HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF THE STOMACH OF INDONESIAN RIVER CATFISH, *Pangasius sp.*

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ABSTRACT. *The stomach of Indonesian river catfish (Pangasius sp.) had studied using standard histological and histochemical methods, which included Alcian Blue/Periodic Acid Schiff (AB/PAS) techniques, to distinguish neutral and acidic glycoconjugates and lectin histochemistry. The stomach was a curve liked sac, measuring about 3 cm in the dorsal length, 6 cm in ventral length and 2.5 cm in width. Histologically, the stomach could be differentiated into 3 glandular regions: the cardiac gland, proper gastric gland (fundic) and pyloric gland regions. The cardiac gland region was narrow with 2-3 glands beneath the columnar epithelium. The proper gastric gland region occupied two thirds of the stomach glands. The pyloric gland region began at one third posterior portion of the stomach and ended at the orifice of pyloric-duodenum. The mucosal surface was lined by the columnar epithelium. Stomach glands showed tubular form and were composed of mucous cuboid cells. By using histochemical techniques, the present study demonstrated the content of complex carbohydrates (glycoconjugates) in the glands and the lumen of the stomach. Columnar epithelium cells in the surface and gastric pits showed abundant of neutral carbohydrates. No reaction was found for the acidic carbohydrates. The adherent mucus gel and the cytoplasm of the gastric glands contained many sugar residues, as revealed by lectin histochemistry that showed positive reaction to Con-A, WGA, PNA, UEA, PHA and RCA. Thus, it was clearly demonstrated that the distribution of mucosubstances in the stomach varied among different locations and regions. The present structural and histochemical results were compared with those of other species of fish. Some specific features observed in this study may be related to specific digestive functions in the stomach of this species, which should be clarified in the future.*

KeyWords: *Indonesian river catfish (pangasius sp.), stomach, lectin histochemistry*

1. INTRODUCTION

The digestive tract of fishes shows a marked diversity in its morphology and functions. The macroscopic and microscopic anatomy of the fish stomach differs among species, although there are some basic structural similarities. The overall gastrointestinal morphology influenced by the nature of the food, the frequency of food intake, as well as the body size and shape. The gross anatomy and histology of the alimentary tract of fishes and their possible related function have been well documented for many species [1, 2], but not for a species of river catfish (*Pangasius* sp.).

Patin fish (*Pangasius sp.*) is an Indonesian river catfish which is included in the order of Ostariophysy, family Pangasidae and genus Pangasius [3]. Taking into account that little is known about the digestive system of the *Pangasius sp.*, this study was designed with the aim to examine the structural features which can be related to some special functional attitudes of the stomach of this species using standard histological, histochemical and lectin histochemical procedures. The result of this work will be the basis for a future comparison on the gut morphology of *Pangasius sp.* Special attention will be paid to the epithelial cells which synthesize glycoconjugates because the stomach mucosubstances are strictly related to physico-chemical environmental conditions and variations [4].

2. MATERIALS AND METHODS

Ten adults *Pangasius sp*. with body weight ranging from 1 to 1.5 kg were used for this study. The fishes were killed by decapitation and the abdomen was incised at the median line, the digestive tract was quickly removed.

Samples were fixed in 4% paraformaldehyde in 0.75% NaCl physiological saline. After fixation, tissues were taken from certain portions of the stomach (cardiac, gastric proper and pyloric regions), dehydrated through a graded series of alcohol, cleared in xylol and embedded in paraffin plastic (paraplast). Serial sections were cut at 4-5 µm.

Sections of tissues were stained for general morphological purpose with hematoxylin-eosin, Azan and Masson Trichrome staining methods. Alcian blue pH 2.5/periodic acid-Schiff (AB/PAS) reaction was performed to demonstrate both neutral and acidic carbohydrate and their mixtures. Lectin histochemistry for investigating the distribution of sugar binding sites in the gland of stomach was conducted using eight biotinylated lectins (10 µg/ml) purchased from Honen Corporation (Japan). Table I shows the specific names, the common names, the acronyms and the preferred binding specificity of the sugar residues of the used lectins.

Briefly, the lectin histochemical procedures were as follow as the sections were incubated in 1% H_2O_2 in methanol for 15 minutes at room temperature to block endogenous peroxidase activity. The sections were then washed in a phosphate-buffered saline (PBS) 0.01 M, pH 7.4 and incubated with biotinylated lectins (10 µg/ml in PBS) for overnight at 4°C. The sections were then washed again in PBS and subsequently treated with avidin-biotin-peroxidase complex (ABC kit, Vector) for 30 minutes at 37°C. After further washing in PBS, the sites of reaction were revealed with diaminobenzidine tetrahydrochloride (DAB) 0.2 mg/ml in 0.05 M Tris Buffer, pH 7.4, containing 0.001 % H_2O_2 for 5-15 minutes at room temperature. Finally, the slide was observed under photograph microscope (NIKON Ltd).

Table I. Biotinylated lectins employed in this study, their preferred binding specificities and blocking sugars.

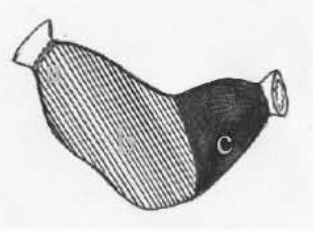
LECTIN: latin name (common name)	ACRONYM	PREFERRED SUGAR RESIDUES BINDING AND BLOCKING SUGAR
Dolichos biflorus (horse gram)	DBA	α-GalNAc
Canavalia ensiformis (Jack bean)	Con-A	α-D-Man
Ricinus communis (caster bean)	RCA-1	β-D-Gal
Ulex europaeus (gorse seed)	UEA-1	L-Fuc
Triticum vulgare (wheat germ agglutinin)	WGA	GlcNAc, NANA
Peanut agglutinin	PNA	Gal,GalNAc
Phaseolus vulgaris (Erythroagglutinin)	PHA-E_4	
Lens culinaris	LCA (LcH)	Man α

Fuc : fucose; Gal : Galactose; GalNAc : N-acetyl-D-galactosamine; GlcNAc : N-acetyl-D-glucosamine; Man : mannose; NANA ; N-acetyl-neuraminic acid (Sialic acid)

3. RESULTS AND DISCUSSION

The stomach of *Pangasius sp*. was a sac with curve-like shaped, measuring about 3 cm in the dorsal length, 6 cm in ventral length and 2.5 cm in width. The stomach was divided into 3 glandular regions, the cardiac gland, proper gastric (fundic) gland and pyloric gland regions (Figure I). The cardiac gland region was narrow with 2-3 glands beneath the columnar epithelium. The proper gastric gland region occupied two third part of the stomach glands. The pyloric gland region began at one third posterior portion of the stomach and ended at the orifice of pyloric duodenum. The internal surface of the stomach possessed mucosal folds. These folds were more prominent in the proper gastric gland gastric region. The folds were ended at the pyloric valve. The muscular layer very prominent at the end portion of the pyloric and made the orifice of the pyloric and duodenum. The present results on the glandular area of the stomach of *Pangasius sp*. were different with those of walking catfish (*Clarias batracus*). The stomach of walking catfish consisted only of fundic gland region in the anterior and pyloric gland region in the posterior part [5]. The study showed that the stomach of *Pangasius sp*. showed a primitive organization of those of mammals.

Figure I. The stomach of *Pangasius sp*. A curve liked sac, three glandular regions: cardiac gland (a), proper gastric gland (b) & pyloric gland (c).



The mucosal surface of the cardiac, proper gastric and pyloric gland regions were lined by columnar type superficial epithelium. Gastric glands were located in the propria layer. The gastric glands were of tubular type, more complex and highly developed in the proper gastric gland region than in the cardiac gland region. The pyloric glands consisted only an epithelial layer. The number of tubular glands increases towards the middle region of the stomach while decreasing towards the posterior portion of the fundic stomach. This finding is similar with those of other fishes [1]. The superficial epithelium were lined by simple columnar cells, and the glandular cells showed a granular appearance. The cytoplasm of these glandular cells showed a markedly granular texture which is similarly known for other fish [6,7]. The cells of glands are stated to synthesize pepsinogen and acid.

The simple columnar cells which composed the superficial epithelium and the gastric pits were covered externally with a thin layer of mucous like substance which stains deep purple with PAS. There were no goblet cells in this areas and that is shown to synthesize a large quantity of neutral glycoconjugates which positive reaction for PAS stains, while more weakly of acidic glycoconjugates (negative reaction for AB stains) (Figure IIA and IIB). Neutral gut mucosubstances are considered to be related with the enzimatic digestion of food, in the transformation of food into chyme, and the absortive functions [3].

The deep tunica propria-submucosa of proper gastric and pyloric glands regions contained voluminous muscularis mucosa which was made up by smooth muscle cells. Other workers have found that the muscularis mucosa is not present in *Llisha filigera* (a plankton feeder), not well formed in *Muraenesox teladon* and *Channa striatus* (carnivora fish) [5], but was well developed in the channel catfish [8], striped bass [9] and the walking catfish [1].

The tunica muscularis of the stomach was divided into inner circular layer and outer longitudinal layer which were arranged by smooth muscle cells layers. The muscular layer of the pyloric gland region was thicker than that in the fundic gland region.

Azan stained sections showed that the tubular glands were covered and separated by connective tissue in the gastric proper region (Figure IIC and IID).

Among the lectins tested, WGA showed intense reaction in the luminal content, microvili, cytoplasm epithelial, lamina propria mucosa and glandular region, while secretions were labeled weakly to negative with LCA. DBA, WGA, PNA, UEA and PHA labeled both cytoplasm and microvili of the superficial epithelium, but Con-A labelled only cytoplasm (Figure III). The sugar binding pattern in the stomach of the *Pangasius* sp. was summarized in table II.

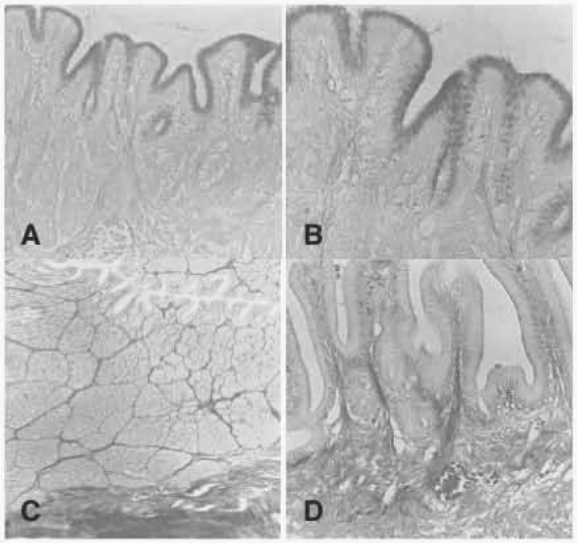
The histochemical observations lectin shows that the stomach of *Pangasius sp*. contained glycoconjugates with some different sugar residues: N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, N-acetyl-neuraminic acid (Sialic acid), galactose or mannose. A previous study reported the content of various composition including carbohydrates and proteins in the stomach of fishes [4]. The lectin binding pattern of the stomach varies among species of fish. Comparison of the glycoconjugate composition with those of the other species of teleost reveals a remarkable variety between species [4]. This variety can be attributed not only to different feeding habits, but possibly also to different taxonomic positions.

Table II. AB/PAS reaction and lectin binding pattern of the stomach *Pangasius sp.*

			Lectin histochemistry							
	AB	**PAS**	**DBA**	**Con-A**	**WGA**	**PNA**	**UEA**	**PHA**	**LCA**	**RCA**
Luminal content	-	-	-	+++	++	+	++	+	-	+
Superficial epithelium										
- Microvili	-	-	+	-	+++	++	+++	+	-	-
- Cytoplasm	-	+++	+/-	++	++	++	+	+	-	-
Lamina propria	-	-	-	++	+++	-	+/-	-	-	+++
Mucosa										
Glandular region										
- Cardiac gland	-	-	-	-	-	-	-	-	-	-
- Proper gastric	-	-	++	++	+++	+++	++	+/-	-	++
- Pyloric gland	-	-	-	-	-	-	-	-	-	-

Note : +++ intense, ++ moderate, + weak

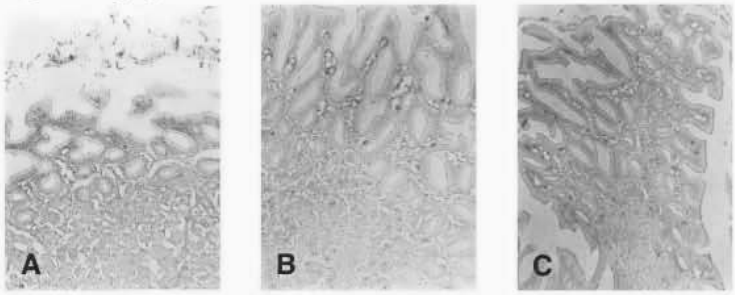
Figure II. A and B. A thin layer of mucous like substance with no goblet cells and large quantity of neutral glycoconjugates (PAS staining). C and D. Tubular gland were covered and separated by connective tissue in the proper gastric region (AZAN staining)



Reference [4] reports lectin binding only in the adherent mucus gel of the stomach of *Sparus aurata* L. The glandular cells which compose the gastric glands are histochemically unreactive, in the larval, juvenile and adult ages of *Sparus aurata* L. The cells are named "oxyntopeptic cells" and are presumed to secrete both enzymes and hydrochloric acid [4]. Our results, however, revealed the presence of some lectins in the glandular cells. These discrepancies may be related with the differences among species and differences in their feeding habit and diet. The presence of lectin bindings in the glandular cells might reflect a more complex secretory function in the glandular cells of *Pangasius sp*.

The presence of N-acetyl-galactosamine is revealed by the staining with DBA and PNA. PNA also reflect the content of galactose. In the present study, positive reactions in the adherent mucous were observed for PNA but not for DBA. The findings explained that the mucous did not contain N-acetyl-galactosamine..

Figure III. The lectin binding specific of the stomach of Indonesian River Catfish, *pangasius sp.* (A. ConA, B. RCA, C. WGA)



In conclusion, the present study revealed the morphological characteristics of the stomach of *Pangasius sp*. The stomach was a curve liked sac and consisted of three distinct glandular regions. These features showed a more highly developed stomach than some other species of fish. Furthermore, this study provided additional information about the morphological and functional organization of the stomach of *Pangasius sp*. and may provide the basis for future nutritional study.

ACKNOWLEDGMENT

The authors thanks to technician of Laboratory of Veterinary Histology, Faculty of Veterinary Medicine for technical assistance of the research.

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