Anatomical, histological and histomorphometric study of the intestine of the northern pike (*Esox lucius*)

Sadeghinezhad, J.[1*], Hooshmand Abbasi, R.[1], Dehghani Tafti, E.[1], Boluki, Z.[2]

[1]*Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[2]*Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

Key words:
Esox lucius, intestine, anatomy, histology, histomorphometry

Correspondence
Sadeghinezhad, J.
Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Tel: +98(21) 61117116
Fax: +98(21) 66933222
Email: Sadeghinezhad@ut.ac.ir

Received: 28 April 2015
Accepted: 10 August 2015

Abstract:

BACKGROUND: The northern pike *Esox lucius* is a fresh water species belonging to the Esocidae family. It is a carnivorous fish which mostly feeds on invertebrates and fishes. The morphology of its intestine is very useful for understanding the fish's digestive physiology, diagnosing some intestinal diseases and formulating suitable feeds. **OBJECTIVES:** This study was designed to determine the anatomical, histological and histomorphometric properties of the intestine of *E. lucius*. **METHODS:** The intestines of five *E. lucius* were examined in this study. After anatomical dissection, the histological specimens were taken and fixed in 10% formalin. Then, tissue passages were stained with hematoxylin-eosin, and Masson's trichrome. **RESULTS:** The anatomical examination showed the short intestine with intestinal coefficient 0.68±0.09 in *E. lucius* which is a characteristic of the carnivorous species. The histological study revealed that the intestinal wall of *E. lucius* is composed of tunica mucosa, submucosa, muscularis, and serosa. The muscularis mucosa was not visible in the intestine. The stratum compactum is present between tunica mucosa and tunica submucosa. The histomorphometric results differentiated between three parts in the intestine of *E. lucius* namely anterior, middle and posterior. The maximum height of mucosal folds was observed in the anterior intestine due to its role in nutrient absorption. The mucosal fold's height then decreased towards the posterior intestine. The tunica muscularis is significantly thicker in the anterior intestine, and the circular muscle layer is thicker than the longitudinal muscle layer throughout the entire length of the intestine. The posterior intestine possessed large numbers of goblet cells in comparison with other parts of the intestine, to promote elimination of unabsorbed particles. **CONCLUSIONS:** The results of this study revealed adaptation for the species feeding habits, so as to protect the intestine and increase absorptive processes.

Introduction

The morphology of fish intestine is important due to its role in digestion and absorption of nutrients. Also, it is closely related to its feeding habits (Cao et al., 2011). There are

several reports available regarding anatomical and histological studies of the intestine of many fish species (Rodríguez et al., 2004; Suíçmez et al., 2005; Chatchavalvanich et al., 2006; Lokka et al., 2013). The data from these studies might contribute to the digestive physiology, formulation of diet and diagnosis of diseases.

The northern pike, *Esox lucius* Linnaeus (1758), a member of the Esocidae is a freshwater species found in rivers, lakes and weakly saline waters throughout the world (Craig, 2008). *E. lucius* is a carnivorous fish which feeds mostly on invertebrates and fishes (Kottelat & Freyhof, 2007). This fish is highly valued for human consumption and it is subject to commercial fishing. It is also considered a spectacular game fish (Laikre et al., 2005). This study was designed to determine the anatomical, histological and histomorphometric properties of the intestine of *E. lucius*.

Materials and Methods

Five *E. lucius* were used for this research. An abdominal wall incision was made on each specimen and the abdominal contents were scrutinized and photographed. The abdominal digestive tract was then removed and gently dissected. The intestinal coefficient (IC), which is the ratio of intestinal length to body length, was calculated.

For histological observation, various sections of the small intestine were collected, cleaned of its contents using 0.01 mol/l phosphate buffer saline (PBS), and fixed in 10% neutral-buffered formalin. The tissues were routinely processed for light microscopy and embedded in paraffin. The paraffin-embedded blocks were cut into 6-µm sections and stained using hematoxylin and eosin (H & E) and Masson's trichrome for general histological examination. The mounted slides were observed under an Axioplan microscope equipped with Zeiss Axiocam MRm and the Axiovision software (Carl Zeiss, Oberkochen, Germany).

The height and width of the mucosal folds, the thickness of the intestinal wall, the thickness of the tunica muscularis (circular and longitudinal muscle layers), and the number of goblet cells were measured in various parts of the intestine. For each specimen, every kind of measurement was made at ten representative points in each section. Evaluation of the goblet cells was made on randomly selected 100 µm length of the mucosal epithelium.

For statistical analysis, group comparisons were performed using the Kruskal- Wallis test. If significant, one-way ANOVA was used to determine the significant differences between the pairs of means. A $p<0.05$ was considered statistically significant.

Results

The anatomical examination showed that the anterior intestine of the *E. lucius* extended from the stomach, turned 180° to continue with the middle intestine and posterior intestine (Fig. 1). The IC of the *E. lucius* was calculated as 0.68 ± 0.09.

The histological study revealed that the wall of *E. lucius* intestine is composed of tunica mucosa, submucosa, muscularis, and serosa. The mucosa is lined by a simple columnar epithelium with a striated border and goblet cells (Fig. 2). There was no muscularis mucosa (mm) between the lamina propria and submocosa. Interestingly, a thick layer of connective tissue fibers, separated the mucosa from the submucosa. Masson's trichrome staining allowed the tunica submucosa to be classified as a dense connective tissue because of the presence of abundant collagenous fibers and few embedded cells (Fig. 3a). The tunica muscularis is composed of an inner circular muscle layer (CML) and an outer longitudinal muscle layer (LML), separated by a thin layer of connective tissue. The tunica serosa is composed of loose connective tissue covered by the mesothelium

Table 1. Histomorphometrical characteristics of the intestine of the *E.lucius*. The measurements from five subjects are expressed as Mean ± SD values. Different superscript letters in the same rows indicate a significant difference, $p<0.05$.

Measurements	Anterior intestine	Middle intestine	Posterior intestine
Height of mucosal folds(µm)	1180.9 ± 215.2[a]	1069.4 ± 240.9[b]	367.4 ± 85.7[c]
Width of mucosal folds(µm)	144.1 ± 33.6[a]	278.3 ± 48.9[b]	147.5 ± 57.5[a]
Thickness of muscularis(µm)	362.9 ± 65.1[a]	167.5 ± 17.2[b]	259±50.6[c]
Thickness of circular muscle (µm)	307.4 ± 52.5[a]	177.1 ± 83.9[b]	175.7 ± 39.3[b]
Thickness of longitudinal muscle(µm)	33.2 ± 15.3[a]	18.4 ± 6.9[b]	59.5 ± 23.8[c]
Thickness of serosa(µm)	4.4 ± 0.9[a]	3 ± 0.8[b]	6.6 ± 3.2[c]
Thickness of intestinal wall(µm)	1779.7 ± 214.5[a]	1823.6 ± 267.8[a]	815.3 ± 167.4[b]
No. goblet cell(number/100µm)	2.6 ± 1[a]	2.8 ± 0.7[a]	3.9 ± 1[b]

Figure 1. The gastrointestinal tract of the *E. lucius*. S: stomach; AI: anterior intestine; MI: middle intestine; PI: posterior intestine (Scale bar 5 Cm).

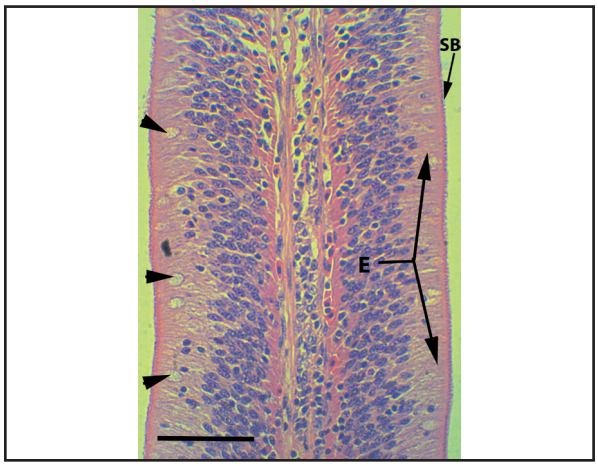

Figure 2. Photomicrograph of a transverse section of the *E. lucius* intestinal mucosal fold. The mucosa is lined with a simple columnar epithelium (E) and goblet cells (arrowheads). On the apical border of the columnar cells, microvilli are arranged as a striated border (SB) (H & E, scale bar 50µm).

(Fig. 3c).

The histomorphometric analysis of the *E. lucius* intestine characterized three regions: anterior, middle and posterior. The comparison of the histomorphometrical results of the various parts of intestine is summarized in Table 1. The mucosal folds showed the maximum height in the anterior intestine. The height of the mucosal folds gradually decreased toward the posterior intestine. The widest mucosal folds were observed in the middle intestine. Measurements of the thickness of the tunica muscularis revealed significant differences ($p<0.05$) between the intestinal regions. The tunica muscularis was the thickest in the anterior region. Furthermore, the CML were thicker than the LML, throughout the entire length of the intestine. Generally, the total thickness of the intestinal walls was significantly thinner in the posterior intestine than in the other regions of the intestine. The number of goblet cells was observed to increase toward the end of the intestine. Significantly ($p<0.05$) the highest average number of goblet cells was found in the posterior intestine.

Discussion

There is a mutual dependence between intestinal length and feeding habits (Kappor et al., 1975). The relationship between the intestinal and corporeal length varies among the carnivores from 0.2 to 2.5 and from 0.6 to 0.8 among the omnivores (Moraes et al., 2004). Therefore, the IC calculated for the *E. lucius* (0.68) classified it into carnivorous or omnivorous fish. It is important to note that Cao et

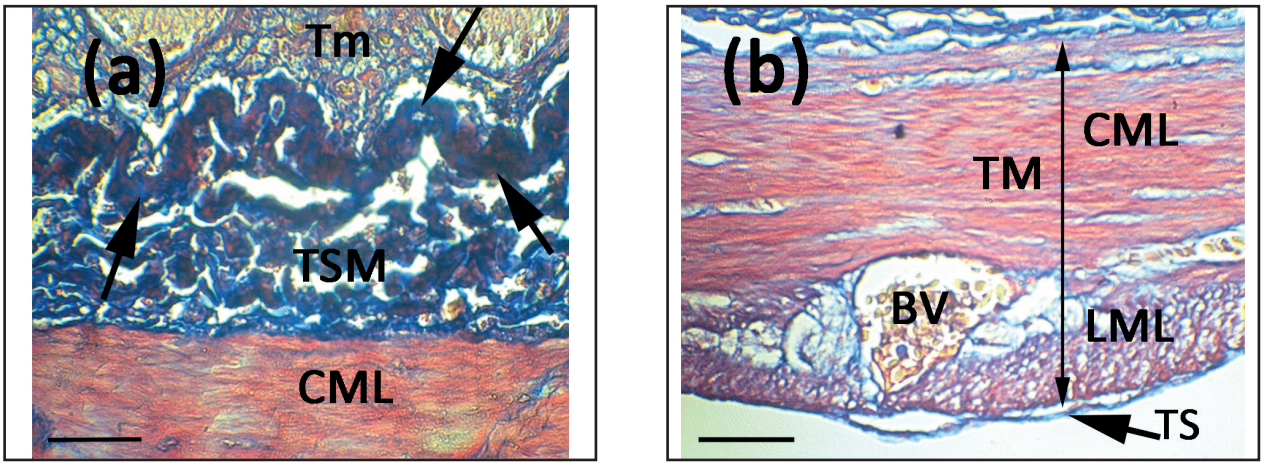

Figure 3. (a) Photomicrograph of the distribution of connective tissue in the intestinal wall. Note the stratum compactum (arrows) between the tunica mucosa (Tm) and tunica submucosa (TSM). The circular muscle layer (CML) is visible. (b) Photomicrograph of a transverse section of the tunica muscularis. The tunica muscularis (TM) is composed of an inner circular muscle layer (CML) and an outer longitudinal muscle layer (LML) separated by a thin layer of connective tissue. One blood vessel (BV) is visible. The tunica serosa (TS) is labeled (Masson's trichrome staining, scale bar 50µm).

al. (2011) suggested that the IC could only be a criterion-reference factor, for the classification of fish feeding habits. The short intestine is a characteristic of carnivorous species (Rodríguez et al., 2004). Thus, based on the short intestinal length, *E. lucius* should be classified as a carnivorous fish.

The general histological features of the intestine of *E. lucius* examined in this study, are in accordance with those described for other fishes, with some differences. Although no mm was seen in the intestine of *E. lucius*, a connective tissue band, the stratum compactum, was present between the tunica mucosa and tunica submucosa. The lack of mm is consistent with the observations made for the intestine of other teleosts (Jaroszewska et al., 2008; Cao et al., 2011). The stratum compactum has been reported in the intestine of some fishes like Dentex dentex (Carrasson et al., 2006).

Due to different histomorphometric characteristics, the intestine of *E. lucius* can be divided into three parts: 1) anterior intestine, 2) middle intestine and 3) posterior intestine. The mucosal folds were long in the anterior intestine and then decreased toward the posterior intestine. The mucosal folds are specialized tissues that provide more surface area for the absorption of nutrient-rich feed particles more efficiently (Nordrum et al., 2000; Bakker et al., 2010). The thickness of the tunica muscularis was significantly different between various regions of the intestine. It was thickest in the anterior intestine. The function of this layer is to promote motility in the intestine, carrying and mixing food with digestive secretions (Vieria-Lopes et al., 2013). Thus, it can be concluded that the anterior intestine in *E. lucius* is a major site of digestion and absorption. In this study, goblet cells were seen scattered throughout the entire length of the intestine, but showed significant increase in number in the posterior intestine. The main function of goblet cells is to secrete mucin that dissolves in water to form mucus, that creates a layer to coat the wall of the intestine (Kim & Samuel, 2010). Thus, this allows the posterior intestine to lubricate the tube to promote the elimination of dehydrated unabsorbed particles.

In conclusion, the anatomical, histological and histomorphometric study of *E. lucius*, revealed adaptation for the species feeding habits, to protect the intestine and increase the absorptive processes. The results of this study offer a baseline for future detailed gastroenterological studies in *E. lucius* and promote fu-

ture investigations in this field.

Acknowledgements

The authors wish to thank Mr. Reza Akbari for his contributions.

References

1. Bakke, A.M., Tashjian, D.H., Wang, C.F., Lee, S.H., Bai, S.C., Hung, S.S. (2010) Competition between selenomethionine and methionine absorption in the intestinal tract of green sturgeon (*Acipenser medirostris*). Aquat Toxicol. 96: 62–69.
2. Cao, X.J., Wang, W.M., Song, F. (2011) Anatomical and histological characteristics of the intestine of the topmouth culter (*Culter alburnus*). Anat Histol Embryol. 40: 292–298.
3. Carrassón1, M., Grau, A., Dopazol, L.R., Crespo1, S. (2006) A histological, histochemical and ultrastructural study of the digestive tract of *Dentex dentex* (Pisces, Sparidae). Histol Histopathol. 21: 579-593.
4. Chatchavalvanich, K., Marcos, R., Poonpirom, J., Thongpan, A., Rocha, E. (2006) Histology of the digestive tract of the freshwater stingray Himantura signifer Compagno and Roberts, 1982 (Elasmobranchii, Dasyatidae). Anat Embryol (Berl). 211: 507-518.
5. Craig, J.F. (2008) A short review of pike ecology. Hydrobiologia. 601: 5-16.
6. Jaroszewska, M., Dabrowski, K., Wilczynska, B., Kakareko, T. (2008) Structure of the gut of the racer goby *Neogobius gymnotrachelus* (Kessler, 1857). J Fish Biol. 72: 1773–1786.
7. Kappor, B.G., Smith, H., Verigina, I.A. (1975) The alimentary canal and digestion in teleosts. Adv mar Biol. 13: 109-239.
8. Kim, Y.S., Samuel, B.H. (2010) Intestinal goblet cells and mucins in health and disease: recent insights and progress. Curr Gastroenterol Rep. 12: 319–330.
9. Kottelat, M., Freyhof, J. (2007) Handbook of European fresh water fishes. Publications Kottelat, Cornol. Switzerland.
10. Laikre, L., Miller, L.M., Palmé, A., Palm, S., Kapuscinski, A.R., Thoresson, G., Ryman, N. (2005) Spatial genetic structure of northern pike (*Esox lucius*) in the Baltic Sea. Mol Ecol. 14: 1955-1964.
11. Lokka., G, Austbo, L., Falk, K., Bjerkås, I., Koppang, E.O. (2013) Intestinal morphology of the wild Atlantic salmon (*Salmo salar*). J Morphol. 274: 859-876.
12. Moraes, M.F., Freitas-Barbola, I., Duboc, L.F. (2004) Feeding habits and morphometry of digestive tracts of *Geophagus brasiliensis* (Osteichthyes, Cichlidae), in a lagoon of high tibagi river, parana state, Brazil. Publ UEPG Biol Health Sci Ponta Grossa. 10: 37–45.
13. Nordrum, S., Bakke-McKellep, A.M., Krogdahl, A., Buddington, R.K. (2000) Effects of soybean meal and salinity on intestinal transport of nutrients in Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol B Biochem Mol Biol. 125: 317–335.
14. Rodríguez Rodríguez, J., González, E., Hernández Contreras, N., Capó, V., García, I. (2004) Morphological and histological comparison of the digestive tract of *Gambusia puncticulata* and *Girardinus metallicus* are the fishes used in the biological control of mosquitoes. Rev Cubana Med Trop. 56: 73-76.
15. Suíçmez, M., Ulus, E. (2005) A study of the anatomy, histology and ultrastructure of the digestive tract of *Orthrias angorae* Steindachner, 1897. Folia Biol (Krakow). 53: 95-100.
16. Vieira-Lopes, D.A., Pinheiro, N.L., Sales, A., Ventura, A., Araújo, F.G., Gomes, I.D., Nascimento, A.A. (2013) Immunohistochemical study of the digestive tract of *Oligosarcus hepsetus*. World J Gastroenterol. 19: 1919-1929.

مجله طب دامی ایران، ۱۳۹۴، دوره ۹، شماره ۳، ۲۱۱-۲۰۷

مطالعه آناتومی، هیستولوژی و هیستومورفومتری روده اردک ماهی (*Esox lucius*)

جواد صادقی نژاد[1*] ریحانه هوشمند عباسی[1] الهه دهقانی تفتی[1] زهرا بلوکی[2]

۱) گروه علوم پایه، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران
۲) گروه بهداشت مواد غذایی و کنترل کیفی، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران

(دریافت مقاله: ۷ اردیبهشت ماه ۱۳۹۴، پذیرش نهایی: ۱۹ مرداد ماه ۱۳۹۴)

چکیده

زمینه مطالعه: اردک ماهی (*Esox lucius*) یک گونه آب شیرین است که متعلق به خانواده Esocidae می‌باشد. این گونه گوشتخوار بوده و از بی مهرگان و ماهیان تغذیه می کند. مورفولوژی روده ماهیان برای درک فیزیولوژی گوارش، تشخیص بیماریهای روده‌ای و فرمولاسیون مواد غذایی بسیار سودمند است. **هدف:** این مطالعه به منظور بررسی خصوصیات آناتومیکی، یافت‌شناسی و هیستومورفومتری روده اردک ماهی صورت گرفت. **روش کار:** در این مطالعه از تعداد ۵ روده اردک ماهی استفاده شد. بعد از بررسی آناتومیکی، نمونه‌های بافت شناسی برداشت و در فرمالین ۱۰٪ ثابت سازی شد و پس از پاساژ بافتی، رنگ‌آمیزی مقاطع به کمک هماتوکسیلین-ائوزین و تری کروم ماسون صورت گرفت. **نتایج:** بررسی آناتومیکی، روده کوتاه اردک ماهی با ضریب روده‌ای ۰/۰۹±۰/۶۸ را نشان داد که از خصوصیات ماهیان گوشتخوار محسوب می‌شود. مطالعه بافت شناسی نشان داد که جدار روده اردک ماهی از لایه‌های مخاطی، زیر مخاطی، عضلانی وسروزی تشکیل شده است. عضله مخاطی در جدار روده مشاهده نشد. لایه متراکم مابین لایه‌های مخاطی و زیرمخاطی دیده شد. نتایج هیستومورفومتری سه قسمت قدامی، میانی و خلفی روده اردک ماهی را از هم تفکیک نمود. بیشترین ارتفاع چین‌های مخاطی در روده قدامی بواسطه نقش آن در جذب مواد غذایی مشاهده شد. ارتفاع چین‌های مخاطی بطرف روده خلفی کاهش یافت. لایه عضلانی در روده قدامی ضخیم‌تر بوده و ضخامت لایه عضلانی حلقوی از لایه عضلانی طولی در سراسر طول روده ضخیم تر بوده است. روده خلفی نسبت به سایر قسمت‌های روده دارای تعداد بیشتری از سلول‌های جامی جهت حذف مواد غیرقابل جذب بوده است. **نتیجه گیری نهایی:** نتایج حاصل از این مطالعه، یک نوع سازگاری این گونه با عادت غذایی را نشان می‌دهد که منجربه حفاظت روده و افزایش مراحل جذب می‌شود.

واژه های کلیدی: اردک ماهی، روده، آناتومی، هیستولوژی، هیستومورفومتری

---

*) نویسنده مسؤول: تلفن: ۶۱۱۱۷۱۱۶ (۲۱)۹۸+ نمابر: ۶۶۹۳۳۲۲۲ (۲۱)۹۸+ Email: Sadeghinezhad@ut.ac.ir