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Existence of Serotonin and Neuropeptides-Immunoreactive Endocrine Cells in the Stomach (Proventriculus and Gizzard) of Long-Legged Buzzard (*Buteo rufinus*): An Immunohistochemical Study

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Absract: This study aimed to determine the existence and distribution of serotonin and certain neuropeptides in proventriculus and gizzard of the long-legged buzzard by using peroxidase anti-peroxidase complex immunohistochemical technique. In both proventriculus and gizzard, serotonin, Somatostatin-14 (SOM-14), Substance P (SP) and Calcitonin Gene Related Peptide (CGRP)-IR endocrine cells were determined. However, Cholecystokinin-8 (CCK-8), neurotensin, Neuropeptide Y (NPY), galanin and Vasoactive Intestinal Polypeptide (VIP)-IR endocrine cells were not found in any region of the stomach. Serotonin-IR endocrine cells were detected in the epithelium and glands of both stomach regions however, they were more intense in the glands of gizzard. Numerous in number SOM-14-IR endocrine cells were detected in the glands of the stomach. A few SP-IR endocrine cells were detected in the epithelium of the whole stomach and in the glands of gizzard. Moderate CGRP-IR endocrine cells were detected only in the epithelium of whole stomach. In conclusion, serotonin, SOM-14, SP and CGRP were detected to exist in the proventriculus and gizzard of the long-legged buzzards for the first time in the present study. It was also found that this species has been found to differ from other types of some avian species.

Key words: Serotonin, neuropeptides, endocrine cell, stomach, long-legged buzzard, immunohistochemistry

INTRODUCTION

The Long-Legged Buzzard (LLB), *Buteo rufinus* is a medium-size raptor that nests in the Southern Palearctic region (Friedemann *et al*., 2011), extend from North Africa through Mid Asia (Atalar *et al*., 2007). The avian stomach consists of two distinct portions: Proventriculus and gizzard. In both proventriculus and gizzard, lamina epithelialis consists of single-layered prismatic epithelium. The proventriculus possess lobular glands in the submucosal layer, contains from cubic to prismatic shaped cells releasing hydrochloric acid and pepsinogen. The gizzard possess ventricular glands consisting of invagination of superficial epithelium into the lamina propia (Yoruk, 2008). There are two types of stomach according to the diet of the bird species. Carnivorous birds that fish and meat eating species have little difference between the two stomachs (Proventriculus and gizzard) having a large easily distensible stomach to contain whole prey. The gizzard in these species is used mainly for allowing time for gastric juices to act on the soft food rather than physical digestion. Granivorous birds eat relatively indigestible foods so need a gizzard for grinding food. Consequently they have a thick-walled, non-distensible and glandular proventriculus and a thick muscular gizzard (O'Malley, 2005).

Gut motility in non-mammalian vertebrates as in mammals is controlled by the presence of food by autonomic nerves and by hormones (Olsson and Holmgren, 2001). Gastrointestinal hormones are secreted by endocrine cells which are distributed throughout the mucosa of the gastrointestinal tract. They play important functions in the overall regulation of digestive processes such as nutrient absorption, the secretion of intestinal and associated glands, gut motility and intestinal blood flow (Agungpriyono *et al*., 2000; Deveney and Way, 1983).

Several immunohistochemical studies have demonstrated the presence and distribution of amin and/or neuropeptide-secreting endocrine cells in the gastro-enteropancreatic tract of human (Meyer and Brinck, 1999), various mammals (Susaki *et al*., 2005; Ali *et al*., 2007; Sari *et al*., 2007; Tarakci *et al*., 2007; Karan *et al*., 2008), some avian species (Alison, 1989; Martinez *et al*., 1993; Mensah-Brown *et al*., 2000; Gulmez *et al*., 2003; Neglia *et al*., 2007; Tarakci *et al*., 2008;

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Shao *et al*., 2010) and long-legged buzzard (Bayrakdar *et al*., 2011). However, as far as we know there have been no reports on the distribution and frequency of neuropeptide-secreting endocrine cells in the stomach of long-legged buzzard, *Buteo rufinus*. In the present study, the regional distribution and relative frequency of endocrine cells in long-legged buzzard stomach were examined by immunohistochemical method using specific antisera against serotonin, Somatostatin-14 (SOM-14), Substance P (SP), Calcitonin-Gene Related Peptide (CGRP), Cholecystokinin-8 (CCK-8), neurotensin, Neuropeptide Y (NPY), galanin and Vasoactive Intestinal Polypeptide (VIP). This study also aimed to ascertain whether their distribution is similar to those of avian species.

MATERIALS AND METHODS

Animals and tissue samples: Two male long-legged buzzards were used in the study. The buzzards had been found by villagers with damaged wings around Elazig province at different times and taken to a veterinarian for treatment however, they could not be cured and euthanasia was inevitably decided for them. Stomach tissues of 61 and 63 cm long birds were excised under deep ether anesthesia. The left carotid artery was cannulated at the base of the neck and allowed to exsanguinations. Tissue samples were taken from stomach and fixed in 10% neutral-buffered formalin for 24 h. They were then dehydrated through graded ethanol and embedded in paraffin. About 5 μm thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemistry: Tissues were incubated in citrate buffer (10 mM citric acid, pH 6.0) for 20 min to retrieve antigenicity. Blocking of endogenous peroxidase activity was done with 3% hydrogen peroxide (H_2O_2) in methanol for 10 min. In order to block un-specific binding incubation with normal goat serum (1:10) in 0.1 M Phosphate Buffered Saline (PBS), pH 7.2 was performed. Immunohistochemical staining was carried out by the Peroxidase Anti Peroxidase (PAP) method.

PAP method: Sections were incubated with primary antibodies for 16-20 h at 4°C as shown in Table 1. The primary antibodies were diluted in PBS containing 0.25% sodium azide and 2.5% Bovine Serum Albumin (BSA). Negative control sections were performed by replacing the primary antibodies with PBS. Sections were then incubated with goat anti-rabbit IgG (Dako, Z0421, Denmark) followed by rabbit PAP complex (Zymed Lab., 61.2003, San Francisco), both at dilution of 1:50 in PBS for 1 h at room temperature. Sections were washed in PBS for 30 min after each incubation step and finally immersed in glucose oxidase-DAB (diaminobenzidine)-nickel ammonium sulphate substrate for 10 min (Shu *et al*., 1988). After washing in distilled water and counterstaining with hematoxylin, sections were dehydrated and cover slips mounted with mounting medium.

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger (1979) including the replacement of specific antiserum by the same antiserum which had been pre incubated with its corresponding antigen. Sections were examined with Olympus BX-51 microscope and photographs were taken. The immuno-reactive endocrine cells on each section were counted by 10×40 times magnification. The mean numbers of immuno-reactive endocrine cells in each sample obtained from proventriculus and gizzard were determined by counting immunoreactive endocrine cells in ten randomly selected microscopic fields (numbers of immunoreactive cells/microscopic field). The density of distribution was subjectively rated into five grades not detected (-), rare (+), a few (+ +), moderate (+ + +) and numerous (+ + + +) (Table 2).

Table 1: Details of antibodies used in this study

Primer antibodies	Dilution	Trade name	Cat. No.
Serotonin	1:500	Zymed (Invitrogen), UK	18-0077
SOM-14	1:1000	Chemicon (Millipore), Canada	AB1976
SP	1:500	Chemicon (Millipore), Canada	AB1566
CGRP	1:200	Chemicon (Millipore), Canada	AB5920
CCK-8	1:100	Chemicon (Millipore), Canada	AB1973
Neurotensin	1:50	Chemicon (Millipore), Canada	AB5496
NPY	1:100	Chemicon (Millipore), Canada	AB1915
Galanin	1:100	Chemicon (Millipore), Canada	AB5909
VIP	1:100	Chemicon (Millipore), Canada	AB982

All antisera were raised in rabbit (Polyclonal). SOM-14, Somatostatin-14; SP, Substance P; CGRP, Calcitonin Gene-Related Peptide; CCK-8, Cholecystokinin-8; NPY, Neuropeptide Y; VIP, Vasoactive Intestinal Polypeptide

Table 2: Regional distribution and relative frequencies of immuno-reactive ECs in the stomach of the long-legged buzzard, *Buteo rufinus*

	Stomach			
	Proventriculus		Gizzard	
Antibodies	Epithelium	Glands	Epithelium	Glands
Serotonin	+	+	+	++
SOM-14	-	++++	-	++++
SP	++	-	++	++
CGRP	+++	-	+++	-
CCK-8	-	-	-	-
Neurotensin	-	-	-	-
NPY	-	-	-	-
Galanin	-	-	-	-
VIP	-	-	-	-

Mean number of detected immunoreactive endocrine cells in one 10×40 field: - = Not detected; + (rare) ≤1; ++ (a few) = 1-4; +++ (moderate) = 5-10; +++ +(numerous) ≥10

RESULTS AND DISCUSSION

Endocrine Cells (ECs) Immuno-Reactive (IR) for serotonin, SOM-14, SP and CGRP were detected in the

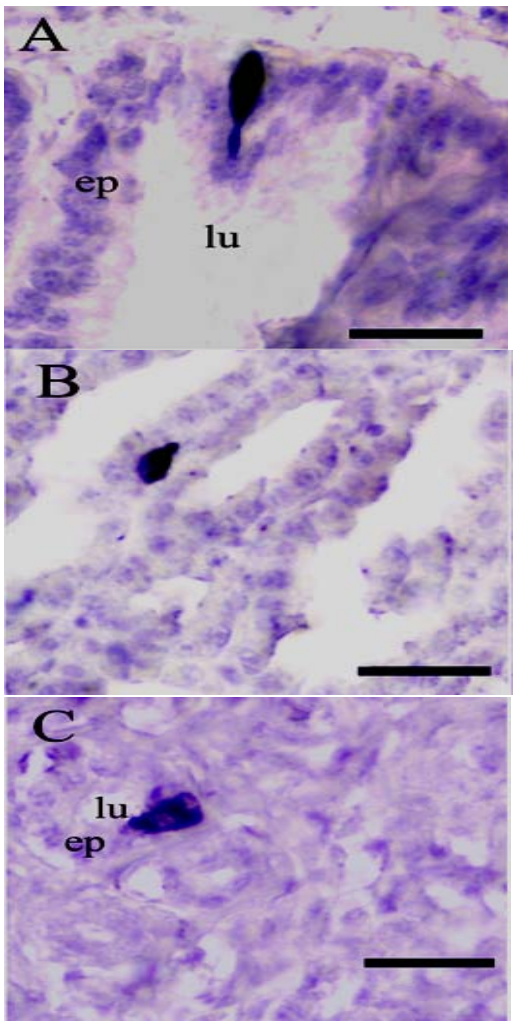

Fig. 1: Photomicrographs of serotonin-IR ECs in the long-legged buzzard stomach; A: Spindle-shaped serotonin-IR EC with long processes was detected in the epithelium of the mucosal folds of proventriculus. Note that intensity of immuno-reactivity was lower in the apical region of EC; B: Shuttle-shaped closed-type serotonin-IR EC was detected in the lobular gland of proventriculus; C: Opened-type serotonin-IR ECs were detected in the crypt of gizzard. (ep, epithelium; lu, lumen), Scale bars = 20 μm

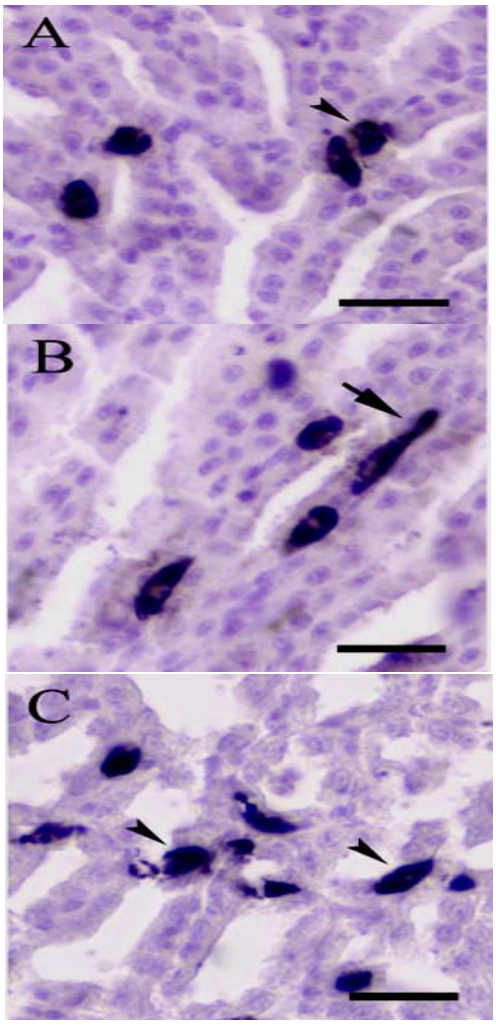

Fig. 2: Photomicrographs of SOM-14-IR ECs in the long-legged buzzard stomach; A and B: SOM-14-IR ECs in the lobular glands of proventriculus. Note that a great majority of SOM-14-IR ECs were closed-type and some of them possessing lateral projections extending along the adjacent glandular cells (B, arrow); C: SOM-14-IR ECs in the glands of gizzard. Limited number of SOM-14-IR ECs were detected as opened-type in the proventriculus and gizzard (Fig. 2, arrowheads); Scale bars = 20 μm

stomach mucosa of the long-legged buzzard for the first time. However, CCK-8, neurotensin, NPY, galanin and VIP were not found in any region of the stomach. The regional distribution and frequency of immuno-reactive ECs varied according to their location in the stomach. The distribution pattern and frequencies of these ECs in the stomach of the long-legged buzzard are shown in Table 2.

Serotonin: Serotonin-IR ECs were detected both of epithelium and glands of the proventriculus and gizzard. These ECs were rare in number throughout the proventriculus and in the epithelium of the gizzard. However, these cells were a few in number in the glands of the gizzard. Most of serotonin-IR ECs in the epithelium of stomach were opened-type and their shape varied from oval to spindle and located in the mucosal

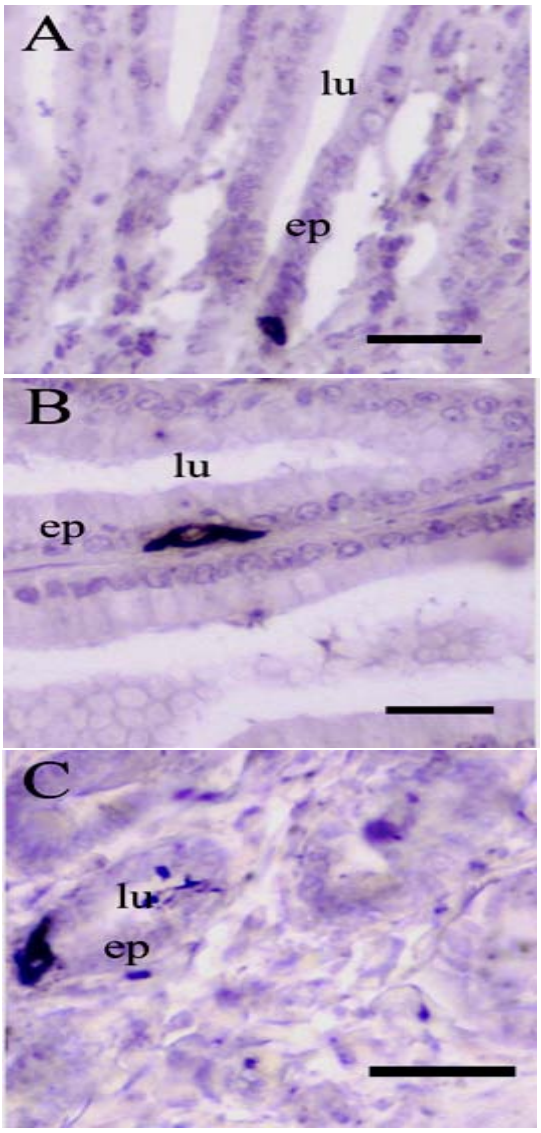

Fig. 3: Photomicrographs of SP-IR ECs in the long-legged buzzard stomach; A: Opened-type SP-IR EC was detected in the fold of proventricular mucosa. Note that intensity of immuno-reactivity was lower in the apical region of EC; B and C: Shuttle-shaped closed-type SP-IR EC was detected in the epithelium of proventriculus (B) and in the crypt of gizzard; C: Scale bars = 20 μm

folds (Fig. 1A). Serotonin-IR ECs in the glands of the proventriculus were mostly closed-type and their predominant shape was shuttle (Fig. 1B).

Serotonin-IR ECs in the crypts of gizzard were mostly opened-type and their shape varied from oval to round (Fig. 1C).

SOM-14: Numerous SOM-14-IR Ecs were detected which scattered in the glands of the proventriculus and gizzard. A great majority of SOM-14-IR ECs were closed type and their shape varied from round to long-shuttle (Fig. 2A and B). However, limited number of immuno-reactive cells were described as opened type ECs (Fig. 2A and C). Long-shuttle shaped SOM-14-IR ECs are usually possessing lateral and apical projections extending along the basal surfaces of adjacent glandular cells (Fig. 2B).

SP: A few SP-IR ECs were detected in the epithelium of the proventriculus and gizzard and in the glands of the gizzard. In the proventriculus, majority of SP-IR ECs were situated in the epithelium of the mucosal folds. Most of SP-IR ECs were from oval to triangular-shaped open type cells which described more intense immuno-reactivity that was restricted to the basal zone of the cytoplasm and they have luminal contact via an apical cytoplasmic process (Fig. 3A). On the other hand, limited numbers of SP-IR ECs were shuttle-shaped close type cells and they located along the basal lamina (Fig. 3B). In the gizzard, shuttle-shaped close type SP-IR ECs were detected in the crypt epithelium and they situated along the basal lamina, too (Fig. 3C).

CGRP: Moderate in number CGRP-IR ECs were detected in the epithelium of the proventriculus and gizzard. Majority of CGRP-IR ECs were located in the epithelium of the mucosal folds. Almost all of CGRP-IR ECs were thin and elongate-shaped open type cells and they extend from basal to luminal surface (Fig. 4A and B).

CCK-8, neurotensin, NPY, galanin and VIP: CCK-8, neurotensin, NPY, galanin and VIP-IR ECs were not found in any region of the proventriculus and gizzard. The present study was revealed the existence and distribution of neuropeptide-secreting ECs in the proventriculus and gizzard of the long-legged buzzards, *Buteo rufinus*. In this study; serotonin, SOM-14, SP and CGRP were determined to exist in the stomach of the long-legged buzzards for the first time. Serotonin is a monoamine and is widely distributed in nervous system in gastrointestinal tract and in endocrine pancreas (El-Salhy *et al*., 1985). Serotonin inhibits gastric acid secretion and contraction of smooth muscle in the gastrointestinal tract (Guyton, 1988). The gastrointestinal ECs are responsible for the production and storage of the largest pool of serotonin in the body (Gershon and Tack, 2007; Bertrand and Bertrand, 2010). Serotonin-IR ECs were detected in the stomach of human (Meyer and Brinck, 1999), babirusa (Agungpriyono *et al*., 2000), dogfish (Chiba, 1998), frog (Ku *et al*., 2003), lizard (Lee and Ku, 2004), axolotl (Maake *et al*., 1999),

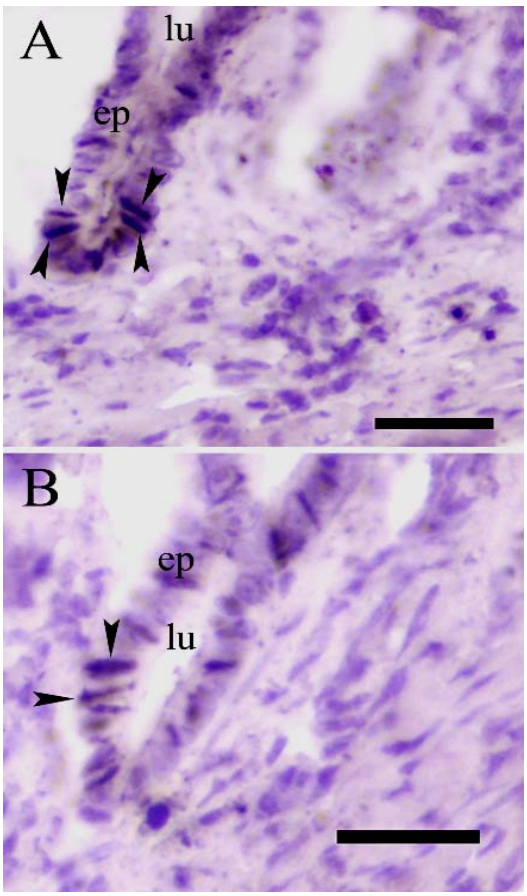

Fig. 4: A and B) Thin and elongate-shaped opened-type ECs (arrowheads) were detected in the epithelium of pro-ventriculus. Note that majority of ECs were located in the mucosal folds; Scale bars = 20 μm

bats (Machado-Santos *et al*., 2009) and *Caiman latirostris* (Yamada *et al*., 1987). Among avian species in ontogenic studies in the chicken revealed that serotonin-IR ECs in the proventriculus reach their peak around the 16th day of incubation then, markedly decrease at hatching and in adults these cells are not so numerous (D'Este *et al*., 1986; Aksoy and Cinar, 2009). In the examinations performed on ostrich (Tarakci *et al*., 2008) and chicken (Yamanaka *et al*., 1989), serotonin-IR ECs were observed in the proventriculus but not in the gizzard. In *Zonotrichia capensis* (Mendes *et al*., 2009), serotonin-IR ECs were not observed in the epithelium of proventriculus and gizzard but they were demonstrated a few in number along the proventricular and ventricular (gizzard) glands.

Differently from ostrich (Tarakci *et al*., 2008), chicken (Yamanaka *et al*., 1989) and *Zonotrichia capensis* (Mendes *et al*., 2009), these ECs were observed in the epithelial and glandular structure both of proventriculus and gizzard in this study.

The straight and cyclic forms of SOM, consisting of 14 amino acids were isolated from the hypothalamus of the sheep for the first time (Brazeau *et al*., 1973). SOM was soon considered a central coordinator of gastrointestinal physiology. Since, the initial demonstration of SOM expression and acitivity in the pancreas, numerous studies have investigated the expression of SOM and its physiological activities in the gastrointestinal tract (Van Op den Bosch *et al*., 2009). SOM-14-IR ECs were detected in the stomach of the avian species including ostrich (Bezuidenhout and van Aswegen, 1990; Tarakci *et al*., 2008), duck (Castaldo and Lucini, 1991), chicken (Martinez *et al*., 1993; Cinar and Diler, 2008), *Zonotrichia capensis* (Mendes *et al*., 2009) and Houbara bustard (Mensah-Brown and Lawrence, 2001). In ostrich (Tarakci *et al*., 2008), SOM-14-IR ECs were numerous and a few in number in the proventriculus and gizzard, respectively. In *Zonotrichia capensis* (Mendes *et al*., 2009), SOM-14-IR ECs were moderate in number and also found only in the submucosal glands of proventriculus and gizzard. In the domestic pigeon (Saito *et al*., 1989), moderate numbers of SOM-14-IR ECs were found in the pro-entriculus but no immunoreactive ECs were detected in the gizzard. In addition to these examinations, Mensah-Brown and Lawrence (2001) were found SOM-14-IR ECs in vast number in the submucosal glands of the proventriculus in *Houbara bustard*. Providing that except frequency of ECs, the results of this study is resemble with the results of *Zonotrichia capensis* (Mendes *et al*., 2009) and ostrich (Tarakci *et al*., 2008) but not with domestic pigeon (Saito *et al*., 1989) stomach. Additionally, as such in *Zonotrichia capensis* stomach, SOM-14-IR ECs with cytoplasmic projections were found in the lobular glands in this study.

SP is a decapeptide which is mostly distributed in brain, spinal cord, peripheral and enteric nervous systems (Pearse and Polak, 1975). In chicken (Brodin *et al*., 1981) and domestic duck (Castaldo and Lucini, 1991), SP-IR ECs were detected in the intestinal tract mucosa from the duodenum to the colorectum but not in the proventriculus and gizzard. In examination performed on ostrich (Bezuidenhout and van Aswegen, 1990), these ECs were found in the gastrointestinal tract. Additionally, the researchers were detected numerous SP-IR ECs in the proventriculus, gizzard and duodenum mucosa of the ostrich but not in the lower intestine (Tarakci *et al*., 2008). In this study, SP-IR ECs were detected a few in number and located in the epithelium of the whole stomach and glands of gizzard. The results of this study is resemble with the results of ostrich (Bezuidenhout and van Aswegen, 1990; Tarakci *et al*., 2008) but different from chicken (Brodin *et al*., 1981) and domestic duck (Castaldo and Lucini, 1991).

CGRP is a neuropeptide constituted by 37 amino acids that is encoded by alternative spilicing products of the *calcitonin* gene (Amara *et al*., 1982). CGRP plays important role in gastrointestinal functions including motility and secretions from the stomach (Qvigstad and Waldum, 2004; Kasacka, 2009). CGRP-IR ECs were described in the stomach of healthy and hypertensive rats (Kasacka, 2009). This peptide was not found in the ECs of the stomach in the neotenic axolotls (Maake *et al*., 1999) and freshwater turtles (Tarakci *et al*., 2005). Among avian species, the researchers were not described CGRP-IR ECs in any region of the gastrointestinal tract in ostrich (Tarakci *et al*., 2008). To the best of the knowledge, there are no reports in the literature that describe CGRP-IR ECs in the stomach of avian species. In the present study, SP-IR ECs were detected in the proventriculus and gizzard epithelium of long-legged buzzards for the first time.

Physiological actions of gastrointestinal CCK include regulation of gallbladder contractions in response to feeding, pancreatic exocrine secretion and gastric emptying (Ruiz-Gayo *et al*., 2006). In the gastrointestinal tract of bats (Machado-Santos *et al*., 2009), CCK-8-IR ECs were observed only in the intestinal portion but not in the stomach. In the frog (Ku *et al*., 2003), CCK-8-IR ECs were observed in the gastric gland regions of antrum. In the examination performed on lizards (Lee and Ku, 2004), moderate CCK-8-IR ECs were observed in the pylorus but no ECs were found in the fundus. In the immunohistochemical studies, CCK-IR ECs were not described in the stomach of the babirusa (Agungpriyono *et al*., 2000), turbot (Bermudez *et al*., 2007), brown trout (Bosi *et al*., 2004), domestic duck (Castaldo and Lucini, 1991) and ostrich (Tarakci *et al*., 2008). The results of this study is resemble with the results of previous studies carried out in ostrich, domestic duck and some others animal species.

Neurotensin is a member of the large family of neurohormonal peptides with localization in both brain and gut (Sundler *et al*., 1980). In the gastrointestinal tract, neurotensin-IR ECs were found predominantly in the distal small intestine and released by intraluminal fats (Evers, 2002). In the dogfish (Cimini *et al*., 1989), no neurotensin-IR ECs were described in the mucosal epithelium of the stomach. Neurotensin-IR ECs were described in the mucosal epithelium throughout the gastrointestinal tract of the axolotls (Maake *et al*., 1999). In the examination performed on porcupine gastrointestinal tract, the researchers were found neurotensin-IR ECs only in the small intestine but not in the stomach and large intestine (Yaman *et al*., 2007). In the ostrich (Tarakci *et al*., 2008), neurotensin-IR ECs were not found in the gastrointestinal tract. In the domestic ducks (Castaldo and Lucini, 1991), neurotensin-IR ECs were present in the intestinal tracts from the duodenum to the colorectum but not in the stomach. In the chicken (Martinez *et al*., 1991), numerous neurotensin-IR ECs were detected in the epithelium and glands of proventriculus. In the chicken and Japanese quail (Atoji *et al*., 1994), there were a few immunoreactive cells in the epithelium of the proventriculus and gizzard. The results of this study is resemble with the results of domestic duck (Castaldo and Lucini, 1991) and ostrich (Tarakci *et al*., 2008) but different from chicken (Martinez *et al*., 1991; Atoji *et al*., 1994) and Japanese quail (Atoji *et al*., 1994).

NPY, galanin and VIP are widely distributed throughout the central and peripheral nervous system with the highest concentrations (first two of them) in the hypothalamus (Fujimiya and Inui, 2000). NPY-IR ECs were found in the stomach mucosa of the dogfish (Cimini *et al*., 1989; Chiba *et al*., 1995). As far as we know, there are no reports in the literature that describe NPY-IR ECs in the stomach of avian species. In this study, NPY-IR ECs were not detected in the proventriculus and gizzard of long-legged buzzards. Both of them, galanin and VIP-IR ECs were not found in the stomach mucosa of brown trout (Bosi *et al*., 2004) and dogfish (Cimini *et al*., 1989). Similarly, in the avian species including Houbara bustard (Mensah-Brown and Lawrence, 2001), chicken (Rawdon, 1984) and ostrich (Tarakci *et al*., 2008), these ECs were not detected in the stomach mucosa. In the present study galanin and VIP-IR ECs were not found in the stomach mucosa of long-legged buzzard. The results of this study were compatible with the findings reported by previous studies.

CONCLUSION

Serotonin, SOM-14, SP and CGRP were detected to exist in the proventriculus and gizzard of the long-legged buzzards for the first time in this study. Serotonin-IR ECs were observed in both epithelium and glands in the proventriculus and gizzard while SOM-14-IR ECs were detected only in the glands. SP-IR ECs were detected in the epithelium of the whole stomach and only in the glands of gizzard. CGRP-IR ECs were detected only in the epithelium of whole stomach. The presence and distribution of serotonin and neuropeptides in the stomach of long-legged buzzard has been introduced in this study and it was also found that this species has been found to differ from other types of some avian species. These difference may be variety in feeding habits (between animal species) and/or caused by the species-specific differences among avian species.

REFERENCES

Agungpriyono, S., A.A. Macdonald, K.Y. Leus, N. Kitamura and I.K. Adnyane *et al*., 2000. Immunohistochemical study on the distribution of endocrine cells in the gastrointestinal tract of the babirusa, *Babyrousa babyrussa* (Suidae). Anat. Histol. Embryol., 29: 173-178.

Aksoy, A. and K. Cinar, 2009. Distribution and ontogeny of gastrin-and serotonin-immunoreactive cells in the proventriculus of developing chick, Gallus gallus domestica. J. Vet. Sci., 10: 9-13.

Ali, M.A., F. Nyberg, S.I. Chandranath, S. Dhanasekaran and S. Tariq *et al*., 2007. Distribution of neuroendocrine cells in the small and large intestines of the one-humped camel (*Camelus dromedarius*). Neuropeptides, 41: 293-299.

Alison, B.C., 1989. The distribution and ontogeny of gastrin/CCK-, somatostatin- and neurotensin-immunoreactive cells in the gastrointestinal tract of the chicken. Histol. Histopathol., 4: 55-62.

Amara, S.G., V. Jones, M.G. Rosenfeld, L.S. Ong and R.M. Evans, 1982. Alternative RNA-processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature, 298: 240-244.

Atalar, O., I. Kurtul and D. Ozdemir, 2007. Morphological and morphometric approach to the bones of the wings in the long-legged buzzard (*Buteo rufinus*). F.U. Saglik Bil. Derg., 21: 163-166.

Atoji, Y., H. Watanabe, N. Nimamoto, M. Sugiyama, Y. Yamamoto and Y. Suzuki, 1994. Neurotensin immunoreactive cells in the gastrointestinal epithelium of the chicken, pigeon and Japanese quail. Eur. J. Histochemistry, 38: 65-72.

Bayrakdar, A., M. Yaman, O. Atalar, B.G. Tarakci and S. Ceribasi, 2011. Distribution of neuropeptides in endocrine and exocrine pancreas of long-legged buzzard (*Buteo rufinus*): An immunohistochemical study. Regul. Pept., 166: 121-127.

Bermudez, R., F. Vigliano, M.I. Quiroga, J.M. Nieto, G. Bosi and C. Domeneghini, 2007. Immunohistochemical study on the neuroendocrine system of the digestive tract of turbot, *Scophthalmus maximus* (L.), infected by Enteromyxum scophthalmi (Myxozoa). Fish Shellfish Immunol., 22: 252-263.

Bertrand, P.P. and R.L. Bertrand, 2010. Serotonin release and uptake in the gastrointestinal tract. Autonomic Neurosci., 153: 47-57.

Bezuidenhout, A.J. and G. van Aswegen, 1990. A light microscopic and immunocytochemical study of the gastrointestinal tract of the ostrich (*Struthio camelus* L.). Onderstepoort J. Vet. Res., 57: 37-48.

Bosi, G., A. Di Giancamillo, S. Arrighi and C. Domeneghini, 2004. An immunohistochemical study on the neuroendocrine system in the alimentary canal of the brown trout, *Salmo trutta*, L., 1758. Gen. Comp. Endocrinol., 138: 166-181.

Brazeau, P., W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier and R. Guillemin, 1973. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science, 179: 77-79.

Brodin, E., J. Alumets, R. Hakanson, S. Leander and F. Sundler, 1981. Immunoreactive subsrance P in the chicken gut: distribution, development and possible functional significance. Cell Tissue Res., 216: 455-469.

Castaldo, L. and C. Lucini, 1991. An immunohistochemical study on the endocrine cells in the gastrointestinal tract of domestic duck. Eur. J. Basic Applied Histochem., 35: 131-143.

Chiba, A., 1998. Ontogeny of serotonin immunoreactive cells in the gut epithelium of the cloudy dogfish, scyliorhinus torazame, with reference to coexistence of serotonin and neuropeptide Y. Gen. Comp. Endocrinol., 111: 290-298.

Chiba, A., Y. Honma and S. Oka, 1995. Ontogenic development of neuropeptide Y-like-immunoreactive cells in the gastroenteropancrearic endocrine system of the dogfish. Cell Tissue Res., 282: 33-40.

Cimini, V., S. van Noorden and V. Nardini, 1989. Peptides of the gastrointestinal tract of the dogfish (*Scyliorhinus stellaris*). J. Exp. Zool., 2: 146-157.

Cinar, K. and D. Diler, 2008. Localizations of cells that secrete bombesin, glucagon, somatostatin-14, in stomach (proventriculus and gizzard) in chicken (*Gallus gallus domestica* L.) during the prenatal and postnatal periods. Ankara Universitesi Veteriner Fakultesi Dergisi, 55: 201-206.

Deveney, C.W. and L.W. Way, 1983. Regulatory Peptides of the Gut. In: Basic and Clinical Endocrinology, Greenspan, F.S. and P.H. Forscham (Eds.). Maruzen, Asian Edn., Singapore, pp: 479-499.

D'Este, L., S. Biancone and T. Renda, 1986. Ontogenesis of 5-hydroxytryptamine-like immunoreactive cells and their relationship with bombesin in chicken proventriculus. Basic Applied Histochem., 30: 109-117.

El-Salhy, M., E. Winder and M. Lundqvist, 1985. Comparative studies of serotonin-like immunoreactive cells in the digestive tract of vertebrates. Biomed. Res., 6: 371-375.

Evers, B.M., 2002. Endocrine gene neurotensin: Molecular mechanisms and a model of intestinal differentiation. World J. Surg., 26: 799-805.

Friedemann, G., Y. Yom-Tov, U. Motro and Y. Leshem, 2011. Shift in nesting ground of the long-legged buzzard (*Buteo rufinus*) in Judea, Israel: An effect of habitat change. Biol. Conserv., 144: 402-406.

Fujimiya, M. and A. Inui, 2000. Peptidergic regulation of gastrointestinal motility in rodents. Peptides, 21: 1565-1582.

Gershon, M.D. and J. Tack, 2007. The serotonin signaling system: fron basic understanding to drug development for functional gastrointestinal disorders. Gastroenterology, 132: 397-414.

Gulmez, N., M. Nazli, S. Aslan and N. Liman, 2003. Immunolocalisation of serotonin, gastrin, somatostatin and glucagon in entero-endocrine cells of the goose (Anser anser). Acta Veterinaria Hungarica, 51: 439-449.

Guyton, A.C., 1988. Secretory Functions of the Alimentary Tract. In: Textbook of Medical Physiology, Guyton, A.G. (Ed.). Saunders WB, Philadelphia, pp: 801-815.

Karan, M., S. Timurkaan and O. Atalar, 2008. The localization of insulin, glucagon, somatostatin immunoreactive cells in the pancreas of the *Martes foina*. F.U. Saglik Bil. Derg., 22: 13-16.

Kasacka, I., 2009. Quantitative distribution and localization of calcitonin gene-related peptide-like cells in the stomach of two kidney, one clip rats. J. Physiol. Pharmacol., 60: 35-39.

Ku, S.K., H.S. Lee, J.K. Koh and J.H. Lee, 2003. An immunohistochemical study on the neuropeptide-producing endocrine cells in the alimentary tract of wrinkled frog, Rana rugosa (Ranidae). Gen. Comp. Endocrinol., 131: 1-8.

Lee, H.S. and S.K. Ku, 2004. An immunohistochemical study of endocrine cells in the alimentary tract of the grass lizard, takydromus wolteri fischer (laceridae). Acta Histochem., 106: 171-178.

Maake, C., W. Kloas, M. Szendefi and M. Reinecke, 1999. Neurohormonal peptides, serotonin, and nitric oxide synthase in the enteric nervous system and endocrine cells of the gastrointestinal tract of neotenic and thyroid hormone-treated axolotls (Ambystoma mexicanum). Cell Tissue Res., 297: 91-101.

Machado-Santos, C., A.A. Nascimento, A.L. Peracchi, J.S. Mikalauskas, P.A. Rocha and A. Sales, 2009. Distributions of the endocrine cells in the gastrointestinal tract of nectarivorous and sanguivorous bats: A comparative immunocytochemical study. Tissue Cell, 41: 222-229.

Martinez, A., J. Lopez and P. Sesma, 1993. Development of the diffuse endocrine system in the chicken proventriculus. Cell Tissue Res., 271: 107-113.

Martinez, A., J. Lopez, M.A. Barrenechea and P. Sesma, 1991. Immunocytochemical and ultrastructural characterization of endocrine cells in chicken proventriculus. Cell Tissue Res., 263: 541-548.

Mendes, R.M., A.A. do Nascimento, C.M. Dos Santos, T.R. Cardoso, N.L. Pinheiro and A. Sales, 2009. An immunocytochemical study of the endocrine cells in the stomach and duodenum of Zonotrichia capensis subtorquata (Passeriformes, Emberizidae). Acta Histochemica, 111: 83-92.

Mensah-Brown, E.P.K. and P.A. Lawrence, 2001. Neurotransmitters regulating acid secretion in the proventriculus of the *Houbara bustard* (*Chlamydotis undulate*): A morphological viewpoint. J. Morphol., 248: 175-184.

Mensah-Brown, E.P.K., T.A. Bailey, D.J. Pallot and A. Garner, 2000. Peptidergic hormones and neuropeptides and aminergic neurotransmitters of the pancreatic islets of the Houbara bustard (*Chlamydotis undulata*). J. Anatomy, 196: 233-241.

Meyer, T. and U. Brinck, 1999. Differential distribution of serotonin and tryptophan hydroxylase in the human gastrointestinal tract. Digestion, 60: 63-68.

Neglia, S., N. Arcamone, G. Gargiulo and P. de Girolamo, 2007. Immunocytochemical detection of leptin-like immunoreactivity in the chicken gastroenteric tract. Gen. Comp. Endocrinol., 155: 432-437.

Olsson, C. and S. Holmgren, 2001. The Control of Gut Motility. Comp. Biochem. Physiol. A, 128: 481-503.

O'Malley, B., 2005. Avian Anatomy and Physiology. In: Clinical Anatomy and Physiology of Exotic Species. O'Malley, B. (Ed.). Elsevier Ltd., USA., ISBN: 9780702027826, pp: 97-161.

Pearse, A.G.E. and J.M. Polak, 1975. Immunocytochemical localization of substance P in mammalian intestine. Histochemistry, 41: 373-375.

Qvigstad, G. and H. Waldum, 2004. Rebound hypersecretion after inhibition of gastric acid secretion. Basic Clin. Pharm. Toxicol., 94: 202-208.

Rawdon, B.B., 1984. Gastrointestinal hormones in birds: Morphological, chemical and developmental aspects. J. Exp. Zool., 232: 659-670.

Ruiz-Gayo, M., M.C. Gonzalez and S. Fernandez-Alfonso, 2006. Vasodilatory effects of cholecystokinin: New role for an old peptide? Regul. Pept., 137: 179-184.

Saito, T., J. Yamada, N. Kitamura and T. Yamashita, 1989. An immunohistochemical study on the distribution of endocrine cells in the gastrointestinal tract of domestic pigeon, (*Columba livia* var domestica). Zeitschrift fur Mikroskopisch Anatomische Forschung, 103: 237-246.

Sari, E.K., M. Nazli, H. Kocamis, N. Gulmez, S. Aslan and T. Deprem, 2007. Immunohistochemical localization of serotonin, gastrin and somatostatin-immunoreactive endocrine cells in the duodenum of the rat (Wistar albino). Kafkas Universitesi Veteriner Fakultesi Dergisi, 13: 133-137.

Shao, Y., S. Liu, X. Tang, J. Gao, G. Wu and Z. Li, 2010. Ontogeny of ghrelin mRNA expression and identification of ghrelin-immunopositive cells in the gastrointestinal tract of the Peking duck, *Anas platyrhychos*. Gen. Comp. Endocrinol., 166: 12-18.

Shu, S., G. Ju and L. Fan, 1988. The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. Neurosci. Lett., 85: 169-171.

Sternberger, L.A., 1979. The Unlabelled Antibody Peroxidase-Antiperoxidase (PAP) Method. In: Immunocytochemistry, Sternberger, L.A. (Ed.). John Wiley Sons, New York, pp: 104-169.

Sundler, F., R. Hakanson and S. Leander, 1980. Peptidergic nervous systems in the gut. Clin. Gastroenterol., 9: 517-543.

Susaki, E., G. Wang, G. Cao, H.Q. Wang, E.W. Englander and G.H. Greeley, Jr., 2005. Apelin cells in the rat stomach. Regul. Pept., 129: 37-41.

Tarakci, B.G., M. Yaman, A. Bayrakdar and I. Yaman, 2008. Immunohistochemical study of the endocrine cells in the oesophagus of the ostrich (*Struthio camelus*). Medycyna Weterynaryjna, 2: 63-67.

Tarakci, B.G., M. Yaman, A. Bayrakdar and O. Atalar, 2007. Immunohistochemical detection of Gonadotropin-Releasing Hormone (GnRH) in porcupine (*Hystrix cristata*) pancreas. Rev. Med. Vet., 3: 134-136.

Tarakci, B.G., S.S. Koprucu and M. Yaman, 2005. An immunohistochemical study on the endocrine cells in the gastrointestinal tract of the freshwater turtle, *Mauremys caspica caspica*. Turk. J. Vet. Anim. Sci., 29: 581-587.

Van Op den Bosch, J., D. Adriaensen, L. van Nassauw and J.P. Timmermans, 2009. The role(s) of somatostatin, structurally related peptides and somatostatin receptors in the gastrointestinal tract: A review. Regul. Pept., 156: 1-8.

Yamada, J., V.J.M. Campos, N. Kitamura, A.C. Pacheco, T. Yamashita and N. Yanaihara, 1987. An immuno-histochemical study of the endocrine cells in the gastrointestinal mucosa of the *Caiman latirostris*. Arch. Histologicum Japonicum, 50: 229-241.

Yaman, M., B.G. Tarakci, A. Bayrakdar, O. Atalar and O. Dabak, 2007. Immunohistochemical study of gastrointestinal endocrine cells in the porcupine (*Hystrix cristata*). Rev. Med. Vet., 158: 196-200.

Yamanaka, Y., J. Yamada, N. Kitamura and T. Yamashita, 1989. An immunohistochemical study on the distribution of endocrine cells in the chicken gastrointestinal tract. Z. Mikrosk. Anatomische Forsch., 103: 437-446.

Yoruk, M., 2008. Sindirim Sistemi II: Sindirim Kanali. In: Veteriner Ozel Histoloji, Aytekin, O. (Ed.). Baski, New York, pp: 161-183.