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Histomorphological Study on the Pyloric Caeca and Intestine of Black Sea Trout (*Salmo labrax* Pallas, 1814)

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Research Article

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A B S T R A C T

This study was conducted to determine about the histomorphology of anterior, middle and posterior intestines and pyloric caeca of fifth-generation Black Sea trout (*Salmo labrax* Pallas, 1814). The adult Black Sea trout were 29 month age and averagely weighted 1106.9±73.88g. Pyloric caeca and intestine tissue of twenty fish were taken in seawater. Intestine was divided into three sections: anterior, middle and posterior. Pyloric caeca and intestine had four layers from the inside to outside: mucosa, submucosa, muscularis and serosa. Muscularis was gradually decreased from the beginning of the anterior intestine to the end of the posterior intestine. While pyloric caeca had the lowest muscularis, anterior intestine had the highest muscularis. The highest villi length was obtained in middle 1, and also posterior intestine had two types of villi as simple and complex villi. Simple villi had lower villi length and fewer villi number. Complex villi, branched out along the intestine, contained a large of simple villi. As a result, the histomorphological structure of the midgut section of the adult Black Sea trout shown an alteration section by section.

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Karadeniz Alabalığı (*Salmo labrax* Pallas, 1814)'nın Pilorik Kese ve Bağırsak Dokusu Üzerine Histomorfolojik Çalışma

M A K A L E B İ L G İ S İ

Araştırma Makalesi

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Ö Z

Bu çalışma beşinci nesil Karadeniz alabalığı (*Salmo labrax* Pallas, 1814)'nın ön, orta ve arka bağırsak ile pilorik kesenin histomorfolojisinin belirlenmesi amacıyla yapılmıştır. Çalışmada kullanılan Karadeniz alabalıkları 29 aylık ve ortalama 1106,9±73,88g ağırlığa sahipti. Balıklar deniz suyunda iken, 20 adet balığın pilorik kese ve bağırsak dokusu alındı. Pilorik kese tek parça olarak, bağırsak üç bölgeye ayrılarak incelendi: ön, orta ve arka. Elde edilen sonuçlara göre, pilorik kese ve bağırsak içten dışa doğru dört tabakaya sahipti: mukoza, altmukoza, muskularis ve serosa. Muskularis ön bağırsağın başlangıcından arka bağırsağın ucuna kadar kademeli olarak azaldığı tespit edildi. Pilorik kese en ince muskularis tabakasına sahipke, ön bağırsak en kalın muskularis tabakasına sahipti. En yüksek villi uzunluğu orta bağırsağın başlangıç kısmında görüldü. Ayrıca, arka bağırsak basit ve karmaşık olmak üzere iki tip villiye sahipti. Basit villi daha düşük villi uzunluğuna ve daha az sayıda villiye sahipti. Karmaşık villi ise bağırsağın lumenine doğru dallanmış olup, çok sayıda basit villiye sahipti. Sonuç olarak, yetişkin Karadeniz alabalığının midgut bölgesinin histomorfolojik yapısı bölüm bölüm değişiklik göstermektedir.

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Introduction

Black Sea trout is a subspecies of the brown trout and is distributed at the Eastern Black Sea. This species is an endemic species for Turkey (Tabak et al. 2002). Black Sea trout has become an important aquaculture species of Turkey in recent years by means of the studies carried out by Central Fisheries Research Institute over the years (Özel et al. 2018). Bioecological and cultural characteristics of Black Sea trout were first determined by the studies of Central Fisheries Research Institute in 1998. By these studies, it was distinguished that the species, produced under culture conditions, was a potential to be reared for the aquaculture sector. Afterwards, selective breeding studies have been continued in the 6th generation.

In fish, as in other vertebrates, the digestive tract is a longitudinally divided hollow tube of varying diameter (Nazlic et al. 2014), which consist of mouth, oesophagus, stomach, pyloric ceaca, intestine and rectum (Merrifield et al. 2011). For teleost fish, this part is divided into four sections: headgut, foregut, midgut and hindgut. The midgut is the greatest part of the alimentary tract and contains the intestine and a variable number of pyloric caeca (Floris, 2010). The fish intestine plays an important role in various important physiological functions such as digestion and absorption of dietary nutrients, and nutrient uptake, and also it is involved in immunological functions (Deshmukh et al. 2015; Khadse and Gladhikar, 2016). The teleost intestine has prevalently separated in two regions by the ileocolonic junction: the anterior and the posterior (Jutfelt, 2006). The anterior part of intestine has some functions such as carrying the feed material from stomach to posterior region, completing the digestion process by secreting enzymes from intestine wall and auxiliary glands, absorbing the end products of digestion to blood and lymph vessels and to secrete some hormones (for stimulating secretin and pancreas secretion). Posterior part of intestine has some other functions like liquid absorption, mucous secretion (more goblet cells), some digestion, and excretion (Mumford et al. 2007). In addition, the posterior region has less nutrient absorptive capacity and more phagocytotic activity (Jutfelt, 2006). The pyloric caeca consist of blind finger-like diverticule which attach to the anterior intestine (Floris, 2010), and open into the lumen of the intestine, and also are called as intestinal caeca (Ray and Ringo 2014). The pyloric caeca are histologically and histochemically similar to the intestine rather than the stomach and have digestive and absorptive functions (Mumford et al., 2007), but don't have a role in fermentation or storage (Khayyami et al., 2015). Therefore, this structure should be monitored enzymologically, histomorphologically and microbiologically, and their physiologic mechanisms should be understood more clearly.

Intestine physiology has an important discipline in understanding the nutritional status of fish. Enzymatic, histomorphologic and microbiotic activities have an important place in this discipline. In the nutrition studies, intestine sections should be well understood histomorphologically to obtain a standard measurement. Therefore, this study was aimed the evaluation of histomorphology of intestine sections and pyloric caeca of Black Sea trout methodologically to figure out and determine the part of the intestine as an indicator in the fish nutrition studies. Also this study has the feature of being the first in histomorphologically monitoring of different parts of midgut section in the fish nutrition.

Material and Methods

Fish and Maintenance

This study was conducted in fifth-generation 29 age months and 1106.9±73.88g weighted Black Sea trout at Central Fisheries Research Institute (Trabzon-Turkey), (Figure 1). From larvae to smolt stage, the fish were kept in the freshwater tanks (10.1-12.5ºC) fed by spring water between January-November 2016. After that, the fish were transferred to the marine cages (6.9-16.6ºC) in November 2016, and where they were kept for six months. In May 2017, the fish were transferred to freshwater ponds, and were kept there for six months. After that, in November 2017, the fish were transferred to seawater tanks (9.7-15.8ºC) and were kept there for six months. After that, twenty fish were selected, and pyloric caeca and intestine tissues samples were taken in June 2018. Equal number of male and female were used. Fish were fed until the feeling of satiety during experiment. Water temperature was saved daily. Seawater salinity was recorded as 18‰.

Figure 1 Black Sea trout (*Salmo labrax*)

Tissue Sampling

Intestine was examined by dividing into three parts: anterior, middle and posterior. Anterior intestine was defined as section attached to the intestine of pyloric caeca. Middle intestine was defined as from final point of section attached to the intestine of pyloric caeca to macroscopic spiral image of the intestine. Posterior intestine was defined as from macroscopic spiral image of intestine to 1.0 cm beyond of anus. Anterior, middle and posterior intestine and pyloric caeca were divided into three parts: beginning, middle and end of each section. Pyloric caeca was examined by taking from the region near to final point of section attached to the anterior intestine (Figure 2). The beginning, middle and end of each section was respectively named 1, 2 and 3.

Tissue samples were taken from pyloric caeca and intestine (anterior, middle and posterior), and placed into 10% formalin for further processing. Tissues sections were placed into tissue cassettes for dehydration process and were embedded in paraffin blocks, and subsequently cut 5-μ thickness and placed on a slide. Each tissue sample was prepared and stained with hematoxylin and eosin solution by using standard paraffin-embedding procedure. After embedding process, villi length (VL), villi width (VW) and muscularis were photographed and evaluated by using an image processing and analysis system (ZEN 2012 SP2) (Xu et al. 2003).

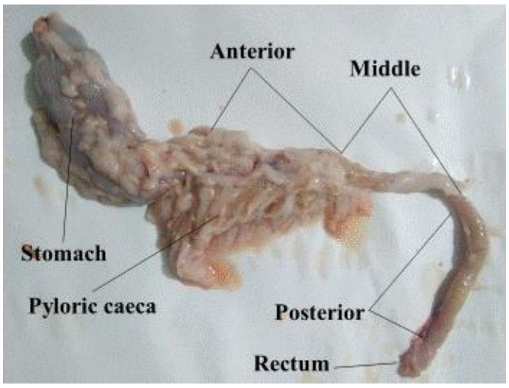

Figure 2 Sampled sections of the pyloric caeca and intestine (Anterior, middle and posterior) tissues of Black Sea trout

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA). Differences between means were compared using Duncan's multiple range test. Differences were considered statistically significant at P<0.05. Statistical analyses were computed SPSS 15.0.

Results

Histomorphological measurements of intestine part and pyloric caeca of Black Sea trout are summarized in Table 1, and shown in Figure 4-5-6-7, respectively. The digestive tract of Black Sea trout was composed of mouth, esophagus, stomach, pyloric caeca, intestine (anterior, middle and posterior) and rectum. Black Sea trout intestine had a straight shape and 21.42±3.96 cm length. Pyloric caeca and intestine had four layers from the inside to outside: mucosa, submucosa, muscularis and serosa. Mucosa layer proceeded as lamina propria, lamina muscularis mucosa and epithelium. The epithelium was compounded by enterocytes, goblet cells and villi. Villi was localized in mucosa layer and covered with epithelium (Figure 3). Crypt depth was not seen in the intestine and pyloric caeca. Also meckel' diverticulum was not seen in the intestine.

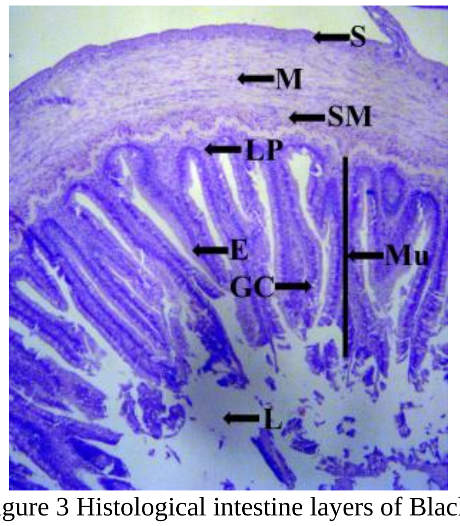

Figure 3 Histological intestine layers of Black Sea trout: S; Serosa, M; Muscularis, SM; Submucosa, Mu; Mucosa, LP; Lamina propria, E; Epithelium, GC; Goblet cells, L; Lumen. (x4, H&E)

Many points of the anterior intestine opened to the pyloric caeca. The anterior intestine had a thicker muscularis than other tissues. Pyloric caeca were number 61.20±11.32 and had 3.34±1.34 cm length. Pyloric caeca had a much thinner muscularis than others, and a lower diameter. Pyloric caeca were localized to approximately 1/3 of the whole intestine. Middle intestine represented to middle of the whole intestine. Anterior, middle and posterior intestines and pyloric caeca had the same histological structure. But intestinal villi were differentiated from the posterior part, which had more a complex physiognomy. Posterior intestine had a larger diameter and proceeded with two types villi: simple and complex villi. Simple villi had lower villi length and fewer villi number. Complex villi, contained a large of simple villi, made branching into the intestine. The complex villi localized in plicae circulares. Villi development in the posterior intestine was histologically evaluated due to its complex structures, but not evaluated morphologically. Each of anterior, middle and posterior parts was held to approximately 1/3 of the whole intestine.

From the beginning of the anterior intestine to the end of the middle intestine, villi length was increased and then decreased consecutively. For the pyloric caeca, the part close to the anterior intestine had a lower villi length than the middle and end parts (P<0.05), but villi width and muscularis were similar to each other for each three parts (P>0.05). Muscularis was gradually decreased from the beginning of the anterior intestine to the end of the posterior intestine (P<0.05). The highest muscularis was seen in the anterior intestine. This was followed middle and then posterior intestine. The lowest muscularis was seen in the pyloric caeca (P<0.05).

Discussion

There are different approaches for histological naming of the fish intestine sections. Intestine sections were named as cranial, intermediate and caudal by Albrecht et al. (2001), anterior, intermediate and posterior by Nazlic et al. (2014), anterior, middle and posterior by Bocina et al. (2017), anterior and posterior by Mumford et al. (2007), proximal and distal by Rodrigues da Silva et al. (2012), and proximal, middle and distal by Khojasteh et al. (2009) and Deshmukh et al. (2015). We preferred to name as anterior, middle and posterior in the histological naming. And so, it can also be respectively used proximal, middle (intermediate) and distal or cranial, intermediate and caudal instead of anterior, middle and posterior.

Our study showed that pyloric caeca or intestine of Black Sea trout (*Salmo labrax*) had mucosa, submucosa, muscularis and serosa layers. This is also supported by the results obtained from different species such as *Oncorhynchus mykiss* (Khojasteh et al. 2009), garfish *Belone belone* (Bocina et al. 2017), *Rastrelliger brachysoma* (Senarat et al. 2015) and *Ctenopharyngodon idella* (Mokhtar et al. 2015). In a previous study, Bocina et al. (2017) stated that anterior, middle and posterior sections of the intestine of garfish *Belone belone* had the same histological characteristic. In a additional study, Khojasteh et al. (2009) stated that the histological structure of intestine of rainbow trout (*Oncorhynchus mykiss*) is similar to other fish species, except for a few variations.

Table 1 Morphology of midgut section of Black Sea trout

Tissues	VL (µm)	VW (µm)	VL/VW	Muscularis (µm)
Anterior 1	447.46 ± 76.53^{bc}	60.43 ± 16.83^{ab}	7.80 ± 1.86^{ab}	185.34 ± 30.71^{a}
Anterior 2	523.89 ± 54.65^{ab}	55.54 ± 8.90^{b}	9.75 ± 2.30^{a}	181.34 ± 30.71^{a}
Anterior 3	426.99 ± 84.52^{bc}	71.45 ± 27.94^{ab}	7.12 ± 3.83^{ab}	169.81 ± 37.99^{a}
Middle 1	549.48 ± 87.33^{a}	58.93 ± 6.72^{b}	9.43 ± 2.62^{a}	127.76 ± 23.89^{b}
Middle 2	391.81 ± 25.96^{c}	71.12 ± 14.37^{ab}	5.73 ± 1.30^{b}	117.79 ± 13.99^{bc}
Middle 3	479.43 ± 127.40^{abc}	83.03 ± 26.36^{a}	6.00 ± 1.24^{ab}	113.86 ± 17.99^{bc}
Posterior 1	NM	NM	NC	101.23 ± 20.54^{cd}
Posterior 2	NM	NM	NC	85.97 ± 18.50^{d}
Posterior 3	NM	NM	NC	55.11 ± 15.68^{e}
Pyloric caeca 1	405.75 ± 45.34^{c}	75.94 ± 13.09^{ab}	5.40 ± 0.51^{b}	51.47 ± 4.66^{e}
Pyloric caeca 2	529.10 ± 75.56^{ab}	67.45 ± 6.19^{ab}	7.94 ± 1.69^{ab}	52.48 ± 8.92^{e}
Pyloric caeca 3	531.59 ± 87.33^{ab}	65.66 ± 9.72^{ab}	8.31 ± 2.25^{ab}	45.66 ± 3.09^{e}

Mean values in column with different superscripts were significantly different ($P < 0.05$), NM: Not measured, NC: Not calculated

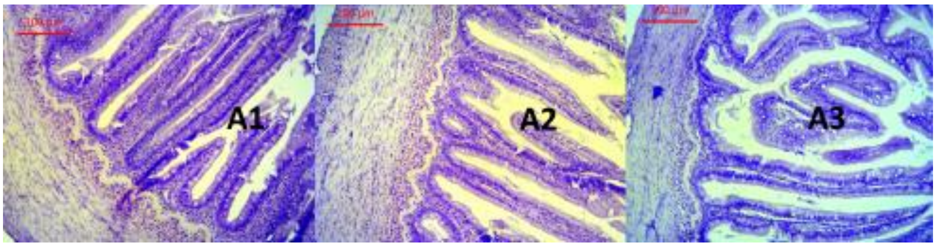

Figure 4 Anterior intestine histology of Black Sea trout: A1, A2, A3 (x10, H&E)

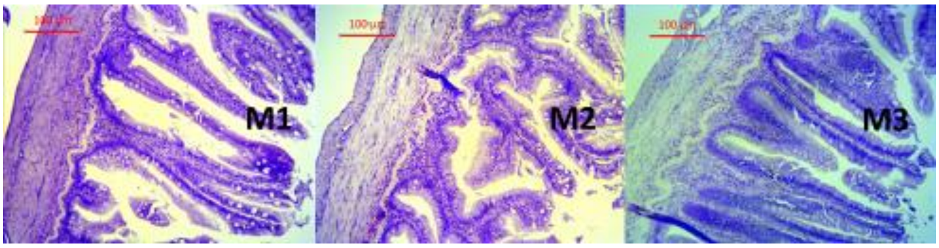

Figure 5 Middle intestine histology of Black Sea trout: M1, M2, M3 (x10, H&E)

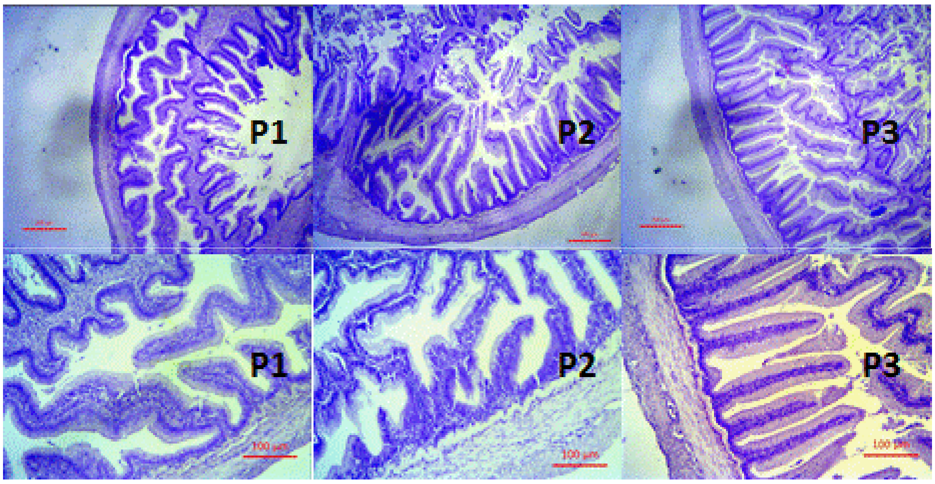

Figure 6 Posterior intestine histology of Black Sea trout: P1, P2, P3 (x4 and x10, H&E)

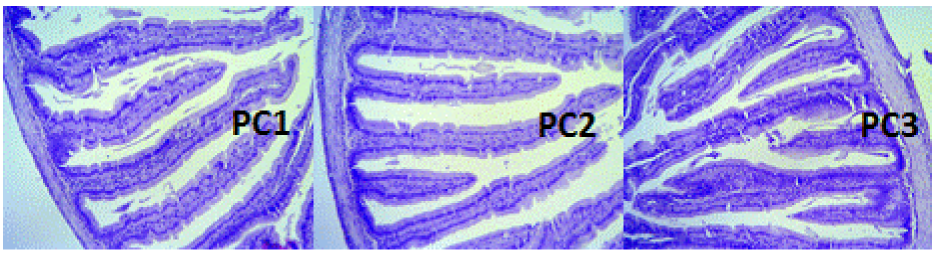

Figure 7 Pyloric caeca histology of Black Sea trout: PC1, PC2, PC3 (x10, H&E)

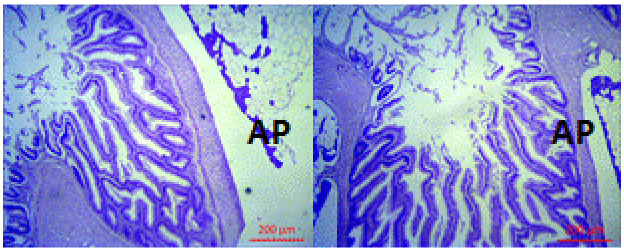

Figure 8 The point attachmented with pyloric caeca of anterior intestine (AP) histology of Black Sea trout (x4, H&E)

A similar observation was made in our study. Three sections (anterior, middle and posterior) of the intestine were histologically the same with each other. But, intestinal villi development only varied in the posterior part. This part had both simple villi and complex villi. Simple villi had lower villi length and fewer villi number compared to anterior intestine and middle intestine. Complex villi contained a large of simple villi. Similar to our study, several studies have shown that the intestinal mucosa layer contained villi of different length (Deshmukh et al., 2015; Kasozi et al., 2017; Khojasteh et al., 2009; Nascimento et al., 2018). Rodrigues da Silva et al. (2012) stated that in the *Satanoperca pappaterra*, the villi were tall and numerous at the proximal intestine but have reduced length and less numerous at the distal intestine. Khojasteh et al. (2009) stated that intestine villi length of rainbow trout (*Oncorhynchus mykiss*) has progressively shorter toward the posterior part. Also, Nascimento et al. (2018) stated that the posterior intestine of *Anablepsoides urophthalmus* had a decrease of the mucosa height according to anterior intestine and middle intestine. Therefore, histological structure of intestine may have a different characteristic according to fish species. And it should be kept in mind that the feeding of intestinal villi is provided while feeding the fish in the fish nutrition studies. In our study, it was found that intestine villi length of Black Sea trout was increased and then decreased consecutively from the beginning of the anterior intestine to the end of the middle intestine. In the fish nutrition studies, intestine of Black Sea trout can be monitoring by dividing into three sections: Anterior, middle and posterior. In our study, pyloric caeca were similar to the intestine tissue in terms of their histological characteristic. A similar expression was stated by Mumford et al. (2007). Also, Canan et al. (2012) and Mitra et al. (2015) stated that the pyloric caeca were histologically similar to anterior intestine. Similar to our study, Murray et al. (1996) stated that the pyloric caeca had a thinner muscularis externa than the intestine, in the Atlantic halibut, the yellowtail flounder and the winter flounder. Also, in our study, the thickness of the muscularis was gradually decreased from anterior intestine to posterior intestine. But, Abdullahi (2005), stated that the terminal part of intestine of sea bream (*Mylio cuvieri*) is characterized by thick tunica muscularis. It can be said that the tunica muscularis of the intestine may have different thickness depending on the fish species and intestine sections (anterior, middle and posterior). Rodrigues da Silva et al. (2012) found that the proximal intestine of the *Satanoperca pappaterra* was a thicker muscle layer than the distal intestine. Deshmukh et al. (2015) found that the proximal intestine of the adult catfishes *Heteropneustes fossilis* was thicker than the middle and distal intestine. A similar result was obtained in the Black Sea trout used in our study. Muscularis layer has the function of contributing to the mixing of feed with digestive enzymes (Mumford et al., 2007). Therefore, the histomorphologically monitoring of muscularis layer can be important for the fish nutrition study.

The pyloric caeca vary in branhing, the gut connection, size (Ray and Ringo, 2014), position and number (Albrecht et al., 2001). In previous studies, Mumford et al. (2007) stated that the number of pyloric caeca in the salmonids can 70 or more. Ray and Ringo (2014) stated that pyloric caeca (intestinal caeca) present in numbers ranging from a few (murrel *Channa punctatus*) to several hundred (Atlantic cod). In additional studies, Ulla and Gjedrem (1985) found that the length and number of pyloric caeca of rainbow trout (2.5 years old) had 50.5±7.1 mm and 52.0±6.8, respectively. Huseyn et al. (2015) found that the length and number pyloric caeca in adult Mugilidae, *Liza aurata*, *Liza abu* and *Mugil cephalus* were 2.08±0.23 cm and 9, 0.72±0.25 cm and 4, 0.98±0.13 cm and 2, respectively. Rahmati-holasoo et al. (2011) found that the length of pyloric caeca of two years old beluga (*Huso huso*) were 3.15±0.09 cm in male and 3.07±0.08 cm in female. We were found that pyloric caeca of the adult Black Sea trout (1106.9±73.88g and 29 month age), were number 61.20±11.32 and had 3.34±1.34 cm length. The number and length of pyloric caeca may vary depending on the species of fish.

Conclusion

It was understood that pyloric caeca make nutrient exchange with anterior intestine and have digestion and absorption roles like intestine. And also, the intestine sections including the pyloric caeca were similar to each other to their histological characteristic. But the posterior part was different from other three sections (anterior intestine, middle intestine and pyloric caeca) in terms of intestinal villi development. Therefore, morphological monitoring of midgut section of the digestive tract of adult Black Sea trout would be more appropriate in the anterior intestine, middle intestine and pyloric caeca. But, the development of villi and muscularis in each part of the midgut section of this species vary. Therefore, in the histomorphological examination of the intestinal villi of the adult Black Sea trout (1106.9±73.88g and 29 month age), the sampling can be suggested obtaining from the middle 1, which can more easily distinguishable with the naked eye due to that part begins with the final point where the pyloric caeca is attached to the intestine, and also has a higher villi length. To reveal a standard measurement region in the nutrition studies, but, such studies should be expanded by carrying out at different ages and in different weights by considering the different environmental conditions.

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