



**HISTOMORPHOLOGIC STUDY ON OSCAR FISH (*ASTRONOTUS OCELLATUS*)
INTESTINE**

MORTAZAVI TABRIZI¹, S. A. MORTAZAVI P^{2*}, POUSTY I³

1: Graduated of Department of Basic sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran

2: Department of pathology, Science and Research Branch, Islamic Azad University, Tehran, Iran

3: Department of Anatomy, Science and Research Branch, Islamic Azad University, Tehran, Iran

***Corresponding Author: E Mail: sp.mortazavi@srbiau.ac.ir**

ABSTRACT

Oscar fish (*Astronotus ocellatus*) is a well-known Carnivorous aquarium fish and is a member of Chichlidae family which lives in freshwater. Therefore, its gastrointestinal recognition is useful in order to percept the physiologic and abnormal conditions. In this study, Oscar Fish intestine histomorphology was studied. For this purpose, 20 healthy Oscar fish average weighing 50 ± 1 g were obtained from Gogan aquarium fish reproduction center. The samples were transferred to the histology lab of the Tabriz Islamic Azad University. Sections of intestine (anterior, midgut, and posterior) were fixed in 10% formalin solution followed by necropsy. Following fixation, the section was prepared with 6-7 thickness using common methods for preparing tissue samples. Then, they were stained and subjected under histological study using optical microscope. The results showed that intestine wall composed of mucous, sub mucous, muscular, and serous layers. Mucous membrane has many folds which become shorter and wider from anterior to posterior part. Mucosal lining is a simple cylindrical type composed of intestinal cells with Brush Border in which there are the mucous secreting cell goblets. The goblets colored in periodic acid schiff staining. A loose connective tissue there is under the mucosal lining. Muscular layer has two parts of smooth muscle: interior annular part is thicker than an exterior longitudinal layer. There are a large number of

nerves between the two layers. The interior longitudinal muscle layer of anterior part of the intestine is thicker than posterior part and finally a serous layer is observed.

Keywords: Oscar Fish, Intestine, Histomorphologic

INTRODUCTION

Oscar Fish with a scientific name of *Astronotus ocellatus* is a well-known Carnivorous aquarium fish and is a member of Chichlidae family which lives in freshwater. This fish is native to the Amazon River, Paraguay, and Venezuela East. The length of Oscar is almost 25 cm and if it lives in natural habitat can survive almost 15 years [40]. The digestive tract is a tubular structure which starts from mouth to the anus. On the whole, the digestive tract of the fishes is divided into four parts [11]. The apical region is the most anterior part of the tract and composed of mouth cavity (gill or pharyngeal). The second region is the anterior part which starts from behind the gills and composed of Esophagus, Stomach, and Pylorus. This part of esophagus and intestine extends to the front of the bile duct. It must be noted that this kind of division is done to ease of anatomical study. The third region is the midgut which starts from the posterior part of pylorus. Midget often has varying numbers of pylorus cecums with pyloric enclosures being near the pylorus. There are no enclosures in fishes lacking stomach (common carp fish). Midgut is the longest part of the intestine, and when its length is longer than

abdominal cavity, some twists appear in midgut. In some fish, the fourth part of digestive tract or posterior intestine is specified by increased intestine diagonal that leading to anus. Compared with mammalian, the cecum, in fish, is seen rarely beside the posterior intestine [3, 7]. The digestive structure in various species of fish is different depend on food type, nutritional behavior, and body shape [21, 25, 36]. Also, maybe some changes are observed in a species, according to its habitat, season, age, and reproduction time [30]. Histological pattern of fish and Land-dwelling vertebrates is similar. The digestive tract wall in fish is formed of four layers that from interior to exterior including: mucosal layer, sub mucosal layer, muscular layer, and serous layer [14, 33, 39].

Mucosal layer is the most interior one on the digestive tract wall that covers its inner surface. The layer itself is formed from various parts; the most exterior part of mucosal layer is epithelial. Mucosal Epithelial is a simple cylindrical type throughout the digestive tract except the first and end parts; these parts are covered with squamous epithelial. In mucosal

Epithelial, besides the epithelial cells, there are mucus-secreting cells, WBC, Lymphocytes [27]. Mucus-secreting cells maybe have spherical, urceolate, or oval shapes that are seen among epithelial cells, sporadically [24]. The secreted material is different according to fish species as well as various routes of the digestive tract [9, 25]. There is a connective tissue under the mucus epithelial which is composed of collagen fibers and Sugar compounds called the basement membrane. A loose connective tissue there is under the basement membrane which is full of capillaries called Paryn [27]. In the mucus layer, there are bunches of smooth muscles called mucous muscles which may be involved in intestine mucous folds movements. Mucousal muscle has a low growth in fish [3]. The sub-mucous layer in true bony fish composed of one or some connective tissue(s). The dense layer is composed of dense collagen fibers. Around and among the collagen fibers the granular eosinophilic cells are seen which form together the granular layer [34]. In true bony fish, the muscular layer composed of two parts that are called circular muscle layer and longitudinal muscle layer in terms of the axis of muscle fibers [34]. The most exterior layer of the digestive tract is the serous layer which composed of a row of squamous epithelial cells that are located along the membrane of the visceral

peritoneum. A fine connective tissue is located under the serous membrane called sub-serous membrane [27, 5]. The structure of the various parts of the digestive tract is different significantly in various species of fish and even maybe there are not some layers in some parts of the digestive tract. Considering the economical importance of aquarium fish breeding, conducting diverse studies on biological features of this species, especially about their intestine is very important, since the stable growth of the fish depends on the intestine natural function. Furthermore, the digestive tract is continuously exposure to the microbial and non-microbial diseases; so, the study on the structure of the tract can be useful in understanding the digestive physiology, disease prevention, and improved feed conversion ration.

METHODS AND MATERIALS

20 healthy Oscar fish average weighing 50 ± 1 g were obtained from aquarium fish breeding center of Gorgan in order to histomorphologic studies on intestine. The macroscopic features were recorded then they were anesthetized by hitting behind their head. The fish were located on their right lateral side on a non-absorbent surface. An incision was made in front of the pelvic girdle to minimize the risk of inadvertent cutting of intestine. It was tried to avoid any damage to the intestines. The second

incision was created between the anus and mandible, the third incision was created from under the mandible to up side and behind the gills, and the fourth incision was created a diagonal from anus to the upper apex of gills. Then the muscles were cut and removed by forceps and scissors; so, all viscera were observable [17]. In order to complete observation of all parts, the digestive tract of the fish was separated carefully from other organs such as liver and genitals. The esophagus is very short in Oscar fish as well as intestine due to being carnivore; so, rectum is not visible at the end part of the intestine. Tissue samples were obtained from the anterior, mid, and posterior parts of the intestine using a sharp scalpel and standard methods. The length of each sample was measured with a caliper to 75 mm. The samples immersed immediately in 10% formalin buffer in order to fix. Following the fixation process, the section serial was obtained from each sample and then samples stained with H&E and Periodic acid–Schiff (PAS) routine methods [22].

Followed by staining, the samples must be covered with a cleaned and dried coverslip with methanol alcohol in order to fix them on the slides; so, a drop of enthelan glue purred on the stained slice and the coverslip was located in such a way that remained no air bubble between slide and coverslip (angle of 45°). In order to uniformity of the

sample and the least distance between the slide and coverslip, a slight force was exerted on coverslip using forceps. The excess glue was removed around the coverslip after drying [26].

RESULTS

All parts of the intestine structure are similar and it has four layers: mucousal layer, sub-mucousal layer, the smooth muscle layer and serous layer. The mucous folds in anterior part are longer than the other parts of the intestine (**Figure 1**). The intestine mucous has simple cylindrical epithelial cells which are seen longitudinally with an oval nuclear in the cell basal and some goblet cells are seen among them sporadically. Most of intestine goblet cells must react positively with PAS if having saccharid mocopoly. In Oscar fish the goblet cells are seen in pink color because of the positive reaction. The smooth muscular layer is composed of two layers: circular (interior part) and longitudinal (exterior part) layers. Circular muscle fibers are visible with a long nuclear and longitudinal fibers are seen with spherical nuclear. In anterior part of the intestine, the interior circular muscle layer is thicker than the exterior longitudinal layer. There is expanding neural network between two muscular layers. The exterior part is covered with serous that composed of a loose connective tissue along with a layer of

simple squamous cells. The midgut is very similar to anterior part of intestine in terms of structure, but the folds of mucosal layer is shorter in midgut and the thickness of circular muscle layer decreases slightly compared with the first part of the intestine. In this part the neural network is seen widely between circular and longitudinal muscle layers (**Figure 3**).

The length of intestine folds and the thickness of circular muscle are much less than other parts of the intestine. By

decreasing the fold number of the mucosal layer in the posterior part, the absorption of nutrition decreases compared to the other parts of the intestine (**Figure 2**), but the goblet cell number increases in this part of the intestine. The increased number of goblet cells in the posterior part of the intestine resulted in increased sliding of undigested foods in the intestine; so, the act of defecation becomes easier (**Figure 3-5 and 3-6**).

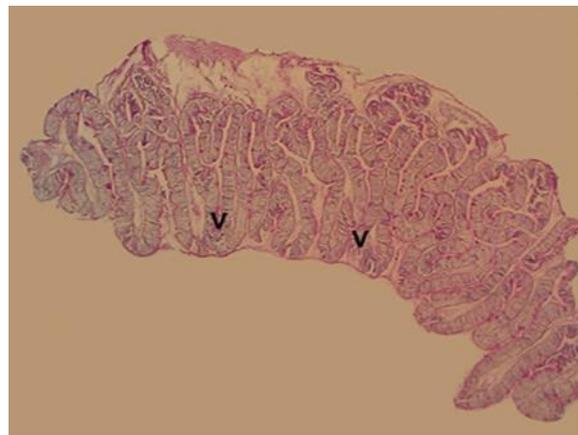


Figure 1: transect of anterior part of the Oscar fish intestine (PAS, X40)

V: observation of the long naps in the first part of the intestine suggesting high absorbance of nutrition in this part

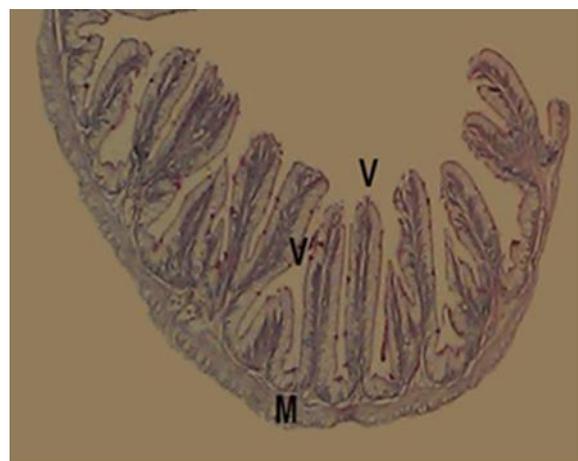


Figure 2: transect of posterior part of the Oscar fish intestine (PAS, X40)

V: observation of the mucous naps with a lower length compared with the first part of the intestine. M: smooth muscular layer

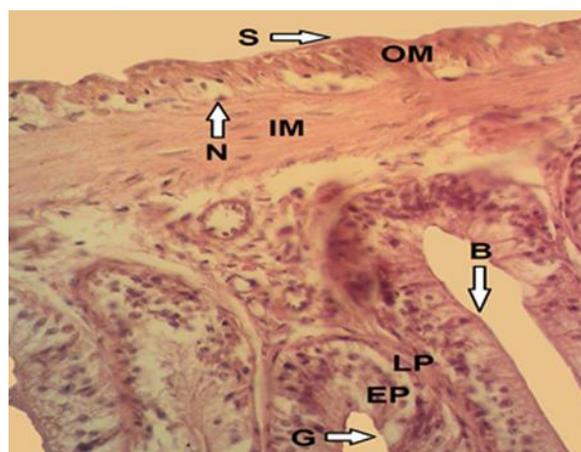


Figure 3: transect of midgut of the Oscar fish (H&E, X400)

G: goblet cells and migrant cells among epithelial tissue cells. EP: simple cylindrical epithelial. B: Brush border LP: lamina propria. IM: interior muscular layer. S: serous layer. OM: outer longitudinal muscular layer. N: neural network between two muscular layers.

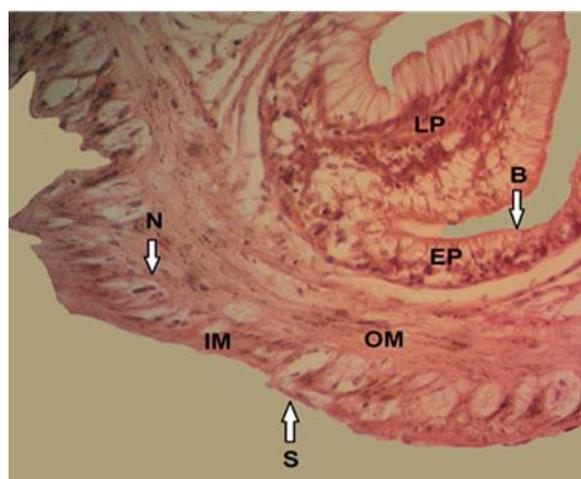


Figure 4: transect of posterior part of the Oscar fish intestine (H&E, X400)

EP: simple cylindrical epithelial. B: Brush border. LP: lamina propria, IM: interior muscular layer. S: serous layer. OM: outer longitudinal muscular layer. N: neural network between two muscular layers.

DISCUSSION

The fish have a digestive tract with an endodermic origin compared with other vertebrates, which originates from the primary digestive tract (Archenteron). According to conducted researches on fishes like Pike, *Oxyeleotris marmoratus*, Summer flounder, Rainbow trout, Hindi carp fish, *Chalcalburnus trichi*, and *Salmo trutta*

caspius, the digestive tract has remained incomplete in fetuses and doesn't distinctly as a direct tube when hatching and placed behind the yolk sac. After a short time of larvae hatching out, the digestive tract structure changes, immediately [14, 16, 28, 35, 38]. The digestive tract of Oscar fish is similar to other bony fishes such as Rainbow Trout, sticky sauce fish, *Tilapia*

Spilurus, White sturgeon, *Strongylura leiura ferox*, and salmon, but there are some small differences between Oscar fish and others. In Oscar fish, like Wolf Fish and Rainbow trout, there are teeth in mouth which are used to crush the food. There are Pharyngeal teeth in some species like Creek fish, Pavel fish, herbivore carp, and needle fish, but in needle fish the use of pharyngeal teeth is to help in food swallowing and have no role in food crushing [23]. The digestive tract in Oscar fish composed of esophagus and intestine and like with tuna fish, Rain bow trout, common sprats and most carnivore fishes the Oscar fish has stomach. There are stomach and Pyloric appendages in Oscar fish. The studies conducted on needle fish suggests that the fish is carnivore but has no stomach [23]. In common carp fish like sticky sauce fish, the bile duct opens to intestine bulb, and the liver lobes cover the intestine bulb and gallbladder near the anterior part of the bulb [19]. The wall of digestive tract in Oscar fish is formed of four main layers, including from inner to outer side: mucosal layer, sub-mucosal layer, muscular layer, and serous layer and this was conformed with the studies conducted on the digestive tract of Rainbow trout, *Caspian Salmon*, *Oxyeleotris marmoatus*, *Tilapia Spilurus*, *Rita rita*, White tuna fish, and most of freshwater fishes. In Oscar fish which is a carnivore,

the intestine length is short like other carnivore fish.

In Oscar fish, the distinction of the area between small and large intestine is difficult in terms of histology. There is no difference among the structure of intestine bulb, the first, mid, and end parts of the intestine, but in fishes like red fish the end part of the intestine has no mucosal folds [9]. In Gold fish, the mucosal folds of large intestine are deeper compared with small intestine [3]. The optical microscope results show that mucosal folds of the Oscar fish intestine become gradually shorter from the first part to the end part of the intestine. This finding is similar to the reports about Rainbow Fish [6], Tuna fish [20], Pike fish, European catfish [25], common carp fish [4], and Silver carp fish [5]. Mucous secreting goblet cells are seen commonly in the bony fish intestine [20]. The secreted material type is different according to the fish species and the various routes of the digestive tract [9, 25]. Based on the conducted studies, the number of goblet cells secreting mucous among cylindrical cells in end part of intestine in Oscar fish is higher than its number at the end part of the intestine, and this has been seen in Rainbow Fish [6], Red Fish [1], Common Carp Fish [4], and Silver Carp Fish [5].

The intestine of Oscar fish contains a muscular layer of smooth muscles that has

two layers: circular and longitudinal. The muscles surround the outer side of the digestive tract like a spring, in such a way that the circular muscles are very spiral than the longitudinal muscles. Also, the circular layer is thicker than the longitudinal layer in inner side [29]. The circular muscular layer is involved in tightening and the longitudinal layer is involved in the shortening of the digestive tract [27]. The movements of muscular layer are controlled by the neural network available in sub-mucosal layer and neural network placed among longitudinal and circular muscle layers. The sub-mucosal neural network of Oscar Fish has no nerve cell bodies. Auerbach neural network of Oscar Fish is well seen between longitudinal and circular muscular layers (**Figure 4**).

The serous layer is the outermost layer of the digestive tract among fishes and other vertebra that composed of a loose connective tissue covered with simple squamous epithelial cells. If there is no simple squamous layer, the serous layer called edvantis that is located around the esophagus, pharynx, anus, at the first and the end part of the digestive tract [27].

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