

## A Histochemical and Immunohistochemical Study on the Gastrointestinal Tract of Sparrowhawk (*Accipiter nisus*)

Estudio Histoquímico e Immunohistoquímico del Tracto Gastrointestinal del Gavilán (*Accipiter nisus*)

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**KARA, H. & BALKAYA, H. A** histochemical and immunohistochemical study on the gastrointestinal tract of sparrowhawk (*Accipiter nisus*). *Int. J. Morphol.*, 40(1):242-250, 2022.

**SUMMARY:** In the present study, we aimed to determine the localization and distribution of entero-endocrine cells in the gastrointestinal tract by immunohistochemical methods and understand the structure of the glycoproteins elaborated by the epithelium the digestive tract regions by histochemical methods. The nine sparrowhawks were euthanized, and gastrointestinal tract tissues were removed and fixed in formalin. The gastrointestinal tract sections were stained with immunohistochemical and histochemical techniques to evaluate the enteroendocrine cells and histomorphometric analysis. The results showed that the numbers of somatostatin in the ventriculus, gastrin in the proventriculus, serotonin in the duodenum and jejunum immunopositivity are higher, remaining segments of the gastrointestinal tract are detected slight positivity in the glucagon, gastrin, serotonin, and somatostatin. In conclusion, some endocrine cells localization and distribution and histomorphometry, and goblet cell counts were revealed in the gastrointestinal tract of the sparrowhawks.

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**KEY WORDS:** Gastrointestinal tract; Enteroendocrine cells; Sparrowhawk; Morphology.

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### INTRODUCTION

Sparrowhawks are birds of prey and have a different digestive system from many other bird species in terms of their feeding habits (Sengul *et al.*, 2015). The digestive system of birds contains a number of differences that reduce body mass to increase flight efficiency. The ventriculus (gizzard) is mechanical digestion takes place. Foods ingested are stored in the proventriculus (glandular stomach) and undergo a chemical digestion. In most species, however, food passes swiftly through the glandular stomach and is rapidly digested by gastric secretions (pepsin, HCl, and mucus) in the muscular stomach (Duke, 1997). These endocrine cells located in the gastrointestinal tract regulate gastrointestinal hormones that play important roles in digestion such as gut motility, nutrient absorption and intestinal blood flow (Budipitojo *et al.*, 2016). Studies conducted have indicated that these endocrine cells show a number of differences among bird species depending on the physiological process, and different types of endocrine cells have been identified in the avian gastrointestinal tract (GIT) (Firmiano *et al.*, 2017). As the localization and distribution of these cells vary among species, it has been reported that there are also differences in terms of the hormones secreted (Firmiano *et al.*).

Twelve different types of diffuse neuro-endocrine system (DNES) cells have been determined in the gastrointestinal tract. These are secretin (S), cholecystokinin (CCK-pancreozymin), serotonin (enterochromaffin-EC-5HT), vasoactive intestinal peptide (VIP, D1), glicentin (enteroglucagon-GLI), neuropeptide Y (NPY), peptide YY (PYY), somatostatin (D), gastrin (G), urogastrone (gastrin inhibitory factor, GIP), ghrelin, and motilin (Kara *et al.*, 2021). Among those hormones, somatostatin controls the gastrointestinal activity with its paracrine and endocrine effects. While gastrin stimulates HCl synthesis from parietal cells, glucagon controls blood sugar (Simsek *et al.*, 2012). It has been reported in the literature that the distribution and localization of these cells in the GIT of avian and mammalian species are demonstrated by immunocytochemical studies.

There is surface mucus (mucin) as a surface protector in the digestive system. This mucus is a secretion released from the mucous gland cells of various organs in the digestive tract. Mucus or mucin may show different chemical properties in different parts of the gastrointestinal tract (Uslu & Yörük, 2015). Mucin-secreting goblet cells are glandular simple

columnar epithelial cells located in the mucosa of the small and large intestine. It is known that mucins is rich in polysaccharides (de Verdal *et al.*, 2010; Uslu & Yörük).

The role of gizzard (ventriculus) in the digestion processes has been extensively described. However, the main site of the nutrient absorption is the small intestine, thus, the structural changes in the small intestine morphology also affect digestion. Differential development of the absorptive epithelium may be responsible for changes in the absorptive capacity of birds. The characteristics of the villi and crypts of the absorptive epithelium are very important for absorption, as they have an important role in the final stages of nutrient digestion and absorption (Wang & Peng, 2008; de Verdal *et al.*; Uslu & Yörük).

The objective of this study is to evaluate the localization and distributions of the enteroendocrine cell, goblet cell counts, and small intestine morphology in the gastrointestinal tract of sparrowhawks.

## MATERIAL AND METHOD

Nine sparrowhawks (five females and four males) brought to Atatürk University, Faculty of Veterinary Medicine Internal Diseases Clinic, due to broken wings they were used in the study. They were irrecoverably injured and did not have any infectious diseases. After clinical examination by internal medicine experts, the hawks were selected and euthanized for the study. All procedures were carried out in accordance with the protocol approved by the Ethical Committee (for Experimental Animal Care and Use) of the Faculty of Veterinary Sciences at Atatürk University.

**Histologic analysis.** All sparrowhawks were euthanized with ether anesthesia, the proventriculus, ventriculus (gizzard), proximal duodenum, proximal jejunum, middle ileum, middle cecum, and proximal colon tissues were collected and approximately 1-cm sections was cut from each segment. The tissue samples were gently flushed with phosphate buffered-solution (PBS) (0.1 M pH 7.1) to wash the intestinal content, and then, all tissues were fixed in 10 % buffered formalin for 72 hours. Afterwards, The tissues were embedded in paraffin, and transversal serial sections (5- $\mu$ m) were cut at 50- $\mu$ m intervals from the tissues. Each section of segments was stained with Crossman's modified trichrome for histological evaluations. Additionally, each section was also stained with the Alcian blue (AB) and periodic acid Schiff's (PAS) reagent for the histochemical demonstration of goblet cells in the GIT tissues.

For the morphometric analysis, the villus length, crypt depth, and epithelial heights were measured in serial sections of large and small intestines by using the Kameran SLR 6.1 image analysis software (Mikro Sistem Co. Ltd., Turkey) and their arithmetic averages were calculated. Then, the goblet cell densities were determined as goblet cell counts per 1000  $\mu$ m. The PAS and AB positivity were evaluated for proventriculus and ventriculus as (-) absent, (+) low-intensity, (++) medium-intensity and (+++) high intensity.

**Immunohistochemical analysis.** For immunohistochemical staining, the serial sections were stained with anti-gastrin (Leica, NCL-GASp, 1/50 dilution), anti-glucagon (Leica, PA0597, 1/50 dilution), anti-serotonin (Dako, M0758, 1/50 dilution), and anti-somatostatin (Dako- A0566, 1/200 dilution) antibodies. Different cells in the same region were identified, and the intensity was evaluated more reliably by serial staining of these sections. For the immunohistochemical density evaluation, four parallel sections of each bird were prepared. Also, in total, 20 different areas were examined in each section of the birds. Then the distribution of these densities throughout the anatomical regions was scored according to the approach described in a previous study (Kara *et al.*, 2014). The densities were scored as (-) absent, (+) low-intensity, (++) medium-intensity and (+++) high intensity.

## RESULTS

**Histochemical results.** In the analysis of the PAS and AB staining, it was found that there were moderate densities in the proventriculus and low densities in the ventriculus in the sparrowhawks. Additionally, the PAS + AB positive goblet cell counts per 1,000- $\mu$ m were found to be  $41.7 \pm 11.1$ ,  $47.8 \pm 15.6$ ,  $56.3 \pm 14.3$ ,  $17.1 \pm 6.6$ ,  $53.9 \pm 15.9$  in the duodenum, jejunum, ileum, cecum and colon, respectively; however, a high intensity was observed in the cecum and colon segments of the gastrointestinal tract of the sparrowhawk (Table I, Figs. 1 and 2).

Morphometric measurements revealed that the villus height (VH) was  $490.3 \pm 56.2$ - $\mu$ m,  $390.5 \pm 45.8$ - $\mu$ m,  $195.1 \pm 23.5$ - $\mu$ m,  $25.4 \pm 5.8$ - $\mu$ m, and  $157.4 \pm 23.2$ - $\mu$ m in the duodenum, jejunum, ileum, cecum, and colon, respectively. The crypt depth (CD) was  $24.4 \pm 6.3$ - $\mu$ m,  $22.9 \pm 4.5$ - $\mu$ m,  $14.7 \pm 5.2$ - $\mu$ m,  $9.1 \pm 3.2$ - $\mu$ m, and  $16.8 \pm 5.1$ - $\mu$ m in the duodenum, jejunum, ileum, cecum, and colon, respectively. The epithelium height was  $9.5 \pm 2.1$ - $\mu$ m,  $8.7 \pm 3.9$ - $\mu$ m,  $9.5 \pm 3.8$ - $\mu$ m,  $8.3 \pm 3.7$ - $\mu$ m, and  $11.2 \pm 4.7$ - $\mu$ m in the duodenum, jejunum, ileum, cecum, and colon, respectively. The VH/CD ratio was  $20.1 \pm 6.4$ ,  $17.1 \pm 8.3$ ,  $13.3 \pm 4.5$ ,  $2.8 \pm 1.8$ , and  $9.4$

Table I. Alcian blue (AB) and Periodic acid schiff (PAS) positive goblet cell counts per 1,000 µm and the histometric measurements of the small and large intestine, and semi-quantitative density scores for the proventriculus and ventriculus of Sparrowhawks.

	Proventriculus	Ventriculus	Duodenum	Sum of PAS+AB	Jejunum	Sum of PAS+AB	Ileum	Sum of PAS+AB	Cecum	Sum of PAS+AB	Colon	Sum of PAS+AB
PAS	++	++	21.3 ± 6.5	41.7 ± 11.1	26.4 ± 8.3	47.8 ± 15.6	28.9 ± 6.9	56.3 ± 14.3	11.7 ± 5.2	17.1 ± 6.6	28.4 ± 8.7	53.9 ± 15.9
AB	+	+	20.4 ± 4.6		21.4 ± 7.3		27.4 ± 7.4		5.4 ± 1.4		25.5 ± 7.2	
µm			490.3 ± 56.2		390.5 ± 45.8		195.1 ± 23.5		25.4 ± 5.8		157.4 ± 23.2	
Villus height (VH)			24.4 ± 6.3		22.9 ± 4.5		14.7 ± 5.2		9.1 ± 3.2		16.8 ± 5.1	
Crypt depth (CD)			9.5 ± 2.1		8.7 ± 3.9		9.5 ± 3.8		8.3 ± 3.7		11.2 ± 4.7	
Epithelium height			20.1 ± 6.4		17.1 ± 8.3		13.3 ± 4.5		2.8 ± 1.8		9.4 ± 3.8	
VH/CD												

The densities were scored as (-) absent, (+) low-intensity, (++) medium-intensity and (+++) high-intensity in the proventriculus and ventriculus. The values were expressed as mean ± standard deviation. The sum of PAS + AB positive goblet cells indicates the total goblet cells.

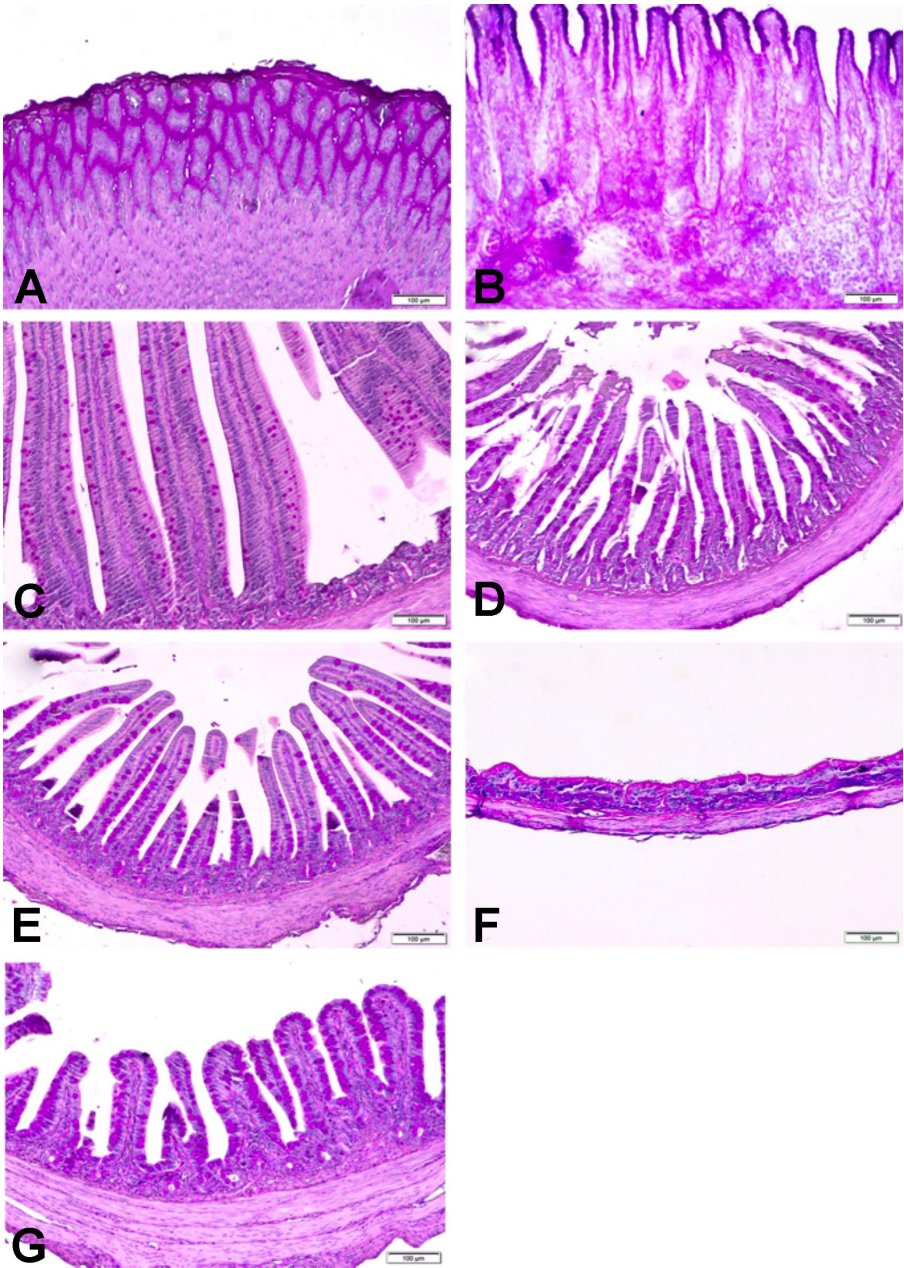


Fig. 1. Histochemical staining of periodic acid Schiff's (PAS) for parts of the gastrointestinal tract of the sparrowhawk, A. proventriculus, B. ventriculus, C. duodenum, D. jejunum, E. ileum, F. cecum, and G. colon tissues, Periodic acid Schiff's (PAS) staining.

± 3.8 in the duodenum, jejunum, ileum, cecum, and colon, respectively. All morphometric measurements of the small and large intestine of sparrowhawks are presented in Table I.

**Immunohistochemical results.** In the analysis of immunohistochemical staining, the density of gastrin-releasing cells was detected to be medium in the proventriculus glands, low in the ventriculus, duodenum, jejunum, and ileum segments (Table II, Fig. 3). But there were no anti-gastrin positive cells in the cecum and colon segments. Anti-glucagon positive cells were in low density in the proventriculus, duodenum and

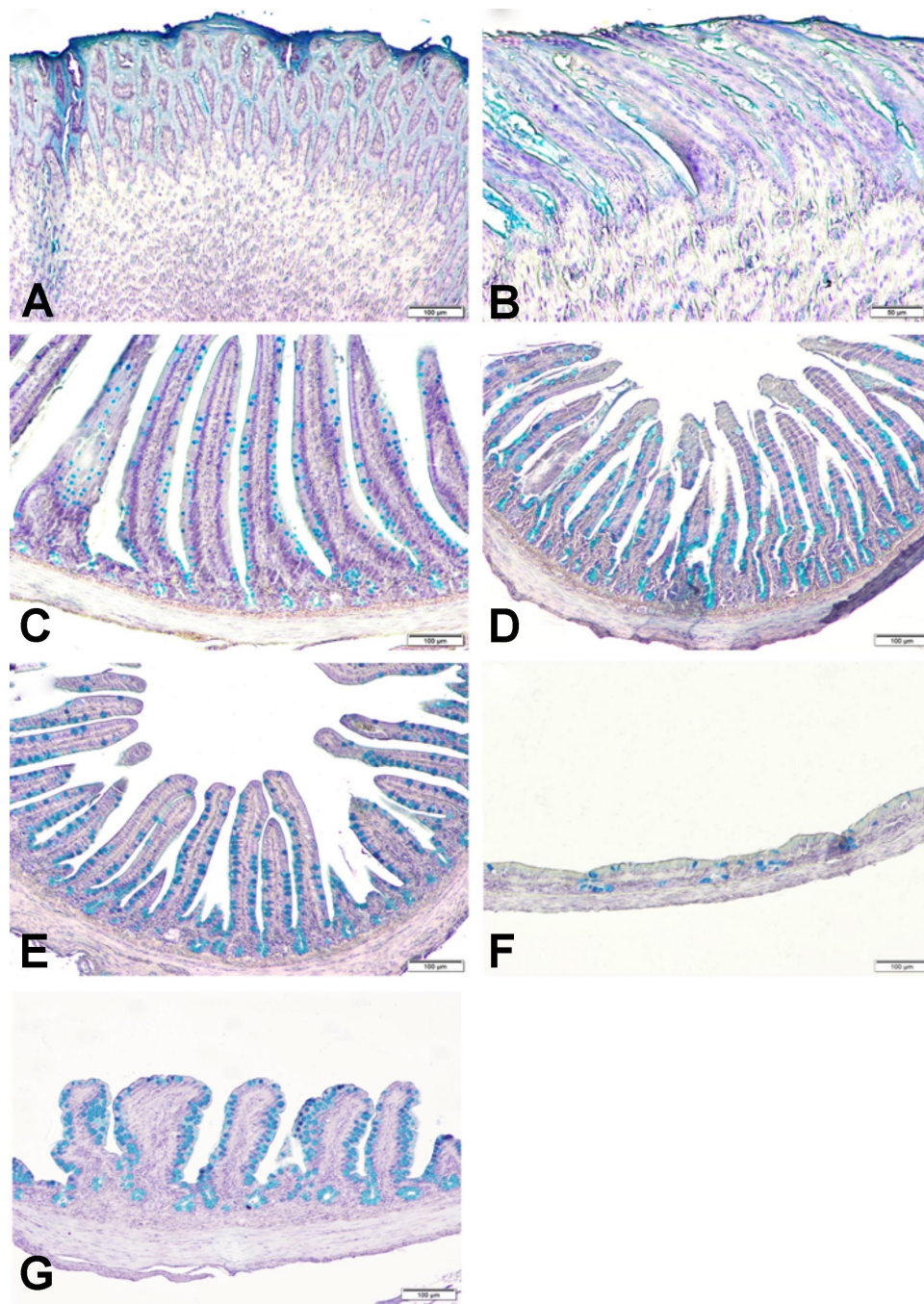


Fig. 2. Histochemical staining of Alcian blue (AB) for parts of the gastrointestinal tract of the sparrowhawk, A. proventriculus, B. ventriculus, C. duodenum, D. jejunum, E. ileum, F. cecum, and G. colon tissues, Alcian Blue pH 2.5 Staining.

jejunum segments (Table II, Fig. 4), but there was not any anti-glucagon positive cells in the ventriculus ileum, cecum, and colon segments. The density of anti-serotonin positive cells were found to be medium in the duodenum and jejunum segments, and low in the ventriculus and ileum segments (Table II, Fig. 5). On the other hand, they were not detected in the proventriculus, cecum, and colon segments. Anti-

somatostatin positive cells in the small and large intestine of sparrowhawks were in medium density in the ventriculus in low density in the proventriculus, duodenum, jejunum, and ileum (Table II, Fig. 6), and they were not observed in the cecum and colon segments. The densities of gastrin, glucagon, serotonin and somatostatin releasing cells are presented in the Table II.

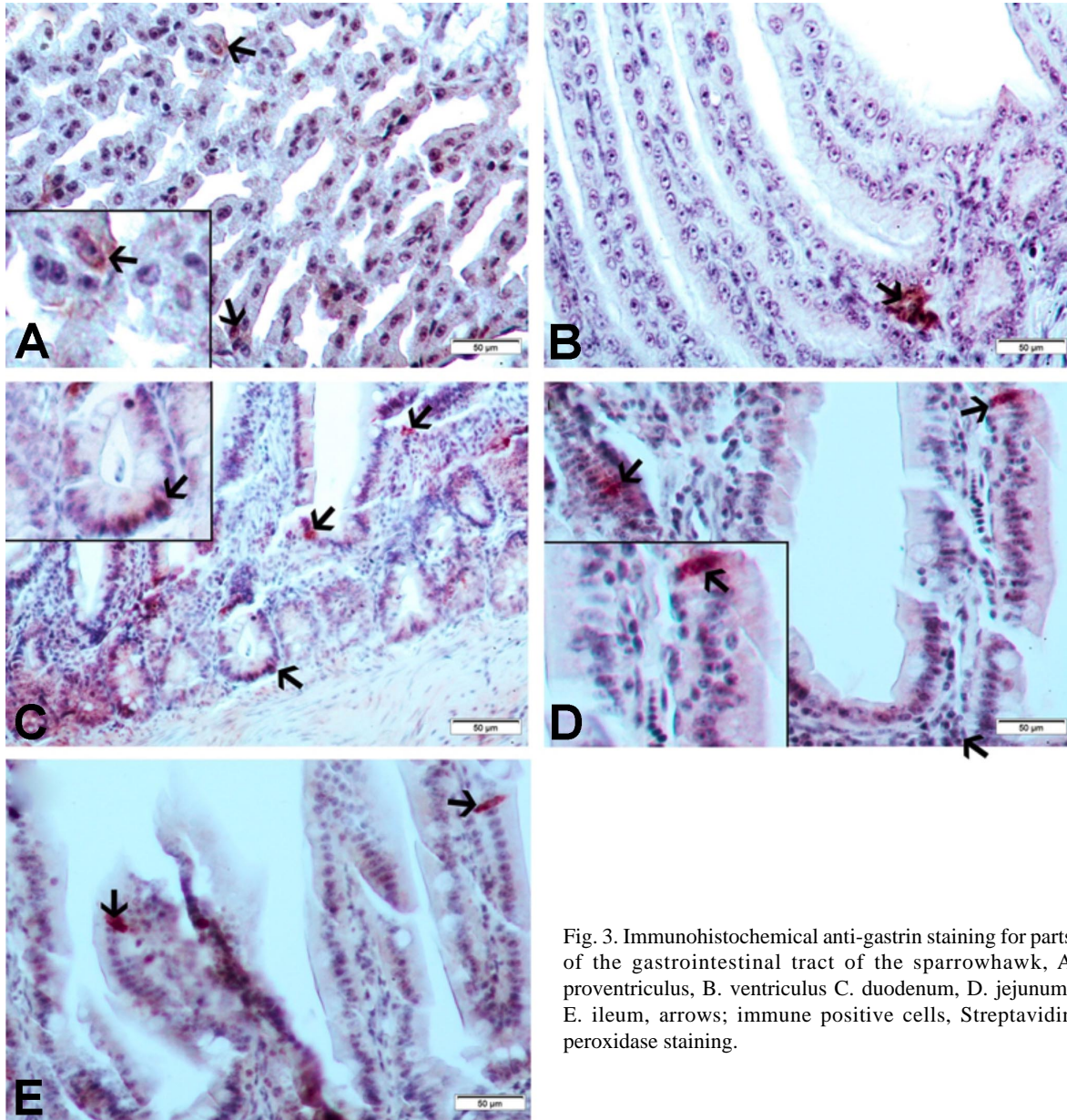


Fig. 3. Immunohistochemical anti-gastrin staining for parts of the gastrointestinal tract of the sparrowhawk, A proventriculus, B. ventriculus C. duodenum, D. jejunum, E. ileum, arrows; immune positive cells, Streptavidin peroxidase staining.

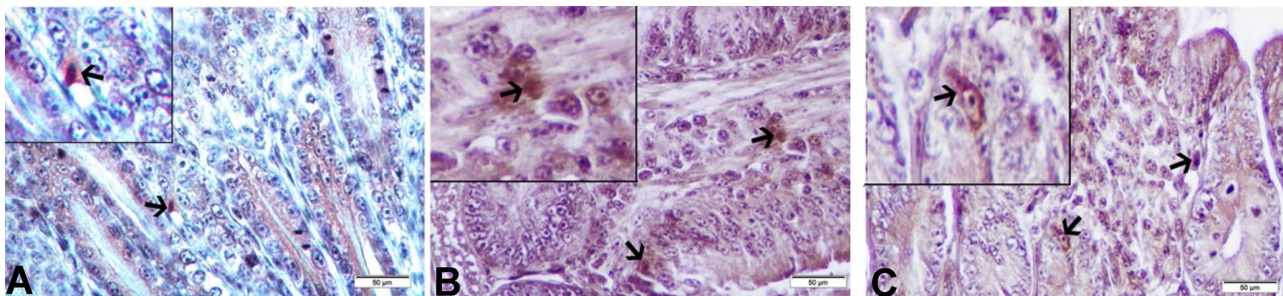


Fig. 4. Immunohistochemical anti-glucagon staining for parts of the gastrointestinal tract of the sparrowhawk, A. proventriculus, B. duodenum, C. jejunum, arrows; immune positive cells, Streptavidin peroxidase staining.

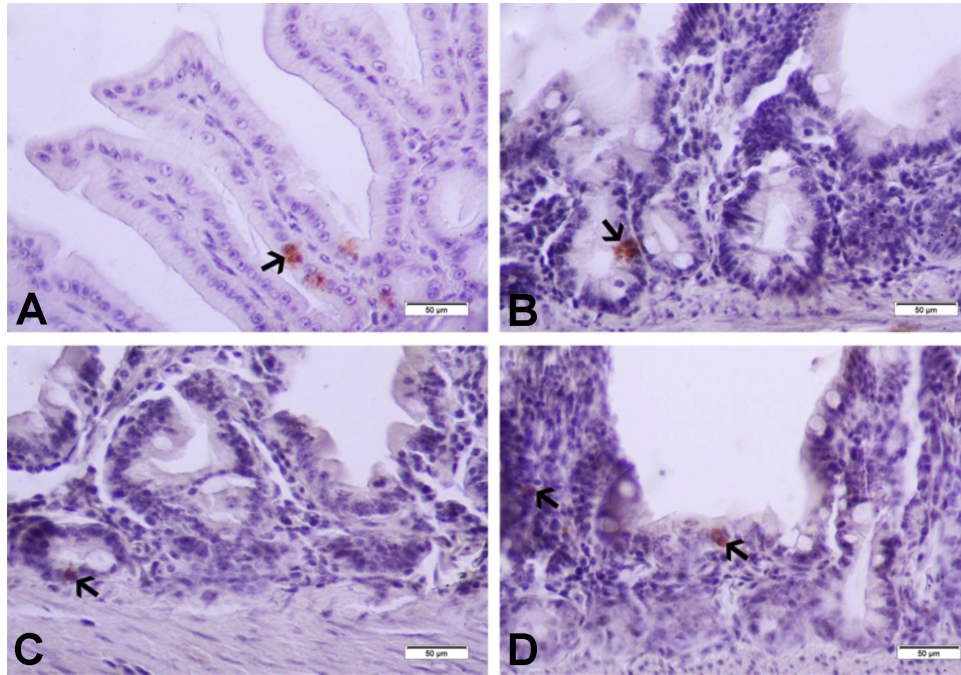


Fig. 5. Immunohistochemical anti-serotonin staining for parts of the gastrointestinal tract of the sparrowhawk, A. ventriculus, B. duodenum, C. jejunum, D. ileum, arrows; immune positive cells, Streptavidin peroxidase staining.

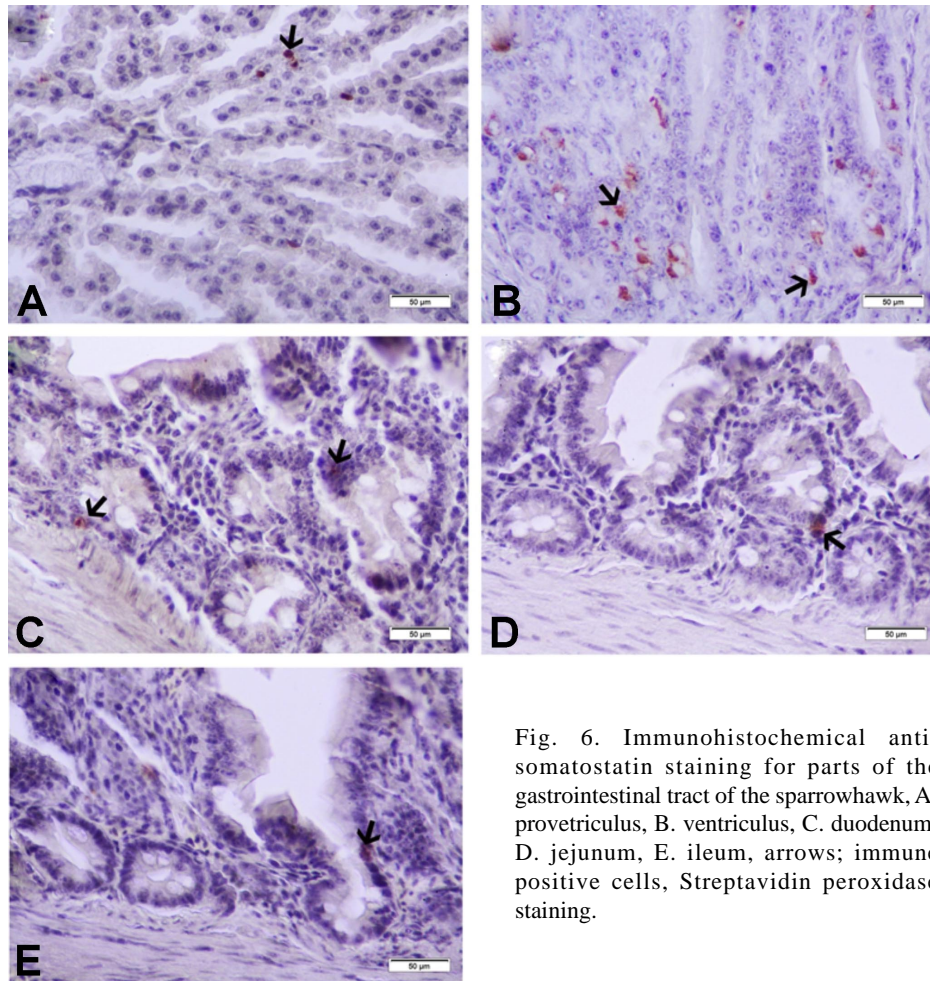


Fig. 6. Immunohistochemical anti-somatostatin staining for parts of the gastrointestinal tract of the sparrowhawk, A. proventriculus, B. ventriculus, C. duodenum, D. jejunum, E. ileum, arrows; immune positive cells, Streptavidin peroxidase staining.

Table II. Density scores of glucagon, somatostatin, gastrin, and serotonin antibodies of sparrowhawks proventriculus, ventriculus, duodenum, jejunum, ileum, cecum, and colon tissues.

Antibody	Proventriculus	Ventriculus	Duodenum	Jejunum	Ileum	Cecum	Colon
Gastrin	++	+	+	+	+	-	-
Glucagon	+	-	+	+	-	-	-
Serotonin	-	+	++	++	+	-	-
Somatostatin	+	++	+	+	+	-	-

Scoring: (-) absent, (+) low-intensity, (++) medium-intensity and (+++) high intensity.

## DISCUSSION

Digestive system organs are elements of the mechanism that begins with a chemical or mechanical digestion of the foods. Nutrients divided into molecular structures in absorption, which starts with mechanical and/or chemical breakdown, are absorbed from the gastric and intestinal mucosa (Özüdogru *et al.*, 2021). The DNES cells, which are endocrine cells that secrete hormones, undertake the important function in this absorption and regulation of digestion (Simsek *et al.*, 2011). The morphology of the digestive organs and the distributions of enteroendocrine cells in the gastrointestinal mucosa have been shown to differ depending on the species and organs (Yamada *et al.*, 1993; Mendes *et al.*, 2009).

It has been stated that there is a relation between the digestive capacity and the VH/CD ratio in the small and large intestines among birds. In the present study, the VH/CD ratio was found to be  $20.1 \pm 6.4$ ,  $17.1 \pm 8.3$ ,  $13.3 \pm 4.5$ ,  $2.8 \pm 1.8$ , and  $9.4 \pm 3.8$  in the duodenum, jejunum, ileum, cecum, and colon, respectively. On the other hand, it was reported in the literature that the VH/CD ratio in the duodenum, jejunum, and ileum was respectively 13.48, 8.44, and 5.87 in turkey hens, (Rahimi *et al.*, 2009),  $10.0 \pm 1.0$ ,  $9.7 \pm 1.4$ , and  $12.3 \pm 0.7$  in quails (Simsek *et al.*, 2012) and 7.73, 6.35, and 4.94 in broilers (De Vardal *et al.*, 2010). From a morphological point of view, it is expected that longer villi result in an increased surface area that allows greater absorption and digestion of the nutrients (Caspary, 1992), the result of the present study showed that sparrowhawks have a higher digestive capacity than other bird species.

Goblet cells are found in the villi epithelium and crypts of the intestinal tract and are responsible for mucin secretion. The production of mucin is required for the formation of mucus layers, which play an important role in intestinal lubrication and the transport of nutrients from the intestinal lumen (Caspary). The number of goblet cells varies among intestinal segments, in addition, the thickness and effectiveness of the mucus layer depend on the integrity of the mucosa and the activity of the microbial flora (Cornick *et*

*al.*, 2015). In the present study, the density of goblet cells was detected to be  $41.7 \pm 11.1$ ,  $47.8 \pm 15.6$ ,  $56.3 \pm 14.3$ ,  $17.1 \pm 6.6$ ,  $53.9 \pm 15.9$  in the duodenum, jejunum, ileum, cecum and colon, respectively. In the literature, the density of goblet cells in the duodenum, jejunum and ileum segments was found to be  $38.0 \pm 3.2$ ,  $46.1 \pm 1.2$  and  $73.7 \pm 4.6$  in quails (Simsek *et al.*, 2012), 11.9, 15.34 and 19.96 in hens (Bozkurt & Sandikçi, 2009) and  $168 \pm 9.8$ ,  $98 \pm 9.9$  and  $714 \pm 38.6$  in 240-day old turkeys (Yovchev & Penchev, 2021). These results indicate that the density of goblet cells varies among avian species.

The gastrin hormone is secreted by enteroendocrine cells in the GIT, and HCl, pepsinogen, and gastric motility is regulated by gastric secretion (Dockray *et al.*, 2001). However, the distribution and localization of gastrin-secreting cells may differ among bird species (Kara *et al.*, 2021; Özüdogru *et al.*). In the present study, the density of immunopositive gastrin cells was found to be medium in the proventriculus, and low in the ventriculus, duodenum, jejunum and ileum segments of the gastrointestinal tract. Okamoto *et al.* (1980) determined that the density of gastrin-positive cells was low in the ventriculus and duodenum of ducks. Although the studies in chickens (Ohmori, 1997) and quails (Yamada, 1980) revealed that the gastrin-positive cells were present only in the proventriculus, another study reported their presence in the duodenum and jejunum in geese (Gülmez *et al.*, 2003). Kara *et al.* (2021) determined them in the proventriculus, duodenum, jejunum and ileum segments in their study on Chukar partridge. Gulmez *et al.* (2003) reported that there were gastrin-immunoreactive cells only in the duodenum, jejunum and colon mucosa.

It was stated in the literature that glucagon immunoreactive (Glu-IR) cells are present in the gizzard and intestines of chickens (Kara *et al.*, 2021). The glucagon hormone regulates food intake in the digestive system (Honda, 2016). Yang *et al.* (2012) reported that in geese, Glu-IR cells were found in the proventriculus, duodenum and jejunum, but not in other tissues. In addition, Gulmez *et al.* determined that, the distribution of glucagon-secreting cells

was especially intense in the duodenum and was also found in the entire digestive system in geese. Kara *et al.* (2021) reported that low-intensity glucagon secretion was detected in the proventriculus, duodenum, jejunum and ileum of chukar partridges. In the present study, glucagon was detected in the proventriculus, duodenum, and jejunum in sparrowhawks, however, it is observed that there are some differences between the results obtained and those in the literature.

Serotonin plays a role in the local regulation of water as well as the electrolyte balance in the gastrointestinal tract (Gershon & Tack, 2007). According to the currently accepted concept, the GIT organs are the second major source of 5-HT Serotonin receptors after the central nervous system (Simsek *et al.*, 2011). In this study, it was determined that the serotonin-positive cell density was medium in the duodenum and jejunum, and low in the ventriculus and ileum. It has been reported by various studies in the literature that serotonin cells were present in different rates and localizations in the GIT (Simsek *et al.*, 2011, 2012; Kara *et al.*, 2021). The study conducted by Simsek *et al.* (2011, 2012) on quails revealed that the serotonin positive cells were present in the epithelium and crypts of the duodenum, jejunum and ileum villus (Dockray *et al.*). Kara *et al.* (2021) determined that they were found in the proventriculus, ventriculus, duodenum, jejunum and ileum in chukar partridges. Similarly, Mendes *et al.* reported the presence of serotonin positive cells in the proventriculus, ventriculus and duodenum in rufous-collared sparrows hawks.

Somatostatin, first isolated from the hypothalamus of sheep, is a hormone consisting of 14 amino acids (Brazeau *et al.*, 1973). It inhibits the secretion of other neuroendocrine hormones (Chang, 2009) and the somatostatin-immunoreactive cells are known to have a wide distribution in the gastrointestinal tract of many bird species (Simsek *et al.*, 2011). It has been reported in the literature that the somatostatin-immunoreactive cells are found in large numbers in the GIT of chicken (Yamanaka *et al.*, 1989), in addition they were detected in quails (Simsek *et al.*, 2012), chukar partridges (Kara *et al.*, 2021) and rufous-collared sparrows hawks (Simsek *et al.*, 2011).

In conclusion, in the study, the regional distribution and relative frequency of gastrin, glucagon, serotonin, and somatostatin endocrine cells, as well as neutral and acidic mucin characterization and histomorphometric assessment in the gastrointestinal tract of sparrowhawks were determined. Also, the findings suggest that the histochemical and immunohistochemical characteristics and distribution of endocrine cells obtained in this study may be a source for future investigations.

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**KARA, H. & BALKAYA, H. A** Estudio histoquímico e inmunohistoquímico del tracto gastrointestinal del gavián (*Accipiter nisus*). *Int. J. Morphol.*, 40(1):242-250, 2022.

**RESUMEN:** El objetivo del presente estudio fue determinar la localización y distribución de células enteroendocrinas en el tracto gastrointestinal de gavián, a través de métodos inmunohistoquímicos y comprender la estructura de las glicoproteínas elaboradas por el epitelio de las regiones del tracto digestivo. Se sacrificaron nueve gavilanes y los tejidos del tracto gastrointestinal se extrajeron y se fijaron en formalina. Las secciones del tracto gastrointestinal se tiñeron con técnicas inmunohistoquímicas e histoquímicas para evaluar las células enteroendocrinas y se realizó análisis histo-morfométrico. Los resultados indicaron que los números de inmunopositividad de somatostatina en el ventrículo, gastrina en el proventrículo, serotonina en el duodeno y yeyuno son más altos, en los segmentos restantes del tracto gastrointestinal, se detecta además una ligera positividad de glucagón, gastrina, serotonina y somatostatina. En conclusión en el tracto gastrointestinal de gavián se observó cierta localización y distribución de células endocrinas e histomorfometría, y recuentos de células caliciformes.

**PALABRAS CLAVE:** Tracto gastrointestinal; Células enteroendocrinas; Gavián; Morfología.

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