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AGE-RELATED ANATOMICAL AND MICROSCOPIC FEATURES OF THE OESOPHAGUS AND STOMACH IN THE RAINBOW TROUT (*ONCHORYNCHUS MYKISS*)

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Summary

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The objective of this study was to describe the most important morphological features of the esophagus and different parts of the stomach in rainbow trouts at different age. Twenty rainbow trouts were divided into 2 groups, 10 numbers in each, at age 1.5 years old (1st group) and 3 years old (2nd group). Anatomical and microscopic measurements were performed. The length of the esophagus and stomach comprises more than one third of the entire gastrointestinal tract in rainbow trouts. The cardiac length was significantly shorter than pyloric one. *Lamina muscularis mucosae* and *submucosa* were not observed in oesophageal wall. The thickness of the inner muscular layer of the cardiac region was 2.8 and 1.5 times greater than the outer longitudinal layer in the 1st and 2nd group. The cardiac circular muscular layer was 22 times greater in the trouts from 1st group and 19 times in the 2nd group. The vascular and myenteric plexuses were better developed in the cardiac region.

Key words: anatomy, histology, oesophagus, rainbow trout, stomach

INTRODUCTION

The rainbow trout is the preferred fish species for consumption in Bulgaria because of the rich and diverse composition of its meat due to the high percentage of essential amino acids and the most favourable ratio between ω6/ω3 fatty acids (Atanassov *et al*., 2009). It is considered that the knowledge of anatomical and histological structure of digestive system is a main indicator of fishes' nutritional status (Miresan *et al*., 2012) and impaired water quality (Yancheva *et al.,* 2015). The monitoring of digestive organs morphology became necessary for optimising the ex-

ploitation technologies, preparation of diets and increasing of economic efficiency in aquaculture (Hernández *et al*., 2009; Miresan *et al*., 2012). The data from the morphological investigations would contribute to supplementing the information concerning digestive organs in the rainbow trout, as well as in a comparative aspect.

The aim of this study was to describe the most important morphological features of the esophagus and different parts of the stomach in rainbow trouts at different age.

MATERIAL AND METHODS

Twenty rainbow trouts collected from Enina fish farm were divided into 2 groups, 10 fish in each, at the age of 1.5 years (1st group) and 3 years (2nd group). The fish were fed commercial pellets (Aqua Dynamic, NOACK, Austria). The experiment was performed according to the legal framework for the protection and welfare of experimental animals. Body weight (g) of each fish and body length (cm), were measured according to Pravdin's scheme (1966). The dissection of the body cavity was carried out according to protocol recommended by Canada Department of Fisheries and Oceans (2004). Digestive tract was removed and the length (mm) of the oesophagus, cardiac and pyloric region of the stomach were measured with caliper (Topmaster professional, accuracy of 0.01 mm). Samples for histology from the middle third of the organs were fixed in 10% formalin solution and embedded in paraffin. The serial sections (3–5 μm thick) were stained with Masson`s trichrome Goldner kit (Merck Millipore, Germany). Microscopic observations and estimations were performed with microscope Leica DM1000 LED (Switzeland), equipped with Software platform Leica Application Suite Core. Fifty thickness measurements of muscle layers of each investigated organs were made.

The data are presented as mean values ± standard error of the mean (mean ± SEM). The statistical processing of data is performed by ANOVA (Statistica v. 6.1, StatSoft Inc., USA).

RESULTS

Anatomical measurements showed that 1st group of fish presented 122.70 ±4.42 g mean body weight and 22.47 ±0.47 cm body length and the 2nd group had 512.50 ±7.01 g body weight and 37.40 ±0.31 cm length. Overall, the length of the oesophagus and stomach made up 32.95% and 33.87% of the length of the entire gastrointestinal tract in 1st and 2nd experimental group, respectively.

Oesophagus. The esophageal length increased from 20.20 ±0.24 mm in 1st group to 27.50 ±0.09 mm in the 2nd group. The epithelial layer was presented by non-stratified columnar epithelium. *Lamina propria* formed a solid layer that showed a strong positive reaction to the presence of collagen and closely connected the esophageal glands with the underlying inner longitudinal muscle layer. *Lamina muscularis mucosae* and *submucosa* were not observed. The propria was richly infiltrated with granulocytes. Striated muscle fibers in the inner layer were longitudinally oriented, and those in the outer – circularly, but the two layers were not clearly limited by each other. Under the serosa, small vascular and nerve plexuses were observed (Fig. 1A,B).

Stomach. The stomach was located in the left cranial part of the body cavity and was V-shaped, with well-defined cardiac and pyloric regions. The lesser stomach curvature was opened cranially. The mass

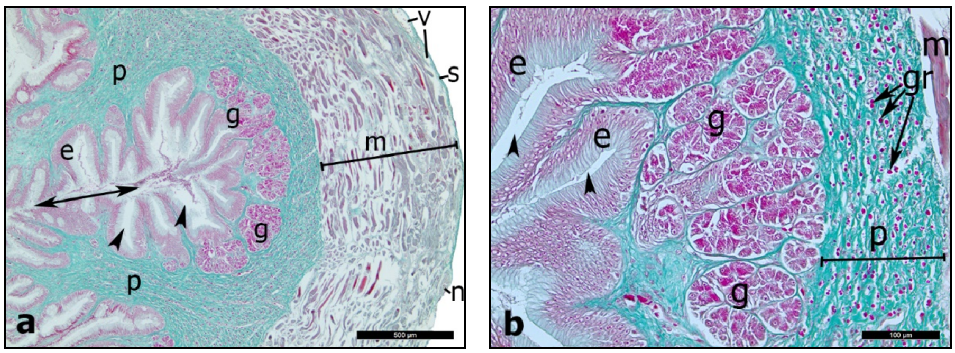

Fig. 1. Cross section through the oesophageal wall in a rainbow trout, 120 g body weight: e – *lamina epithelialis*, g –esophageal glands, arrows ↔ indicates the formation of common excretory glandular duct, arrowheads - tubule, p - propria, gr – granulocytes, m – *tunica muscularis*, s – serosa, v – blood vessels, n – nerves. Masson-Goldner's stain. **A.** Bar=500 µm; **B.** Bar=100 µm.

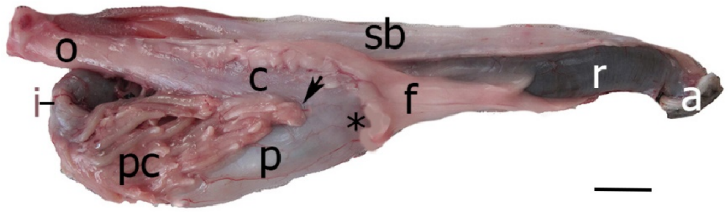

Fig. 2. Anatomy of gastrointestinal tract and connection with swim bladder in a rainbow trout, 510 g body weight. Left lateral view: o – esophagus, c – cardiac region, p – pyloric region, arrow - lesser stomach curvature, * - greater stomach curvature, f – regional fat depot, pc - pyloric caeca, I - intestine, r – rectum, a – anus, sb - swim bladder. Line=1 cm.

of pyloric caeca and pancreas among them occupied the space between cardiac and pyloric region. Regional fat depot was attached to the greater stomach curvature. Cranially, the stomach reached the liver, caudally was in contact with the spleen, dorsally – bordered the swim bladder, ventrally – the soft abdominal wall and to the right side – the small intestine (Fig. 2).

The cardiac length in 1st group was 24.71±0.12 mm and increased significantly in 2nd group – 38.32±0.28 mm ($P<0.001$). The mean pyloric length in 1st group, 22.50±0.13 mm was shorter ($P<0.001$) than that in 2nd group, 42.61±0.09 mm. A prominent *stratum compactum* in the mucosa showed intense blue-green colour and the granulocytes in *stratum granulosum* appeared with a saturated pink-red coloring, when stained with Masson-Goldner's kit. *Plexus myentericus* was much better developed in the cardia than in the pylorus (Fig. 3A–C). The thickness of the inner muscular layer of the cardiac region was 2.8 and 1.5 times greater than the outer longitudinal layer in 1st and 2nd group, respectively. Cardinally different was the ratio in the pyloric region, where the circular layer was 22 times greater in the trouts from the 1st group and 19 times in those from group 2. Especially distinct were six to eight bundles of collagen fibres separated the circular smooth muscles (Table 1, Fig. 3B).

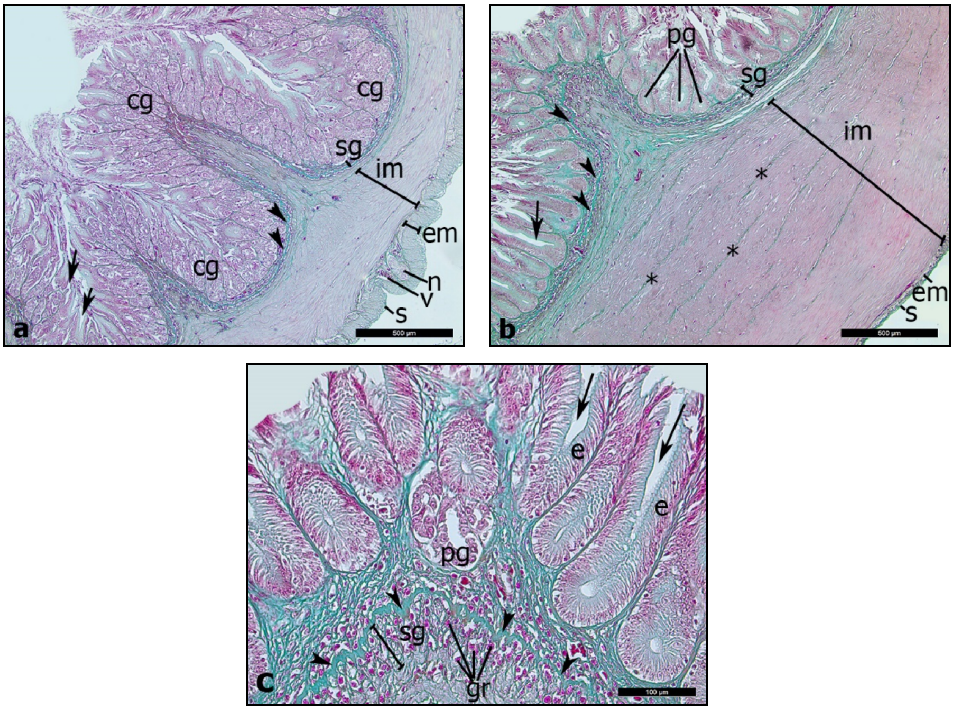

Fig. 3. Cross section of the stomach in a rainbow trout, 120 g body weight: **A.** through the cardiac region; **B, C.** through the pyloric region: cg – cardial glands, pg – pyloric glands, arrow - tubule, arrowheads – *stratum compactum*, sg – *stratum granulosum*, gr – granulocytes, im –circular muscle layer, * – collagenous bundles, em – longitudinal muscle layer, v – blood vessels, n – *plexus myentericus*, s – serosa. Masson-Goldner's stain. **A, B.** Bar = 500 µm; * Bar = 100 µm.

Table 1. Thickness of the muscle layers of esophagus and stomach in rainbow trouts at a different age (mean ± SEM)

Organ/Layer (µm)	1st group	2nd group
Esophagus/muscle layer	682.46±5.78	1245.48 ±28.84*
Cardia/ circular muscle layer	271.50±3.21	572.50±11.02*
Cardia/longitudinal muscle layer	97.38±2.83	366.35±35.42*
Pylorus/ circular muscle layer	913.62±4.01	1802.41±31.97*
Pylorus/longitudinal muscle layer	40.77±1.47	93.79±2.26*

*P<0.001

DISCUSSION

Despite the four-fold increase in body weight in the trouts from 2nd group, the esophageal length increased only 1.3 times while the prolongation of the cardia and the pylorus was 1.6 and 1.9 times, respectively. The overall length of the esophagus and stomach consists more than one third of the entire gastrointestinal tract in rainbow trout at the age of 1.5 and 3 years. As in other fish species (Nazlić *et al*., 2014), *lamina muscularis mucosae* in

the esophagus were not observed. Although Burnstock (1959) mentioned lack of *stratum granulosum* in the esophagus of brown trouts, we found that diffuse scattered granulocytes between the esophageal glands and the muscle layer.

Hernández *et al.* (2009) and Dos Santos (2015) describe that herbivorous and omnivorous fish possess very well developed esophageal submucosa, in contrast to data we obtained in rainbow trouts, despite the similarity in the digestive tract anatomy. The cardiac length was significantly less than pyloric one. Similarly to Miresan *et al*. (2012), we found a significant greater thickness of the circular layer in the pyloric region compared to this one in the cardia. Vascular and myenteric plexuses were better developed in the cardiac region, compared to those in the esophagus and pylorus wall.

CONCLUSIONS

We conclude that rainbow trouts increased more than 4 times their body weight and 1.6 times their body length from 1.5 to 3 years at age. The esophageal length increased only 1.3 times with age, while the prolongation of the cardia and pylorus was 1.6–1.9 times which means that the cranial segment of alimentary tract grows linearly and proportionally to the length of the body. *Lamina muscularis mucosae* and *submucosa*) were not observed in the esophageal wall. Circular muscle layer in the pyloric region was 19–22 times thicker, than the longitudinal one. Vascular and myenteric plexuses were better developed in the cardiac region.

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