

# Regional Distribution and Mucosal Localization of Some Gastrointestinal Endocrine Cells in Quail (*Coturnix coturnix*)

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## Abstract

The purpose of this study was to investigate the regional distribution and mucosal localization of serotonin-containing endocrine cells in the pancreas and small intestine (duodenum, jejunum, and ileum) of quails. Endocrine cells in the digestive tract were identified using the immunohistochemical peroxidase-antiperoxidase (PAP) method. Most serotonin-secreting endocrine cells were observed in the basal regions of the glands, exhibiting oval or spindle-shaped morphology. Two main types of endocrine cells were identified based on their contact with the intestinal lumen: *open-type* cells, which extend to the lumen and can sense luminal contents, and *closed-type* cells, which do not reach the lumen and are entirely embedded within the epithelium or glandular tissue. In the duodenal glands, some *closed-type* immunoreactive cells were detected, while *open-type* immunoreactive cells were mainly found in the lamina propria epithelium. Endocrine cells with luminal contact were present in the epithelial layer of the small intestine, whereas cells within the glands lacked such contact. Serotonin immunopositive reactions were most abundant in the duodenum, followed by a gradual decrease in the jejunum and ileum. In the pancreas, serotonin-positive cells were also detected, but at a lower density compared to the intestinal segments. In the pancreas, serotonin-positive cells were more concentrated in the endocrine regions compared to the exocrine tissue.

## Keywords

Quail, *Coturnix coturnix*, Digestive System, Histology, Histochemistry, Endocrine Cells, Immunohistochemistry

## 1. Introduction

The digestive system comprises the alimentary canal and associated glands such as the salivary glands, liver, and pancreas. Its primary function is to break down complex macromolecules like proteins, lipids, carbohydrates, and nucleic acids into absorbable units to meet the body's metabolic and energy demands. Among vertebrates, structural and functional variations in digestive organs reflect dietary adaptations. Birds exhibit highly specialized gastrointestinal systems adapted for rapid and efficient nutrient absorption due to their high metabolic demands.

In avian species, the digestive tract lacks teeth and certain salivary glands but includes unique adaptations such as the crop, proventriculus, and muscular gizzard. The small intestine, divided into the duodenum, jejunum, and ileum, is the principal site of digestion and absorption. Additionally, the pancreas plays a dual role in producing digestive enzymes (exocrine) and hormones regulating glucose metabolism (endocrine). Unlike in mammals, glucagon-producing A cells often outnumber insulin-producing B cells, and two types of islets—dark and light—are observed due to differential staining of these endocrine cells.

The mucosal immune system of the avian intestine includes diffuse and organized lymphoid tissues, including Peyer's patches and the bursa of Fabricius, which are critical for immune defense. Serotonin (5-HT), a biogenic amine, is one of the key modulators of gastrointestinal motility, secretion, and local immune responses. It is secreted by enterochromaffin (EC) cells dispersed along the gastrointestinal mucosa and plays a significant role in the gut-brain axis.

Despite the growing interest in avian gastrointestinal physiology, data on the regional distribution and mucosal localization of serotonin-immunoreactive (IR) cells, particularly in quails (*Coturnix coturnix*), remain limited. Therefore, this study aimed to investigate the immunohistochemical organization of the pancreas and small intestine (duodenum, jejunum, ileum) in quails and to characterize the regional distribution and mucosal localization of serotonin-IR cells.

Recent studies have increasingly highlighted the complex roles of serotonin and other gastrointestinal hormones in avian species, particularly concerning digestive physiology and neuroimmune interactions. For example, Jadhav *et al.* [1] emphasized the influence of gut microbiota on serotonin production in *Gallus gallus*, linking serotonergic signaling with overall gut health and behavior. Similarly, Lyte *et al.* [2] reported elevated serotonin concentrations in the jejunum, ileum, and cecum of broiler chickens, suggesting region-specific synthesis and activity. Additionally, enteroendocrine hormones, including serotonin, have been shown to regulate appetite and nutrient absorption in chickens, with important implications for poultry production under stress conditions [3]. Moreover, serotonin's functions extend beyond neurotransmission to modulate inflammation and immunity within the gastrointestinal tract [4]. These advancements underscore the importance of understanding serotonin-containing endocrine cells' distribution and function in avian gastrointestinal physiology.

## 2. Materials and Methods

This study was conducted using digestive system samples collected from ten adult quails (*Coturnix coturnix*), obtained from the Farmer Training Center of the Faculty of Agriculture, Süleyman Demirel University. Tissue samples from the pancreas and regions of the small intestine (duodenum, jejunum, ileum) were fixed in Bouin's solution for 18 - 24 hours, dehydrated through graded alcohol series, cleared in xylene, and embedded in paraffin.

Immunohistochemical detection of serotonin-immunoreactive (IR) cells in mucosal layers of the small intestine and pancreatic tissue was performed using the peroxidase-antiperoxidase (PAP) method [5]. After deparaffinization and rehydration, sections were washed in phosphate-buffered saline (PBS, 0.01 M, pH 7.2). Endogenous peroxidase activity and non-specific background staining were blocked with 3% hydrogen peroxide (20 min) and 10% normal goat serum (host animal: goat) (30 min), respectively.

The sections were incubated overnight at 4°C with a primary antibody against serotonin (anti-5-HT, Sigma S5545, dilution 1:400). Following PBS washes, the sections were incubated for 30 minutes at room temperature with a secondary antibody (Goat Anti-Rabbit IgG, dilution 1:10), followed by PAP complex incubation. Immunoreactivity was visualized using 0.05% diaminobenzidine (DAB) for 10 minutes. Slides were counterstained, dehydrated, cleared in xylene, and mounted with Entellan.

To assess antibody specificity, negative controls were performed by omitting the primary antibody, which resulted in no detectable staining. Although absorption tests were not conducted in this study, the anti-serotonin antibody used has been previously validated in avian tissue and is widely cited in related immunohistochemical literature.

Microscopic evaluation was carried out using an Olympus CX41 light microscope, and microphotographs were taken using a Leica DM2500 system. Morphometric analyses were performed on digital images using the ImageJ software (NIH), calibrated with a stage micrometer, and photographed under the same magnification.

The density of serotonin-positive endocrine cells was semi-quantitatively scored at ×20 and ×40 magnification as follows: (-) absent, (+) low, (++) moderate, (+++) strong, and (++++) very strong.

## 3. Results

Immunohistochemical staining of sections from different regions of the small intestine and pancreas was performed to determine the regional and mucosal distribution of serotonin-immunoreactive (IR) cells. The analysis revealed significant differences in both the distribution and density of serotonin-IR cells within the intestinal mucosa. Serotonin-IR cells were primarily located in the glands of the duodenum, while in the jejunum and ileum, they were more concentrated in the lamina propria. These IR cells exhibited various morphologies, including oval,

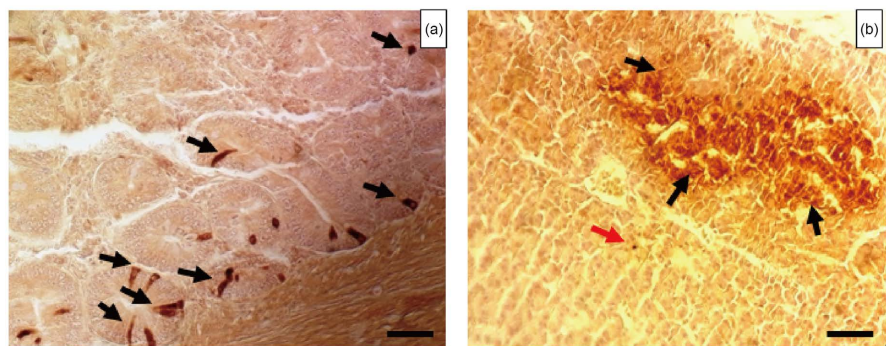
triangular, and spindle-shaped forms. Some elongated serotonin-IR cells extending toward the lumen were observed in the jejunal and ileal lamina propria. Additionally, IR cells were also localized within connective tissue components of the lamina propria and submucosa, with a decreasing gradient in density from the duodenum to the ileum. These findings are summarized in **Table 1**.

**Table 1.** Relative densities of serotonin-IR cells in different regions of the quail (*Coturnix coturnix*) small intestine and pancreas.

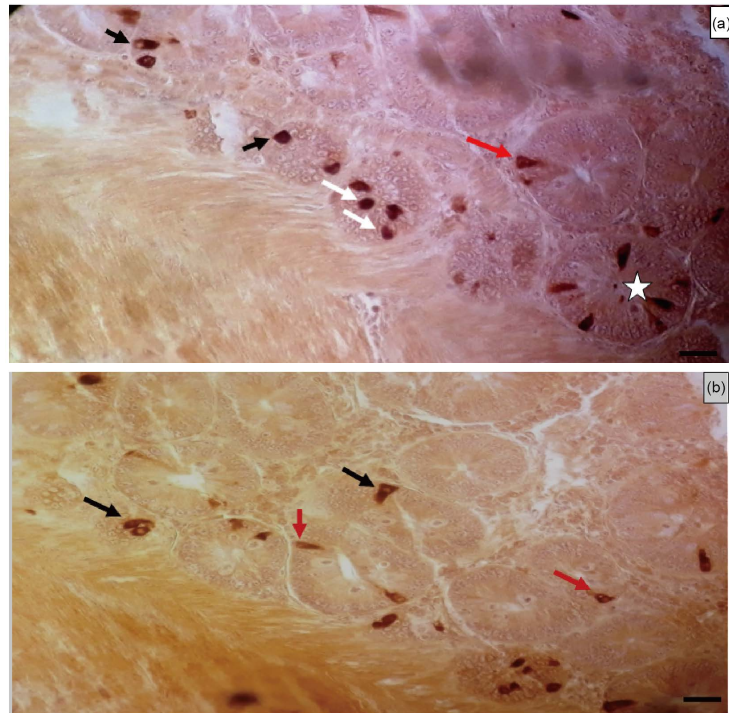
Endocrine cell Histological Localization	Duodenum			Jejunum			İleum			Pancreas	
	EP	CT	G	EP	CT	G	EP	CT	G	END	EXO
Serotonin	+++	+++	++++	+++	++	+++	++	++	++	++++	++

Relative Density: (-), no cells detected; (+), rare; (++) moderate density; (+++), strong; (++++), very strong. Lamina Epithelium (EP); Connective Tissue (CT); Glands (G); Endocrine Pancreas (END); Exocrine Pancreas (EXO).

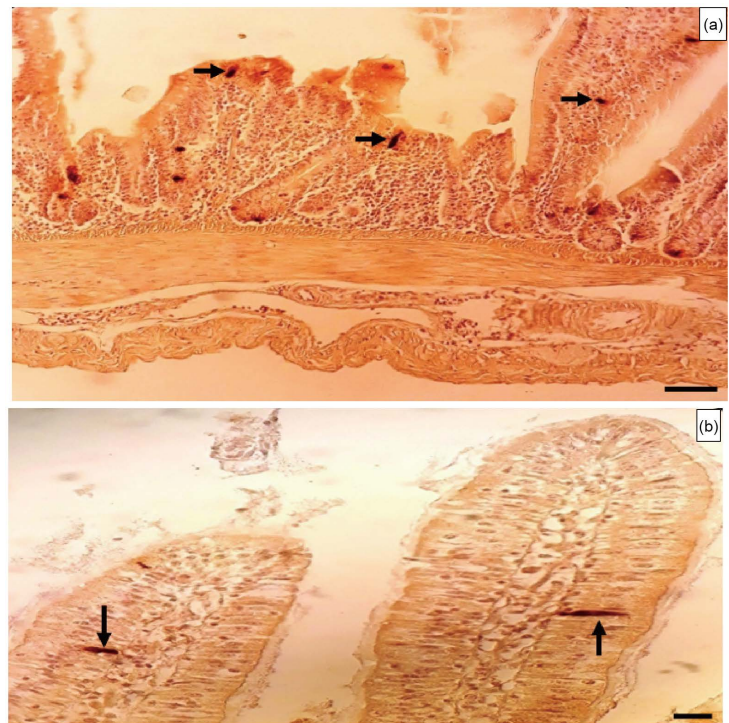
Quantitative analysis showed that serotonin-IR cells were most abundant in the duodenum and least in the ileum. Compared to the pancreas, these cells were more densely distributed in the lamina epithelialis and glands of the small intestine, while fewer were found in the lamina propria (**Figure 1**). In the duodenum, cells were primarily located in the glands, with both lumenally open and closed types identified. These cells were oval, spindle-shaped, or triangular (**Figure 2**). In the jejunum and ileum, serotonin-IR cells were more prominent in the lamina epithelialis, primarily spindle-shaped, and extended toward the lumen (**Figure 3(a)**, **Figure 3(b)**). While the duodenal IR cells were found in both the lamina epithelialis and glands, those in the jejunum were mainly concentrated in the glands. In the ileum, the cells were predominantly localized in the lamina epithelialis. Both open-type (luminal contact) and closed-type (no luminal contact) serotonin-IR cells were observed in the intestinal mucosa (**Figure 4**). These cells were mostly oval, or spindle shaped. In the pancreas, serotonin-IR cells were detected in both the exocrine and endocrine compartments, where they also appeared primarily in oval or spindle forms (**Figure 5**).



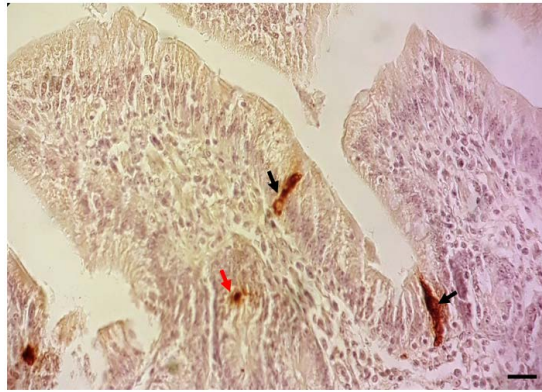
**Figure 1.** (a) Serotonin-immunoreactive (IR) cells localized in the Brunner's glands (arrows). (b) Serotonin-IR cells in the pancreas; cells located in the endocrine region (black arrows) and exocrine region (red arrows). PAP staining. Scale bar: 200  $\mu$ m.



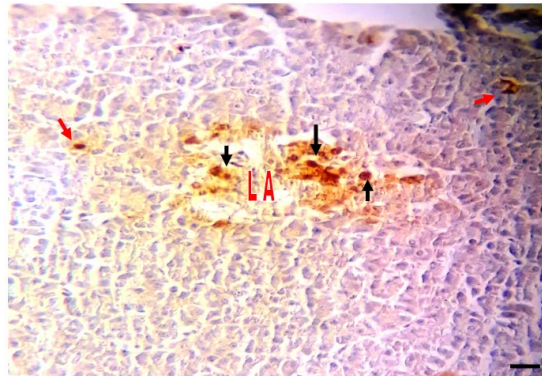
**Figure 2.** Duodenum. (a) Serotonin-immunoreactive (IR) cells localized in the glands (black arrows); oval (white arrows), triangular (red arrow), and spindle-shaped (white star) serotonin-IR cells. (b) Serotonin-IR cells in the glands (black arrows); spindle-shaped serotonin-IR cells opening into the lumen (red arrows). PAP staining. Scale bar: 200 µm.



**Figure 3.** (a) Jejunum (arrows), (b) Spindle-shaped serotonin-immunoreactive (IR) cells localized in the lamina epithelialis of the ileum (arrows). PAP staining. Scale bars: (a) 100 µm, (b) 200 µm.

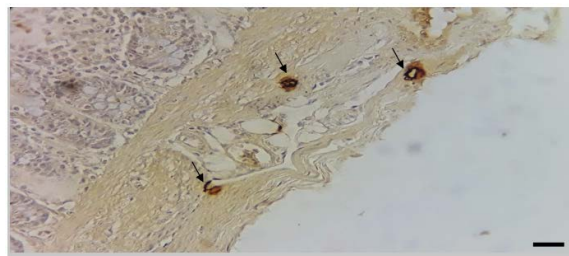


**Figure 4.** Small intestine. Serotonin-immunoreactive (IR) cell extending to the lumen in the villus lamina epithelialis (arrows), and an oval-shaped serotonin IR cell in the gland not reaching the lumen (red arrow). PAP staining. Scale bar: 200  $\mu\text{m}$ .

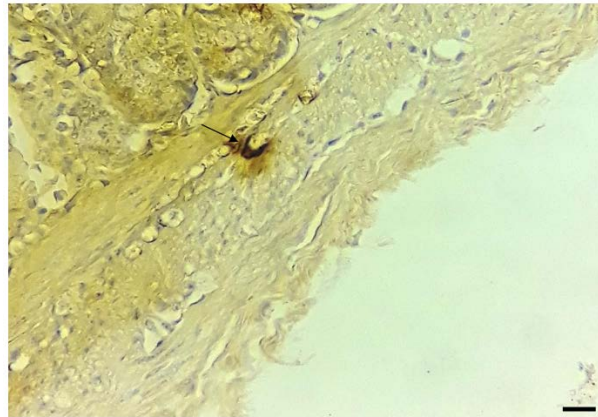


**Figure 5.** Pancreas. Islet of Langerhans (LA); oval-shaped serotonin-secreting immunoreactive (IR) cells in the endocrine region (thin arrows). Oval and spindle-shaped serotonin-IR cells in the exocrine region (red arrows). PAP staining. Scale bar: 200  $\mu\text{m}$ .

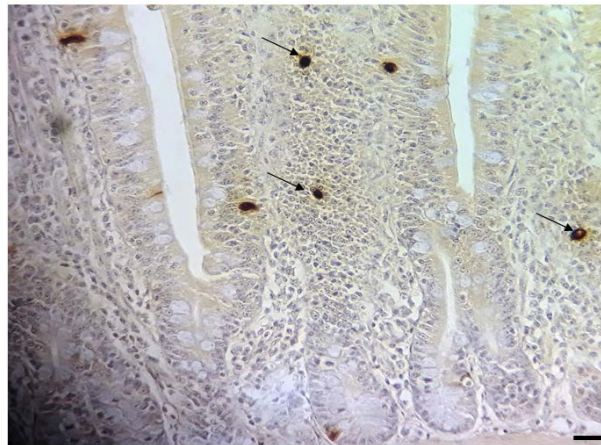
Serotonin-IR cells were observed in some connective tissue cells of the lamina propria and submucosal connective tissue layers of the small intestine (**Figure 6** and **Figure 7**). Serotonin-secreting endocrine cells were more frequently detected in the villous connective tissue of the ileum (**Figure 8**), and immunoreactivity was also observed in basophils (**Figure 9**).



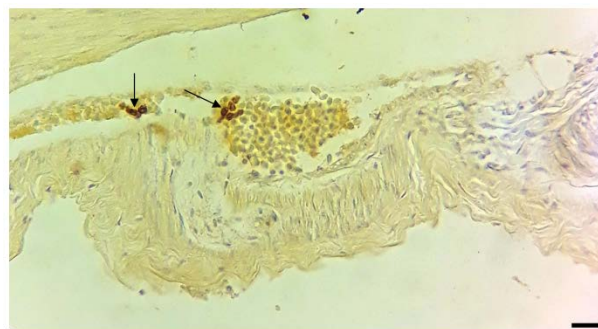
**Figure 6.** Duodenum. Serotonin-immunoreactive (IR) cells (arrows) in some connective tissue cells of the lamina propria and submucosal connective tissue layers. PAP staining. Scale bar: 200  $\mu\text{m}$ .



**Figure 7.** Jejunum. Serotonin-immunoreactive cell (arrow) in some connective tissue cells of the lamina propria layers. PAP staining. Scale bar: 200  $\mu$ m.



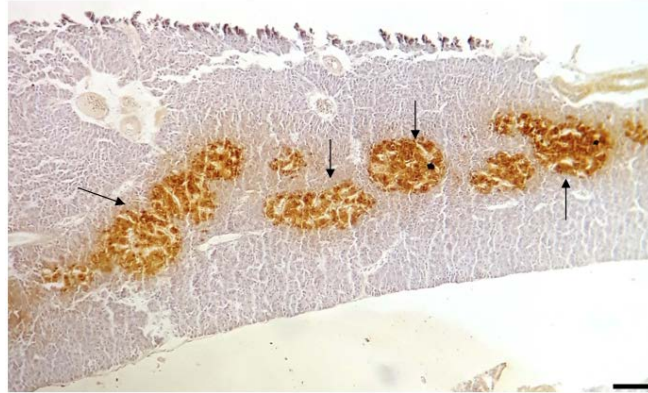
**Figure 8.** Ileum. Serotonin-secreting endocrine cells (arrows) located in the villous connective tissue. PAP staining. Scale bar: 100  $\mu$ m.



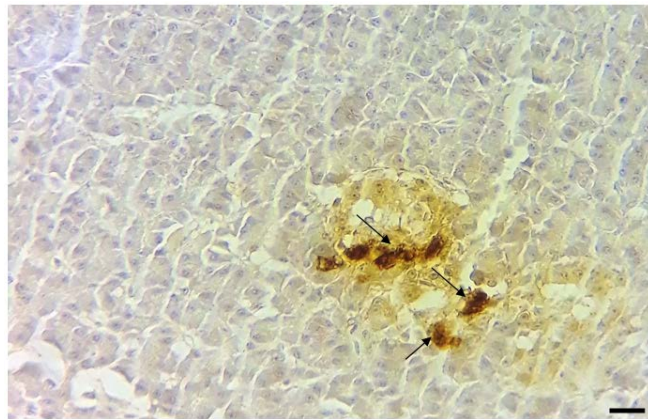
**Figure 9.** Duodenum. Positive immunoreactivity in basophils (arrows). PAP staining. Scale bar: 100  $\mu$ m.

Serotonin-secreting immunoreactive cells in the pancreas were observed to be predominantly distributed in the endocrine region (**Figure 10**). In the islets of Langerhans, serotonin-IR cells were found to be localized in various shapes and

arranged in clusters (Figure 11).

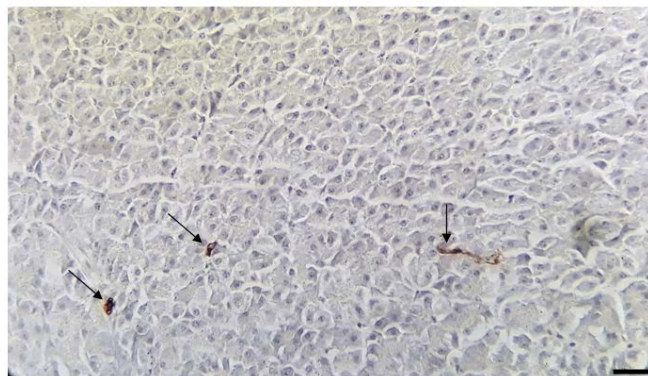


**Figure 10.** Pancreas. Islets of Langerhans. Serotonin-immunoreactive cells (arrows). PAP staining. Scale bar: 100  $\mu$ m.

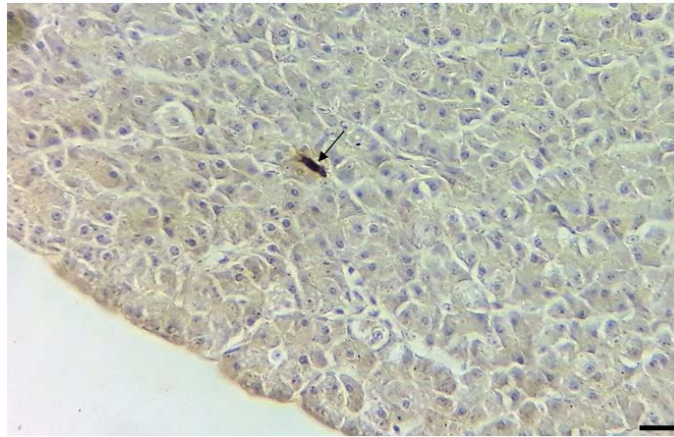


**Figure 11.** Endocrine pancreas. Irregularly shaped serotonin immunoreactive cells localized in the Islets of Langerhans (arrows). PAP staining. Scale bar: 100  $\mu$ m.

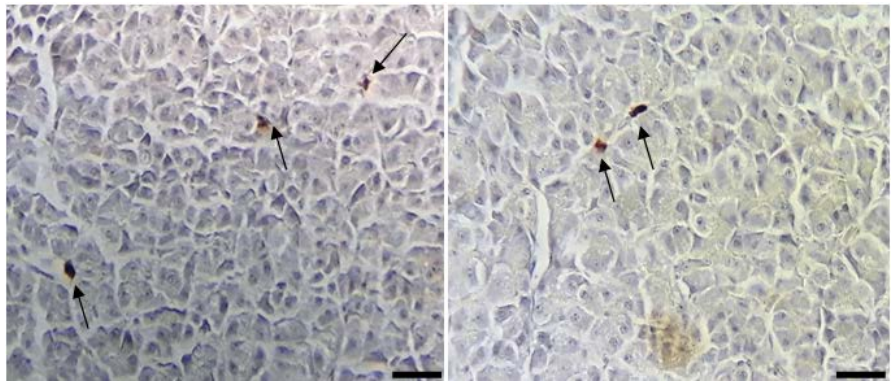
Serotonin immunoreactive (IR) cells were detected at a lower density in the exocrine pancreas compared to the endocrine pancreas (Figures 12-14).



**Figure 12.** Exocrine pancreas. Serotonin-IR cells (arrows). PAP staining. Scale bar: 100  $\mu$ m.



**Figure 13.** Exocrine pancreas. Serotonin-IR cells (arrows). PAP staining. Scale bar: 100  $\mu$ m.



**Figure 14.** Exocrine pancreas. Serotonin-IR cells (arrows). PAP staining. Scale bar 100  $\mu$ m.

#### 4. Discussion

This study demonstrated that serotonin-immunoreactive (IR) endocrine cells in the digestive tract of the quail (*Coturnix coturnix*) exhibit distinct regional distributions and mucosal localizations, varying in shape and density. The observed morphological diversity, including oval, pyramidal, spindle, triangular, and lanceolate forms, aligns with previous findings in various avian species [6]-[9].

Similar to reports in domestic chickens (*Gallus gallus domesticus*) [10], we observed lanceolate serotonin-IR cells predominantly in the lamina epithelialis of the small intestine. Both closed and open types of serotonin-secreting cells were detected in the lamina epithelialis and glands of quail small intestines, consistent with earlier findings in adult quail and other birds [11]. The duodenal glands showed a notably high density of serotonin IR cells, corroborating observations in ducks and chickens [11].

Our results further align with comparative studies reporting similar densities and distributions of serotonin-secreting endocrine cells across various bird species such as quail, ducks, and pigeons [12], as well as findings in passerines and other avian species indicating the presence of serotonin-positive cells in the lamina epithelialis, propria, glands, and submucosa of the small intestine [13].

Consistent with previous reports in geese (*Anser anser*) [14] and black Australorp chicks [15], we found the highest serotonin IR cell density in the duodenum, decreasing towards the ileum. This gradient parallels the pattern observed in mammalian gastrointestinal systems, although serotonin cells are less frequent in avian stomachs [15].

Moreover, the abundant localization of serotonin-IR cells in duodenal glands corresponds with findings in other species such as hedgehogs and rodents [16]. The distribution of serotonin-immunoreactive cells within the lamina propria and connective tissue layers showed a gradual decrease from duodenum to ileum, supporting data from rainbow trout and rabbits [17] [18].

In contrast to reports of absence in the pancreas of some raptors [19], serotonin IR cells were moderately distributed in both endocrine and exocrine regions of the quail pancreas, consistent with observations in ducks and other mammals [20] [21]. Our findings confirm that serotonin-secreting cells are more abundant in the endocrine pancreas, with moderate presence in exocrine tissue, as reported in rodents [22].

An unexpected yet noteworthy observation in this study was the presence of serotonin immunoreactivity in basophils. This finding aligns with reports that basophils, like mast cells, can store and release serotonin, contributing to immunomodulatory functions in the gastrointestinal tract [23] [24]. The presence of serotonin in basophils may reflect a role in mediating local immune responses, influencing gut motility, or participating in neuroimmune interactions. Further studies are needed to clarify the functional significance of this association in avian species.

Overall, this study enriches the understanding of serotonergic endocrine cell distribution in avian digestive systems, highlighting species-specific patterns and supporting comparative gastrointestinal endocrinology.

## 5. Conclusion

In conclusion, this study demonstrated that serotonin-immunoreactive (IR) cells in the digestive tract of quail (*Coturnix coturnix*) exhibit variations in distribution and mucosal localization both among different bird species and across different regions of the digestive system. Serotonin IR cells were widely distributed throughout the digestive tract, with particularly high densities observed in the small intestinal glands. The immunohistochemical pattern in quail was largely like that reported in other bird species; however, interspecies differences likely reflect variations in feeding habits and environmental conditions. This distribution pattern suggests a functional specialization of serotonin-secreting cells along different regions of the gastrointestinal tract. The high density of serotonin-IR cells in the duodenum may be linked to its role in initiating digestive processes, as serotonin is known to regulate gut motility, stimulate the release of digestive enzymes, and influence local neural reflexes. Lower densities in the jejunum, ileum, and pancreas may reflect region-specific differences in serotonin-mediated signaling, sup-

porting a more modulatory rather than initiating role in these segments. Serotonin-secreting IR cells were identified at varying densities in the lamina propria, mucosa, glands, and crypt epithelia of the small intestine, as well as in both the exocrine and endocrine parts of the pancreas. The highest serotonin immunoreactivity was observed in the duodenum, followed by a gradual decrease through the jejunum and ileum. In the pancreas, serotonin-positive cells were also present, primarily localized in the endocrine regions, but at a lower density compared to intestinal segments. These findings provide a valuable foundation for future studies in avian physiology, nutrition, histomorphology, and histophysiology within the context of poultry science.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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