

Scanning Electron Microscopic Examination of Rainbow Trout Gastrointestinal Mucosa

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Abstract

Aim to study: The aim of this study was to determine the morphological characteristics of the gastrointestinal mucosa of the rainbow trout.
Material and methods: This study was carried out on 10 rainbow trout's stomach and intestinal tissues using scanning electron microscope. In the study, 2.5% glutaraldehyde fixation and routine scanning electron microscopy procedures were applied.
Results: The surface architecture of the gastrointestinal tract was examined in detail. It was observed that the mucosal folds in the stomach increased in number towards the pyloric region and were arranged in a configuration that allowed food to be directed to the intestine. The columnar cells of the digestive tract mucosa and the mucosal openings that allow mucus secretion were shown in detail.
Conclusion: The gastrointestinal tract of trout, a carnivorous species, was studied in detail. The structure of the columnar cells, gastric glands and mucus openings of this system was revealed in three dimensions. The detailed anatomy of the mucosal surface, which is rapidly affected by food variation or pathological changes in fish, was revealed. This study will shed light on studies affecting the morphology of the digestive system.
Keywords: Intestine, rainbow trout, scanning electron microscopy, stomach.

Gökkuşağı Alabalığı Gastrointestinal Mukozasının Taramalı Elektron Mikroskobik İncelemesi

Öz

Çalışmanın amacı: Bu çalışmada gökkuşağı alabalığının gastrointestinal mukozasının morfolojik özelliklerinin incelenmesi amaçlanmıştır.
Materyal ve yöntemler: Bu çalışma 10 gökkuşağı alabalığının mide ve bağırsak dokuları üzerinde gerçekleştirilmiştir. Dokular taramalı elektron mikroskobu ile incelenmiştir. Çalışmada %2,5 gluteraldehit fiksasyon ve rutin taramalı elektron mikroskobu prosedürü uygulanmıştır.
Bulgular: Gastrointestinal sistemin yüzey mimarisi ayrıntılı olarak incelenmiştir. Midedeki mukozal kıvrımların pilorik bölgeye doğru sayıca arttığı ve gıdanın bağırsağa yönlendirilmesini sağlayacak bir konfigürasyonda düzenlendiği gözlendi. Sindirim sistemi mukozasının kolumnar hücreleri ve mukus salgılanmasını sağlayan mukozal açıklıklar ayrıntılı olarak gösterildi.
Sonuç: Etçil bir tür olan alabalığın gastrointestinal sistemi ayrıntılı olarak incelendi. Bu sistemin kolumnar hücrelerinin, mide bezlerinin ve mukus açıklıklarının yapısı üç boyutlu olarak ortaya kondu. Balıklarda besin çeşitliliği veya patolojik değişikliklerden hızla etkilenen mukozal yüzeyin detaylı anatomisi ortaya çıkarıldı. Bu çalışma sindirim sistemi morfolojisini etkileyen çalışmalara ışık tutacaktır.
Anahtar kelimeler: Bağırsak, gökkuşağı alabalığı, taramalı elektron mikroskobu, mide.

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Introduction

Rainbow trout has an extensive distribution in freshwater around the world and is one of the most widely cultivated species in aquaculture due to its many advantages, such as adaptability and productivity (Crawford & Muir, 2008). Although ever-increasing costs and sustainability are influential in the search for the most suitable aquafeed, the main focus should be on the knowledge of the function of gut structures and the effects of the feeds used on the gut (Barker et al., 2012). Since the beginning of the digestive system, dental structures, oral mucosa, and oropharyngeal structures have been studied in detail and associated with dietary preferences (Abbate et al., 2006; El Bakary, 2011; Abbate et al., 2012a; 2012b; Guerrera et al., 2015). Food ingestion and food preference are linked to taste buds located on the oral mucosa. Moreover, teeth play a crucial role in masticating food within the mouth and transforming it into manageable bites. In particular, molar-like and capped teeth are present in species that prefer crusted foods and facilitate food crushing (Whitehead, 1977; Bond, 1979).

Microridge structures on the oral mucosa are associated with mucus fluidity. Microridges are also thought to provide resistance to the oral mucosa (Ezeasor, 1982). Microridge structures are found in the esophageal mucosa as well as the oral mucosa of the digestive tract (Mahmoud et al., 2016). The mucus facilitates the passage of food in the digestive tract and protects the epithelial layer from mechanical influences. Mucus cells responsible for mucus production are found throughout the entire mucosa of the digestive tract and distributed throughout the oral cavity, esophagus, and digestive tract (Harabawy et al., 2008; Baoom, 2012; Guerrera et al., 2015). Stosik et al., (2023) mentioned that changes in the anatomical and histological structures of different intestinal sections in teleost fishes, which have different characteristics from mammals, are related to the immune system. Intestinal mucosal immunity in teleost fish, where lymphoid tissue is absent, is a controversial and current topic.

Intestinal morphology in fish is rapidly affected by diet and adapted to external factors. Dietary ingredients have a direct proportional effect on intestinal morphology in terms of digestibility and feed utilization. Fish intestinal morphology changes in a very short period to provide maximum benefit from feeds (Demirci et al., 2021). Extensive studies of intestinal morphology have been carried out in different livestock species (Elia et al., 2018), such as poultry and cattle (Verdile et al., 2019). However, the knowledge about the morphology of the internal structure of the gut wall in fish is still limited. There is a wide variability in gut characteristics among Teleostei species according to their feed preferences (Fagundes et al., 2016). The digestive tract in fish can also adapt rapidly and reversibly to the environment, depending on their physiological requirements. Several factors, such as taxonomy, feeding habitats, food type, and feeding frequency, affect the morphology of the digestive tract. Previous studies have shown that changes in rainbow trout intestinal morphology are directly related to fish diet. Research on fish intestinal morphology is mostly based on histopathological studies such as changes in villus length, thickening of the villus lamina propria, and lymphocyte infiltration (Li et al., 2019). The morphology of the fish digestive tract varies considerably in the proximal and distal parts of the intestine. Although the proximal parts have been studied in recent years, there are no descriptive morphological studies on the distal parts (Abbate et al., 2006; El Bakary, 2011; Abbate et al., 2012a; 2012b; Guerrera et al., 2015). This situation is related to the inadequacy of analysis due to the complex structure, especially in the distal intestinal parts (Ray & Ringø, 2014). Therefore, in order to establish accurate reference values in rainbow trout, individuals between 220-280 g were studied. The aim of this study was also to determine the intestinal morphology of rainbow trout and to reveal the three-dimensional structure of the intestinal mucosa by scanning with scanning electron microscopy (SEM).

Material and Methods

In this study, tissues obtained from ten rainbow trout (average weight 220-280 g; n = 10) were used. The digestive tract tissues of the fish were fixed in 2.5% glutaraldehyde solution and postfixed in 1% osmium tetroxide for 1 hour for electron microscopy. Samples were serially dehydrated in graded acetone and dried in a critical point dryer (Quorum Technologies, E3100) and then coated with gold-palladium in a coater for SEM (Cressington, Sputter Coater 108 Auto). After tissue preparation, the samples were examined by SEM (FEI, Quanta FEG 250) under Everhart–Thornley detector-2.00Kv.

Results

Macroscopically, the gastrointestinal parts of rainbow trout were examined as stomach, pyloric caeca, small intestine, and large intestine. It was observed that the stomach was shaped like a hollow J character and its pyloric part was short. The cardiac part was separated with a circular thickening from the esophagus, and its inner surface was observed to have longitudinal mucosal folds. Immediately posterior to the pyloric part of the stomach, the pyloric caeca was observed with numerous bluntly terminated finger-like projections. The small intestine behind the pyloric caeca also had several single rows of finger-like projections. Although the transition of the small intestine to the large intestine was unclear, the caudal part of the intestine was wider.

SEM examination

Stomach

Scanning electron micrographs revealed that the mucosal folds of rainbow trout's cardiac part of the stomach were irregular. The gastric mucosa surface epithelium was observed close to each other like a flower bouquet (Figure 1A). There are no micro ridge structures on gastric mucosa epithelial cells. Mucosal folds were very dense and caudally oriented in the pyloric region (Figure 1C). Also, there were irregular-shaped polygonal areas with columnar cells in the pyloric caeca and fundus.

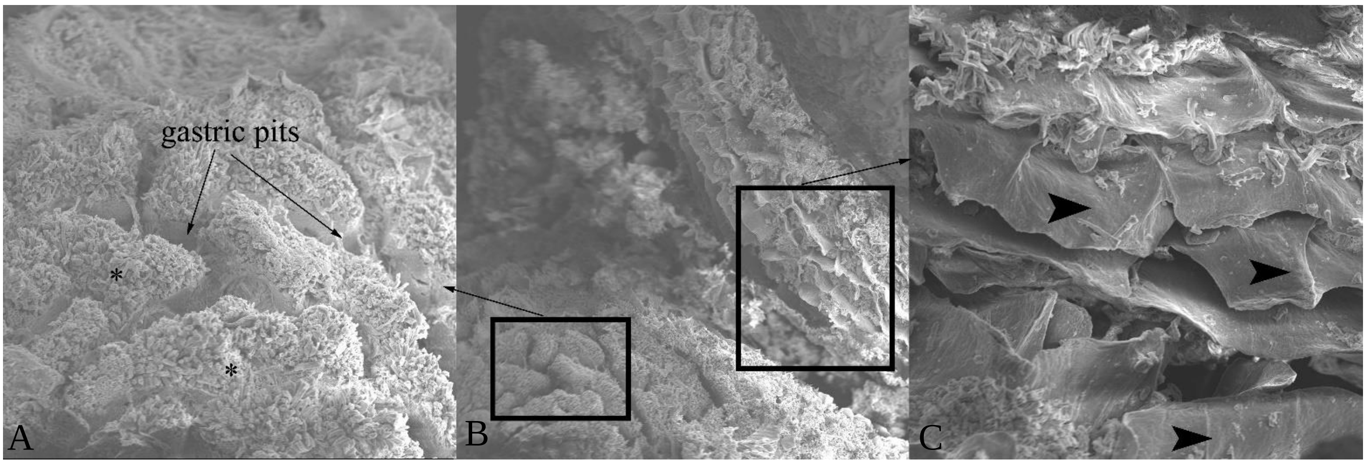

Figure 1. Scanning electron micrograph of stomach pyloric part surface. A) Left oblong area in B. B) Pyloric part surface. C) Right oblong area in B. *: Columnar cells. Arrowhead: Mucosal fold.

Pyloric caeca

The pyloric ceaca had mucosal folds in the inner surface (Figure 2A, B). The mucosal folds had an interconnected honeycomb appearance on the inner surface of the pyloric caeca (Figure 2A). Also, there were micro folds at the ends of the mucosal folds facing the lumen (Figure 2C). In places, the mucosa overlapped each other. On the transversal section of the finger-like blind processes, mucosal folds extending to the inner surface were observed; the inner surface was covered with columnar epithelium, and mucus openings were dense (Figure 2D).

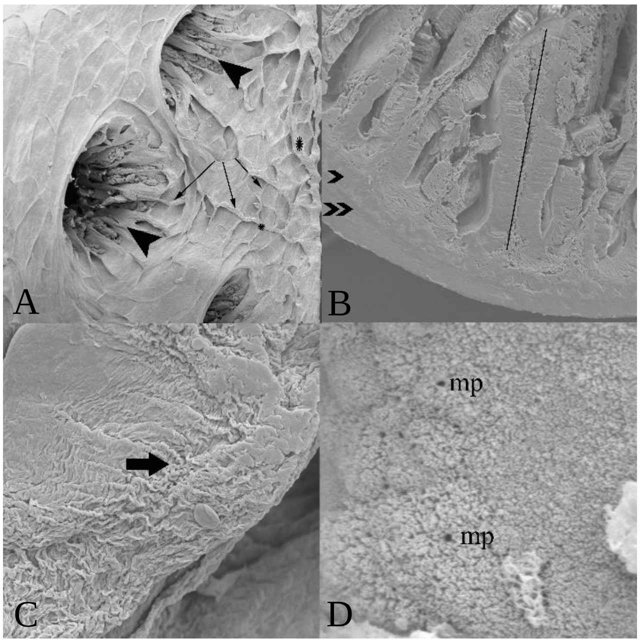

Figure 2. A) Scanning electron micrograph of pyloric caeca surface. B) Transversal slice area of the pyloric caeca. C) close-up view of mucosal fold in A. D) Columnar epithelial surface of the pyloric caeca. Thin arrows: mucosal folds, star: main mucosal fold, thick arrowhead: cecum of pyloric caeca, thin arrowhead: circular muscular layer, double thin arrowhead: longitudinal muscular layer, line: mucosal fold, thick arrow: micro folds, mp: mucosal pore.

Intestine

Scanning electron microscopy observations revealed that the luminal mucosa in the anterior regions of rainbow trout intestine exhibited thin, longitudinal mucosal folds. Above these mucosal folds, transverse and short band-shaped folds formed pouches on the mucosal folds (Figure 3A). Mucous openings were observed on the mucosa of the intestinal canal (Figure 3B, E). The mucosal folds of the large intestine were thicker than the small intestine’s (Figure 3C), and mucosal folds had micro folds at the end of the facing the lumen (Figure 3D).

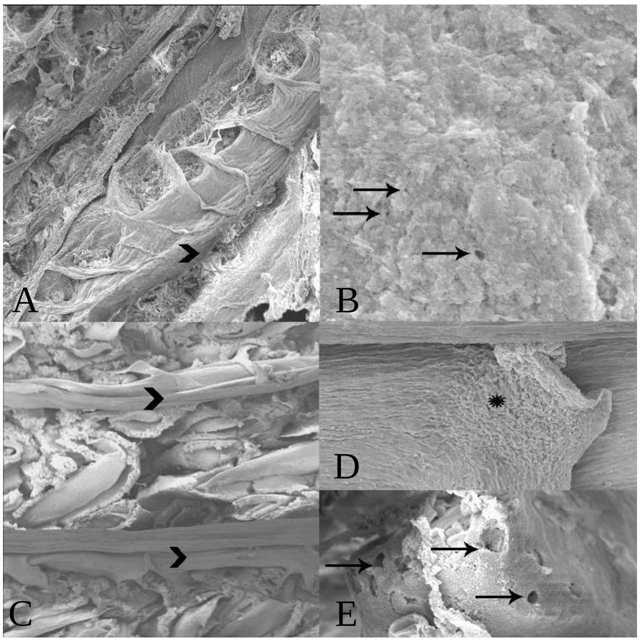

Figure 3. A) Scanning electron micrograph of small intestine surface. B) Close-up view of the small intestine surface. C) Scanning electron micrograph of large intestine surface. D) Close-up view of the mucosal folds surface of the large intestine. E) Close-up view of the small intestine surface. Arrowhead: mucosal folds, arrow: mucosal pores, * micro folds on the mucosal folds of the large intestine.

Discussion

The stomach varies considerably among fish species and is absent in some teleost species. The stomach is divided into three regions: cardia, fundus, and pylorus (Wilson & Castro, 2010). It is easy to define the stomach in the gastrointestinal system. However, in some species, such as Cyprinidae, Gobiidae, and Blennidae, the stomach appears to be an enlarged part of the gastrointestinal tract. Therefore, it is difficult to distinguish the stomach macroscopically. These species have adaptations such as glands in the esophagus and thickening of the muscular layer (Stevens & Hume, 2004; Flores et al., 2020). Depending on the feeding habits, the stomach is present in many fish species and has different shapes, such as straight, U, J, and Y. The U-shaped stomach is found in herbivores, while the Y-shaped stomach is found in carnivores (Johnson & Clements, 2022). It has been reported that the stomach is j-shaped in trout (De Felice et al., 2021). In our study, the rainbow trout's stomach was J-shaped, similar to previously reported (Wilson & Castro, 2010; De Felice et al., 2021).

In fish, the stomach also releases hydrochloric acid (HCl) for food storage and modulation of digestion. Parietal cells from the gastric glands secrete HCl and create the acidic environment essential for the activation of digestion, which is the task of the stomach (Okuthe & Bhomela, 2021). These cells are more numerous in the cranial parts of the stomach than in the caudal parts. Therefore, the caudal part of the stomach is thought to have a food storage function rather than digestion. The presence of columnar cells in the gastric mucosa protects the gastric mucosa against the low pH of the stomach (Alves et al., 2021; Johnson & Clements, 2022). It is argued that mucus in the stomach enhances digestive activity and forms a barrier against physical and pathological factors (Pedini et al., 2005; Sharba et al., 2022). In the detailed SEM examination of the gastric surface in the study, parietal cells were seen between polygonal-shaped mucosal folds, columnar cells covering the gastric surface, and gastric glands were shown in detail (Figure 4).

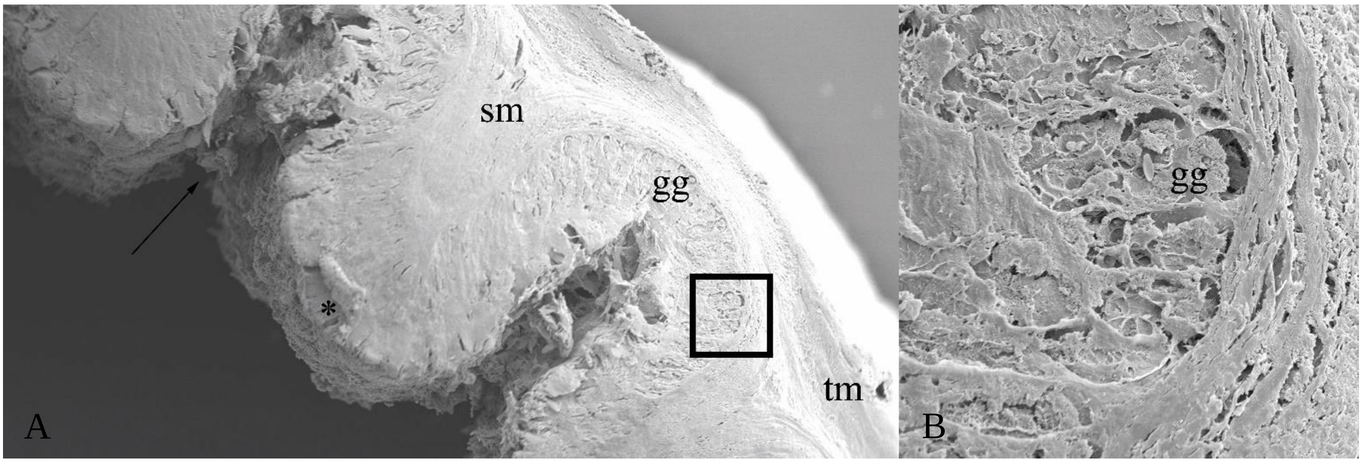

Figure 4. A) Scanning electron micrograph of transversal slice area of the stomach. B) Close-up view of oblong area in A. Arrow: gastric pit, sm: submucosa, gg: gastric glands, tm: muscular layer

The cranial parts of the intestines have finger-like pyloric caeca. Pyloric caecae are thought to shape the continuation of digestion in the stomach and increase the intestinal absorption surface without fermentation. The number and size of pyloric cecae vary according to animal species and the nature of the food (Farrag et al., 2020; Demirci et al., 2021). Our study observed that the columnar epithelium, a continuation of the gastric mucosa, was on the surface of the pyloric caeca, and there were mucosal folds. Furthermore, the honeycomb-shaped mucosal folds on the inner surface of the pyloric caeca may provide resistance to the mucosa during the digestive activity of the pyloric caeca.

Conclusion

In conclusion, the gastrointestinal tract of trout was analyzed in detail and ultrastructurally by SEM. The structure of columnar cells, gastric glands, and mucus openings on the gastric mucosal surface were revealed. The detailed anatomy of the mucosal surface affected by food diversity or pathological changes was revealed.

This study will shed light on studies affecting the morphology of the digestive system.

Financial Support

This study did not receive a grant by any financial institution/sector.

Ethical Statement

The study was conducted in accordance with the guidelines of the Kastamonu University Experimental Animals Local Ethics Committee (Decision no: 2023-9/39).

Author Contributions

Investigation: B.D.; Material and Methodology: B.D.; Supervision: B.D.; Visualization: B.D.; Writing-Original Draft: B.D.; Writing- review & Editing: B.D.

Conflict of Interest

The author declared that there is no conflict of interest.

Data Availabilty Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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