

Histochemistry of the Alimentary Canal of the Rudd (*Scardinius erythrophthalmus* (L., 1758)) in Lake Uluabat-Turkey

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This study involves the determination of mucous histochemistry at various regions of the alimentary canal of the *Scardinius erythrophthalmus* using light microscopy. Histochemical analysis showed that the gastrointestinal mucous content included sulphate-esters and/or carboxylic [Alcian blue (AB) pH 2.5 +], glycogene and/or oxidable diols [periodic acid/Schiff+ (PAS+)], neutral or acid-rich (PAS/AB pH 2.5+) and strong acid sulphated [(AF+) (AF/AB pH 2.5+)] glycoproteins (GPs). But, in stomach and anterior intestine epithelium sulphated glycoproteins were not observed. As a result of the content of the glycoprotein was found to be more neutral property.

[**Keywords:** Alimentary canal, Glycoproteins, Histochemistry, *Scardinius erythrophthalmus*]

Introduction

The morphology and histology of the teleostean digestive tract have been described for numerous species^[1-4]. Many authors have focused on the importance of muco-substances present in the fish digestive system and correlated these with the absorption and transportation of macromolecules, increase of digestive efficiency, prevention of proteolytic damage to the epithelium and defenses against bacteria^[2,5-6]. Presences of glycoproteins is correlated with different functions^[1-4]. Histological and histochemical studies indicate that fishes with parasitic infection display, like mammals, goblet cell hyperplasia and hypertrophy, whereby their intracellular mucins shifted towards acidic and sulphated glycoprotein types^[6]. Morphology of the fish alimentary canal is so related to the physical characteristic of food and to feeding habits that alimentary canals typical of carnivorous, herbivorous and omnivorous species are found in the same family. In addition, for a given species,

the feeding habits differ according to locality, season, age or sex^[7].

Rudd (*Scardinius erythrophthalmus*) fish has been oval-shaped body and usually is covered with large scales. Head length is shorter than body height. The mouth is located end. Pectoral, ventral, anal and caudal fins are orange-red color. Eyes of rudd are carried a small speck red^[8]. Present study is to determinate of mucous histochemistry at various regions of the alimentary canal of the *Scardinius erythrophthalmus* using light microscopy.

Materials and Methods

Ten fish species, Rudd (*Scardinius erythrophthalmus*) were selected for this study. Species were provided from adult roach fish located in Uluabat lake. Stomach and intestine sampling was performed with abdominal dissection after application of anesthesia clove oil. Samples were taken from stomach, anterior, middle and posterior intestine.

All samples were fixed for 12 h in Bouin’s fluid. After dehydration by passing tissues through a series of alcohol solutions, the samples were vacuum-embedded in paraffin.

Sections (6-7 µm) were stained for general morphological purposes with

haematoxylin and eosin (H & E) ^[9] and Masson trichromes ^[10] stains. Histochemical techniques were performed for the localization and differentiation of carbohydrate moieties (Table 1).

Table 1— Histochemical reactions in the digestive tract mucosa of Rudd (*Scardinius erythrophthalmus*)

Procedures	References
1. PAS (Periodic acid/Schiff) GPs (Glycoproteins) with oxidizable vicinal diols and/or glycogen	Mc Manus ^[11]
2. PAS/AB pH 2.5 Neutral and/or acid rich GPs	Mowry ^[12]
3. AB(Alcian blue) pH 2.5 GPs with carboxyl groups (sialic acid or uranic acid) and/or with sulphate esters	Lev and Spicer ^[13]
4. AF (Aldehyde fuchsin) GPs with sulphate	Gomari ^[14]
5. AF/AB pH 2.5 Strong sulphated GPs	Spicer and Mayer ^[15]

Results

Histochemical reactions of the glycoproteins from the epithelial surface and goblet cells of the stomach, anterior, middle and posterior intestines are summarized in Table 2. Histochemical analysis showed that the gastrointestinal mucous content included sulphate-esters and/or carboxylic [Alcian blue (AB) pH 2.5 +], glycogene and/or oxidable dioles [periodic acid/Schiff+ (PAS+)] (Fig. 1), neutral or acid-rich (PAS/AB pH 2.5+) and strong acid sulphated [(AF/AB pH 2.5+)] glycoproteins (GPs). But, in stomach and anterior intestine epithelium sulphated glycoproteins were not observed. Especially neutral mucosubstance was found strong reaction in goblet cells and

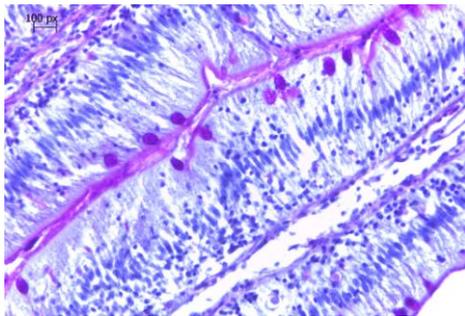
epithelial surface of the digestive tract (Figure 1a,b). (PAS/AB pH 2.5+) satining showed the PAS (+) and combination mucosubstances dominance (Figure 1c). Neutral and acidic glycoproteins in the stomach and posterior intestinal epithelial were medium density and these mucosubstance was decreasing in anterior and middle intestinal epithelial. Combination and PAS goblet cells of the gastric mucosa were medium reaction and acidic goblet cells were weak reaction. In anterior intestine PAS and AB pH 2.5 reaction were negative but combination reactin was positive. Acidic mucosubstance was weak reaction in middle intestine despite combination staining was very dominant. Acidic mucosubstance was

negative reaction but combination staining was strong reaction in posterior intestine.

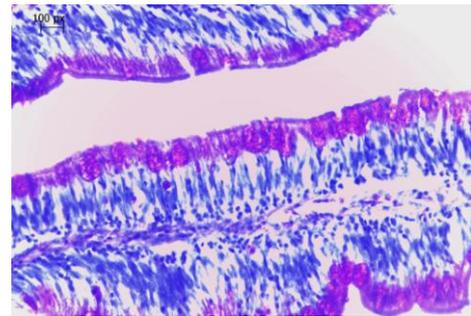
Furthermore, sialic acid residues including GPs were determined in goblet cells, while epithelial cells lacked these mucosubstances. Strong reaction was anterior and middle intestine (Figure 1d).

Sulphated mucosubstance of stomach mucosa was determined negative reaction.

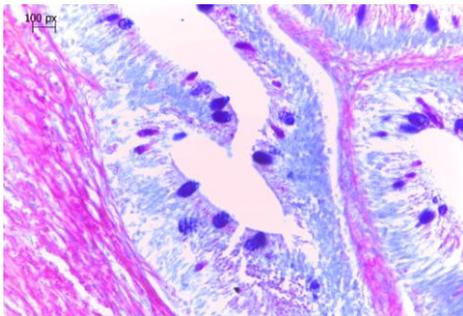
While AF staining was moderate reaction in intestine, density was increased towards anterior intestine to posterior intestine (Figure 1e). Applied AF/AB pH 2.5 results were observed with the weak reaction in stomach and intestine epithelium but no reaction was in gastric mucosa (Figure 1f).



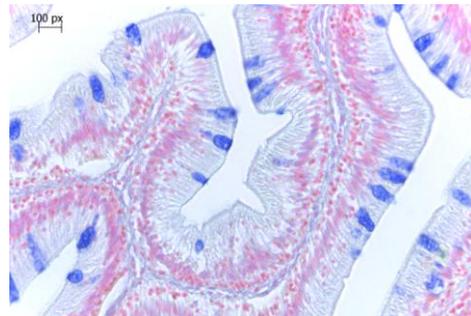
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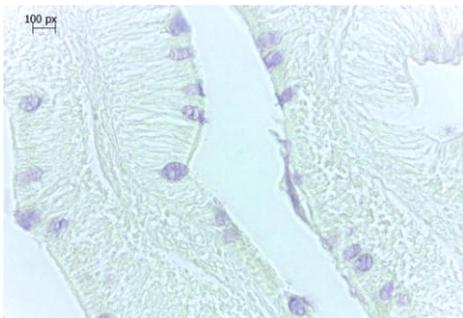
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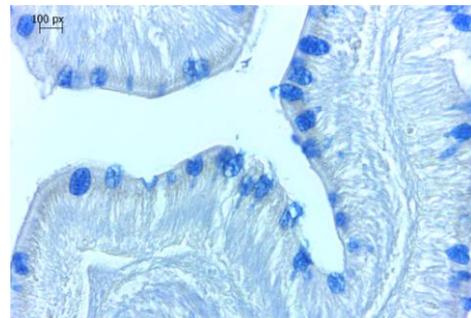
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Figure 1— a) PAS positive cells in stomach, b) PAS positive cells in anterior intestine, c) PAS/AB and PAS positive cells in anterior intestine d) AB pH 2.5 positive cells in posterior intestine e) AF positive cells in anterior intestine f) AF/AB pH 2.5 and AB pH 2.5 positive cells in anterior intestine

Table 2—Histochemical reaction in gastrointestinal canal

RUDD METOD	STOMACH			ANTERIOR INTESTINE			MIDDLE INTESTINE			POSTERIOR INTESTINE			
	EPITHELIUM SURFACE	GOBLET CELL		EPITHELIUM SURFACE	GOBLET CELL		EPITHELIUM SURFACE	GOBLET CELLS		EPITHELIUM SURFACE	GOBLET CELLS		
		REACTION	DISTRIBUTION		REACTION	DISTRIBUTION		REACTION	DISTRIBUTION		REACTION	DISTRIBUTION	
PAS	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
PAS/ AB pH 2.5	++	PAS ++ AB + COMB ++	++ + ++	PAS - AB - COMB +++	COMB +++	+++	+	PAS - AB + COMB +++	- + +++	+	++	PAS + AB - COMB +++	+ - ++
AB pH 2.5	+	++	++	+++	+++	+++	+	+++	+++	+	+	++	++
AF	-	-	-	+	++	++	+	+	+	+	+	++	+++
AF/ AB pH 2.5	+	AF - AB - COMB -	-	AF - AB ++ COMB -	- + -	+++	+	AF - AB + COMB -	- ++ -	+	+	AF - AB ++ COMB -	- ++ -

Discussion

Rudd (*Scardinius erythrophthalmus*) is a teleostean fish belonging to the familia Cyprinidae, possessing a digestive system not different from other teleosts. Digestive tract histochemical studies, mucus is secreted by lamina epithelialis cells and some glandular epithelial cells [16-17]. Presence of mucous-secreting cells are a general character of teleosts, while mucosubstances secreted changes between species and different regions at digestive tract [18-19]. In this study, although denser in goblet cells at whole tract lamina epithelialis various mucosubstances were determined in lamina epithelialis cells. Denser goblet cells in large intestine are similarly observed in some fish species [20-21]. As in *Oncorhynchus mykiss* [1]. Goblet cells were found along the entire intestine and their number increased in the distal portion, possibly indicating the need for mucosal protection [4]. Similar results were obtained in this study.

While neutral glycoproteins were observed in the whole intestine of *Arrhamphus sclerolepis krefftii* [22], *Acipenser transmontanus* [23] and *Misgurnus anguillicaudatus* [24] and in the posterior intestine of *Tilapia spp.* [7], in the anterior intestine of *Tilapia spp.* [7]. Murray *et al.* [5] found a few of these glycoproteins were present in all regions of *Hippoglossus hippoglossus*, *Pleuronectes americanus*, *Pleuronectes ferruginea* intestine. In the present study, high neutral glycoproteins were determined in the epithelial surface and goblet cells of the intestine.

As in the present study, reaction of AB pH 2.5 have been determined in the intestine of many fish species, such as *Umbrina cirrosa* [4], *Tilapia spp.* [7], *Hippoglossus hippoglossus* [5], *Misgurnus anguillicaudatus* [24], *Acipenser transmontanus* [23]. In the present study, strong reaction of PAS/AB pH 2.5 were observed in the whole gastrointestinal canal. These findings agree well with those of previous reports in *Hippoglossus hippoglossus*, *Pleuronectes americanus*,

Pleuronectes ferruginea species [5], *Cyprinus carpio* [6], *Tilapia spp* [7], *Misgurnus anguillicaudatus* [24].

Reaction of AF and AF/AB pH 2.5 were determined in the gut of *Misgurnus anguillicaudatus* [3]. Similar results were obtained in this study.

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