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A Histochemical Study of Glycoconjugates in the Gills of the Sea Bass (*Dicentrarchus labrax* L. 1758)

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ABSTRACT

The characteristics of glycoconjugates in the mucous cells of the sea bass (*Dicentrarchus labrax* L. 1758) gill were investigated by histochemical staining procedures. Histochemical results showed that mucous cells in the primary filament (primary lamellae) epithelium contain acidic, neutral and sulphated glycoconjugates and also mucous cells were stained weakly with AB pH 0.5 were detected in the primary filament epithelium.

Key Words: *Dicentrarchus labrax,* Gill, Glycoconjugates, Histochemistry, Mucous cells.

1. INTRODUCTION

The gills which are one of the most important organs of the body of the fish serve a variety of functions to fish such as gas exchange, acid-base balance, osmoregulation and ionic regulation [1-5].

Different cell types such as mitochondrion-rich, chloride, pavement and mucous cells in the gills were identified in several studies [4, 6, 7]. Gills are exposed directly to the water and because of their direct contact with environment mucus secreted by mucous cells is a physical barrier which inhibits entry of disease microorganisms from the environment into the fish [8, 9]. Also mucus has an important role in lubrication ion regulation and diffusion [10, 11].

Mucins the main substance of mucus are high molecular weight glycoconjugates [12]. The characteristics of the mucous glycoconjugates in the gills of the different fish species were investigated [7, 11, 13-15, 21].

The aim of this work was to analyze the glycoconjugate composition in the mucous cells of the gills of the sea bass (*D. labrax*) by using a series of histochemical techniques.

2. MATERIALS AND METHODS

In this study ten-specimen-sea bass (*D. labrax*) (approximately weight between 350-400 g, total length between 20-25 cm) were obtained from a fish farm Ege-Mar Su Ürünleri Ltd. Şti. in Akbük/Aydın. Fish were killed by decapitation. Gill tissue samples were immediately dissected fixed in 10% buffered formalin solution, dehydrated in a graded series of ethanol, cleared in xylene and embedded in paraffin. Serial sections (5 µm thick) were stained with conventional techniques for histochemical identification of glycoconjugates (Table 1).

3. RESULTS

It was observed that mucous cells were located in primary filament epithelium. Mucous cells were present towards the outer surface in primary filament and at the basis of primary filament epithelium as single cells or in groups.

Mucous cells showed the presence of glycoconjugates strong reactivity to PAS staining. The combinations of PAS/AB pH 2.5 staining procedure showed that most mucous cells showing the presence of a mixture of neutral and acidic glycoconjugates (Figure 1).

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Table 1. Conventional histochemical staining procedures for identification of glycoconjugates in mucous cells of *D. labrax* gill; AB, Alcian blue; PAS, Periodic acid/Schiff; AF, Aldehyde fuchsin; GCs, glycoconjugates.

Procedures	References	GCs revealed
PAS	[16]	GCs with neutral
PAS/AB pH 2.5	[17]	Neutral and/or acid rich GCs
AB pH 2.5	[18]	Acidic GCs with carboxylated and sulphated esters
AB pH 1.0	[18]	GCs with O-sulphate esters
AB pH 0.5	[18]	Very sulphated GCs
AF	[19]	GCs with sulphate
AF/AB pH 2.5	[20]	To separate sulphated GCs from acidic GCs

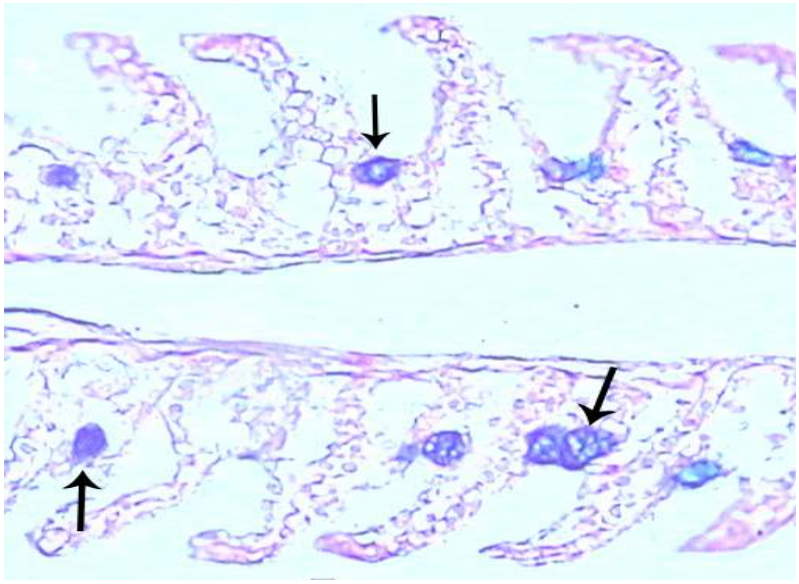

Figure 1. Mucous cells (arrows) in the primary lamellae show a mixed reaction. X200 PAS/AB pH 2.5 staining procedure.

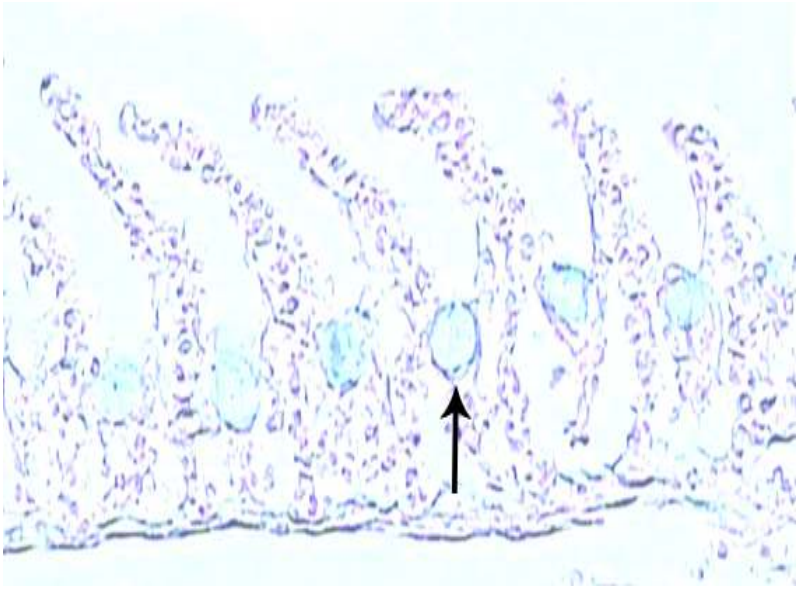

Figure 2. Mucous cell (arrow) in the primary lamellae show weak AB reaction. X200 AB pH 0.5 staining procedure.

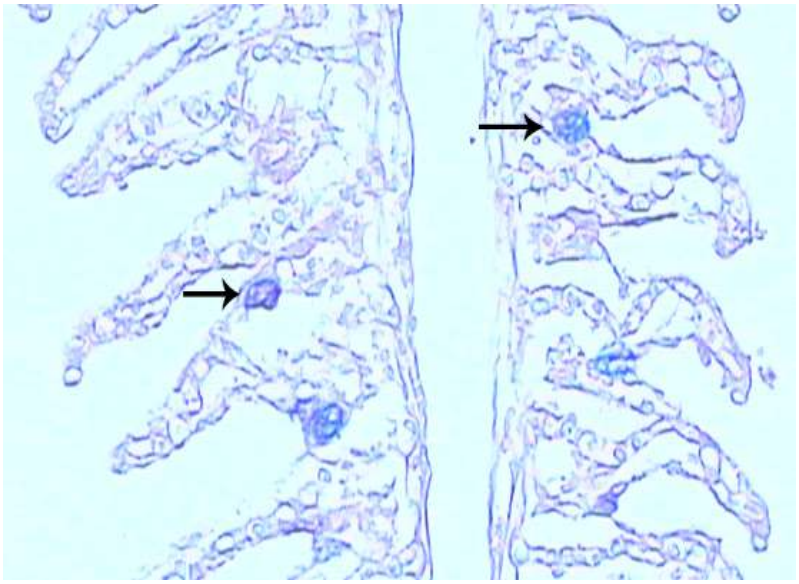

Figure 3. Mucous cells (arrows) in the primary lamellae show a mixed reaction to AF/AB pH 2.5 staining procedure. X200.

Whereas mucous cells were weakly stained with AB pH 0.5 (Figure 2) and strongly stained with AB pH 2.5 they were negative with AB at pH 1.0. and indicated that mucous cells had not contained glycoconjugates with O-sulphate esters in all areas.

The histochemical properties of the contents of the mucous cells revealed by the AF staining method confirmed that glycoconjugates with sulphate were present. It was detected that acidic glycoconjugate were dominant in the mixture of sulphated and acidic glycoconjugates when treated with AF/AB pH 2.5 (Figure 3).

4. DISCUSSION AND CONCLUSION

Mucous cells in secondary lamellae contained different glycoconjugates [14, 15, 21] were showed with different fish species. But in this study, mucous cells were identified to be broadly distributed in the primary filament epithelium.

The fact that mucous cells in the gills reacted positively to PAS indicated that cells contained neutral glycoconjugates. This has been demonstrated in other fish species in which cells reacted in the same way with PAS method [11, 14, 15, 21]. The majority of the mucous cells in the gills of *D. labrax* were stained violet by the combined PAS/AB pH 2.5 staining as similar to findings observed in *M. furnieri* [11]. Contrary to these results authors [15, 22] observed that most mucous cells stain blue.

As observed in *C. carpio* [15] weak AB pH 0.5-positive feature was detected in some mucous cells in contrast to these results mucous cells gave a moderate positive reaction in *C. guatucupa* [21]. Though reported that glycoconjugates with O-sulphate esters were present in mucous cells [15, 21], no AB pH 1-positive mucous cells were found in this study. This study revealed that mucous cells had acidic glycoconjugates in agreement with the findings of Calabró et al. [14], Çınar et al. [15] and Diaz et al. [21].

In this study the combined AF/AB pH 2.5 staining showed that acidic glycoconjugates were predominant. But authors [15] showed that AF/AB pH 2.5 application yielded the AF-positive glycoconjugate dominance.

The data obtained in this study could serve as a basis for further histopathological and aquatic toxicological studies and to get information which would be necessary in order to characterize glycoconjugates structure and determine their biological roles.

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