

An anatomical, histological, and immunohistochemical investigation on the intestines of the Eurasian lynx (*Lynx lynx*)

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Abstract

In this study, it was aimed to perform an anatomical, histological, and immunohistochemical investigation of the intestines of the lynx. After performing macro-anatomical measurements of the tissues obtained from the small and large intestines of the lynx which was brought to the Kafkas University Veterinary Faculty Clinics and Wild Life Preservation and Rehabilitation Center for treatment but could not be saved despite all interventions, the routine histological procedure was followed. Triple staining was applied to examine the general histological structure of intestinal tissues, and PAS staining was applied for identifying goblet cells. Somatostatin distribution was examined immunohistochemically. The lengths and layer thicknesses of intestinal segments were measured and statistically evaluated. It was determined that the total length of the intestines of the lynx was similar to that of *Canis* species, mucosa thickness decreased from the duodenum to the jejunum, and muscularis thickness was higher in the jejunum in comparison to the other segments. Somatostatin immunoreactivity was determined to be at varying intensities in the mucosa, muscularis, and serosa throughout the intestine.

Keywords: anatomy and histology, intestine, immunohistochemistry, lynx

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Introduction

The Eurasian lynx (*Lynx lynx*) is a medium-sized carnivorous cat species from the Felidae family. Its relatively long legs have a strong structure. It has furred paws and a short tail with an all-black tip, and there are black tufts of hair on its ears (Tumlison, 1987). Its head and body length ranges between 80 and 130 cm, its tail length varies between 11 and 24.5 cm, and its shoulder height is between 60 and 75 cm. The Eurasian lynx is twice the size of the Iberian lynx (*L. pardinus*) and the Canada lynx (*L. canadensis*), and it is the largest species of its genus. Males weigh 18-30 kg, while females weigh 8-21 kg (Jackson, 1997; Nowak, 1999). The Eurasian lynx is commonly found in Asia and Europe. Factors such as the destruction of forests, absence of its preys, illegal hunting, and car accidents are a significant threat for this genus. There are no clear data on the distribution and ecology of the Eurasian lynx in the east of Turkey (Chynoweth *et al.*, 2015).

The intestines play significant roles in the digestion and absorption of foods and the elimination of undigested foods, microorganisms, and microbial products. For the epithelial cells in the intestinal mucosa to fulfill their function, the mucous layer, the tight connection between cells, epithelial cells, and innate and adaptive immune response must be regulated (Lievin-Le Moal and Servin, 2006; Dharmani, 2009). The small intestine is where digestion processes are completed, and foods (products of digestion) are absorbed by epithelial cells. It is composed of three segments, namely the duodenum, jejunum, and ileum. The large intestine, which enables the excretion of undigested foods allows water and electrolytes to be absorbed, is made up of the cecum, colon, and rectum (Mescher, 2018). The intestinal mucosal epithelium consists of four main types of cells. These are absorbing enterocytes, goblet cells, Paneth cells, and enteroendocrine cells. These cells continuously pass through restoration cycles (van der Flier and Clevers, 2009).

Somatostatin is a 14-amino acid inhibiting peptide which is secreted from D cells of the islets of Langerhans in the pancreas and the hypothalamus, it has a wide spectrum of biological activities, and it is known as the factor inhibiting the secretion of growth hormone from the hypothalamus. It is found in the pancreas, stomach, intestinal mucosa, and myenteric neurons. It reduces hepatobiliary, pancreatic, and gastric acid secretion and slows down intestinal passage (Demircan *et al.*, 2003; Timurkaan *et al.*, 2009; Narin *et al.*, 2014). It was reported that there are somatostatin-endocrine cells (like SST-producing-D cells) in the stomach, intestine, and pancreas that release gastrointestinal hormones and regulate gastrointestinal functions. It is also involved in the regulation of secretory activity and intestinal motility in the gastrointestinal tract, blood flow, inflammatory response, transmission of pain and sensation, and the release of hormone factors and other neurotransmitters. Somatostatin and its receptors mediate the release of gastric juice, intestinal juice, gastric acid, and other hormones via other endocrine

factors (Morisset, 2017; Schubert and Rehfeld, 2019; Engevik *et al.*, 2020).

In the literature review that was carried out for this study, it was seen that studies conducted on the digestive system of wild carnivores were limited in numbers (Yıldız *et al.*, 2006; Sapundzhiev *et al.*, 2017). It was also found that there was no anatomical, histological, and immunohistochemical study conducted on the intestines of the lynx. Knowing about the normal appearance of the intestinal tissue of a certain species and identifying variations is important in terms of diagnosing diseases. Accurate diagnosis is an important step towards appropriate treatment. Hence, it was aimed in this study to investigate the anatomical, histological, and immunohistochemical characteristics of the intestines of the lynx, compare the findings obtained in this study to the results of studies previously conducted on particularly the lynx and other carnivorous animals and reveal similarities and differences in this regard. In addition, it is thought that examining the distribution of somatostatin in the small and large intestines will contribute to basic and clinical research on the role of somatostatin in digestion and absorption.

Materials and Methods

Materials: A conditional permission to conduct the study was firstly obtained from the Kafkas University Animal Experiments Local Ethics Committee (KAU-HADYEK/2022-019). With this conditional permission, a recourse was made to the Nature Preservation and National Parks General Directorate under the Ministry of Agriculture and Forestry, and the study was approved with the decision number E-21264211-288.04-4752856/2022. Intestinal parts obtained from a wounded lynx (*Lynx lynx*), 1.5 years old and female, that was brought to the Kafkas University Wild Life Preservation and Rehabilitation Center for treatment after a gunshot wound but could not be saved in spite of all medical interventions were used as the study material.

Anatomical examinations: The lynx cadaver brought to the anatomy laboratory for examination was dissected, and its intestines were removed. Some measurements were taken from organ sections fixed in formaldehyde. The length, width, and thickness of each intestinal segment starting from the pylorus of the stomach were measured with the help of a digital caliper (stainless steel 1 to 150 mm). NAV 2017 was used in labelling the anatomical terms.

Histological examinations: After obtaining the macro-anatomical findings, tissue samples were taken from the intestinal material (duodenum, jejunum, ileum, cecum, colon) and fixed in 10% formaldehyde solution. The tissue samples were subjected to routine tissue processing procedures and embedded in paraffin. By taking sections at thicknesses of 5 µm from the paraffin blocks, Crozman's triple staining and H&E staining was applied for investigating the general structure of the intestines, and PAS (Periodic acid-Schiff) staining was applied for identifying goblet cells.

Immunohistochemical examinations: The streptavidin-biotin peroxidase method was applied to the sections placed on slides covered with Chrome Alum-Gelatin. Throughout the procedure, PBS (0.1 M, PH, 7.2) buffer was used for the irrigation processes. The sections were incubated for 15 minutes in 3% H₂O₂ prepared in 0.1 M PBS to prevent endogenous peroxidase activity. To expose antigens, the sections were subjected to heat at the maximum temperature (800 watt) in a microwave oven for 10 minutes (once) in a citrate buffer solution. After this, they were incubated for 10 minutes with Large Volume V Block solution. Then, somatostatin (1/500 dilution) (abcam ab183855) primary antibody was applied to the sections at room temperature and in a humid environment for one hour. Next, the sections were incubated with Biotinylated Goat Anti B Polyvalent solution and Streptavidin Peroxidase solution, respectively, at room temperature for 30 minutes each. DAB-H₂O₂ (Diaminobenzidine hydrogen peroxide) Substrate Solution was added for chromogen application. For counterstaining, modified Gill III hematoxylin solution was used. To determine whether somatostatin immunoreactivity was specific or not, all procedures were exactly applied to the sections which were kept in PBS without adding the primary antibody. Rat brain tissue was used as positive control for somatostatin. For immunohistochemical evaluations, the staining properties and intensities of the target cells were considered. The evaluations were made by two independent observers by assigning scores from 0 to 3, (0) for no staining, (1) for weak staining, (2) for moderate staining, and (3) for strong staining. The sections prepared for the histological and immunohistochemical examinations were evaluated, and their images were taken with an optical microscope (Olympus BX43, JAPAN).

Statistical analyses: The mucosa, submucosa, and muscularis layer thicknesses of the intestinal tissue separated based on the segments of the intestine were measured at 4- μ m magnification by using the Image J (v1.50i) software. The measurements were made on five different regions on the five serial sections taken from each region. The measurements in the submucosa were made by choosing regions without lymph follicles. The SPSS (20.0) package software was used in the analyses of the obtained data. The significance of the differences in layer thicknesses in different intestinal parts was analyzed using One-Way ANOVA. When significant differences were found, the sources of these differences were identified using Duncan's test.

Results

Anatomical results: As seen in Figure 1, the small intestine was composed of the duodenum, jejunum, and ileum. The duodenum started from the pylorus of the stomach. The part of the duodenum progressing up to shaping the cranial duodenal flexura was the descending portion. The descending portion's length of the duodenum was measured as 36.20 cm, its width was measured as 1.24 cm, and its canal width was found as 0.42 cm. It was determined that while the flexura progressed towards the caudal after shaping

the cranial duodenal flexura, it formed an elbow. The length of the transverse portion of the duodenum after the caudal duodenal flexura was found to be 7.51 cm, its width was 0.8 cm, and its canal width was 0.45 cm. The length of the ascending portion was 30.93 cm, its width was 1.42 cm, and its canal width was 0.4 cm. The length of the jejunum was measured as 157.46 cm, its width was found as 0.69 cm, and its canal width was 0.49 cm. The jejunal folds on the cadaver were rather prominent. The jejunum was suspended from the roof of mesojejunum and abdominal cavity. The length of the ileum was determined as 6.52 cm, its width was 1.48 cm, and its canal width was 0.97 cm. The large intestine was examined in three parts as the cecum, colon, and rectum. The length of the cecum was measured as 4.67 cm, its width was 1.56 cm, and its canal width was 1.66 cm. Colon length was measured as 9.93 cm. The colon-rectum length was 19.46 cm, the width at this portion was 1.70 cm, and the canal width here was 1.34 cm. The total lengths of the intestinal segments are presented in Table 1 in comparisons to different species.

Histological results: The mucosa, submucosa, muscularis, and serosa layers in the intestinal tissue were identified. In the small intestine, mucosal layer consisted of epithelial layer, lamina propria and muscularis mucosa. In the lamina propria, the villi intestinales were observed to have a narrow and long structure, and there were numerous goblet cells located between single-layer cylindrical epithelial cells on their surfaces. Lieberkühn crypts were determined at the base of the villi intestinales. It was determined that the muscularis mucosa also separates the crypts into the submucosa and has a smooth muscle structure (Figures 2, 3 and 4).

It was determined that the submucosa was composed of mucous-structured Brunner's glands and connective tissue in the duodenum. Numerous blood vessels were noted in the connective tissue. In the jejunum and ileum, no gland structure was encountered in the submucosa, and they were seen to consist of connective tissue. In addition, autonomic nerve plexuses were observed in the submucosa along the small intestinal tract. Aggregated Lymphatic Nodules were detected especially in jejunum and ileum. The muscularis layer in the duodenum had a circular structure on the inside and longitudinal structure on the outside as a thick layer, whereas it had a similar structure in the jejunum and ileum. Autonomic nerve plexuses were present along the small intestinal tract in the muscularis layer (Figures 2, 3 and 4).

In the cecum and colon, the mucosa layer was seen to have a thin structure, there was no villus, and the surface of the mucosa facing the lumen was found to be smooth. It was also determined that there were crypts consisting of numerous goblet cells with a tubular shape, there were several blood vessels, and there were lymph follicles located in the submucosa in the cecum. It was seen that the muscularis layer had a circular structure on the inside and longitudinal structure on the outside in both the cecum and colon, and it was thicker compared to the mucosa. Autonomic nerve plexuses were present along the large intestinal tract in the muscularis layer. It was determined that the

serosa was located in the outermost part of all intestinal sections and consisted of a loose connective tissue layer covered with mesothelium. As a result of PAS staining performed in small and large intestines, PAS positive reaction was observed in goblet cells of villi and crypts and submucosal glands (Figures 5, 6 and 7).

Immunohistochemical results: Strong somatostatin immunoreactivity was determined in the cytoplasm of the villi and crypt epithelial cells in the duodenum, jejunum, and ileum, and moderate somatostatin immunoreactivity was determined in the cytoplasm of the crypt epithelial cells in the cecum and colon. In all segments of the small intestine and large intestine, strong immunoreactivity was determined in the

muscularis and serosa layers (Figures 8 and 9). The positive and negative control tissues was shown in Figure 10.

Statistical analysis results: The thicknesses of the mucosa, submucosa, and muscularis in the duodenum, jejunum, ileum, and cecum are presented in Table 2. A statistically significant difference was determined among the segments in terms of their mucosal thicknesses ($p<0.05$), and these thicknesses decreased especially from the duodenum towards the cecum. It was also found that submucosal thickness varied especially between the duodenum and the cecum, and this thickness decreased from the duodenum to the cecum. Muscularis thickness showed a statistically significant difference between the jejunum and the other segments ($p<0.05$), and this thickness increased particularly in the jejunum.

Table 1 Comparison of total lengths of intestinal segments to different species.

Intestinal segments (cm)	Canis (Bahadır and Yıldız 2014; Eva'nsa and de Lahunta 2013)	Felis (Bahadır and Yıldız 2014; Eva'nsa and de Lahunta 2013)	<i>Lynx lynx</i>
Intestinum tenuie	180-480	80-130	238.62
Duodenum	20-60	10-12	74.64
Jejunum	160-420	70-120	157.46
Ileum			6.5
Intestinum crassum	28-90	20-45	24.06
Cecum	8-30	2-4	4.6
Colon			9.93
Colon-rectum	20-60	20-40	19.46
Total intestinal length	200-570	100-180	262.68

Table 2 Statistical analyses of layer thicknesses according to intestinal segments.

Intestinal segments (μm)	Duodenum	Jejunum	Ileum	Cecum	p
Mucosa	608.09 \pm 24.11 ^a	361.12 \pm 21.57 ^b	135.27 \pm 12.65 ^c	76.71 \pm 5.43 ^d	.000
Submucosa	123.39 \pm 39.54 ^a	96.93 \pm 37.06 ^{ab}	48.47 \pm 13.57 ^{ab}	31.29 \pm 1.38 ^b	.114
Muscularis	171.67 \pm 9.13 ^a	374.83 \pm 49.01 ^b	207.46 \pm 24.89 ^a	128.42 \pm 3.16 ^a	.000

^{a,b,c,d}There is a statistically significant difference between the mean values shown with different letters in the same row.

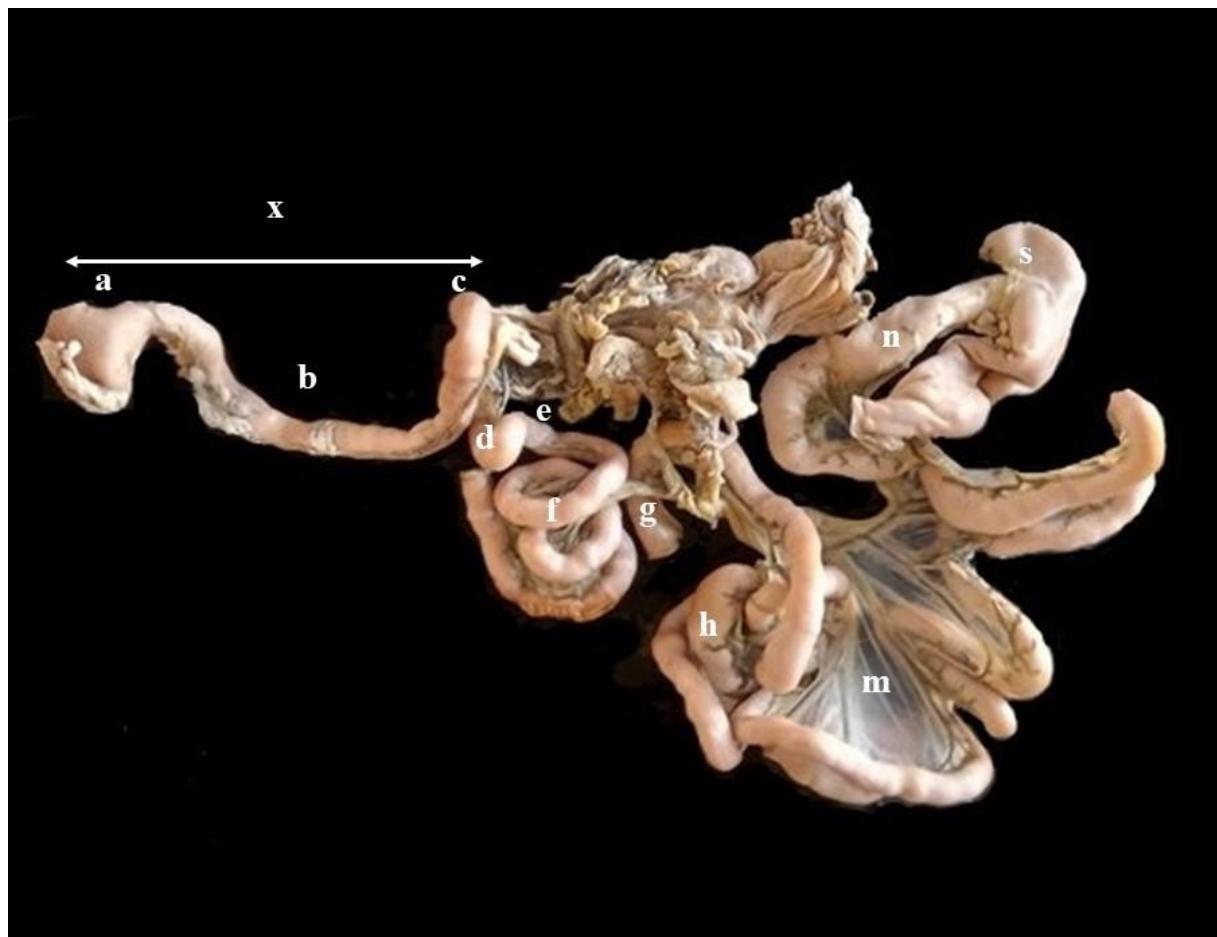


Figure 1 Intestinal sections in lynx (*Lynx lynx*). a: pylorus, b: pars descendens of duodenum, fold between a and x: flexura duodeni cranialis, c: flexura duodeni caudalis, d: flexura duodenojejunalis, e: pars transversa, f: first jejunal loops, g: jejunum, h: ansa jejunalis, m: mesojejunum, n: ileum, s: cecum.

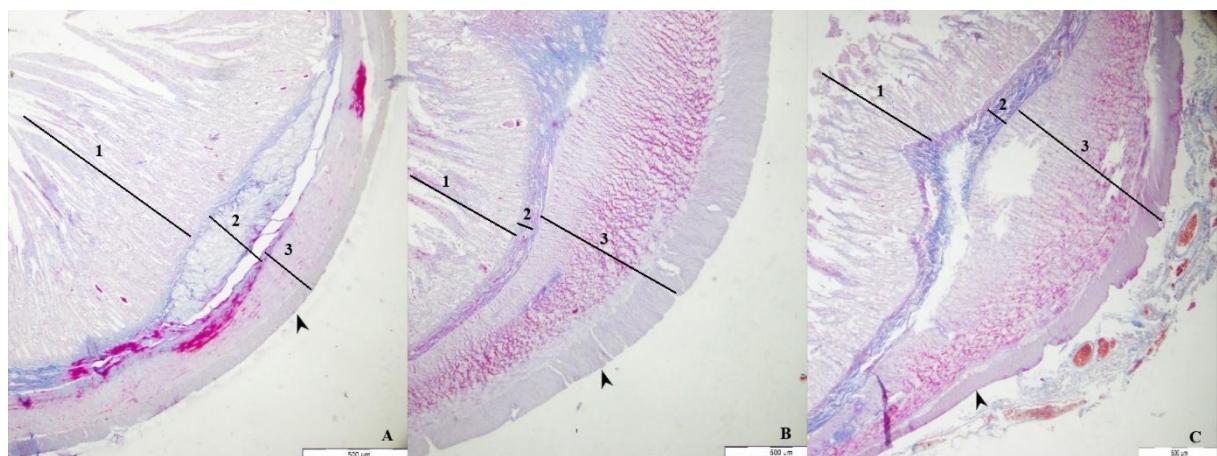


Figure 2 Lynx small intestine tissue. A: Duodenum, B: Jejunum, C: Ileum. Mucosa (1), submucosa (2), muscularis (3), serosa (arrow). Triple staining. 500 μm.

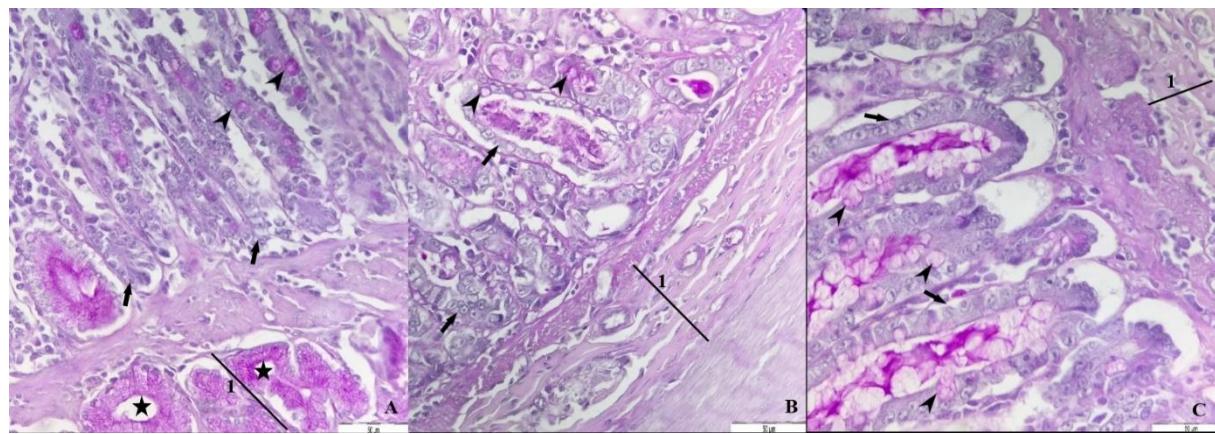


Figure 3 PAS staining of lynx small intestine tissue. PAS positive reaction in goblet cells and submucosal glands. A: Duodenum, B: Jejunum, C: Ileum. Crypt epithelium (arrow), goblet cell (arrowhead), submucosa (1), mucous glands (asterisk). 50 μ m.

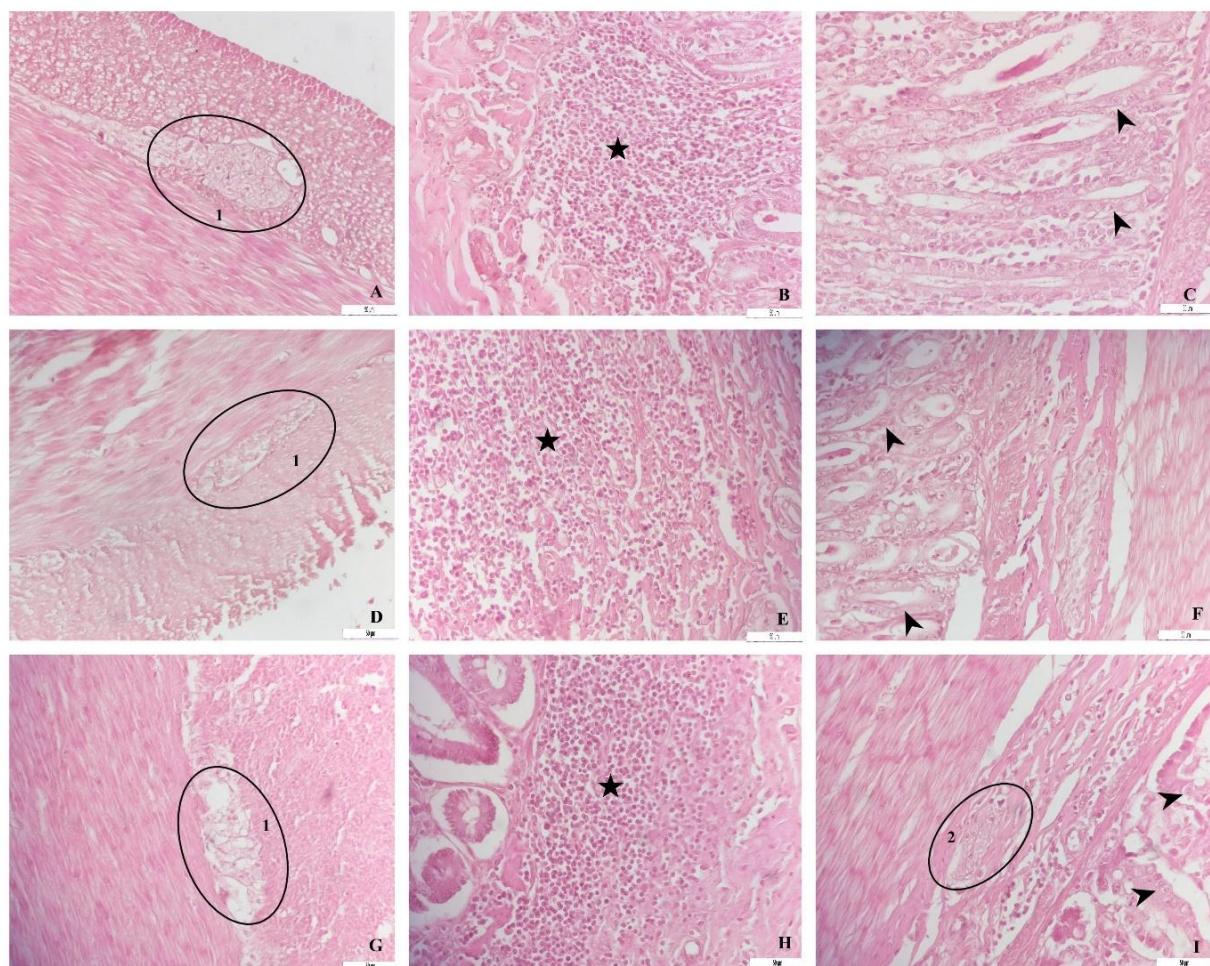


Figure 4 Lynx small intestine tissue. A,B,C: Duodenum, D,E,F: Jejunum, G,H,I: Ileum. Autonomic nerve plexuses (tunica muscularis) (1), autonomic nerve plexuses (submucosa) (2), lymphoid nodule in the submucosa (star), crypt epithelium (arrow head). H-E staining. 50 μ m

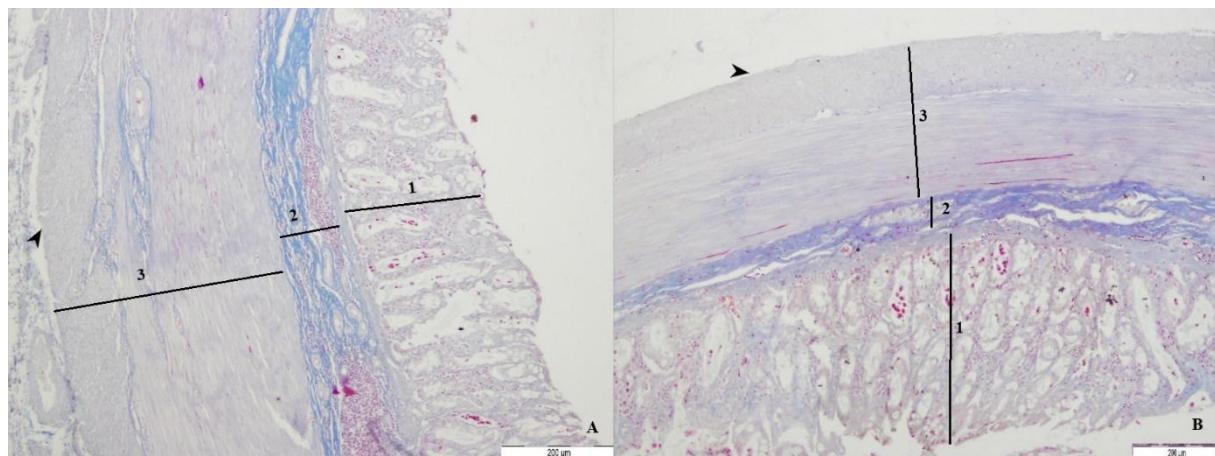


Figure 5 Lynx large intestine tissue. A: Cecum, B: Colon. Mucosa (1), submucosa (2), muscularis (3), serosa (arrow). Triple staining. 200 μ m.

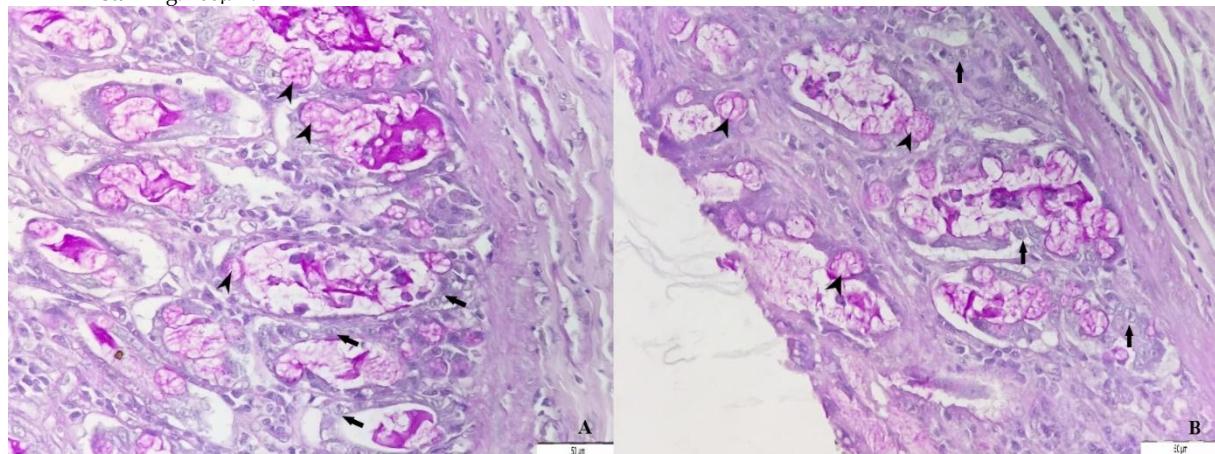


Figure 6 PAS staining of lynx large intestine tissue. PAS positive reaction in goblet cells. A: Cecum, B: Colon. Crypt epithelium (arrow), goblet cell (arrowhead). 50 μ m.

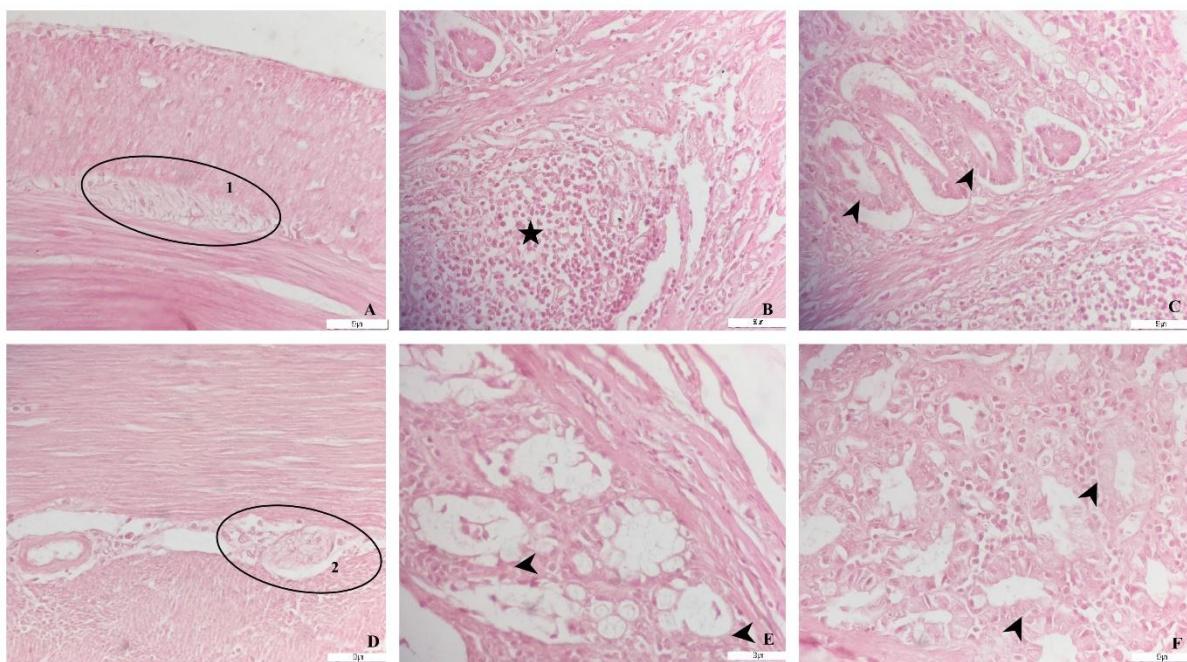


Figure 7 Lynx large intestine tissue. A,B,C: Cecum and D,E,F: Colon. Autonomic nerve plexuses (tunica muscularis) (1, 2), lymphoid nodule in the submucosa (star), crypt epithelium (arrowhead). H-E staining. 50 μ m

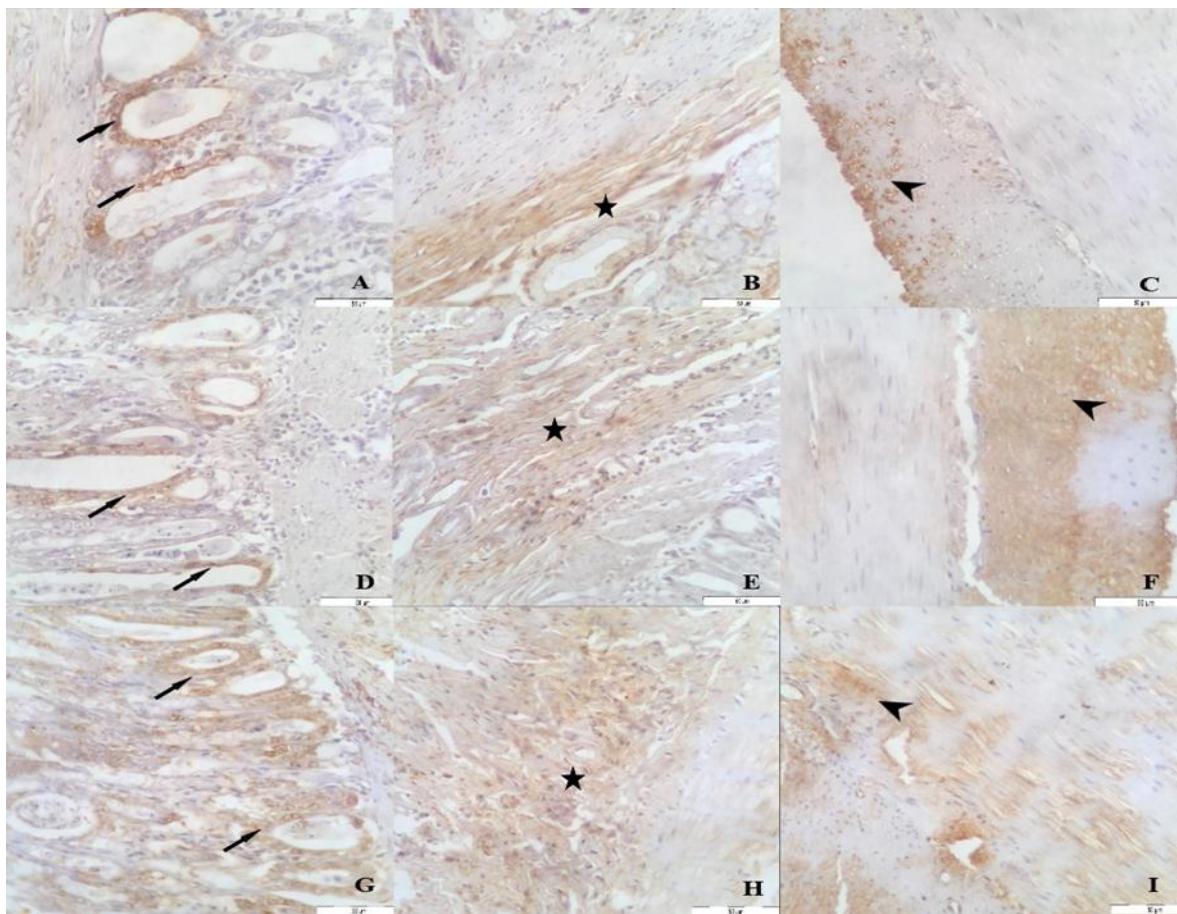


Figure 8 Somatostatin immunoreactivity in lynx small intestine tissue. A,B,C: Duodenum, D,E,F: Jejunum, G,H,I: Ileum. Crypt epithelium (arrow), submucosa (star), muscularis (arrowhead). 50 μ m.

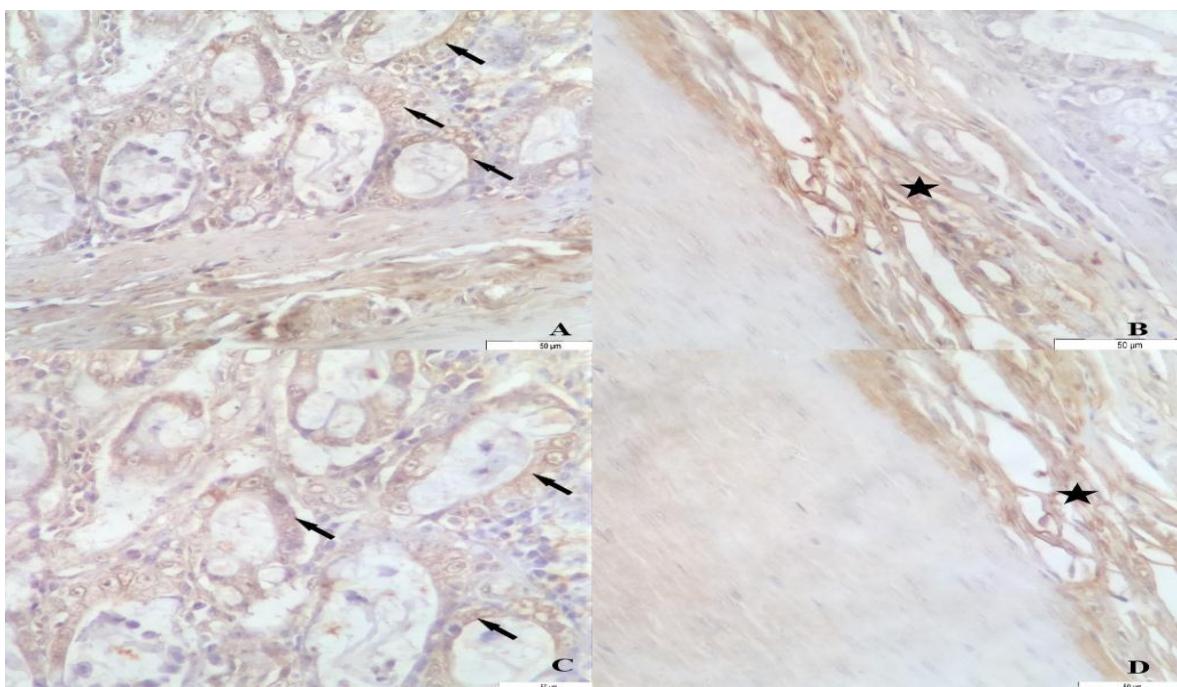


Figure 9 Somatostatin immunoreactivity in lynx large intestine tissue. A,B: Cecum, C,D: Colon. Crypt epithelium (arrow), submucosa (star). 50 μ m.

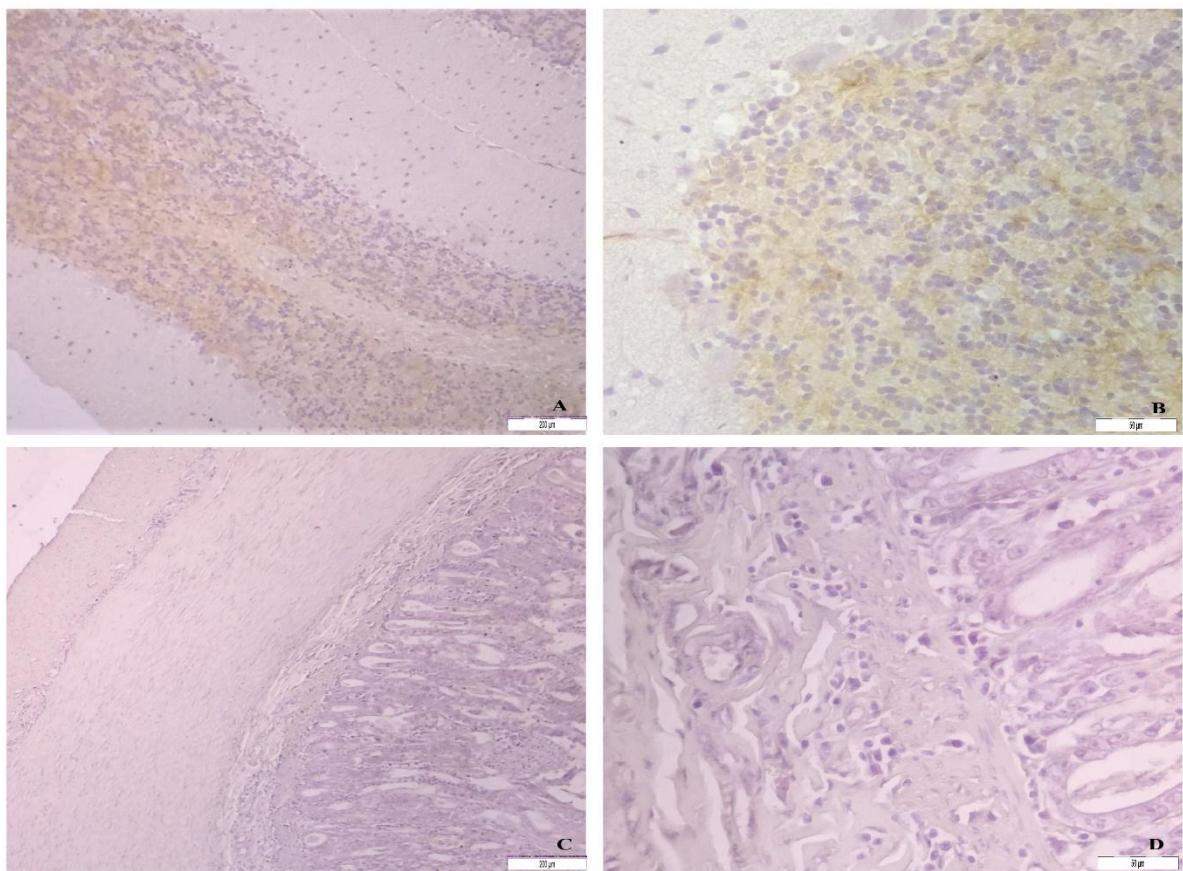


Figure 10 Somatostatin immunoreactivity in lynx large intestine tissue. A,B: Cecum, C,D: Colon. Crypt epithelium (arrow), submucosa (star). 50 μ m.

Discussion

The intestines are organs of the gastrointestinal system with varying structures and lengths depending on the nutritional characteristics of different species. They start from the pylorus of the stomach and continue up to the anus. The length of the intestine can be roughly calculated by taking different ratios of the body length according to animal species. It has been reported that this length is 3-4 times the body length in domestic animals and generally 5 times the body length in the *Canis* genus (Bahadir and Yıldız, 2014; Dyce, 2015). The intestines are divided into two parts as the *intestinum tenue* (small intestine) and the *intestinum crassum* (large intestine) according to their lumen diameter. The segments of the small intestine are determined based on their location in the abdominal cavity, their relation to other organs, their mesentery, and incoming blood vessels (Bahadir and Yıldız, 2014). The segments of the small intestine are the duodenum, jejunum, and ileum, respectively. Most nutrient absorption takes place in the small intestine (Demirşalan and Orhun Dayan, 2021). At the beginning of the small intestine is the short and fixed duodenum, which is followed by the jejunum and ileum suspended by the mesojejunum and mesoileum. The jejunum is usually the longest small intestinal segment with hollow insides in a cadaver. It is shaped by the curves named as jejunal folds. However, it is shorter in carnivores than in other species, and it is located in the ventrolateral of the abdomen. The jejunum is separated from the duodenum through the

duodenocolical fold and from the ileum by a band called the ileocecal fold. The ileum is the shortest segment of the small intestine. In carnivores, it opens to the inside of the junction between the cecum and colon. Next to the outside of the hole where the ileum opens to the cecum, there is the cecocolic orifice between the cecum and colon (Dursun, 2008). The large intestine consists of the cecum that ends in a blind pouch, the colon separated by the cranial pelvic aperture, and the rectum, respectively (Bahadir and Yıldız, 2014). In carnivores, the cecum is located to the right of the abdominal cavity and is folded on. It is 5-6 cm long and has a round end. The paralumbar fossa is in the ventral of the 3rd and 4th Lumbar vertebrae in the dextra (Dursun, 2008). In carnivores, the colon segment following the cecum is on the right, and the colon that proceeds towards the cranial is the ascending portions. It ends with the rectum and anal canal (Bahadir and Yıldız, 2014). As reported in the literature (Evans and de Lahunta, 2013; Demirşalan and Orhun Dayan, 2021), the intestinal segments of the lynx that were examined in this study were composed of the duodenum's cranial portion, descending portion, transverse portion, and ascending portion. There was an elbow structure called the cranial duodenal flexura between the cranial portion and descending portion. Between the descending portion and transverse portion, there was the caudal duodenal flexura. After the ascending portion, the duodenum was connecting to the jejunum through the duodenojejunal flexura. The duodenum length in

carnivores is approximately 25 cm. It starts from the dorsal hypochondriac region opposite the 10th intercostal void (Evans and de Lahunta, 2013). In a previous study, the total length of the small intestine was reported to be 194 cm in the red fox, 396 cm in the wolf, and 251 cm in the lynx, while in this study, it was measured as 238.62 cm. In the lynx, the duodenum was between the 9th and 10th costa at a length of 74.64 cm. In comparison, this value was closer to those of the *Canis* genus among other carnivores but longer. The length of the jejunum was 157.46 cm. The animal genus that it was the closest to was again *Canis*. The length of the ileum was 6.5 cm. No comparison could be made as the literature review did not reveal any findings reported on other carnivores. While the total length of the large intestine was reported to be 44 cm in the red fox, 87 cm in the wolf, and 48 cm in the lynx (McGrosky *et al.*, 2016), it was 24.06 cm in this study, which was lower than the reported values. While McGrosky (2016) reported the length of the cecum as 55 cm in the red fox, 12.5 cm in the wolf, and 5.9 cm in the lynx, it was found to be 4.6 cm in this study, which was close to the reported values but still lower. The total length of the intestine was reported to be 238 cm in the red fox, 484 cm in the wolf, and 300 cm in the lynx in the literature (McGrosky *et al.*, 2016), while it was measured as 262.68 cm in this study. It was seen that among the values reported, the shortest measurement values regarding intestinal lengths were obtained in this study.

From a histological perspective, the digestive system is composed of the layers of mucosa being the innermost, and submucosa, muscularis, and serosa (or adventitia) being the outermost, respectively. The mucosa consists of the inner epithelium, middle lamina propria, and outer muscularis mucosa. In most parts of the small and large intestines, the mucous epithelium is simple cylindrical epithelium. Goblet cells located between the mucous epithelium contribute to the secretion of lubricating material by the small and large intestines. The intestinal villi are limited to the small intestine in mammals. They are short and thick in ruminants and long and thin in carnivores. At the base of the villi, there are epithelial invaginations, that is, Lieberkühn (intestinal glands) crypts. A muscularis mucosae, consisting of two layers of smooth muscle, separates the crypts from the underlying submucosa. In the submucosa, there are compound Brunner's glands (duodenal glands, submucosal glands). While the submucosa is located at the beginning or in the middle region of the duodenum in carnivores, sheep, and goats, it extends to the jejunum in horses, pigs, and cows (Bacha and Bacha, 2012). It was determined in the lynx that the villi intestinales had a narrow and long structure, there were numerous goblet cells located between the single-layer cylindrical epithelial cells on the surfaces, their length decreased from the duodenum to the ileum, and the crypts had a narrow tubular shape. While Brunner's glands were observed in the duodenum, they were not found in the jejunum or ileum.

There is no villus in the three segments of the large intestine, but Lieberkühn crypts are found in the lamina propria. The mucosa is made up of cylindrical epithelial and several goblet cells. While the length of

the cecum, which is the first segment of the large intestine, is longer in herbivores (e.g., horses), it is shorter in carnivores. In all domestic mammals, there are numerous lymph follicles dispersed along the length of the cecum. While lymph follicles are found in the cecum apex, particularly in pigs, ruminants, and dogs, they are concentrated close to the peak point (distal) of the cecum in horses and cats (Eurell and Frappier, 2006; Gartner and Hiatt, 2014). The mucosa of the colon is thicker than that of the small intestine. As there is no villus, the mucosal surface is smooth. The number of goblet cells is increased compared to the small intestine. There is frequently lymph tissue in the submucosa that extends up to the muscularis. The tunica muscularis in pigs and horses is composed of smooth muscles whose outer layers have thickened, longitudinally directed, and elastic fibers (Eurell and Frappier, 2006). In the lynx, it was determined that the cecum and colon mucosa had a thin structure, there was no villus, the mucosa surface facing the lumen was smooth, there were tubular shaped crypts consisting of numerous goblet cells, and there were numerous blood vessels. Additionally, lymph follicles located in the submucosa in the cecum were identified. In both the cecum and colon, the muscularis layer was found to have a circular structure on the inside and longitudinal structure on the outside, and it was thicker compared to the mucosa.

Regarding the evaluations made on the thicknesses of intestinal segments in carnivores, it was reported that the mucosal thickness in the small intestine in dogs gradually decreased from the proximal of the small intestine towards the distal, the submucosal thickness changed a little from the duodenum towards the jejunum, but it significantly increased in the ileum depending on the increase in the amount of lymphoid tissue (Sarriá *et al.*, 2012). In cat small intestines, it was reported that the mucosa layer was thicker in the duodenum and jejunum in comparison to the muscularis and was equal in the ileum. It was stated that muscularis thickness showed an increase from the duodenum towards the ileum (Martínez *et al.*, 2018). It was expressed that the crypts in the cecum had a narrow tubular shape, the parts other than the surface epithelium were filled with goblet cells, and enterocytes were squeezed in between. It was reported that lymph follicles were distributed in the cecum along the submucosa (Robert, 1984). It was determined in this study that the mucosal and submucosal thicknesses in the lynx decreased from the duodenum towards the cecum, and the muscularis thickness was at the highest level in the jejunum. It is thought that the thickness of the mucosa and submucosa decreases as digestion and absorption events are completed as one moves from the duodenum to the cecum, and the thickness of the muscularis increases to facilitate the excretion of waste materials.

Somatostatin is commonly found in the brain, pancreas, and peripheral autonomous nervous system. However, the most important source of somatostatin in the periphery is the gastrointestinal system. In the gastrointestinal system, as a result of the functioning of different translations of prosomatostatin, two biologically active peptides are produced. These peptides are widely found in the stomach, duodenum,

distal jejunum, and ileum (Patel *et al.*, 1981; Trent and Weir, 1981; Baldissera *et al.*, 1985; Francis *et al.*, 1990). Additionally, they are distributed throughout the length of the small intestine in the myenteric plexus (neural network formed by autonomous nerve threads distributed in the muscular layer of the stomach and intestines). Both peptides have strong inhibiting effects on digestive functions including exocrine secretions in the stomach and pancreas, neuro/endocrine peptide expression, and intestinal growth (Newman *et al.*, 1987).

When the immunohistochemical distribution of somatostatin in the digestive system was examined, positive prosomatostatin immunoreactivity in the pig gastrointestinal canal was reported in the mucosal glands in the stomach, in numerous endocrine cells in the duodenum, in a few cells in Brunner's glands, and in myenteric and submucosal nerve fibers. Immunoreactivity was also reported in the jejunum and ileum, in mucosal endocrine cells, in myenteric and submucosal nerve fibers, and in few cells in mucosal crypts in the colon (Skak-Nielsen *et al.*, 1987). In the cat intestinal wall, the immunohistochemical localization of somatostatin was observed in the pyloric sphincter, ileum, ileocecal sphincter, and nerve fibers in the proximal colon (Lolova *et al.*, 1984). Intense immunoreactivity was reported in rats in the stomach's cardia, fundus, and pylorus mucosa (Eliş Yıldız *et al.*, 2019). The determination of somatostatin immunoreactivity at varying intensities in the small and large intestines of the lynx, which is a carnivorous species, especially in the layers of mucosa, muscularis, and serosa, suggested that somatostatin may play certain roles in maintaining digestive functions.

The digestive system, where nutrients are digested and absorbed, has a significant place for all living species. In the anatomical examinations performed on the digestive system of the lynx in this study, it was determined that the total length of the intestines of the lynx was similar to those of *Canis* species. The histological structure of the small and large intestinal segments was found to be similar to those in other carnivorous species. It was determined that mucosal and submucosal thicknesses decreased from the duodenum towards the cecum, and somatostatin immunoreactivity at varying intensities was found in the small and large intestines, particularly in the layers of mucosa, muscularis, and serosa. It is believed that the findings obtained in this study will contribute to the determination of similarities and differences by comparing them to the findings obtained in previous studies on carnivorous animals and constitute a stepping stone for future studies.

References

Bacha WJ and Bacha LM 2012. Color atlas of veterinary histology. (3rd edition). John Wiley & Sons, Ltd.

Bahadir A and Yıldız H 2014. Veteriner Anatomi, Hareket Sistemi & İç Organlar. 5. Baskı. Ezgi kitabevi, Bursa. 287-295.

Baldissera FGA, Holst JJ, Jensen SL and Krarup T 1985. Distribution and molecular forms of peptides containing somatostatin immunodeterminants in extracts from the entire gastrointestinal tract of man and pig. *Biochim Biophys Acta.* 838: 132-143.

Chynoweth MW, Çoban E and Şekercioğlu ÇH 2015. Conservation of a new breeding population of Caucasian lynx (*Lynx lynx dinniki*) in eastern Turkey. *Turkish Journal of Zoology.* 39: 541-543.

Demiraslan Y and Orhun Dayan M 2021. Veteriner Sistematiğ Anatomi. (1. Baskı). Atlas Kitabevi.

Demircan C, Kapıcıoğlu S, Kuşkonmaz İ, Taşkin A, Günaydin M and Kaya N 2003. Mekanik intestinal obstruksiyonlu ratlarda somatostatin analogu SMS 201- 995 (Octreotide) ve omeprazolun etkileri. *Uludağ Üniversitesi Tıp Fakültesi Dergisi.* 29 (1): 11-14.

Dharmani P, Srivastava V, Kissoon-Singh V and Kris C 2009. Role of intestinal mucins in innate host defense mechanisms against pathogens. *J Innate Immun.* 1: 123-135.

Dursun N 2008. Veteriner Anatomi II. (12. Baskı). Medisan yayinevi, 53-61.

Dyce KM, Sack WO and Wensing CJG 2010. Textbook of Veterinary Anatomy. (4th edition). Saunders, Elsevier.

Eliş Yıldız S, Bakır B, Yediel Aras Ş, Dağ S and Karadağ Sarı E 2019. Immunohistochemical distribution of somatostatin in gastric tissue of diabetic rats treated with cinnamon extract. *Kafkas Univ Vet Fak Derg.* 25 (3): 427-433.

Engevik AC, Kaji I and Goldenring JR 2020. The physiology of the gastric parietal cell. *Physiol Rev.* 100: 573-602.

Eurell JA and Frappier BL 2006. Textbook of veterinary histology. (6th edition). Blackwell Publishing Ltd.

Evans HE and de Lahunta A 2013. Miller's Anatomy of the Dog. (4th edition). WB Saunders Company, Philadelphia, 345-358.

Francis BH, Baskin DG, Saunders DR and Ensink JW 1990. Distribution of somatostatin-14 and somatostatin-28 gastrointestinal-pancreatic cells of rats and humans. *Gastroenterology.* 99: 1283-1291.

Gartner LP and Hiatt JL 2014. Color atlas and text of histology. (6th edition). Lippincott Williams & Wilkins, a Wolters Kluwer Business.

Jackson P 1997. "The status of cats in the wild." *International Zoo Yearbook,* 35(1): 17- 27.

Lievin-Le Moal V and Servin AL 2006. The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. *Clin Microbiol Rev.* 19: 315-337.

Lolova I, Itzhev D and Davidoff M 1984. Immunocytochemical localization of substance p, methionine-enkephalin and somatostatin in the cat intestinal wall. *J Neural Transm.* 60: 71-88.

Martinez M, Pallaresb FJ, Solera M and Agut A 2018. Relationship between ultrasonographic and histopathological measurements of small intestinal wall layers in fresh cat cadavers. *The Veterinary Journal.* 237: 1-8.

McGrosky A, Navarrete A, Isler K, Langer P and Clauss M 2016. Gross intestinal morphometry and allometry in carnivorans. *Eur J Wildl Res.* 62: 395-405.

Mescher AL 2018. Junqueira's Basic Histology text and atlas. (15th edition). McGraw-Hill Education, 314-320.

Morisset J 2017. Somatostatin: One of the rare multifunctional inhibitors of mammalian species. *Pancreas*. 46: 8-18.

Narin S, Pişkin İE and Üstündağ G 2014. 2014' te Somatostatin'in tip'ta kullanımı (Oktreotid). *Güncel Gastroenterology*. 18 (2): 272-276.

Newman JB, Lluis F and Townsend CM 1987. Somatostatin. In: Thompson JC, Greeley GH, Rayford PL, Townsend CM (eds). *Gastrointestinal Endocrinology*. McGraw-Hill, New York, 286-299.

Nowak RM 1999. *Walker's mammals of the world*. (8th edition). JHU Press.

Patel YC, Wheatley T and Ning C 1981. Multiple forms of immunoreactive somatostatin and portal plasma of the rat. *Endocrinology*. 109: 1943-1949.

Robert L 1984. Snipes. Anatomy of the cecum of the cat. *Anat Embryol*. 170: 177-185.

Sapundzhiev E, Zahariev P and Stoyanov S 2017. Histological structure of the Grey wolf (*Canis lupus*) stomach. *Tradit Mod Vet Med*. 2(3): 66-68.

Sarriá R, Latorre R, Henroteaux M, Henroteaux N, Soria F, Pérez-Cuadrado E and López Albors O 2012. Morphometric study of the layers of the canine small intestine at five sampling sites. *The Veterinary Journal*. 192: 498-502.

Schubert ML and Rehfeld JF 2019. Gastric peptides-gastrin and somatostatin. *Compr Physiol*. 10: 197-228.

Skak-Nielsen T, Hoist JJ, Baldissera FGA and Poulsen SS 1987. Localization in the gastrointestinal tract of immunoreactive prosomatostatin. *Regul Pept*. 19: 183-195.

Timurkaan S, Timurkaan N, Ozkan E and Girgin M 2009. Immunohistochemical distribution of somatostatin, glucagon and gastrin in the gastric fundus of the citellus (*Spermophilus xanthoprymnus*). *J Anim Vet Adv*. 8 (11): 2210-2214.

Trent DF and Weir GC 1981. Heterogeneity of somatostatin-like peptides in rat brain, pancreas, and gastrointestinal tract. *Endocrinology*. 108 (6): 2033-2038.

Tumlison R 1987. "Felis lynx." Mammalian species. American Society of Mammalogists. 269: 1-8.

van der Flier LG and Clevers H 2009. Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu Rev Physiol*. 71: 241-260.

Yıldız H, Arslan K, Coşkun İ and Yıldız B 2006. A Geometric Modeling of dog intestine. *Turkish J Vet Anim Sci*. 30: 483-488.