Süleyman Demirel Üniversitesi Fen Edebiyat Fakültesi Fen Dergisi
Süleyman Demirel University Faculty of Arts and Sciences Journal of Science
2021, 16(2): 424-433
DOI: 10.29233/sdufeffd.895449
Atıf için / For Citation: M. Öztop, K. Çınar, E. Demirbağ, "Distribution of glycoconjugates in the gills of Oscar fish (*Astronotus ocellatus*)", *Süleyman Demirel Üniversitesi Fen Edebiyat Fakültesi Fen Dergisi*, 16(2), 424-433, 2021.

Distribution of Glycoconjugates in the Gills of Oscar Fish (*Astronotus ocellatus*)

Mustafa ÖZTOP[1], Kenan ÇINAR[2], Emel DEMİRBAĞ[*3]

[1]*Mehmet Akif Ersoy University, Faculty of Science and Art, Department of Biology, 15100, Burdur, Türkiye*
[2]*Süleyman Demirel University, Faculty of Science and Art, Department of Biology, 32100, Isparta, Türkiye*
[3]*Süleyman Demirel University, Isparta Vocational School of Health Services, Department of Medical Laboratory Techniques, 32100, Isparta, Türkiye*

**yazışılan yazar e-posta: emeldemirbag@sdu.edu.tr*

(Received: 12.03.2021, Accepted: 02.09.2021, Published: 25.11.2021)

Abstract: Glycoconjugates involved in many functions are produced by many cell types. This study aimed to investigate the glycoconjugate expression in the Oscar fish (*Astronotus ocellatus*) gills using five horseradish peroxidase-conjugated lectins. This study revealed that gill epithelial cells react with *Canavalia ensiformis* agglutinin (Con A) and *Arachis hypogaea* agglutinin (PNA). Strong reaction to Con A was seen in the epithelial cells of the gill primary lamellae. Epithelial cells of primary and secondary lamellae and eosinophilic granular cells had varying intensity of PNA binding. *Ulex europaeus* agglutinin-I(UEA-I) positive eosinophilic granular cells were observed in the connective tissue of primary lamellae. Epithelial cells of the gill primary filament had Con A reaction in varying intensities. Superficial epithelial cells reacted with PNA in various degrees. Epithelial cells of the secondary lamellae had Con A and PNA positive reaction. These results indicate that glycoconjugate distribution may show interspecific variations, suggesting that the high heterogeneity of naturally occurring glycoconjugates in the epithelial cells of Oscar fish gills might be attributed to the various functional roles of glycoconjugates.

Key words: Lectin, Oscar fish, Glycoconjugate, *Astronotus ocellatus*

Oskar Balığının (*Astronotus ocellatus*) Solungaçlarında Glikokonjugatların Dağılımı

Özet: Pek çok fonksiyona katılan glikokonjugatlar, birçok hücre tipi tarafından üretilir. Bu çalışmada Oscar balığı (*Astronotus ocellatus*) solungaçlarındaki glikokonjugat varlığının beş horseradish peroksidaz ile konjuge lektin kullanarak araştırılması amaçlandı. Bu çalışma, solungaç epitel hücrelerinin *Canavalia ensiformis* agglutinin (Con A) ve *Arachis hypogaea* agglutinin (PNA) ile reaksiyona girdiğini ortaya çıkardı. Solungaç primer lamellerinin epitel hücrelerinde Con A'ya karşı güçlü reaksiyon görüldü. Primer ve sekonder lamel epitel hücreleri ve eozinofilik granüler hücreler, değişen yoğunlukta PNA bağlanmasına sahipti. Primer lamellerin bağ dokusunda *Ulex europaeus* agglutinin-I (UEA-I) pozitif eozinofilik granüler hücreler gözlendi. Solungaç primer filamentinin epitel hücreleri, çeşitli yoğunluklarda Con A reaksiyonuna sahipti. Yüzeysel epitel hücreleri PNA'ya karşı çeşitli derecelerde reaksiyon göstermekteydi. Sekonder lamellerin epitel hücreleri Con A ve PNA pozitif reaksiyona sahipti. Bu sonuçlar, glikokonjugat dağılımının türler arası varyasyonlar gösterebileceğine işaret etmektedir, bu da Oscar balık solungaçlarının epitelyal mukoza hücrelerinde doğal olarak oluşan glikokonjugatların yüksek heterojenitesinin, glikokonjugatların çeşitli fonksiyonel rollerine dayandırılabileceğini düşündürmektedir.

Anahtar kelimeler: Lektin, Oskar balığı, Glikokonjugat, *Astronotus ocellatus*

Mustafa ÖZTOP, mustafamalatya@gmail.com, ORCID: https://orcid.org/0000-0002-2923-9280
Kenan ÇINAR, kenancinar@sdu.edu.tr, ORCID: https://orcid.org/0000-0003-3410-6180
Emel DEMİRBAĞ, emeldemirbag@sdu.edu.tr, ORCID: https://orcid.org/0000-0002-8873-4513

1. Introduction

Fish are much more adversely influenced than terrestrial animals by the environmental changes. This results mainly from the use of water rather than air as the respiratory gas exchange medium. The composition of water differs to a much larger extent than that of air, thanks to many natural or contamination-related factors [1]. Fish are exposed directly to water and the environment through their gills, the main respiratory organs in which the exchange of gases essentially occurs [2,3]. Therefore, mucus secreted chiefly by superficial epithelial cells present in the gills composes an initial line of defence against changes in nature and chemical composition of the surrounding water [4,5].

Mucous cells of the gill epithelium produce a mucous secretion that plays important roles in many vital cellular processes [6]. The key building blocks of mucus are mucins whose components are glycoproteins known to possess many functions, including antimicrobial, respiration, mechanical, ion regulation, osmoregulation and antiviral [7,8]. Roughly half of the dry weight of mucins present in mucus can be composed of carbohydrate chains capable of covalently binding to many different proteins to form glycoproteins and glycoconjugates [9]. On the other hand, lectins are carbohydrate binding proteins and are implicated in biological activities through the process of glycoconjugation [10]. In this regard, the existence of glycoconjugates and glycoproteins can be identified by different histochemical techniques, including lectin histochemistry, a versatile tool that is commonly being used in the detection of modifications in mucosubstances [10]. Lectin histochemistry approach has advantages over other histochemical approaches to identifying modifications that occur on cellular surfaces. For example, this approach is useful in especially detecting alterations in cellular glycosylation associated with transformation of normal cells to malignant, and changes associated with cancer progression [11].

The histochemistry and lectin-based histochemistry of the different types of gill epithelial cells in the fish and of their glycoconjugates have been studied by some researchers [12-15]. Oscar fish, *Astronotus ocellatus* (Cuvier, 1829), is a cichlid species with omnivorous feeding habits. It is a freshwater fish species found in habitats with warm water temperatures. Since it is an ornamental fish, it is transported alive. Therefore, it may be mediated the transmission of many pathogenic diseases [16-17]. Fish gills are organs being exposed to various noxious factors, including chemicals and pathogens. So, they may be a model for exploring abnormal changes caused by many adverse factors. Since no study regarding lectin-based histochemistry of glycoconjugates in gills of Oscar fish exists, the objective of this study was to examine the distribution of the glycoconjugates in the Oscar fish gills using five horseradish peroxidase-conjugated lectins.

2. Material and Method

2.1 The study protocol

The approval for this study was obtained from Animal Experiments Local Ethics Committee of Süleyman Demirel University (approval number #03.04.2012-04). Use of the animals was performed in accordance with the guidelines of the International Association for the Study of Pain.

2.2 Tissue processing

The gill specimens harvested from six Oscar fish, *Astronotus ocellatus*, were used for the lectin histochemical study. The fish were purchased from the ornamental fisher in Isparta, Turkey. After anesthetizing with 25-30 ppm / l quinaldine sulphate for 1-4 minutes, the fish were killed by decapitation. The gills were rapidly excised and fixed by immersion in 10% of formalin for 24 h at room temperature for light microscope studies. After fixation, tissues were passed through series of ascending ethanol, followed by clearing in xylene and embedding in paraffin. Paraffin sections cut at 5-µm-thickness were placed onto the slides that had been pre-coated [18].

2.3 Lectin histochemistry (LHC)

Horseradish Peroxidase (HRP)-conjugated lectins were employed to explore specific sugar moieties of glycoconjugates. As described in previous study [18], the slides were dewaxed in xylene and rehydrated in descending grades of ethanol, followed by washing in distilled water. The slides were pre-incubated with 0.3% H_2O_2 (v/v) in absolute methanol at room temperature for 10 minutes so as to eliminate endogenous peroxidase activity. After washing in distilled water and 0.01 M PBS (Phosphate Buffered Saline) (pH 7.2) with 1% BSA (Bovine Serum Albumin), the slides were exposed to HRP-conjugated lectins at room temperature for 30 minutes and rinsed with PBS. The HRP-conjugated lectins employed in this study, their carbohydrate binding specificities and optimal concentrations were given in Table 1. Staining was then visualized with the incubation of DAB (3,3-diaminobenzidine tetrahydrochloride) at room temperature for 10 minutes. Positive staining was considered as brown or dark-brown in colour. The slides were rinsed in distilled water, passed through ascending alcohol series, followed by clearing in xylene, and permanently coverslipping with entellan. They were analysed under light microscope (Olympus, CX 41) and photographed with the help of a digital camera (DP26, Olympus, Tokyo, Japan) affixed to the microscope.

2.4 Negative control

The slide had been incubated for 30 minutes at room temperature without using the lectins. Then, the same procedure was pursued.

2.5 Semi-quantitative assessment

Intensity of staining was graded as negative – 0, weak – 1, moderate – 2 and strong – 3, as described in previous study [18].

Table 1. Lectins, their carbohydrate binding specificities and used optimal concentrations

Lectins	Origin	Carbohydrate specificity[a, b]	Optimal Concentration
UEA-I	*Ulex europaeus*	αL-Fuc	25 µg/ml [18]
Con A	*Canavalia ensiformis*	αMan>αGlc>αGlcNAc	50 µg/ml [18]
PNA	*Arachis hypogaea*	Galß1,3GalNAc>α and βGal	20 µg/ml [19]
MAA-I	*Maackia amurensis*	Galβ4GlcNAc	50 µg/ml [19]
BSA-I-B_4	*Bandeiraea simplicifolia*	αGal>αGalNAc	25 µg/ml [19]

[a]Carbohydrate specificities of lectins [20]. [b]Abbreviations: Glc, glucose; GlcNAc, N-acetylglucosamine; Gal, galactose; Fuc, fucose; GalNAc, N-acetylgalactosamine; Man, mannose.

3. Results

The lectin histochemistry revealed that the epithelial cells present in the gills of the Oscar fish, *Astronotus ocellatus*, react with the lectins *Canavalia ensiformis* agglutinin (Con A) and *Arachis hypogaea* agglutinin (PNA), except for *Ulex europaeus* agglutinin-

I (UEA-I), *Maackia amurensis* agglutinin-I (MAA-I) and *Bandeiraea simplicifolia* agglutinin (BSA-I-B_4), in various degrees. UEA-I reacted with granular cells considered to be eosinophilic possibly (Figures 1-6). Table 2 summarizes the lectin labelling patterns in the gill epithelial cells of Oscar fish, *Astronotus ocellatus*.

Con A positivity was detected in varying intensities from moderate to strong in the mucosal epithelium of gill primary filament. A strong reaction to Con A was determined in the epithelial cells at the end parts of the gill primary lamellae (Figure 1). Con A reaction varying from weak to strong was seen in the epithelial cells of the secondary lamellae (Figure 2). Cells localized in the superficial and deep regions of primary filament epithelium, secondary filament epithelial cells (Figure 3) and granular cells considered to be eosinophilic possibly present in the connective tissue of primary lamellae reacted with PNA in various degrees ranging from moderate to strong. In addition, strong PNA reaction was detected in the deep epithelial layer cells of the secondary lamellae (Figure 4). UEA-I positive glycoconjugate was not detected in epithelial cells of primary and secondary filaments. Moreover, UEA-I positive granular cells considered to be eosinophilic possibly were observed in the connective tissue of primary lamellae, and these positive reactions were from weak (Figure 5) to strong (Figure 6).

Table 2. Lectin-binding patterns in the gill structures of Oscar fish

	UEA-I	**Con A**	**PNA**	**MAA-I**	**BSA-I-B_4**
Primary lamellae					
Superficial epithelial cells	0	2-3	2-3	0	0
Epithelial cells in deeper layers	0	2-3	2-3	0	0
Secondary lamellae					
Epithelial cells	0	1-3	2-3	0	0

Intensity of staining: 0, negative; 1, weak; 2, moderate; 3, strong

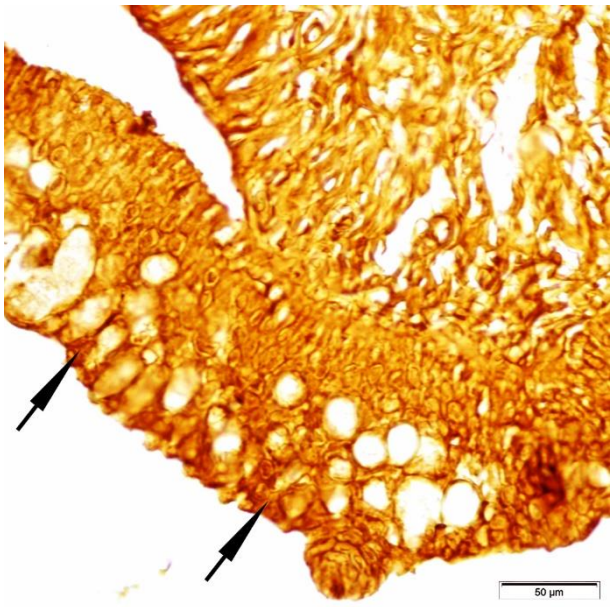

Figure 1. Strong reaction to Con A in epithelial cells (arrows) at the end parts of primary lamellae. Scale bar: 50µm.

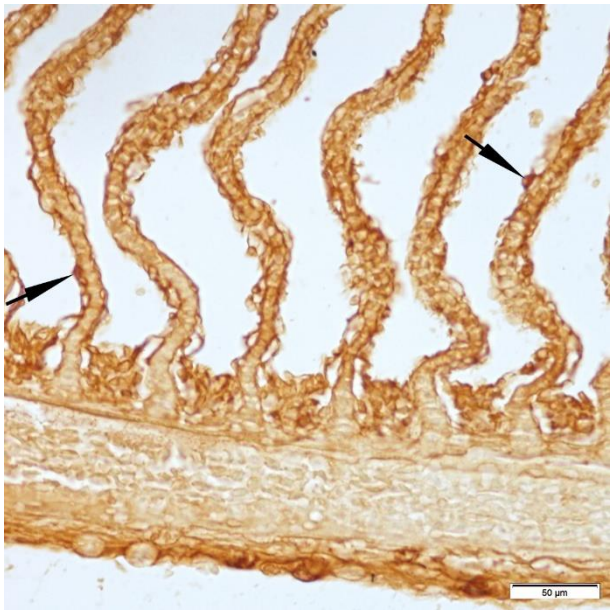

Figure 2. Strong reaction to Con A in epithelial cells (arrows) of secondary lamellae. Scale bar: 50 µm.

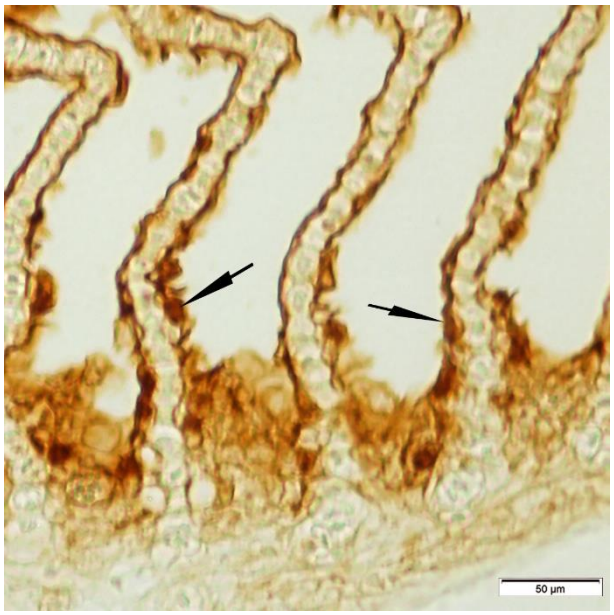

Figure 3. Strong reaction to PNA in epithelial cells (arrows) of secondary lamellae. Scale bar: 50 µm.

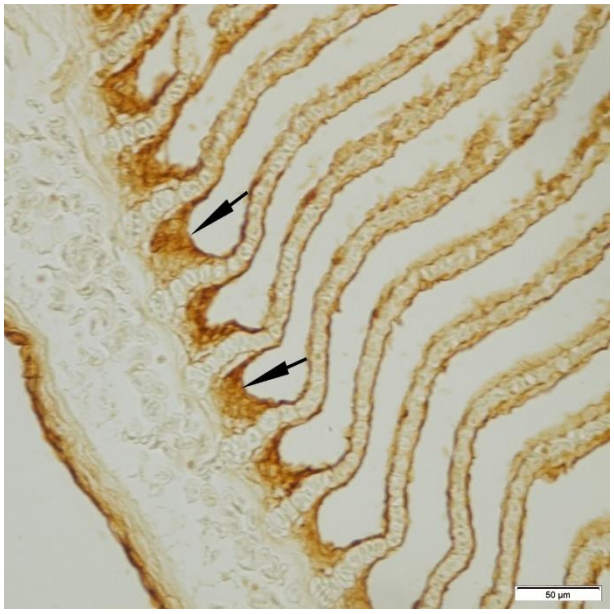

Figure 4. Strong reaction to PNA in deep epithelial layer cells (arrows) of secondary lamellae. Scale bar: 50 µm.

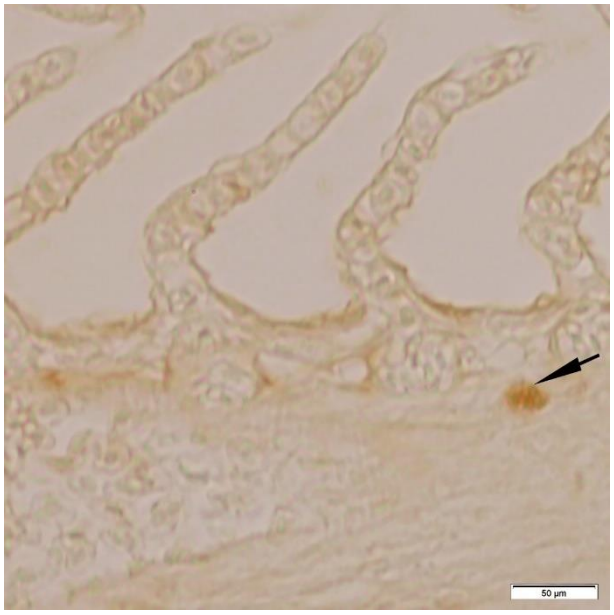

Figure 5. Weak reaction to UEA-I in granular cell (arrow) considered to be eosinophilic possibly in the connective tissue of primary lamellae. Scale bar: 50µm.

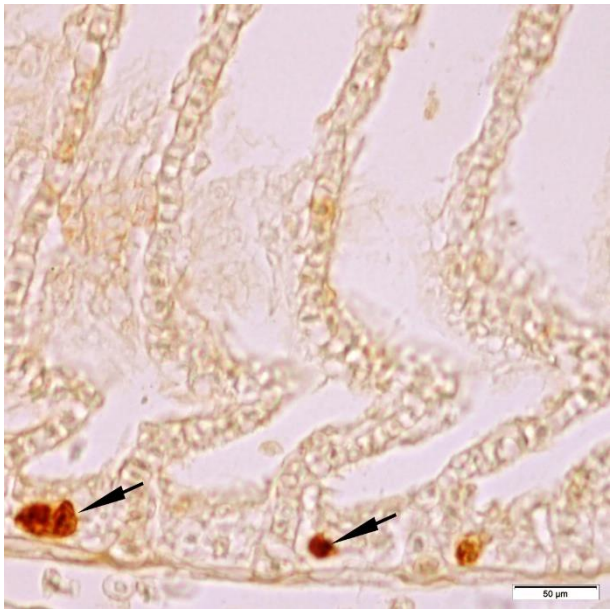

Figure 6. Strong reaction to UEA-I in granular cells (arrows) considered to be eosinophilic possibly in the connective tissue of primary lamellae. Scale bar: 50µm.

4. Conclusion and Comment

The present study investigates the heterogeneity of glycoconjugates found in the gill epithelial cell of the Oscar fish, *Astronotus ocellatus* using lectin histochemistry. We found that Con A and PNA positive glycoconjugates were predominant in the epithelial cells of gill primary and secondary lamellae. In addition, our results revealed that granular cells considered to be eosinophilic possibly in the gills contain UEA-I positive glycoconjugates. However, staining with MAA-I and BSA-I-B_4 lectins were not observed in these cells. The absence of reaction to MAA-I and BSA-I-B_4 indicates that these cells did not produce the residues of galactose-β1,4-acetylglucosamine and α-D-galactose or N-acetyl-α-D-galactosamine.

It has been demonstrated that PNA, Con A and UEA-I did not bind to any cell types or structures in the gill of tilapia, *Oreochromis mossambicus* [21], which is inconsistent with our results. A study on *Odontesthes bonariensis* showed [22] that gill filaments and secondary lamellae have the same distribution pattern as lectins of the epithelial cells, and that the lectins PNA, Con A and UEA-I give a moderate, weak and negative reaction, respectively. These findings agree with our results to a certain degree. It has been reported that PNA showed a strong reaction with epithelial cells in the gill of the trout *Salmo trutta* [23, 24]. Besides, another study [25] showed that PNA is bound to all the surface epithelial cells of the primary lamellae, as well as to a few cells in the deeper layers of the primary lamellae epithelium. They also reported that most of the epithelial cells in the primary lamellae did not bind to PNA but the epithelial cells of the secondary lamellae were homogeneously labelled with PNA. These findings are inconsistent with the results of our study. This may be due to interspecific differences. The same researchers [24] found that Con A strongly labelled superficial epithelial cells and a few cells deeper in the primary lamellae, and some cells of the secondary lamellae were labelled with Con A. In fact, this finding is almost in accordance with our results. Another study showed no reaction to ConA in the gill epithelium in the abyssal teleost fish *Coelorhynchus coelorhynchus* [26], which is inconsistent with our findings.

A study on *Micropogonias furnieri* indicated that epithelial cells of both primary lamellae and secondary lamellae in *Micropogonias furnieri* are not labelled with UEA-I [14], which is in line with our findings. In addition, the reaction to UEA-I and PNA was detected in epithelial cells of gill filament in *Paralichthys olivaceus* that is a temperate marine species [27]. A recent study has revealed that UEA-I staining was present in the gill epithelial cells of rainbow trout [28]. The results in [26] is consistent with our results of PNA. However, the results of UEA-I reported by [27] and [28] is not in parallel with our findings of UEA-I. These discrepancies can be attributed to an interspecific difference.

Con A showed no staining or weak staining with the epithelial cells of the primary and secondary lamellae of *Cynoscion guatucupa* [16]. The present results partly agree with those of *Cynoscion guatucupa*, with our findings of more prominent reactions in the epithelial cells of both primary lamellae and secondary lamellae. In many fish species, the surface epithelial cells of the gills have mannose, generally found in N-linked glycoproteins [25]. Moreover, Con A-binding protein secreted by a serous cell was found in the carp skin [29]. A serum cell type which occurs intermittently in the fish epidermis [30] might be expected to be present in the gill as well. This cell type is increased during a chronic parasitic infestation, suggesting that it provides protection against bacterial infections [31]. Furthermore, it is most likely that UEA-I positive granular cells considered to be eosinophilic possibly in the gill filament in this study participate in inflammatory processes.

In conclusion, fishes have the cells producing glycoconjugates on the gill primary and secondary lamella although the distribution of these cells may show interspecific variations. The high heterogeneity of naturally occurring glycoconjugates in the epithelial cells of Oscar fish gills could be attributed to the various functional roles of glycoconjugates. This study would contribute to understanding the physiological and functional significance of the glycoconjugates in the gills of the fishes, which are used for ornamental purposes.

Author Statement

Mustafa ÖZTOP: Investigation, Methodology, Original Draft Writing, Review and Editing, Visualization.
Kenan ÇINAR: Investigation, Methodology, Original Draft Writing, Review and Editing.
Emel DEMİRBAĞ: Investigation, Methodology, Original Draft Writing, Review and Editing.

Acknowledgment

As the authors of this study, we declare that we do not have any support and thank you statement.

Conflict of Interest

As the authors of this study, we declare that we do not have any conflict-of-interest statement.

Ethics Committee Approval and Informed Consent

The approval for this study was obtained from Animal Experiments Local Ethics Committee of Süleyman Demirel University (approval number: 03.04.2012-04).

References

[1] N. Heisler, "Acid-base regulation in response to changes of the environment characteristics and capacity," in *Fish Ecophysiology*, J. C. Rankin, F. B. Jensen, Eds. London: Chapman-Hall, 1993, pp. 207-230.

[2] W. S. Hoar and D. J. Randall, "Gills-Anatomy, gas transfer, and acid-base regulation," in *Fish Physiology*, vol. XA, New York: Academic Press, 1984, p. 456.
[3] W. S. Hoar and D. J. Randall, "Gills-Ion and water transfer," in *Fish Physiology*, vol. XB, New York: Academic Press, 1984, p. 416p.
[4] E. M. Zuchelkowski, R. C. Lantz, and D. E. Hinton, "Effects of acid-stress on epidermal mucous cells of the brown bullhead *Ictalurus nebulosus*: A morphometric study," *The Anatomical Record*, 200, 33-39, 1981.
[5] E. M. Zuchelkowski, C. A. Pinkstaff, and D. E. Hinton, "Mucosubstance histochemistry in control and acid-stressed epidermis of brown bullhead cat fish, *Ictalurus nebulosus*," *The Anatomical Record*, 212, 327-335, 1985.
[6] K. L. Shephard, "Functions for fish mucus," *Reviews in Fish Biology and Fisheries*, 4, 401-29, 1994.
[7] A. Allen, A. Bell, M. Mantle, and J. P. Pearson, "The structure and physiology of gastrointestinal mucus," *Advances in Experimental Medicine and Biology*, 144, 115-133, 1982.
[8] M. E. V. Johansson, H. Sjövall, and G. C. Hansson, "The gastrointestinal mucus system in health and disease," *Nature Reviews-Gastroenterology and Hepatology*, 10, 352-361, 2013.
[9] G. J. Strous and J. Dekker, "Mucin-type glycoproteins," *Critical Reviews in Biochemistry and Molecular Biology*, 27, 57-92, 1992.
[10] F. Genten and A. Danguy, "Light microscopic characterization of glycoconjugates and cell proliferation in teleost epidermis exposed to acute levels of pH," *Zeitschrift fur Mikroskopisch-Anatomische Forschung*, 104, 119-139, 1990.
[11] S.A. Brooks, "Lectin Histochemistry: Historical Perspectives, State of the Art, and the Future," *Methods in Molecular Biology*, 1560, 93-107, 2017.
[12] S. M. T. Saboaia- Moraes, F. J. Hernandez- Blazquez, D. L. Mota, and A.M. Bittencourt, "Mucous cell types in the branchial epithelium of the euryhaline fish *Poecilia vivipara," Journal of Fish Biology*, 49 (3), 545-548. 1996.
[13] K. Çınar, N. Şenol, and M. R. Özen, "Histochemical characterization of glycoproteins in the gill of the carp (*Cyprinus carpio*)," *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 55, 61-64, 2008.
[14] A. O. Diaz, A. M. Garcia, C. V. Devincenti, and A. L. Goldemberg, "Ultrastructure and histochemical study of glycoconjugates in the gills of the white croaker (*Micropogonias furnieri*)," *Anatomia, Histologia, Embryologia*, 34, 117-122, 2005.
[15] A. O. Diaz, A. M. Garcia, A. H. Escalante, and A. L. Goldemberg, "Glycoconjugates in the branchial mucous cells of *Cynoscion guatucupa*," *Scientia Marina*, 69 (4), 545-553, 2005.
[16] D. M. Fracalossi, M. E. Allen, D. K. Nichols, and O. T. Oftedal, "Oscars, *Astronotus ocellatus*, have a dietary requirement for vitamin C," *The Journal of Nutrition*, 128 (10), 1745-1751, 1998.
[17] M. E. de Jesus Trindade, and H. L. de Queiroz, "Feeding ecology and morphometry of the digestive tract of *Astronotus ocellatus* (Cichlidae) in Várzea environments of the Middle Solimões Region, Central Amazon, Brazil," *Scientific Magazine UAKARI*, 8, 45-57, 2012.
[18] K. Çınar, M. Öztop, and B. Özkarasu, "Glycoconjugate composition of ovine parotid glands elucidated by lectins," *Journal of Morphological Sciences*, 33 (1), 8-13, 2016.
[19] M. Öztop, K. Çınar, and E. Demirbağ, "Lectin histochemistry of the glycoconjugates in partridge and quail conjunctival epithelia," *Van Veterinary Journal*, 32(1), 1-6, 2021.
[20] I. E. Liener, N. Sharon, and I. J. Goldstein, *The Lectins: Properties, Functions, and Applications in Biology and Medicine*. Orlando, Florida: Academic Press, 1986, p. 618.
[21] C. Purbomartono, A. Takemura, and K. Takano, "Histochemical study of mucus lectin distribution on several tissues of tilapia (*Oreochromis mossambicus*)," *Jurnal Perikanan* (*Journal of Fisheries Sciences)*, 9 (1), 1-7, 2007.
[22] A. O. Díaz, A. M. García, A. H. Escalante, and A. L. Goldemberg, "Glycoproteins histochemistry of the gills of *Odontesthes bonariensis* (Teleostei, Atherinopsidae)," *Journal of Fish Biolology*, 77 (7), 1665-1673, 2010.
[23] M. C. Rojo, M. J. Blanquez, and M. E. Gonzalez, "A histochemical study of the distribution of lectin binding sites in the developing branchial area of the trout *Salmo trutta*," *Journal of Anatomy*, 189 (3), 609-621, 1996.
[24] P. Burkhardt-Holm, "Lectin histochemistry of rainbow trout (*Oncorhynchus mykiss*) gill and skin," *The Histochemical Journal*, 29, 893-899, 1997.
[25] G.G. Goss, S. Adamia, and F. Galvez, "Peanut lectin binds to a subpopulation of mitochondria-rich cells in the rainbow trout gill epithelium", *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 281, 1718-1725, 2001.
[26] C. Calabrò, M. P. Albanese, E. R. Lauriano, S. Martella, and A. Licata, "Morphological, histochemical and immunohistochemical study of the gill epithelium in the abyssal teleost fish *Coelorhynchus coelorhynchus*," *Folia Histochemica et Cytobiologica*, 43(1), 51-56, 2005.

[27] K. S. Jung, M. J. Ahn, Y. D. Lee, G. M. Go, and T. K. Shin, "Histochemistry of six lectins in the tissues of the flat fish *Paralichthys olivaceus*," *Journal of Veterinary Science*, 3 (4), 292-301, 2002.
[28] G. Kato, H. Miyazawa, Y. Nakayama, Y. Ikari, H. Kondo, T. Yamaguchi, M. Sano, and U. Fischer, "A novel antigen-sampling cell in the teleost gill epithelium with the potential for direct antigen presentation in mucosal tissue," *Frontiers in Immunology*, 9, 1-12, 2018
[29] C. Lemaitre, N. Orange, P. Saglio, N. Saint, J. Gagnon, and G. Molle, "Characterization and ion channel activities of novel antibacterial proteins from the skin mucosa of carp (*Cyprinus carpio*)," *European Journal of Biochemistry*, 240, 143-149, 1996.
[30] N. Blackstock and A. D. Pickering, "Acidophilic granular cells in the epidermis of the brown trout, *Salmo trutta* L.," *Cell and Tissue Research*, 210 (3), 359-369, 1980.
[31] A. D. Pickering and J. M. Fletcher, "Sacciform cells in the epidermis of the brown trout, *Salmo trutta*, and the Arctic char, *Salvetinus alpinus*," *Cell and Tissue Research*, 247 (2), 259-265, 1987.