Pakistan J. Zool., vol. 49(2), pp 445-453, 2017. **DOI:** http://dx.doi.org/10.17582/journal.pjz/2017.49.2.445.453

Histochemical Characterization of Convict Cichlid (*Amatitlania nigrofasciata*) Intestinal Goblet Cells

Sezgi Arman* and Sema İşisağ Üçüncü
Department of Biology, Ege University, 35100 Bornova-Izmir, Turkey

ABSTRACT

In the present study, some histochemical features of goblet cells of the intestine of convict cichlid (*A. nigrofasciata*) were described. In order to reveal the main histological construction, transverse sections of different parts of intestine were firstly stained with hematoxylin eosin (H-E). Sections of anterior, mid and posterior intestinal segments were also treated with different staining methods of alcian blue (AB) at pH 2.5, aldehyde fuchsin (AF), periodic acid-Schiff (PAS), KOH/PAS and PAS/AB (pH 2.5) and investigated. Goblet cells of anterior intestine were colored strongly by AB (pH 2.5), AF and PAS; and moderately by PAS/AB (pH 2.5) (AB dominant). In the mid part of the gastrointestinal tract, goblet cells were also stained strongly with AB, PAS and PAS/AB (pH 2.5) (AB dominant), however, their reactions to KOH/PAS treatment were recorded as negative. Because of numerous supranuclear vacuoles of the epithelial cells, only a few goblet cells could be differentiated in posterior parts of alimentary channel, with their weakly reaction to AB (pH 2.5), PAS and PAS/AB (pH 2.5). Moreover, these cells were not stained with AF and KOH/PAS. According to their affinities, goblet cells oriented in anterior and the mid intestine were mainly classified as acidic (AB-positive) and neutral (PAS-positive). Statistical analysis were confirmed that, the numbers of acidic and neutral cells of per unite square of epithelial area were significantly different.

Article Information
Received 08 October 2015
Revised 09 September 2016
Accepted 20 September 2016
Available online 30 January 2017

Authors' Contributions
SA designed the study, performed experimental work and analyzed the data. SİÜ helped in microscopic examinations. SA and SİÜ were involved in manuscript write up.

Key words
Goblet cell, Intestine, Glycoconjugate, Glycoprotein, *Amatitlania nigrofasciata.*

INTRODUCTION

Inspite of some morphological and functional alterations due to feeding habits, body form, weight and sex (Grosh and Das, 1987; Boglione *et al*., 1992; Murray *et al*., 1994; Cinar and Şenol, 2006), the main histological organization of the alimentary channel of teleosts is similar. In order to determine the complicated activities of intestine, understanding of the coordinated interactions of cells within different tissue layers is required. In addition to their essential functions noted as absorption and transportation, goblet cells, the main secretory units of the intestinal mucosa, have some important defense mechanism against irritants, microbial attachments and invasion (Phalipon *et al*., 2002; Bruno *et al*., 2005; Strugnel and Wijburg, 2010; Hasnain *et al*., 2013; Kim and Khan, 2013).

Mucoid substance of goblet cells is mainly responsible for hydrating and protecting the intestinal mucosa. It is composed of both organic and inorganic materials such as lipids, proteins, glycoproteins, nucleic acids, salt and water (Hasnain *et al*., 2010, 2013; Bosi and Dezfuli, 2015). As a physiological barrier, mucus is an essential layer of the innate immune system and plays an important role in the regulation and protection of intestinal mucosa.

* Corresponding author: sezgiarman@gmail.com
0030-9923/2017/0002-0445 $ 9.00/0

The functional integrity of the intestinal mucosal epithelial cells depends on the regulation of the mucus layer, the intercellular tight junction, epithelial cells, and host innate and adaptive immune response (Kim and Ho, 2010).

Methods to identify the contents of goblet cells may be helpful for expanding the data on complex intestinal functions. The distribution and histochemical properties of goblet cells of gastrointestinal tract of some teleosts have previously been investigated by various histochemical techniques (Sarasquete *et al*., 2001; Díaz *et al*., 2003; Leknes, 2010, 2015). However, the reports are still limited and further studies are needed.

The present study is based on histochemical demonstration of glycoproteinic contents of the intestine of a popular aquarium fish *Amatitlania nigrofasciata*, which has also been used for behavioral studies (Townshend and Wootton, 1985; Budaev *et al*., 1999; Grant *et al*., 2002; Foam *et al*., 2005). For comparison of the content of mucosubstances of different parts of digestive tract, the cells oriented in anterior, mid and posterior parts of intestine were identified by their specific staining properties. Some statistical data related to goblet cell distribution was also evaluated.

MATERIALS AND METHODS

All experiments were performed in accordance with the guidelines for animal research established by the

Local Ethics Committee of Animal Experiments at Ege University (Certificate number: 000018).

Fish maintenance

Ten specimens of adult *A. nigrofasciata* were obtained from commercial dealers. Fishes were maintained in well aerated glass aquaria at 26±2°C, 14 h light:10 h dark photoperiod and fed twice a day with *Artemia* sp. for two weeks before the experiment. Specimens were euthanatized with overdose of MS-222 (Sigma). The whole intestine was removed and equally divided into three segments as anterior, mid and posterior.

Histochemistry

Tissue samples were fixed in Bouin's fluid for 24 h at 4ºC, rinsed in distilled water, dehydrated in ethanol, treated with xylene and embedded in paraffin. In order to demonstrate the general morphology of the intestine, 5 µm-thick transverse sections were stained with hematoxylin-eosin (H-E). To identify different contents of the goblet cells, the staining methods of alcian blue (AB) at pH 2.5 for acidic glycoconjugates with carboxylated and sulfate esters; aldehyde fuchsin (AF) for glycoconjugates with sulfate; periodic acid (0.5%)-Schiff reagent (PAS) for neutral glycoconjugates; potassium hydroxide/PAS (KOH/PAS) for glycoconjugates with sialic acid residues; and PAS/AB (pH 2.5) to distinguish between neutral and acidic glycoconjugates (Çınar and Şenol, 2006) were performed (Presnell *et al*., 1997). Slides were examined and photographed by Leica DM300 microscope equipped with a Leica digital camera (DFC290) and Zeiss Axio Scope. A1 equipped with Zeiss AxioCam ERc5s.

Goblet cells: differentiation and counting

By using the grid plugin of Image J software, AB-positive and PAS-positive cell populations in per unit (mm^2) of the epithelial area of randomly choosen from five to ten microphotographs of both anterior and the mid intestine were examined. For each specimen, AB-positive and PAS-positive goblet cells in random 100 grids were counted. The total grid area was presented in mm^2.

Statistical analysis

The analysis were performed with SPSS 20.0 software and the data was evaluated by 2-tailed *t*-test for independent samples ($p \leq 0.05$).

RESULTS

The intestinal wall of *A. nigrofasciata* is composed of the four layers called as serosa (S), muscularis externa (ME), submucosa (SM) and mucosa (M) (Fig. 1). Serosa, the outmost layer of the intestine was composed of a thin layer of simple squamous epithelium. Muscularis externa was consisted of smooth muscle fibers. In all of the intestinal segments investigated, numerous eosinophilic granule-containg cells and blood vessels were observed at the loose connective tissue forming submucosa. The mucosa was displayed of single layer of characteristic columnar epithelium (E) and lamina propria (LP). More folds (villi) were observed in the mucosa of the anterior part than those of the mid and posterior intestine, and the height of villi was gradually decreased towards mid and posterior. The epithelial cell layers of the anterior intestine were consisted mainly of columnar enterocytes, sac-shaped goblet and a few APUD cells (Figs. 1, 2).

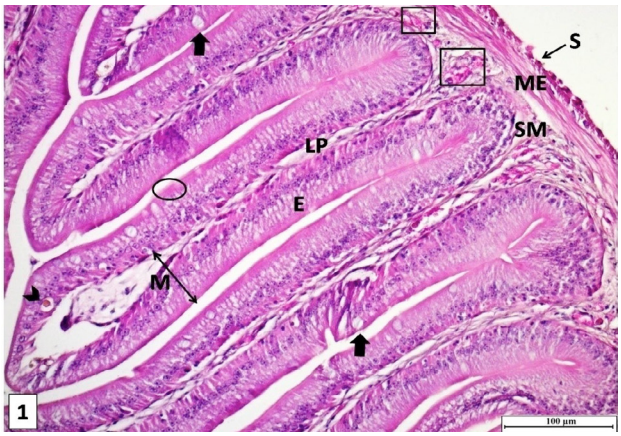

Fig. 1. Transverse section of the anterior intestine of *Amatitlania nigrofasciata*. Serosa (S), muscularis externa (ME), submucosa (SM) with numerous eosinophilic granule-containing cells (rectangle), and mucosa (M) with epithelial layer (E) and lamina propria (LP), note the brush border (encircled), APUD cells (arrowhead) and goblet cells (arrows). Stain: H-E.

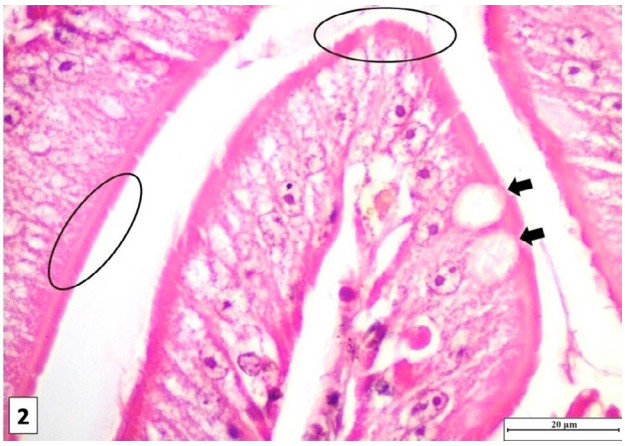

Fig. 2. Transverse section of mid intestine of *Amatitlania nigrofasciata*. Goblet cells (arrows) and thick brush border (encircled). Stain: H-E.

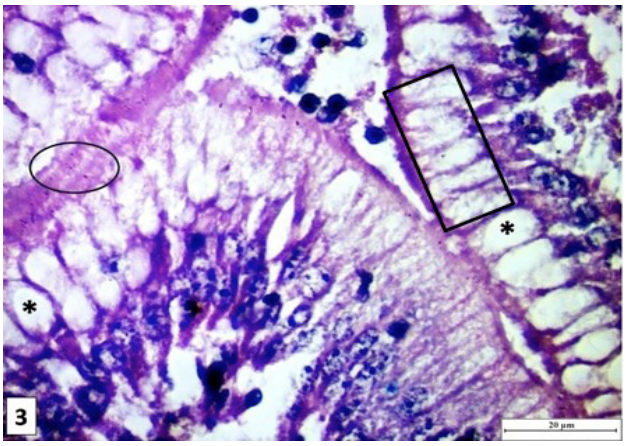

Fig. 3. Transverse section of posterior intestine of *Amatitlania nigrofasciata*. Epithelial cells occupied with numerous, heterogenous supranuclear vacuoles (rectangled) and filled with giant ones (asterisks); note also brush border (encircled). Stain: H-E.

Goblet cells which were characterized with their typical shape, basally located nucleus and scarce organelles, were abundant in the epithelial layer of the anterior intestine. The middle intestine was exhibited similar histological features to the anterior intestine. The most striking feature of the enterocytes of posterior intestine was noted as the presence of large and clear supranuclear vacuoles. Although most of the posterior enterocytes were exhibited a number of heterogen vacuoles, some of them had fusioned, giant and homogenous ones (Fig. 3). Only a few and small goblet cells could be observed in this segment, moreover, APUD cells were not seen. The dense brush border of enterocytes was easily observed in all of the segments investigated (Figs. 1, 2, 3), but Paneth cells were not identified. The differences in reaction intensities of the goblet cells of three intestinal segments are given in Table I.

Anterior and mid intestine

The strong reaction of goblet cells with AB at pH 2.5 indicated acidic glycoprotein content of the mucus (Figs. 4a, 5a).

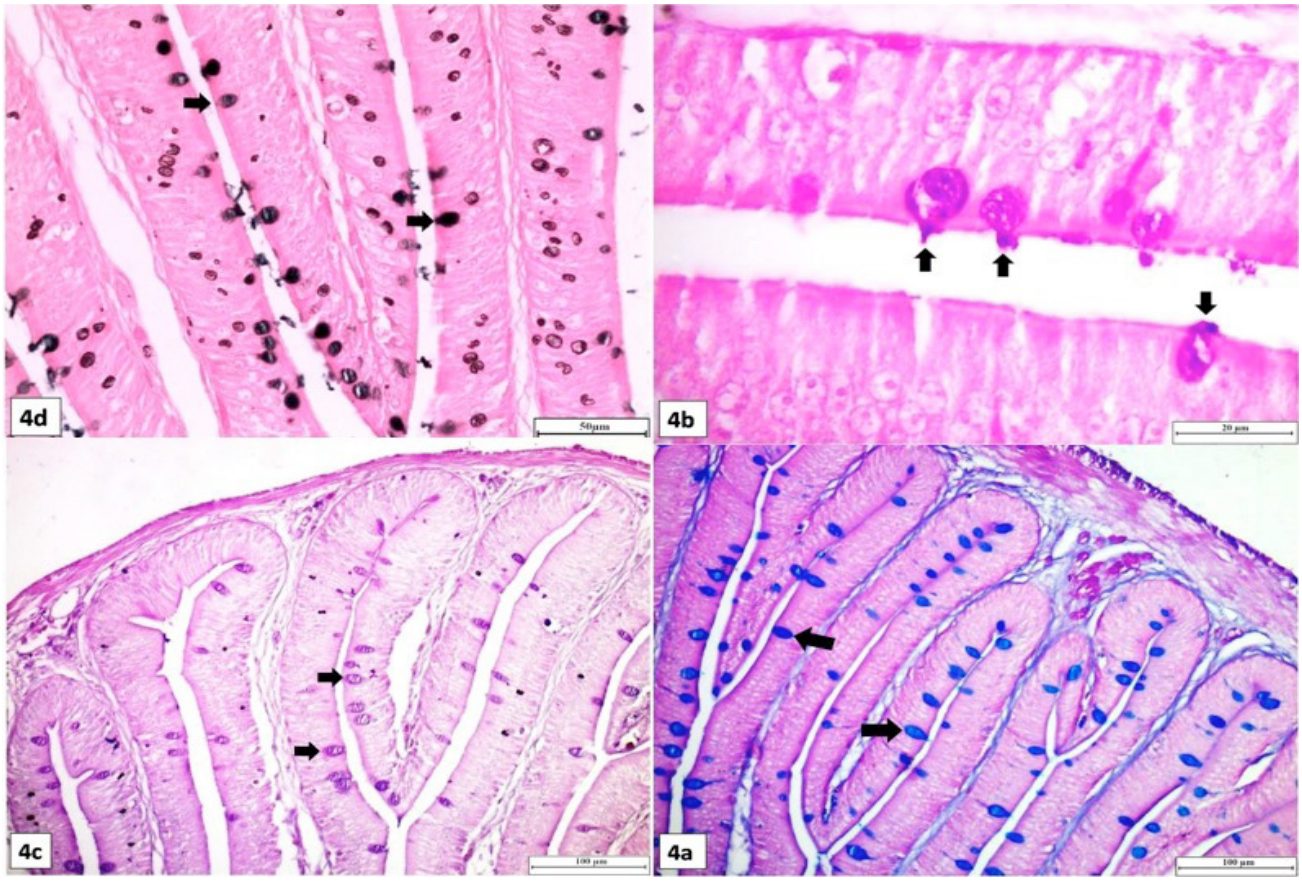

Fig. 4. Goblet cells (arrows) in transverse sections of anterior intestine of *Amatitlania nigrofasciata* stained with different methods: **a,** AB (pH 2.5); **b,** PAS; **c,** AF; **d,** AB (pH 2.5) followed by PAS.

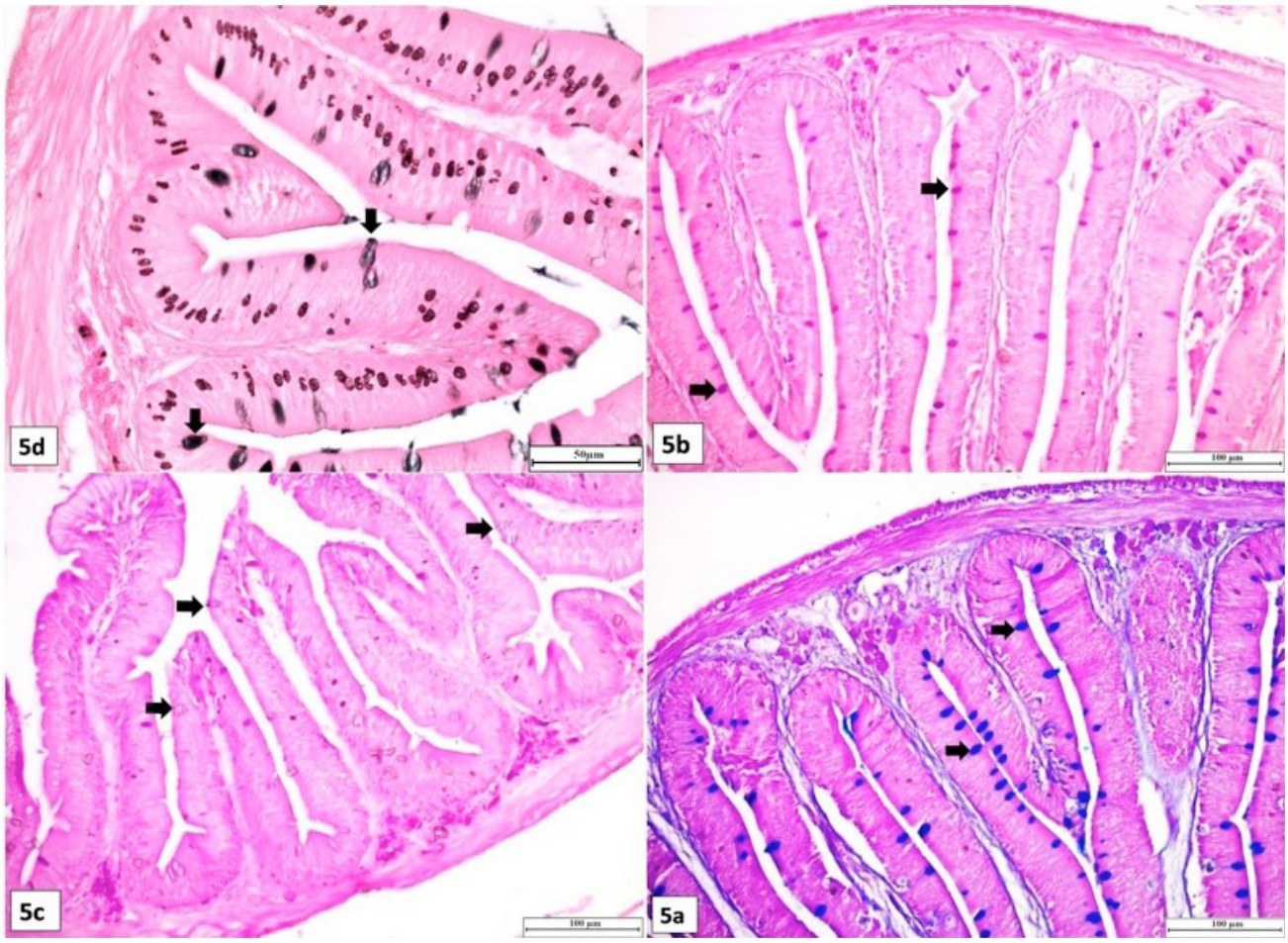

Fig. 5. Goblet cells (arrows) in transverse sections of mid intestine of *Amatitlania nigrofasciata* stained with different methods: **a,** AB (pH 2.5); **b,** PAS; **c,** AF; **d,** AB (pH 2.5) followed by PAS.

Table I.- Reaction intensities of goblet cells of anterior, mid and posterior intestine *of A. nigrofasciata.*

	Ant. Int.	Mid. Int.	Post. Int.
AB (pH 2.5)	+++	+++	±
AF	+++	++	-
PAS	+++	+++	±
KOH/PAS	+	-	-
PAS/AB/ (pH 2.5)	++	+++	±

Degree of intensity: -, negative; ±, poorly; +, weakly; ++, moderately, +++, strongly stained. AB, alcian blue; AF, aldehyde fuchsin; KOH/PAS, potassium hydroxide/ periodic acid-Schiff's reagent; PAS, periodic acid-Schiff's reagent; PAS/AB, periodic acid-Schiff's/alcian blue.

Since mucus consists of glycoproteins with oxidizable vicinal diols and/or glycogen, goblet cells were stained as magenta in color with PAS method (Figs. 4b, 5b). Reactions to PAS/AB (pH 2.5) staining of the goblet cells of these parts were slightly different, while AB was dominant for both of them. When compared to anteriorly oriented ones which were moderately stained, mid intestinal cells showed strong reaction (Figs. 4d, 5d).

In the anterior intestine, goblet cells were strongly stained with AF, whereas mid intestinal ones were moderately stained (Figs. 4c, 5c). The content of the AF-positive, purple cells was composed of sulfated glycoproteins.

The anteriorly embedded goblet cells were weakly stained as pale pink by KOH/PAS method, the cells of mid intestine were however, not colored. The low affinity to KOH/PAS proved that the mucous content lacked sialic acid residues.

Posterior intestine

Only a few, small goblet cells which could rarely be observed, were poorly stained AB at pH 2.5 (Fig. 6), PAS and PAS/AB (pH 2.5), however they did not react with

AF and KOH/PAS methods. Because of scarce distribution of posteriorly oriented goblet cells, only anterior and mid intestine data was statistically compared (Table II). One way-ANOVA analysis indicated that the number of acidic and neutral goblet cells in the anterior and mid intestine were significantly different. Both of the acidic and neutral cells of mid intestine were higher in number than anteriorly oriented ones.

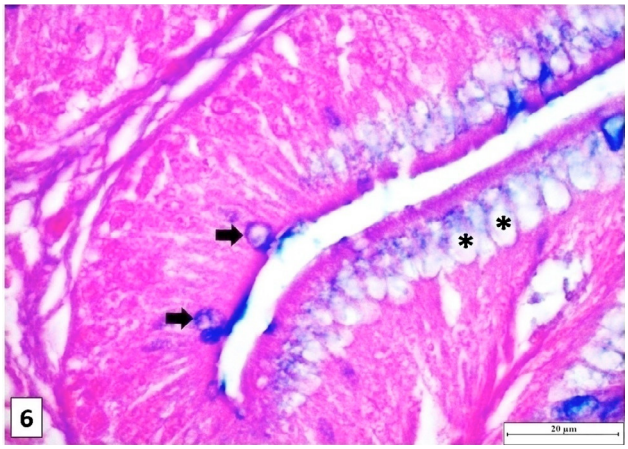

Fig. 6. Transverse section of the posterior intestine of *Amatitlania nigrofasciata* stained with AB; note vacuolar enterocytes (asterisks) and a few, poorly stained goblet cells (arrows).

Table II.- Comparison of acidic and neutral goblet cells per mm² of anterior and mid intestine of *A. nigrofasciata.*

	Mean	SD	SEM	Sig. (2-tailed)	*t*-value
Acidic goblet cells (AB-Positive)					
Anterior intestine	1392.00	806.99	80.69	0.001	3.345
Mid intestine	1616.00	691.46	69.14	0.000	3.614
Neutral goblet cells (PAS-Positive)					
Anterior intestine	1060.00	578.04	57.80	0.001	3.345
Mid intestine	1272.00	654.13	65.413	0.000	3.614

SD, standard deviation; SEM, standard error of mean. $p \leq 0.05$ by 2-tailed *t*-test.

DISCUSSION

Although the alimentary canal shows striking differences in its morphology and function due to variation of habitats and feeding behavior, the histological structure of intestine of different species comprises of four layers which is common in all fishes. Several authors have shown that post-gastric parts of alimentary canal of fishes are responsible for nutrient absorption (Kapoor *et al*., 1975; Fänge and Grove, 1979; Morrison, 1987; Murray *et al*., 1996; Cinar and Şenol, 2006; Leknes, 2010). The histochemical characterization of this part was generally associated with feeding habits (Martin and Blaber, 1984; Grosh and Das, 1987; Murray *et al*., 1996). Consequently, the numbers and types of the cells located in post-gastric parts were differed due to the feeding behavior (Tyler, 1972; Kapoor *et al*., 1975; Martin and Blaber, 1984; Anderson, 1986; Kuperman and Kuz'mina, 1994; Murray *et al*., 1994, 1996). Intestinal parts can also be distinguished by villi sizes which were decreased in length, distinctly (Albrecht *et al*., 2001; Cinar and Şenol, 2006).

Main histological organization of the intestine of *A. nigrofasciata* is considerably similar to other teleosts (Wallace *et al*., 2005) except the abundancy of vacuolar enterocytes of posterior intestine in *Ictalurus punctatus,* Krementz and Chapman (1975) formerly noted that there were large and clear vacuoles present in the apical cytoplasm of epithelial cells of the posterior half of intestine after feeding. Enterocytes with supranuclear vacuoles were also reported in the larvae of *Sparus aurata* (Calzada *et al*., 1998), *Pelteobagrus fulvidraco* (Yang *et al*., 2010), and the juveniles of *Culter alburnus* (Cao *et al*., 2011). While their function is still unclear, some studies indicate that they may be associated with nutrition type or protein digestion (Smith and Sepúlveda, 1989; Cyrino *et al*., 2008; Cao *et al*., 2011). It was also assumed that the supranuclear vacuoles of the posterior enterocytes of *A. nigrofasciata* also might be involved in intracellular digestion.

The essential function of the gastrointestinal epithelium is to regulate counteractions between the external environment and the body (Loretz, 1995; Domeneghini *et al*., 2005). As concluded by Delashoub *et al*. (2010), intestinal digestive activity depends on goblet cell secretions, proteolytic action of pancreatic juice and/or intracellular digestion. Goblet cells as common structural elements of the alimentary tract and histochemical properties of the mucoid substance have been investigated in several teleosts (Domeneghini *et al*., 1998; Pedini *et al*., 2001; Bucke, 1971; Groman, 1982; Tibbetts, 1997; Díaz *et al*., 2003; Leknes, 2011).

Various glycoproteins (neutral, acidic and sulfated) in mucoid substance of different species had special functions such as lubrication, regulation of viscosity, entrapping of food particles, buffering the fluids at the epithelial surface, preclusion of proteolytic epithelial damage, antimicrobial activity and immunological defense (Reid *et al*., 1988; Yashpal *et al*., 2007; Díaz *et al*., 2008; Zhu, 2015). Neutral glycoproteins were involved mainly in absorption and transportation of molecules through the membranes

(Reifel and Travill, 1979; Sarasquete *et al*., 2001; Pedini *et al*., 2005; Díaz *et al*., 2010). As a lubricative and protective secretion, acidic glycoproteins were associated with protection of epithelial damage against the high acidic gastric juices. Sulfated glycoproteins were also lubricative and usually related with the protection against microorganisms (Ferraris *et al*., 1987; Mittal *et al*., 2002; Park *et al*., 2003; Díaz *et al*., 2010; Leknes, 2015).

Domeneghini *et al*. (2005) have shown the presence of neutral and acidic glycoconjugates in intestinal mucus cells of *Anguilla anguilla*. The intestinal goblet cells of *Sparus aurata* were also shown to secrete both neutral and acidic glycoconjugates, especially sulfated forms (Domeneghini *et al*., 1998). Leknes (2011) has noted an ingredient composed of sulfated and neutral mucins in *Hyphessobrycon anisitsi*.

In this study, positive reactions of the goblet cells of anterior and mid intestine of *A. nigrofasciata* to AB, PAS and AF staining methods indicated intense acidic and neutral content, which is an evidence for relatively less sulfated glycoprotein.

PAS/AB (pH 2.5) staining, showed densely acidic glycoconjugates both in the anterior and mid intestinal goblet cells. The fact that the cells stained dominantly with AB at pH 2.5, showed occurrence of glycoproteins with carboxyl groups and/or with sulfate esters. However, negative reaction to KOH/PAS staining was evaluated as absence of sialic acid residues in this content. Presence of glycoproteins with sialic acid residues was correlated with protection against microorganism (Suprasert *et al*., 1987; Díaz *et al*., 2010).

It has also been noted that only anterior intestinal goblet cells exhibited too low affinity to KOH/PAS staining. Neutral glycoconjugates were also observed in the anterior and mid intestine of *A. nigrofasciata.*

As it was mentioned above, goblet cells were rarely distributed in posterior intestine of *A. nigrofasciata.* Several authors have shown that goblet cells increased in number in the posterior intestine, due to ease of defecation (Martin and Blaber, 1984; Cataldi *et al*., 1987; Grau *et al*., 1992; Murray *et al*., 1996; Domeneghini *et al*., 1998; Veggetti *et al*., 1999; Pedini *et al*., 2001; Cinar and Bilgin, 2001; Cinar and Şenol, 2006; Banan Khojasteh *et al*., 2009). At this point, this kind of disagreement could only be explained by abundance of vacuolar enterocytes.

Although, only two parts of intestine could be compared, the results presented in this study are in accordance with those reported in literature. The number of acidic and neutral goblet cells of the mid part of intestine was higher than those in anterior part.

It is concluded that the distribution of goblet cells and glycoconjugate composition of the gut of *A. nigrofasciata* depends on the intestinal region.

ACKNOWLEDGMENTS

We would like to thank Dr. Esra Akat for her contributions and technical support throughout the study.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Albrecht, M.P., Ferreira, M.F.N. and Caramasch, E.P., 2001. Anatomical features and histology of the digestive tract of two related neotropical omnivorous fishes (Characiformes; Anostomidae). *J. Fish Biol*., **58**: 419-430. https://doi.org/10.1111/j.1095-8649.2001.tb02261.x

Anderson, T.A., 1986. Histological and cytological study of the gastro-intestinal tract of the Luderick, *Girella tricuspidata* (Pisces, Kyphosidae), in relation to diet. *J. Morphol*., **190**: 109–119. https://doi.org/10.1002/jmor.1051900110

Banan Khojasteh, S.M., Sheikhzadeh, F., Mohammadnejad, D. and Azami, A., 2009. Histological, histochemical and ultrastructural study of the intestine of rainbow trout (*Oncorhynchus mykiss*). *World appl. Sci. J*., **6**: 1525-1531.

Boglione, B., Bertolini, B., Russiello, M. and Cataduella, S., 1992. Embryonic and larval development of thick-lipped mullet (*Chelon labrosus*) under controlled reproduction conditions. *Aquaculture*, **101**: 349-359. https://doi.org/10.1016/0044-8486(92)90037-L

Bosi, G. and Dezfuli, B.S., 2015. Responses of *Squalis cephalus* intestinal mucous cells to *Pomphorhynchus laevis*. *Parasitol. Int.,* **64**: 167-172. https://doi.org/10.1016/j.parint.2014.11.018

Bruno, L.S., Li, X., Wang, L., Soares, R.V, Siqueira, C.C., Oppenheim, F.G., Troxler, R.F. and Offner, G.D., 2005. Two-hybrid analysis of human salivary mucin MUC7 interactions. *Biochim. biophys. Acta*, **1746**: 65–72. https://doi.org/10.1016/j.bbamcr.2005.08.007

Bucke, D., 1971. The anatomy and histology of the alimentary tract of the carnivorous fish the pike *Esox lucius* L. *J. Fish Biol*., **31**: 421–431. https://doi.org/10.1111/j.1095-8649.1971.tb05914.x

Budaev, S.V., Dmitry, D.Z. and Andrei, D.M., 1999. Individual differences in parental care and behavior profile in the convict cichlid: a correlation study. *Anim. Behav*., **58**: 195–202. https://doi.org/10.1006/anbe.1999.1124

Calzada, A., Medina, A. and Gonzales De Canales, M.L., 1998. Fine structure of the intestine development in cultured sea bream larvae. *J. Fish Biol*., **53**: 340–365. https://doi.org/10.1111/j.1095-8649.1998.tb00985.x

Cao, X.J., Wang W.M. and Song, F., 2011. Anatomical and histological characteristics of the intestine of the topmouth culter (*Culter alburnus*). *Anat. Histol. Embryol*., **40**: 292–298. https://doi.org/10.1111/j.1439-0264.2011.01069.x

Cataldi, E., Ctaudella, S., Monaco, G., Rossi, A. and Tancioni, L., 1987. A study of the histology and morphology of the digestive tract of Sea bream, *Sparus aurata*. *J. Fish Biol*., **30**: 135-145. https://doi.org/10.1111/j.1095-8649.1987.tb05740.x

Cinar, K. and Bilgin, F., 2001. Gökkuşağı alabalığı (*Oncorhynchus mykiss* Walbaum, 1792) bağırsaklarının farklı gelişim dönemlerindeki histolojik ve histokimyasal yapısı. *SDÜ Fen. Bil. Ens. Der*., **5**: 72-85.

Cinar, K. and Şenol, N., 2006. Histological and histochemical characterization of the mucosa of the digestive tract in flower fish (*Pseudophoxinus antalyae*). *Anat. Histol. Embryol*., **35**: 147-151. https://doi.org/10.1111/j.1439-0264.2005.00629.x

Cyrino, J.E.P., Bureau, D.P. and Kapoor, B.G., 2008. *Feeding and digestive functions in fishes*. CRC Press, Boca Raton, Florida.

Delashoub, M., Pousty, I. and Banan Khojasteh, S.M., 2010. Histology of bighead carp (*Hypophthalmichthys nobilis*) intestine. *Glob. Vet.*, **5**: 302-306.

Díaz, A.O., García, A.M., Devincenti, C.V. and Goldemberg, A.L., 2003. Morphological and histochemical chaeacterization of the mucosa of the digestive tract in *Engraulis anchoita*. *Anat. Histol. Embryol*., **32**: 341-346. https://doi.org/10.1111/j.1439-0264.2003.00490.x

Díaz, A.O., García, A.M., Devincenti, C.V. and Goldemberg, A.L., 2008. Glycoconjugates in the mucosa of the digestive tract of *Cynoscion guatucupa*: a histochemical study. *Acta Histochem*., **110**: 76-85. https://doi.org/10.1016/j.acthis.2007.08.002

Díaz, A.O., García, A.M., Esca Lante, A.H. and Goldemberg, A.L., 2010. Glycoproteins histochemistry of the gills of *Odontesthes bonariensis* (Teleostei, Atherinopsidae). *J. Fish Biol*., **77**: 1665–1673. https://doi.org/10.1111/j.1095-8649.2010.02803.x

Domeneghini, C., Ponnelli Staini, R. and Vaggetti A., 1998. Gut glycoconjugates in *Sparus aurata* L. (Pisces, Teleostei). A comparative histochemical study in larval and adult ages. *Histol. Histopathol*., **13**: 359-372.

Domeneghini, C., Arrighi, S., Radaelli, G., Bosi, G. and Vaggetti, A., 2005. Histochemical analysis of glycoconjugate secretion in the alimentary canal of *Anguilla anguilla* L. *Acta Histochem*., **106**: 477-487. https://doi.org/10.1016/j.acthis.2004.07.007

Fänge, R. and Grove, D., 1979. Digestion. In: *Fish physiology* (eds. W.S. Hoar, D.J. Randall and J.R. Brett). Academic Press, Toronto, pp. 162–241. https://doi.org/10.1016/s1546-5098(08)60027-8

Ferraris, R.P., Tan, J.D. and De La Cruz, M.C., 1987. Development of the digestive tract of milkfish, *Chanos chanos*. *Histol. Histochem. Aqua*., **61**: 241–257.

Foam, P.E., Harvey, M.C., Mirza, R.S. and Brown, G.E., 2005. Heads up: juvenile convict cichlids switch to threat-sensitive foraging tactics based on chemosensory information. *Anim. Behav*., **70**: 601-607. https://doi.org/10.1016/j.anbehav.2004.12.011

Grant, J.W.A., Girard, I.L.G., Breau, C. and Weir, L.K., 2002. Influence of food abundance on competitive aggression in juvenile convict cichlids. *Anim. Behav*., **63**: 323-330. https://doi.org/10.1006/anbe.2001.1891

Grau, A., Crespo, S., Saraguete, N.C. and Gonzales d'Canales, N.L., 1992. The digestive tract of the Amberjack, *Seriola dumerili*, Risso: A light and scanning electron microscope study. *J. Fish Biol*., **41**: 387-390. https://doi.org/10.1111/j.1095-8649.1992.tb02658.x

Groman, D.B., 1982. *Histology of the striped bass*. American Fisheries Society Monograph Number 3.

Grosh, A. and Das, K.M., 1987. Morphohistology of the digestive tract of a mullet, *Liza parsia* (Ham) in relations to its food habits. *J. Indian Soc. Coast. Agric. Res*., **5**: 437-444.

Hasnain, S.Z., Wang, H., Ghia, J.E., Haq, N., Deng, Y. and Velcich, A., 2010. Mucin gene deficiency in mice impairs host resistance to an enteric parasitic infection. *Gastroenterology*, **138**: 1763–1771. https://doi.org/10.1053/j.gastro.2010.01.045

Hasnain, S.Z., Gallagher, A.L., Grencis, R.K. and Thornton, D.J., 2013. A new role for mucins in immunity: insights from gastrointestinal nematode infection. *Int. J. Biochem. Cell Biol*., **45**: 364–374. https://doi.org/10.1016/j.biocel.2012.10.011

Kapoor, B.G., Smit, H. and Verighina, I.A., 1975. The alimentary canal and digestion in teleosts. *Adv. Mar. Biol*., **13**: 109–239. https://doi.org/10.1016/S0065-2881(08)60281-3

Kim, J.J. and Khan, W.I., 2013. Goblet cells and mucins: Role in innate defense in enteric infections. *Pathogens,* **2**:55-70. https://doi.org/10.3390/pathogens2010055

Kim, Y.S. and Ho, S.B., 2010. Intestinal goblet cells and mucins in health and disease: recent insights and progress. *Curr. Gastroenterol. Rep*., **12**: 319-330. https://doi.org/10.1007/s11894-010-0131-2

Krementz, A. B. and Chapman, G. B., 1975. Ultrastructure of the posterior half of the intestine of the channel catfish *Ictalurus punctatus*. *J. Morphol*., **145**: 441–482. https://doi.org/10.1002/jmor.1051450405

Kuperman, B.I. and Kuz'mina, V.V., 1994. The ultrastructure of the intestinal epithelium in fishes with different types of feeding. *J. Fish Biol*., **44**: 181–193. https://doi.org/10.1111/j.1095-8649.1994.tb01197.x

Leknes, I.L., 2010. Histochemical study on the intestine goblet cells in cichlid and poecilid species (Teleostei). *Tissue Cell*, **42**: 61-64. https://doi.org/10.1016/j.tice.2009.09.001

Leknes, I.L., 2011. Histochemical studies on mucin-rich cells in the digestive tract of a teleost, the Buenos Aires tetra (*Hyphessobrycon anisitsi*). *Acta Histochem*., **113**: 353-357. https://doi.org/10.1016/j.acthis.2010.01.010

Leknes, I.L., 2015. Mucin in epithelial cells in oesophagus and stomach of black tetra, *Gymnocorymbus ternetzi* (Characidae, Teleostei). *Zoomorphology*, **134**: 269-277. https://doi.org/10.1007/s00435-015-0256-9

Loretz, C.A., 1995. Electrophysiology of ion transport in teleost intestinal cells. In: *Cellular and molecular approaches to fish ionic regulation* (eds. C.M. Wood, T.J. Shuttleworth). Academic Press, London. https://doi.org/10.1016/s1546-5098(08)60241-1

Martin, T.J. and Blaber, J.M., 1984. Morphology and histology of the alimentary tract of Ambassidae (Teleostei) in relation to feeding. *J. Morphol*., **182**: 295-305. https://doi.org/10.1002/jmor.1051820305

Mittal, S., Pinky and Mittal, A.K., 2002. Characterisation of glycoproteins in the secretory cells in the operculum of an Indian hill stream fish *Garra lamta* (Hamilton) (Cyprinidae, Cypriniformes). *Fish Physiol. Biochem.,* **33**: 35–48.

Morrison, C.M., 1987. Histology of the Atlantic Cod, *Gadus morhua*: *An Atlas. Part one. Digestive tract and associated organs*. Canadian Special Publications in Fisheries and Aquatic Science, pp. 98.

Murray, H.M., Wright, G.M. and Goff, G.P., 1994. A comperative histological and histochemical study of the stomach from three species of *Pleuronectid*, the Atlantic halibut, *Hippoglossus hippoglossus*, the yellowtail flounder, *Pleuronectes ferruginea*, and the winter flounder, *Pleuronectes americanus*. *Can. J. Zool*., **72**: 1199-1210. https://doi.org/10.1139/z94-161

Murray, H.M., Wright, G.M. and Goff, G.P., 1996. A comparative histological and histochemical study of the post-gastric alimentary canal from three species of pleuronectid, the atlantic halibut, the yellowtail flounder and the winter flounder. *J. Fish Biol*., **48**: 187–206. https://doi.org/10.1111/j.1095-8649.1996.tb01112.x

Park, J.Y., Kim, I.S. and Kim, S.Y., 2003. Structure and histochemistry of the skin of a torrent catfish, *Liobagrus mediadiposalis*. *Environ. Biol. Fish*, **66**: 3–8. https://doi.org/10.1023/A:1023298520696

Phalipon, A., Cardona, A., Kraehenbuhl, J.P., Edelman, L., Sansonetti, P.J. and Corthésy, B., 2002. Secretory component: a new role in secretory IgA-mediated immune exclusion *in vivo*. *Immunity*, **17**: 107–115. https://doi.org/10.1016/S1074-7613(02)00341-2

Pedini, V., Scocco, P., Radaelli, G., Fagioli, O. and Ceccarelli, P., 2001. Carbohydrate histochemistry of the alimentary canal of the shi drum, *Umbrina cirrosa*. L. *Anat. Histol. Embryol*., **30**: 345-349. https://doi.org/10.1046/j.1439-0264.2001.00345.x

Pedini, V., Dall'Aglio, C., Parillo, F. and Scocco, P., 2005. Glycoconjugate distribution in gastric fundic mucosa of *Umbrina cirrosa* L. revealed by lectin histochemistry. *J. Fish Biol*., **66**: 222–229. https://doi.org/10.1111/j.0022-1112.2005.00596.x

Presnell, J.K., Schreibman, M.P. and Humason, G.L., 1997. *Humason's animal tissue techniques*. Johns Hopkins University Press.

Reid, P.E., Volz, D., Cho, K.Y. and Owen, D.A., 1988. A new method for the histochemical demonstration of O-acyl sugar in human colonic epithelial glycoproteins. *Histochem. J.,* **20**: 510-518. https://doi.org/10.1007/BF01002649

Reifel, C.W. and Travill, A.A., 1979. Structure and carbohydrate histochemistry of the intestine in ten teleostean species. *J. Morphol.,* **162**: 343–360. https://doi.org/10.1002/jmor.1051620305

Sarasquete, C., Gisbert, E., Ribeiro, L., Vieira, L. and Dinis, M.T., 2001. Glycoconjugates in epidermal, branchial and digestive mucous cells and gastric glands of gilthead sea bream, *Sparus aurata*, Senegal sole, *Solea senegalensis* and Siberian sturgeon, *Acipenser baeri* development. *Eur. J. Histochem*., **45**: 267-278. https://doi.org/10.4081/1637

Smith, M.W. and Sepúlveda, F.V. (Eds.), 1989. *Adaptation and development of gastrointestinal function*. Manchester University Press.

Strugnell, R.A. and Wijburg, O.L., 2010. The role of secretory antibodies in infection immunity. *Nat. Rev. Microbiol*., **8**: 656–667. https://doi.org/10.1038/nrmicro2384

Suprasert, A.T., Fujioka, T. and Yamada, K., 1987. The histochemistry of glycoproteins in the colonic epithelium of the chicken. *Histochemistry,* **86**: 491–497. https://doi.org/10.1007/BF00500622

Tibbets, I.R., 1997. The distribution and function of mucous cells and their secretions in the alimentary tract of *Arrhamphus sclerolepis krefftii*. *J. Fish Biol*., **50**: 809–820. https://doi.org/10.1111/j.1095-8649.1997.tb01974.x

Townshend, T.J. and Wootton, R.J., 1985. Adjusting parental investment to changing environmental conditions: the effect of food ration on parental behavior of the convict cichlid, *Cichlasoma nigrofasciatum*. *Anim. Behav*., **33**: 494-501. https://doi.org/10.1016/S0003-3472(85)80072-5

Tyler, A.V., 1972. *Alimentary tract morphology of selected North Atlantic fishes in relation to food habits*. Fisheries Research Board of Canada Technical Report Nos, 360–370.

Veggetti, A., Rowlerson, A., Radaelli, G., Arrighi, S. and Domeneghini, C., 1999. Posthatching development of the gut and lateral muscle in the Sole, *Solea solea* (L). *J. Fish Biol*., **59**: 44–65. https://doi.org/10.1111/j.1095-8649.1999.tb01045.x

Wallace, K.N., Akhter, S., Smith, E. M., Lorent, K. and Pack, M., 2005. Intestinal growth and differentiation in zebrafish. *Mech. Dev*., **122**: 157-173. https://doi.org/10.1016/j.mod.2004.10.009

Yang, R., Xie, C., Fan, Q., Gao, C. and Fang, L., 2010. Ontogeny of the digestive tract in yellow catfish *Pelteobagrus fulvidraco* larvae. *Aquaculture*, **302**: 112-123. https://doi.org/10.1016/j.aquaculture.2010.02.020

Yashpal, M., Kumari, U., Mittal, S. and Mittal, A.K., 2007. Histochemical characterization of glycoproteins in the buccal epithelium of the catfish, *Rita rita*. *Acta Histochem*., **109**: 285-303. https://doi.org/10.1016/j.acthis.2007.03.002

Zhu, L., 2015. Histological and histochemical study on the stomach (Proventriculus and Gizzard) of black-tailed crake (*Porzana bicolor*). *Pakistan J. Zool*., **47**: 607-616.