VETERINARSKI ARHIV 75 (4), 317-327, 2005

Mucosubstances of the digestive tract mucosa in northern pike (*Esox lucius* L.) and european catfish (*Silurus glanis* L.)

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PETRINEC, Z., S. NEJEDLI, S. KUŽIR, A. OPAČAK: Mucosubstances of the digestive tract mucosa in northern pike (*Esox lucius* L.) and european catfish (*Silurus glanis* L.). Vet. arhiv 75, 317-327, 2005.

ABSTRACT

The distribution and quality of mucosubstances in the digestive tract of pike (*Esox lucius*) and catfish (*Silurus glanis*) is described. The digestive tract of pike and catfish is short, in accordance with their predatory nature, and consists of oesophagus, stomach and intestine. The oesophagus is short, with longitudinal folds. The mucosa of pike oesophagus is stratified and contains two types of mucous cells: in contrast to catfish oesophagus there is only one type of mucous cell. The stomach is an elongated sac-like structure with glands in lamina propria. In pike the gastric glands are simply tubular, but in catfish they are branched tubular and surrounded by connective tissue. The intestines in both fishes are short with many intestinal villi. Many goblet cells are situated between superficial epithelial cells.

Key words: pike, catfish, digestive tract, mucosubstances, histochemistry

Introduction

The digestive tract of fishes show marked diversity in its morphology and function. This is in correlation with taxonomy and the different feeding habits, as well as with body shape (VERMA and TYAGI, 1974; VERMA et al., 1974; KAPOOR et al., 1975; RAY and MOITRA, 1982; BUDDINGTON et al., 1997). The presence of mucosubstances in mucosa

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ISSN 0372-5480
Printed in Croatia

of the digestive tract has been observed in most teleosts (OSTOS GARRIDO et al., 1993; NACHI et al., 1998; DOMENEGHINI et al., 1998; DOMENEGHINI et al., 1999; DOMENEGHINI et al., 2002). The gut mucins lubricate and protect the tunica mucosa against chemicals, parasites and acidity and form a diffusion barrier for various ions (PABST, 1987; GUPTA, 1989; VEGETTI et al., 1999). MURRAY et al. (1994) suggests that mucins in the posterior oesophagus of the same fish may play a role in pregastric digestion. FIERTAK and KILARSKI (2002) concluded that the glycoconjugate composition of cyprinid digestive tract depends on the species as well as on the intestinal region, and that there is no correlation between its composition and type of ingested food.

Both investigated fishes are active predators. Pike feed exclusively on small fish, while catfish have a broad range of prey, such as fish, frogs and crayfish and sometime small mammals (BEKBERGENOV and SAGITOV, 1984; OMAROV and POPOVA, 1985; STEPHENSON and MOMOT, 1991; ELVIRA et al., 1996; KANGUR and KANGUR, 1998).

The purpose of the present study was to describe histochemically the gut mucosubstances of two carnivorous fish species which display different feeding habits.

Materials and methods

Adult northern pike (*Esox lucius*) and catfish (*Silurus glanis*) specimens collected from the Lonja River (NW Croatia) were used for investigation. Body weight ranged between 1-2 kg. Samples of digestive tract (oesophagus, stomach and middle part of intestine) were fixed in buffered 10% formalin and embedded in paraffin. Dewaxed sections (8 μm) were stained for general morphological purposes with haematoxylin and eosin (HE). Periodic acid-Shiff (PAS) (McMANUS, 1948) was used for demonstration of neutral glycoconjugates. Alcian blue (AB) at pH 2.5, (MOWRY, 1956) and metachromasia with toluidine blue (TB) were used for demonstration of various kinds of acid glycoconjugates (PEARSE, 1968).

Results

Pike. Large numbers of distinct longitudinal folds extend along the entire length of the oesophagus. A differentiation exists between major and minor folds. The mucosa is lined with a stratified epithelium and two basic mucous cells are present. One type is round, the other elongated. Round cells are typically mucous and they are numerous on the basal part of the epithelium. Elongated cells, on the other hand, have a fine granular cytoplasm and are dominant in the superficial part of the epithelium. Both cell types were stained purple with periodic acid-Shiff (Fig. 1). But with AB and TB, only round mucous cells were stained (Fig. 2).

The mucosa of the stomach is raised into thick longitudinal folds. The epithelium of mucosa consists of very high, simple columnar cells with large nuclei located on the basal

Fig. 1. Pike, oesophagus, PAS-positive stained cells (arrows), scale bar = 200 µm

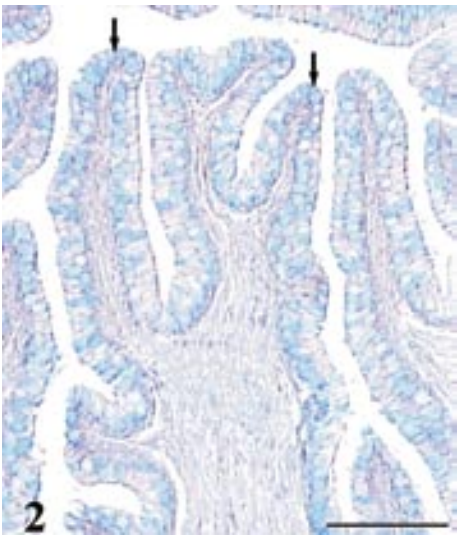

Fig. 2. Pike, oesophagus, AB-positive stained cells (arrows), note unstained cells in apical part of mucosa, scale bar = 200 µm

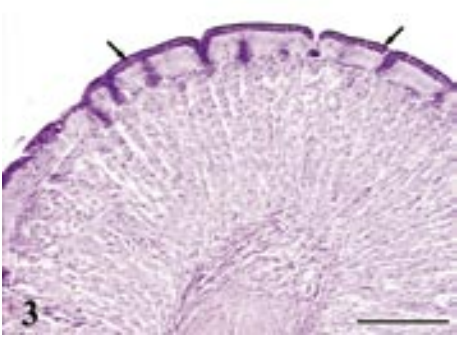

Fig. 3. Pike, stomach, PAS, epithelial cells show strong PAS reaction (arrows), scale bar = 200 µm

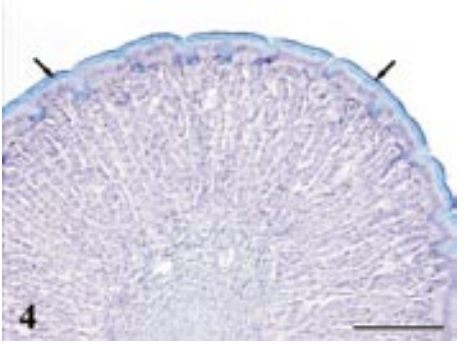

Fig. 4. Pikef, stomach AB-positive mucosubstances in apical part of epithelial cells (arrows), scale bar = 200 µm

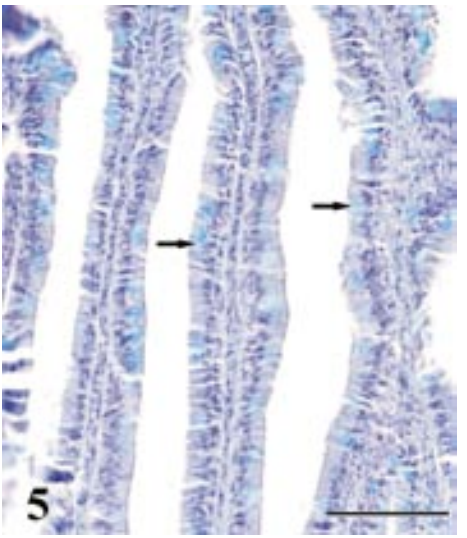

Fig. 5. Pike, intestine, AB, goblet cells show acid mucosubstances stained AB positive (arrow), scale bar = 100 µm

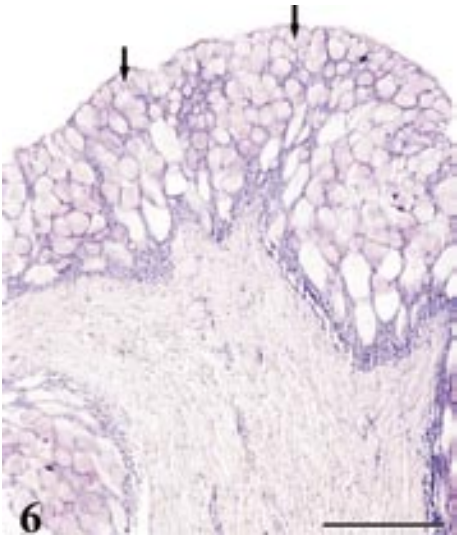

Fig. 6. Catfish, oesophagus, PAS-positive mucous cells in oesophageal mucosa (arrows), scale bar = 200 µm

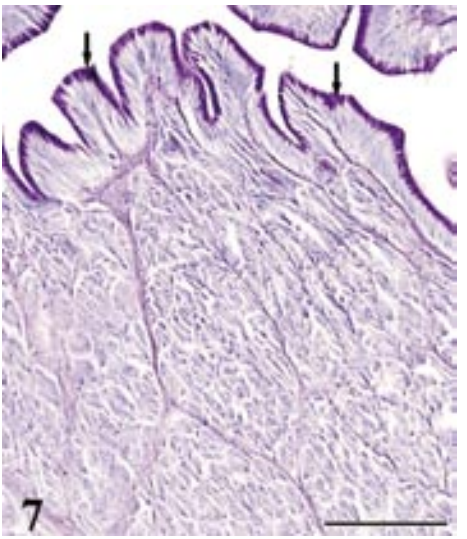

Fig. 7. Catfish, stomach, PAS-positive mucosubstance present in apical part of epithelial cells (arrows), scale bar = 200 µm

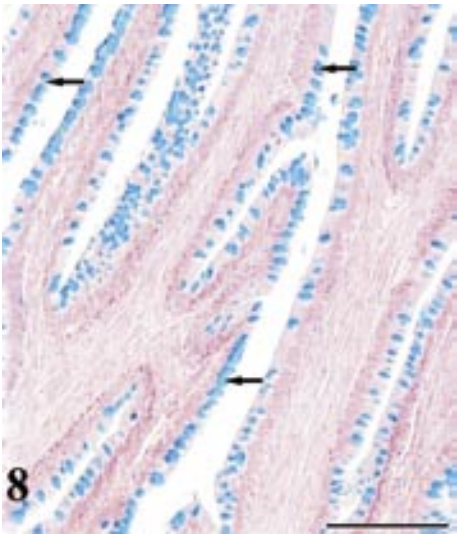

Fig. 8. Catfish, intestine, AB, numerous goblet cells stained AB-positive (arrows), scale bar = 200 µm

side of the cells. The apical cytoplasm of epithelial cells shows presence of substances stained strongly with PAS and AB, and slightly with TB (Fig. 3 and 4).

The glandular cells are arranged as straight tubular glands and occupy the entire mucosal layer beneath the superficial epithelium. Tubular gastric glands are composed of one type of polygonal cell, with fine granular cytoplasm. There is no presence of any kinds of mucous substance in these glands.

The intestine is short and shows a uniform histological structure throughout its entire length. The mucosa is folded into long and thin intestinal villi consisting of a single layer of columnar epithelial cells. Mucous goblet cells are interspersed among them and connective lamina propria. Mucosubstances are present only in goblet cells and all were stained very strongly with PAS and AB (Fig. 5), but only of them is also stained with TB.

Catfish. The mucosa of the oesophagus is very thick and lined with stratified epithelium with only one type of intraepithelial mucous cell. All mucous cells show a moderate to high content of PAS-positive stained mucosubstances (Fig. 6). But only some of them were positively stained by AB, and metachromatic by TB.

The mucosa of the stomach is raised into fine longitudinal folds. The epithelium of the mucosa consists of simple columnar cells. The apical cytoplasm of the epithelial cells shows the presence of mucous substances stained strongly with PAS (Fig. 7), and slightly with AB and metachromatic with TB. The gastric glands were branched tubular and surrounded by a layer of connective tissue and opened into the bottom of gastric pits. The glands consisted of a single layer of cells of uniform appearance. The high content of PAS-positive, as well as TB-metachromatic substances, are present in the surface epithelial cell. A small amount of PAS-positively stained substances is observed in lamina propria. Glandular cells show no presence of mucosubstances.

The intestine is short and its mucosa has an almost uniform structure throughout its entire length, with some minor difference in the shape of the villi. The villi are long, branched and lined by columnar cells with rare goblet cells, which are found at all levels of the intestinal villi. The goblet cell contents stain intensively with AB (Fig. 8) and they are also PAS-positive. The epithelial cells show presence of a small amount of TB metacromatic substances, which are also present in some goblet cells.

Discussion

Mucous is an important layer protecting the epithelia either from mechanical injuries or bacterial invasion (HUMBERT et al., 1984).

Mucous cells in fish oesophagus differentiated early during embryonic development and their appearance corresponds to the opening of the mouth. Mucous cells were found

early in the developing oesophagus of *Sparus aurata* (DOMENEGHINI et al., 1998), in *Chanos chanos* (FERRARIS et al., 1987) and *Solea solea* (BOULHIC and GABAUDAN, 1992). In this period these cells are PAS negative but stained by alcian blue, and contained acid mucosubstances only.

The mucosa of pike oesophagus is lined by the epithelium with two types of mucous cell. These two mucous cells appear to have different secretions, since the round mucous cells are PAS and AB positive, and TB-metachromatic, but elongated cells stained with AB only. Mucous finds in round mucous cells exhibit properties of neutral and acid sialosulfoglycoproteins as well as carboxylated and weak sulfated mucosubstances. Periodic acid-Schiff and alcian blue positive mucous cells in oesophagus of fishes were described by AL-HUSSAINI and KHOLY (1953), YAMADA and YOKOTE (1975), GARGIULIO et al. (1996), SCOCCO et al. (1998), and DOMENEGHINE et al. (1999). In elongated mucous cells there are neutral mucosubstances only. Two types of mucous cell described here were found in the oesophagus of tilapia (PASHA, 1964; MORRISON and WRIGTH, 1999). Mucous cells in catfish oesophagus were mainly PAS positive, although some contain AB positive and TB metacromatic mucosubstances. These histochemical staining properties of mucosubstances in catfish mucous cells point to the presence of a high content of neutral mucosubstances. Acid mucosubstances stained with AB and TB metacromatic suggest the presence of sialic acid-rich and weak sulfated mucosubstances. As reported by TIBBETTS (1997), mucous cells containing sialo- and sulfoglycoproteins lead to an increase in the viscosity of secretion, which is likely to be related to a higher protection role (SUPRASERT et al., 1987).

The stomach of pike displays uniform histological features. Superficial epithelial cells and simple tubular gastric glands in lamina propria form the mucosa. In catfish, gastric glands are arranged as tubular branched glands surrounded by connective tissue. The apical borders of the superficial epithelia in both fish show strong PAS reaction and mild AB and TB reaction. These reactions mostly indicated the presence of neutral mucosubstances and a small amount of acid mucosubstances. The acid mucosubstances are shown to be chiefly of the carboxylate and sialylated type. In fish, neutral mucosubstances secreted by the stomach epithelium have been related to the absorption of easily digested molecules, such as disaccharide and short-chain fatty acids (GRAU et al., 1992). The mucosubstances facilitate the movement of large-sized food particles, as well as protecting the gastric mucosa from mechanical injury. The presence of both neutral and acid mucosubstances was observed in the superficial gastric epithelium of the white sturgeon (DOMENEGHINI et al., 1999) and in developing larvae and adults of *Sparus aurata* (DOMENEGHINI et al., 1998).

The tubular gastric glands of pike and catfish show the presence of only one type of secretor cells, but they show no presence of any kind of mucosubstances. Many authors agree that the gastric glands of teleosts contain only one type of secretory cell (WESTERN and JENNINGS, 1970; VERMA and TYAGI, 1974; RAY and MOITRA, 1982). ARELLANO

et al. (2001) reported the presence of light and dark cells in the gastric glands of *Solea senegalensis*. It is generally thought that fish gastric glands synthesise both pepsinogen and acid (SMITH, 1989) and that neutral mucosubstances have a buffering effect on the high acidity of the stomach content (SCOCCO et al., 1996).

The intestine of both investigated fish shows uniform histological structure throughout its entire length. The mucosa of pike intestine consists of a single layer of columnar cells with many mucous goblet cells. The catfish intestinal mucosa is similar to the pike, but there are more mucous cells than in pike. The intestinal goblet cells of both investigated fishes synthesise neutral and acidic mucosubstances. The acid mucosubstances are made by carboxylated and sulfated mucoconjugates. The presence of TB metacromatic substances, in particular goblet cells, shows in the presence of a small amount of sialic-rich nonsulfated mucoconjugates.

The first goblet cells can be observed early during the differentiation of the intestinal mucosa of fish (BOULHIC and GABAUDAN, 1992; MIYASHITA et al., 1998; GARCIA HERNANDEZ et al., 2001; MORRISON et al., 2001). The presence of mucous-producing goblet cells in the intestinal mucosa has been reported in many fish studied earlier (NARASIMHAM and PARVATHESWARAO, 1974; CHATURVEDI and GUPTA, 1976; RAY and MOITRA, 1982; DOMENEGHINI et al., 1998; NACHI et al., 1998; DOMENEGHINI et al., 1999; MORRISON and WRIGHT, 1999). The mucous secreted by goblet cells in the intestine has many functions. For example, it lubricates undigested materials for onward progression into the rectum. NARASIMHAM and PARVATHESWARAO (1974) suggested a possible role of intestinal mucins in osmoregulation. The studies of RIBELLES et al. (1995) have shown that the quality of gut mucosubstances is directly related to environmental conditions, which in turn may directly affect the function of the alimentary tract. 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 (STROMBAND and VAN DER VEEN, 1981; GUPTA, 1989; SIRE and VERNIER, 1992; SEGNER et al., 1994; DOMENEGHINI et al, 1998). The possible role of mucosubstances in intestinal absorption processes is supported by the findings of BOŽIĆ et al. (2001) who observed that starvation induced an increase in the number of intestinal goblet cells in carp.

This investigation, as well as previous investigations on other fishes, demonstrates that the quality of gut mucosubstances varies in different regions of pike and catfish alimentary canals and that it sustains functional harmony of the digestive tract. It appears that the quality of mucoconjugates in particular parts of the digestive tract is similar in both investigated carnivorous fish, and that there is no correlation between mucous composition and food habits.

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Received: 16 June 2004
Accepted: 28 June 2005

PETRINEC, Z., S. NEJEDLI, S. KUŽIR, A. OPAČAK: Mukopolisaharidi u sluznici probavnog trakta štuke (*Esox lucius* L.) i soma (*Silurus glanis* L.). Vet. arhiv 75, 317-327, 2005.

SAŽETAK

Istraživana su svojstva i raspored mukosubstancija u probavnom traktu štuke i soma. Probavni trakt istraživanih riba je jednostavne građe i sastoji se od jednjaka, želuca i relativno kratkog crijeva. U sluznici štuke opažaju se dva tipa stanica koje pokazuju prisutnost neutralnih mukopolisaharida, a kiseli mukopolisaharidi nađeni su samo u mukoznim stanicama bazalnog dijela epitela. U jednjaku soma opisan je samo jedan tip okruglih mukoznih stanica u kojima su nađeni neutralni mukopolisaharidi, a samo neke od njih sadrže i kisele mukopolisaharide. Želudac je vrećastog oblika sa žlijezdama u lamini propriji. U žlijezdama nisu dokazani mukopolisaharidi, no nađeni su u površinskom epitelu. U štuke su neutralnih i kiselih osobina, a u soma su uglavnom neutralni s manjom količinom kiselih. Mješavina neutralnih i kiselih mukopolisaharida prisutna je u vrčastim stanicama crijeva. Broj vrčastih stanica veći je u crijevu soma nego štuke.

Ključne riječi: štuka, som, probavni trakt, mukopolisaharidi, histokemija