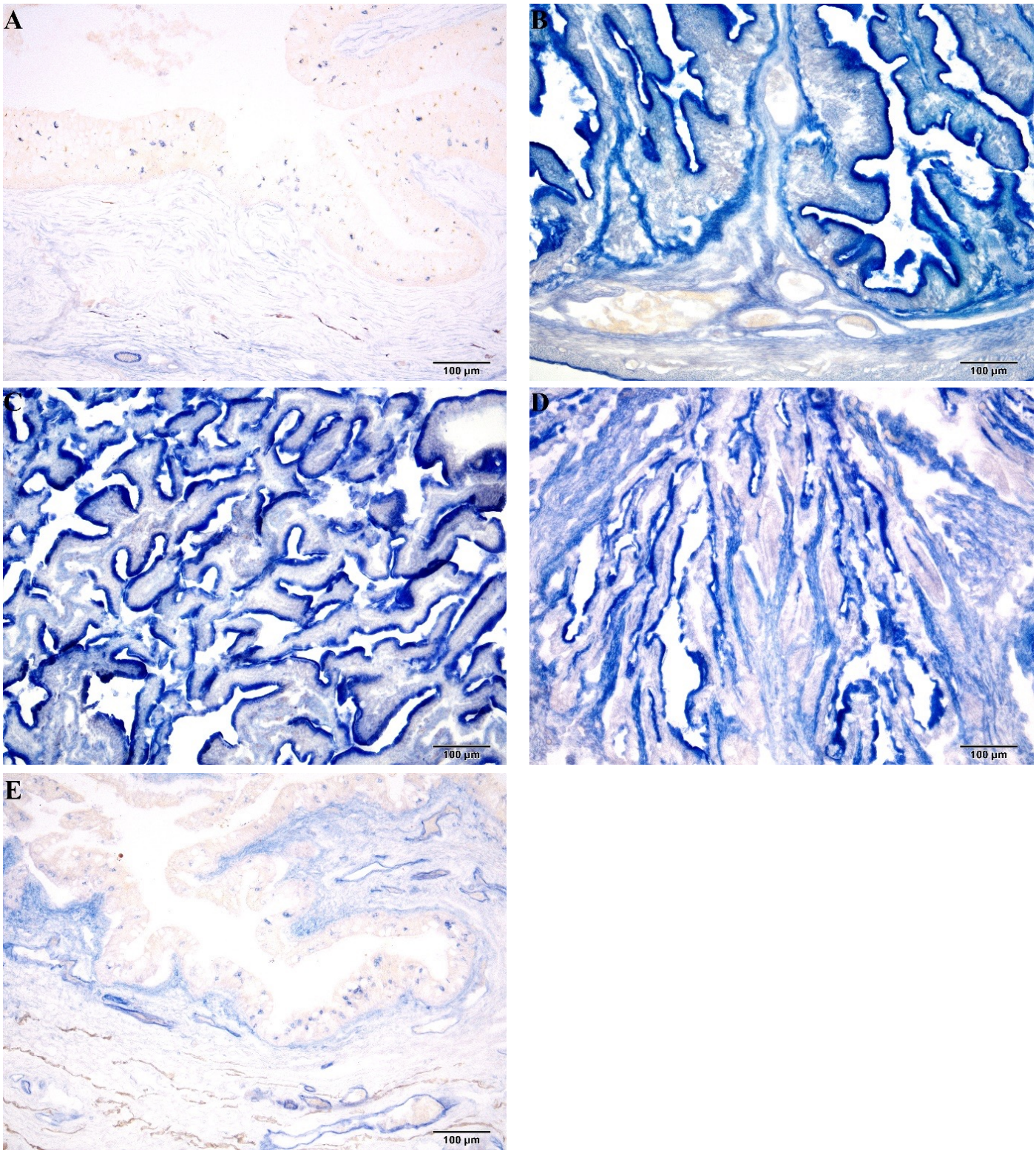

Figure 4: Positive reactions of ALP in the digestive tract of the European hake (A – E). The figure shows reactions in the: esophagus (A), anterior (B), middle (C) and posterior (D) part of the intestine as well as in the rectum (E)

pseudostratified columnar epithelium with microvilli. In this epithelium, cells had nuclei at different levels. However, the highest columnar cells did not reach the free surface because cuboidal cells with round nuclei were embedded between them. The unicellular mucous glands were numerous and were filled with AB+PAS- secretion. The lamina propria consisted of loose connective tissue. The muscular layer of the mucosa was not present, so the dense connective tissue of the submucosa lay directly on the loose connective tissue of the lamina propria. The muscularis was built of an inner circular and an outer longitudinal layer

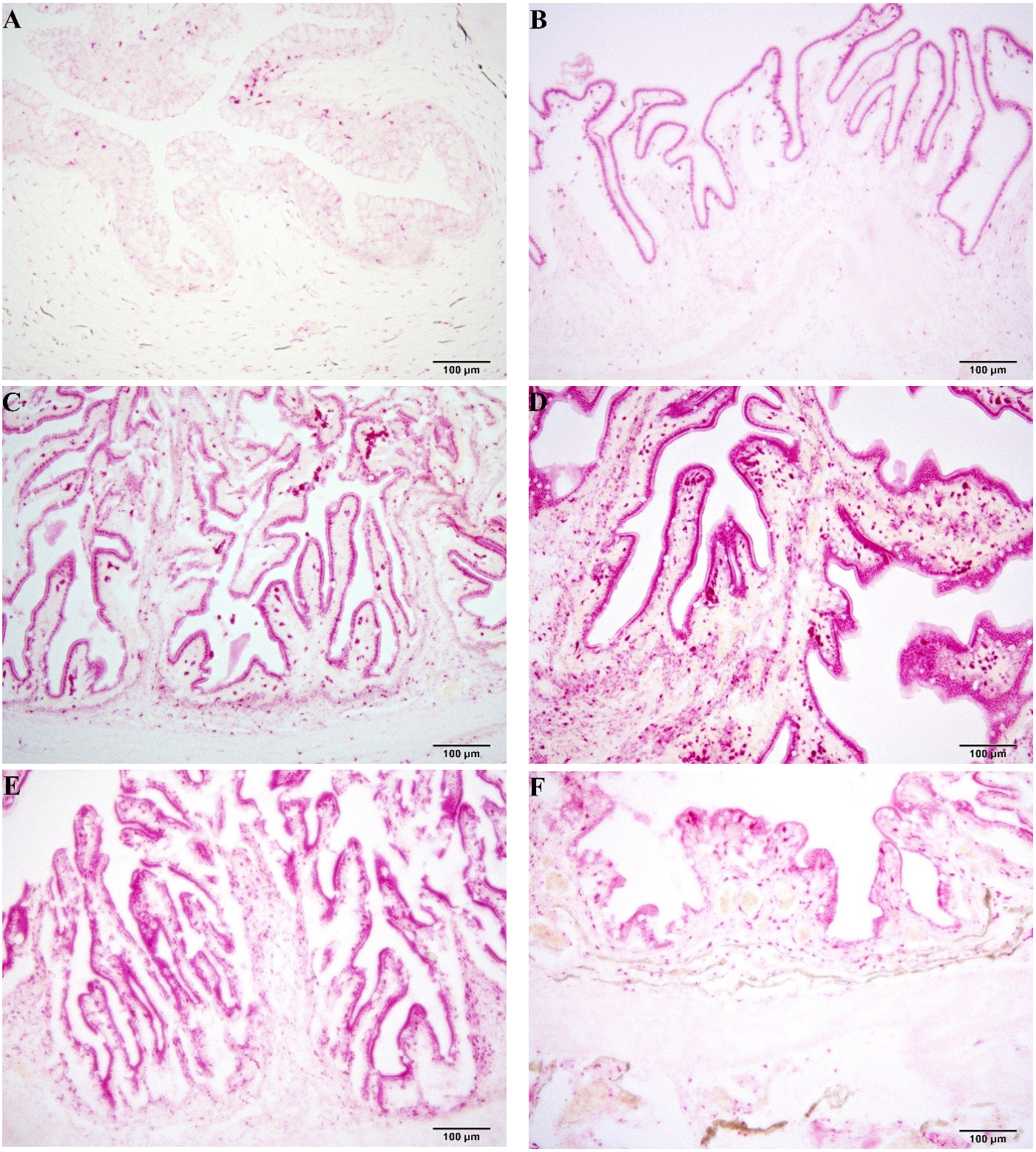

Figure 5: Positive reactions of AP in the digestive tract of the European hake (A – F). The figure shows reactions in the: esophagus (A), posterior part of the stomach (B), anterior (C), middle (D) and posterior (E) part of the intestine as well as in the rectum (F)

of smooth muscle cells. The outer surface of the rectum was covered with serosa which was pigmented.

Enzyme localization in the digestive tract

Esophagus

Positive reactions of ALP (Figure 4A), AP (Figure 5A) and NSE were observed in the epithelial cells of the esophagus.

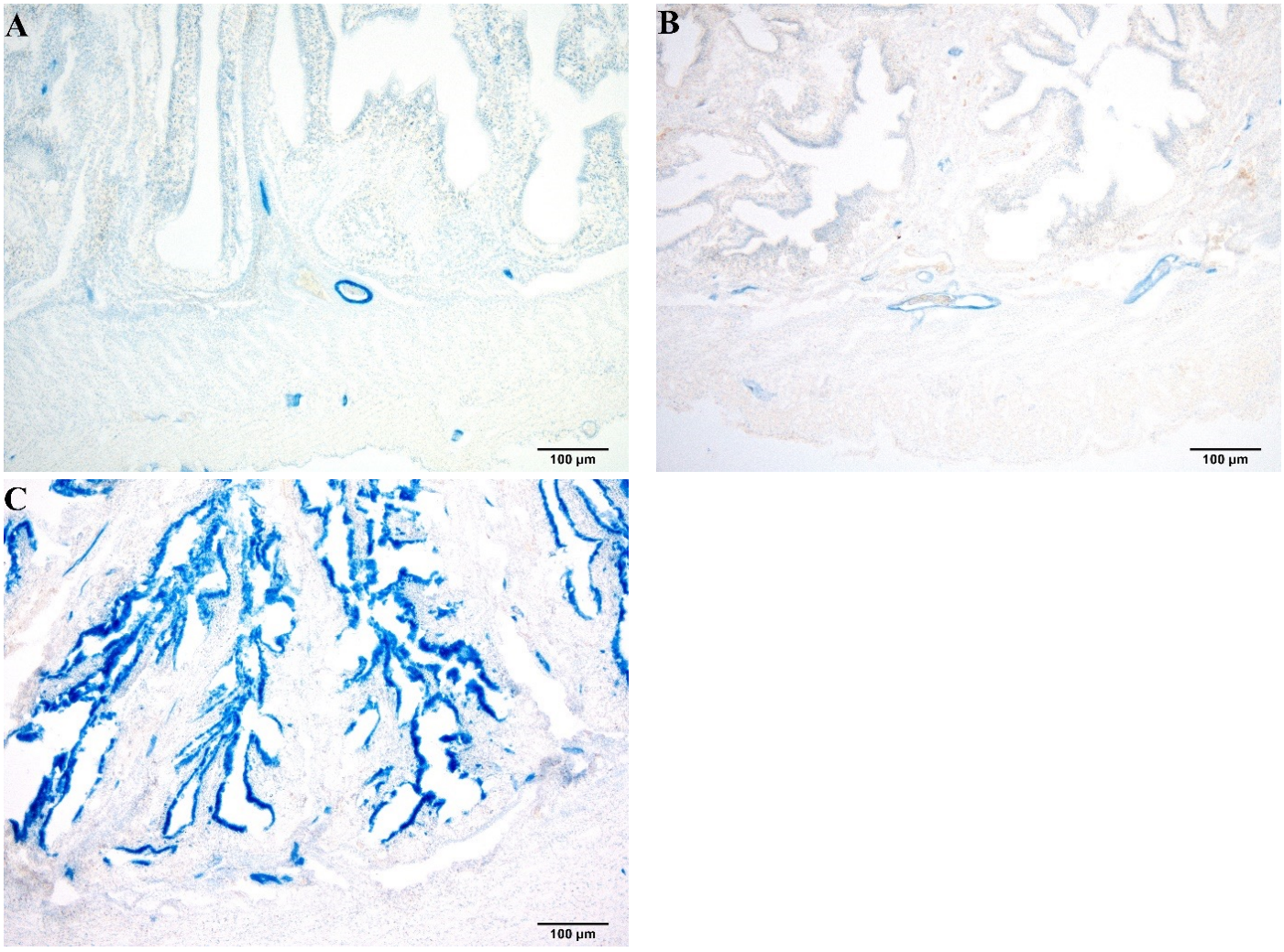

Figure 6: Positive reactions of NSE in the digestive tract of the European hake (A – C). The figure shows reactions in the: anterior (A), middle (B) and posterior (C) part of the intestine

ALP and NSE (rare) reactions were noted throughout the epithelium, while AP reactions were observed perinuclearly. Granular reactions of AP were observed in the lamina propria, while ALP reactions were found around the blood capillaries.

Stomach

Anterior part of the stomach

In the epithelial cells of the anterior part of the stomach, AP activity was observed from the supranuclear to the middle part of the cells. Occasionally, the reaction was also noted in the apical parts of the cells. Numerous AP responses were found in the connective tissue of the lamina propria. Within this layer, ALP and NSE reactions were observed around the blood vessels.

Posterior part of the stomach

AP and NSE activities were observed in the epithelial cells of the posterior part of the stomach. AP reactions were visible in the supranuclear and perinuclear parts of the cells (Figure 5B). In the same layer, ALP and NSE reactions were rare. In the lamina propria, ALP and NSE reactions were found around capillaries, while AP reactions were detected in connective tissue cells.

Intestine

Positive reactions of ALP (Figure 4B – 4D) and A (Figure 7A – 7C) were observed in the brush border of enterocytes in the anterior, middle and posterior parts of the intestine. NSE reactions (Figure 6A – 6C) were found in the apical part of the cells, while AP reactions were observed in the cytoplasm (Figure 5C – 5E). Single reactions of ALP and AP were detected in the connective tissue layers.

Rectum

Positive reactions of ALP (Figure 4E) and A (Figure 7D) were found in the brush border of epithelial cells in the rectum. AP reactions (Figure 5F) were abundant in the superficial parts, whereas they were rare in the rest of the

Table 1: The intensity of alkaline phosphatase measured by MOD

Digestive tract	Epithelium		Connective tissue
	Brush border	Cytoplasm	
Esophagus	/	$0.062^{b} \pm 0.005$	/
Intestine proper			
anterior part	$0.565^{a} \pm 0.124$	/	/
middle part	$0.493^{ab} \pm 0.118$	/	/
posterior part	$0.380^{b} \pm 0.080$	/	/
Rectum	$0.159^{c} \pm 0.116$	$0.158^{a} \pm 0.022$	0.131 ± 0.035

The values were shown as mean ± SD. Means within columns with different lowercase superscripts (^{a,b,c}) were significantly different (P<0.05). If a positive reaction is not or only rarely observed, it was marked with /.

Table 2: The intensity of acid phosphatase measured by MOD

Digestive tract	Epithelium	Connective tissue	Gastric glands
Esophagus	$0.138^{cA} \pm 0.018$	$0.049^{dB} \pm 0.019$	/
Stomach			
anterior part	$0.268^{adA} \pm 0.072$	$0.078^{bdC} \pm 0.035$	$0.156^{B} \pm 0.039$
posterior part	$0.287^{adA} \pm 0.044$	$0.182^{aB} \pm 0.039$	/
Intestine proper			
anterior part	$0.327^{aA} \pm 0.079$	$0.173^{acB} \pm 0.044$	/
middle part	$0.288^{adA} \pm 0.123$	$0.155^{acB} \pm 0.065$	/
posterior part	$0.327^{aA} \pm 0.063$	$0.159^{acB} \pm 0.034$	/
Rectum	$0.212^{bcdA} \pm 0.096$	$0.113^{bcB} \pm 0.063$	/

The values were shown as mean ± SD. Means within columns with different lowercase superscripts (^{a,b,c,d}) were significantly different (P<0.05). Means within rows with different uppercase superscripts (^{A,B}) were significantly different (P<0.05). If a positive reaction was not or only rarely observed, it was marked with /.

epithelium. Rare ALP reactions were observed throughout the epithelium. ALP and AP reactions were observed in the connective tissue layers.

Statistical analysis of enzymatic activity

Alkaline phosphatase

In the digestive tract of the European hake, the OD of ALP was measured in the brush border of the intestine and rectum, in the cytoplasm of the epithelial cells in the esophagus and rectum, and in the connective tissue of the rectum (Table 1). In the brush border the highest OD was measured in the anterior part of the intestine and it decreased towards the posterior parts. Accordingly, a statistically significant difference was found between the MOD in the brush border of the anterior intestine and the values measured in the posterior part of the intestine and rectum. In addition, a statistically significant difference was found between the MOD in the brush border of the middle and posterior parts of the intestine and compared to the rectum. A statistically significant difference in MOD was also found in the cytoplasm of the epithelial cells of the esophagus and rectum.

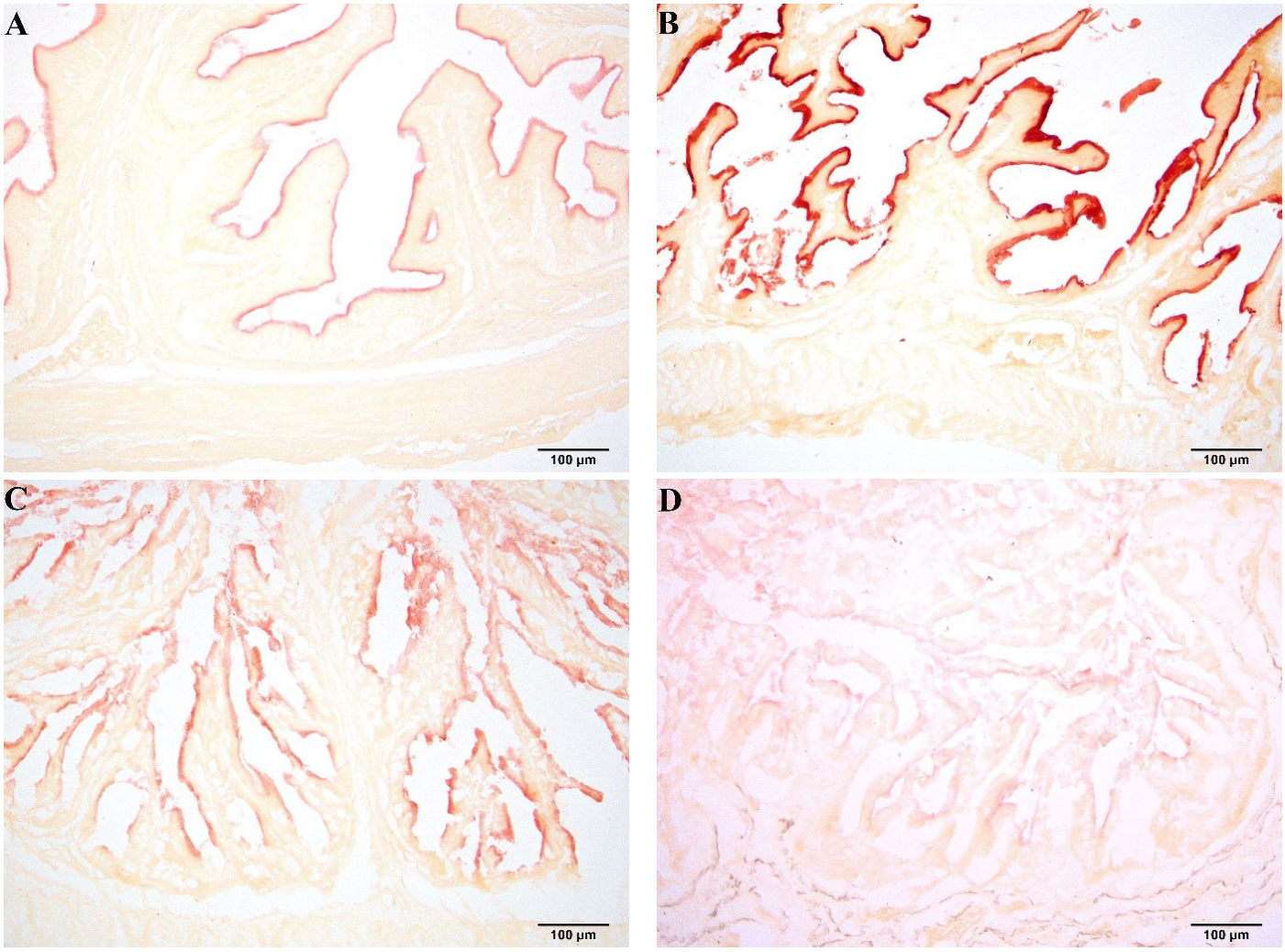

Figure 7: Positive reactions of A in the digestive tract of the European hake (A – D). The figure shows reactions in the: anterior (A), middle (B) and posterior (C) part of the intestine as well as in the rectum (D)

Acid phosphatase

The MOD of AP was shown in Table 2. In the digestive tract of the European hake, the OD of AP was measured in the epithelium and connective tissue of all parts of the digestive tract as well as in the gastric glands. The highest OD was measured in the epithelium of the anterior and posterior parts of the intestine, while the lowest OD was measured in the epithelium of the esophagus. A statistically significant difference was found between the MOD of the enzyme in the esophagus and the MOD measured in the posterior parts of the digestive tract. A statistically significant difference was also found between the MOD in the epithelium of the anterior and posterior part of the intestine compared to the values measured in the esophagus and rectum. Within the connective tissue, the highest MOD was measured in the posterior part of the stomach and the lowest in the esophagus.

Non-specific esterase

The MOD of NSE was shown in Table 3. In the digestive tract of the European hake, the OD of NSE was measured in the epithelium of the anterior, middle and posterior parts of the intestine. The highest MOD was measured in the epithelial cells of the posterior part of the intestine. Accordingly, a statistically significant difference was found between this value and the values measured in the anterior and middle parts of the intestine.

Aminopeptidase

In the digestive tract of the European hake the OD of A was measured in the brush border of the intestine and rectum (Table 4). The highest MOD was found in the epithelium of the middle part of the intestine and the lowest in the epithelium of the rectum. A statistically significant difference was found between the values measured in the anterior and posterior parts of the intestine compared to the values measured in the rectum.

Discussion

The European hake (*Merluccius merluccius*) is an important commercial species, widely distributed throughout the

Table 3: The intensity of non-specific esterase measured by MOD

Digestive tract	Epithelium
Intestine proper	
anterior part	$0.111^{b} \pm 0.060$
middle part	$0.106^{b} \pm 0.054$
posterior part	$0.269^{a} \pm 0.087$

The values were shown as mean ± SD. Means within columns with different lowercase superscripts (^{a,b}) were significantly different (P<0.05).

Table 4: The intensity of aminopeptidase measured by MOD

Digestive tract	Brush border
Intestine proper	
anterior part	$0.290^{a} \pm 0.050$
middle part	$0.314^{a} \pm 0.093$
posterior part	$0.280^{ab} \pm 0.147$
Rectum	$0.168^{b} \pm 0.056$

The values were shown as mean ± SD. Means within columns with different lowercase superscripts (^{a,b}) were significantly different (P<0.05).

Mediterranean Sea (14). The aim of this study, which sampled specimens from the Adriatic Sea, was to investigate the posterior part of the digestive tract (from the esophagus to the rectum) in adult European hake.

The esophagus of the European hake was short and lined with stratified squamous non-keratinized epithelium. This is consistent with findings described in many fish species, such as European hake (23), Argentine hake (24) and Argentine anchovy (25). In agreement with the previous findings in the European hake (16), mucous cells with acidic secretion in this part could have a role in the maceration of the food and general protection of the mucosa during feeding. The stomach of the European hake was divided into an anterior and a posterior part. Simple columnar epithelium with microvilli is most commonly found in mucosa of the stomach in fish (26, 27, 28, 29). The neutral composition of the mucins in this part is related to the protection of the mucosa against the acidic content of gastric juice, protection against mechanical injury and support for the breakdown of food components. Based on the morphological characteristics of the intestinal wall, the intestine of the European hake was divided into an anterior, middle and posterior part. The diversity in the division of the intestine proper and the lack of a precise definition of the sampling locations make it difficult to compare certain parts in different fish species. The mucosa of the intestine was lined with a simple columnar epithelium with microvilli. In agreement with a previous study on the European hake (23), unicellular mucous glands containing acid secretions were found between the enterocytes. Acidic mucins in the intestine lubricate the mucosa and facilitate the passage of undigested food particles towards the rectum. In general, the mucus prevents mechanical damage to the microvilli and thus contributes to better absorption. The mucus forms a diffusion barrier for ions and fluids, enabling their absorption. It acts as a physical barrier between enzymes in the lumen and serves as a cofactor for enzymatic hydrolysis. By preventing the degradation of glycoproteins, acidic mucins are involved in protein digestion (30). Although it was reported that no submucosa is found in the intestine of the European hake (23), the results of this study suggest the opposite. Despite the fact that the muscular layer of the mucosa was not differentiated, the lamina propria could be distinguished from the submucosa based on the type of connective tissue proper. In some specimens, however, isolated muscle cells were found in the posterior part of the intestine proper. These results suggest not only variability in the structure of the digestive tract between different fish species, but also diversity between individual specimens within the same species. In the present study, a special form of pseudostratified columnar epithelium was found lining the rectum of the European hake. In a previous study on European hake (23), the specific locations where samples were taken from within the digestive tract were not specified. The term "posterior part of the intestine" was not clearly defined, and therefore may not have included the rectum. This highlights the importance of accurately defining terminology and sampling locations for histological and histochemical analyses to ensure research repeatability and avoid misinterpretation. To address this, a precise illustration of the sampling sites on the digestive tract has been provided in this study to ensure clarity and consistency. The acidic mucins in the rectum facilitate the excretion of undigested food particles and protect the mucosa.

In fish, ALP has crucial role in the digestion and absorption of nutrients by hydrolyzing phosphates from carbohydrates, fats and proteins (5, 10, 11, 30). Compared to the results of a previous study on the European hake (13), enzymatic activity was found in this study not only in the brush border of the intestine proper, but also in the brush border of the rectum, in the cytoplasm of epithelial cells in the esophagus and rectum, and in the connective tissue of the digestive tract. In contrast, ALP activity was not found in the cytoplasm of enterocytes. If this enzyme helps to maintain the protective barrier, regulate pH, control tight junctions, detoxify microbial components, and modulate the gut microbiota (11, 12), then its presence at these locations is closely linked to these functions in addition to digestion.

In the previous study on European hake (13), the intensity of enzymatic activity was assessed subjectively by visual inspection, rather than using an objective method that includes precise numerical values for the reaction intensity. The data of this study related to the MOD of ALP are not consistent with the results previously described for the reared European eel (5), European hake (13), half-smooth tongue sole (30) and tub gurnard (31). Acid phosphatase activity was found throughout the entire digestive tract. Similar results have been described in the European eel (5), European hake (13), large-scaled gurnard (17), porthole shovelnose catfish (32), half-smooth tongue sole (30) and tub gurnard (31). Since AP is involved in protein degradation (5, 10, 13), it could be concluded that the stomach and the entire intestine proper are the main locations for protein metabolism in the European hake. The finding of its activity in the gastric glands agrees with the previous study in the European hake (13) and confirms connection of the enzyme with the process of secretion. The activity of NSE was measured in the epithelium of all parts of the intestine proper. A similar finding is also reported in the European hake (13) and half-smooth tongue sole (30). Since the secretory duct of the hepatopancreas opens into the intestine proper, the increased activity of NSE is associated with the emulsification of fat, making this the location of most intense lipid digestion. Similar to previous studies on European eel (5), large-scaled gurnard (17) and tub gurnard (31), the activity of A was measured in the brush border of the intestine proper. In addition, the enzyme activity was also found in the brush border of the rectum. These data indicate that all the mentioned parts have an important role in the hydrolysis of amino acids and thus contribute to the protein metabolism.

Conclusion

In conclusion, this study provides a detailed insight into the digestive tract of the European hake. It reveals important anatomical, histological and enzymatic features that support its role in the digestion and absorption of nutrients. All parts of the digestive tract consist of mucosa, submucosa, muscularis and serosa. The mucosa is the most variable layer in the entire posterior part of digestive tract. All parts of the digestive tract are involved in the digestion and absorption of nutrients. The most important location for lipid digestion in the European hake is intestine. Although the intestine is the main part for protein digestion, both the stomach and the rectum are also important locations for this process. The results of this research highlight the need for standardized sampling locations to improve the comparability of future studies of the digestive tract in fish.

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Celostna študija prebavil evropskega osliča (*Merluccius merluccius*)

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Izvleček: Evropski oslič (*Merluccius merluccius*) je pomembna komercialna riba, ki je razširjena v Jadranskem morju. Je mesojeda riba, ki se prehranjuje predvsem z ribami. Namen raziskave je bil opraviti podrobno analizo prebavil evropskega osliča, zato sta bili opisani anatomija in histologija prebavil (od požiralnika do danke). Ribe so bile ulovljene s parangalom v Jadranskem morju ob hrvaški obali. Skupaj je bilo analiziranih 33 odraslih osebkov. Komponente tkiva so bile vizualizirane z uporabo hematoksilin-eozina, Malloryjevega trihroma, Verhoeff-Van Giesona, kompleta Alcian Blue-PAS in kompleta za barvanje z mrežastimi vlakni. Da bi raziskali celično prebavo, smo izmerili lokalizacijo in aktivnost alkalne fosfataze, kisle fosfataze, nespecifične esteraze in aminopeptidaze. Vsi deli prebavnega trakta so sestavljeni iz sluznice, podsluznice, mišične plasti in seroze. Vrsta epitelija se razlikuje od dela do dela. V požiralniku, črevesu in danki nismo ugotovili mišične plasti sluznice. Mišična plast je sestavljena iz gladkih mišičnih celic, razen požiralnika, kjer jo tvorijo prečno progasta mišična vlakna. Vsi deli prebavnega trakta sodelujejo pri prebavi in absorpciji hranil. Glavno mesto za prebavo lipidov in beljakovin je sicer črevo, vendar sta želodec in danka prav tako pomembna za prebavo beljakovin. Čeprav sta anatomija in histologija prebavnega trakta pri evropskem osliču delno opisani, v razpoložljivi literaturi ni podatkov o optični gostoti encimov. Opisana raziskava, opravljena na 33 osebkih, prinaša izčrpne ugotovitve in nova spoznanja, ki bistveno razširjajo doslej znane in se delno razlikujejo od njih, ter poudarja potrebo po nadaljnjih študijah na tem področju.

Ključne besede: evropski oslič; *Merluccius merluccius*; histologija; histokemija; prebava; encimi