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Morphological and Immunohistochemical Study on the Distribution of Endocrine Cells in the Gastrointestinal Tract of Partridge, *Alectoris chukar*

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ZÖ, HK and AK designed the study. HK, AK, DÖ and HB conducted the study. HK, AK, ZÖ, DÖ and HB analyzed the data. HK and AK wrote the manuscript. HK and AK revised the manuscript.

Key words
Endocrine cells, Birds, Gastrointestinal tract, Alectoris chukar

ABSTRACT

This study investigates the distribution and relative frequencies of the endocrine cells in the gastrointestinal tract of the partridge (*Alectoris chukar*). These cells which secrete coloestokinin (CCK), motilin, ghrelin, gastrointestinal polypetide (GIP) and vasointestinal polypeptide (VIP) in gastrointestinal tract were examined by using immunohistochemistry. CCK-IR, motilin-IR, ghrelin-IR, GIP-IR and VIP-IR cells were found to be more densely populated than other immunostained endocrine cells in the proventriculus and ventriculus sections. The distribution of CCK-IR, ghrelin-IR, and VIP-IR endocrine cells in the gastrointestinal tract of the red legged partridge was of an uncommon pattern. Our findings show that CCK-IR, motilin-IR, ghrelin-IR, GIP-IR and VIP-IR cells might be produced by all the proventriculus, ventriculus, duodenum, jejenum and ileum regions. These distribution patterns also provide further evidence of species-specific differences.

INTRODUCTION

The entero-endocrine cells located in the gastrointestinal tract produce many gastrointestinal hormones that play key roles in various physiological functions during digestion, including gut motility, nutrient absorption and intestinal blood flow (Bell, 1978; Budipitojo *et al*., 2016). Previous studies have demonstrated that the frequencies and distributions of these endocrine cells vary depending on the animal species and the feeding habits (Solcia *et al*., 1975; Lee *et al*., 2014; Firmiano *et al*., 2017).

Entero-endocrine cells in the alimentary tract in avian species was the subject of several previous studies employing various methods including immunohistochemistry (Solcia *et al*., 1975; Lee *et al*., 2014; Firmiano *et al*., 2017). Gastrointestinal tract houses many types of endocrine cells including coloestokinin (CCK), motilin, ghrelin, gastrointestinal polypetide (GIP) and vasointestinal polypeptide (VIP). CCK is transported from the duodenum in response to the presence of food, particularly fatty acids and amino acids (Wilding, 2002). Ghrelin has been identified in non-mammalian species including chickens and stimulates growth hormone (GH) release (Kaiya *et al*., 2002) and suppresses feeding behaviors in neonatal chicks (Saito *et al*., 2002). Motilin is secreted from endocrine cells of the duodenal mucosa to help regulate motility of the digestive tract. GIP has two main roles in the digestive tract: Firstly is to inhibit the gastric acid secretion and secondly is to stimulate the insulin secretion from the pancreas. VIP is a hormone that stimulates the secretion of electrolytes and water by the intestinal mucosa.

The morph-physiology of the gastrointestinal tract of the partridge has not yet been fully examined. There is, therefore, a need to investigate the gastrointestinal endocrine cells of this species to acquire basic data to pave the way to a better understanding of the digestive processes of chukar partridge. The aim of this study was to determine relative distribution and localizations of entero-endocrine cells that secrete CCK, motilin, ghrelin, GIP and VIP in gastrointestinal tract of partridge by using immunohistochemistry. The obtained data would be useful

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for better understanding of the digestive function of the gastrointestinal system of this bird species.

MATERIALS AND METHODS

Proventriculus, ventriculus and small intestines (duodenum, jejunum, ileum) of Chukar patridges procured from Merzifon, Amasya Patridge Hutchery were removed from the animals under ether anesthesia, fixed in the 10% formaldehyde solution for 72 h and dehydrated through different gradations of alcohal. Finally, the tissues were embedded in paraffin wax and 7-µm thick sections were cut. The serial sections were examined under light microscopy, after staining with Mallory's triple staining of Crossman.

Immunohistochemical staining of serial sections was done by the streptavidin peroxidase method with anti-CCK, anti-motilin, anti-ghrelin, GIP and VIP antibodies. Different types of cells in the same region were identified and the desired intensity was evaluated more reliably by staining these sections serially. Then the distribution of these densities throughout anatomical regions was scored. The densities were scored as (-) absent, (+) low-intensity, (++) medium-intensity and (+++) very intense.

RESULTS

Motilin positive cell densities are shown in different parts of GI tract in Figure 1. In proventriculus, medium density among glandular cells found in lamina propria. In ventriculus, a small amount of motilin-positive cells was seen between the lamina propria glands. In duodenum, very few motilin-positive cells are present among the villus crypts. In the jejunum and ileum, motilin-positive cells are located among the villus crypts and the villus epithelium cells at slightly higher density than the duodenum. Motilin cell density is presented in Table I and Figure 1.

Table I. Density score of motilin, vasoactive intestinal polypeptide (VIP), gastrointestinal polypeptide (GIP), ghrelin and cholecystokinin (CCK) antibodies of chukar partridge proventriculus (Provent), ventriculus (Vent), duodenum (Duod), jejunum (Jej) and ileum.

Antibody	Provent	Vent	Duod	Jej	Ileum
Motilin	++	+	+	++	++
VIP	+	+	+	+	+/-
GIP	+	++	+	+	+
Grelin	+	+	+	+	+
CCK	+	+	+	+	+

Score: as (-) absent, (+) low intense, (++) medium intense, (+++) very intense.

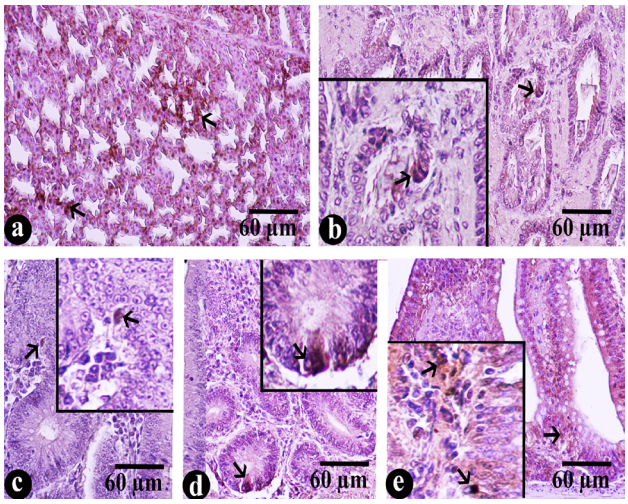

Fig. 1. Histological structure of gastrointestinal tissues of red legged partridge stained with anti-Motilin antibodies; (a) proventriculus; (b) ventriculus; (c) duodenum; (d) jejenum and (e) ileum. Arrows show immunoperoxidase staining positive stained cells.

Figure 2 shows that low-intensity VIP-positive cells are located around the secretory glands in the lamina propria of proventriculus. In ventriculus, a small number of VIP positive cells are present between epithelium cells and among gland cells in lamina propria. VIP-positive cells are present in villus lamina propria in duodenum and jejunum, and positive cells with few densities are located among crypts in ileum. VIP cell density is presented in Table I and Figure 2.

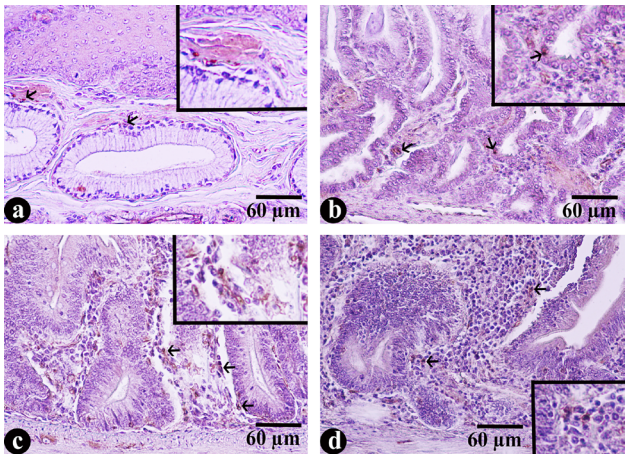

Fig. 2. Histological structure of gastrointestinal tissues of red legged partridge stained with anti-VIP antibodies; (a) proventriculus; (b) ventriculus; (c) duodenum and (d) jejenum. Arrows show immunoperoxidase positive stained cells.

Regarding GIP-positive cell density the low-intensity positive cells are located in secretory epithelium cells in lamina propria of proventriculus. Medium-intensity positive cells are located among surface epithelium cells in ventriculus. In duodenum and jejunum, low density GIP positive cells are present in villus epithelium. GIP positive cells are among villus crypts and in lamina propria in ileum. GIP cell density is presented in Table I and Figure 3.

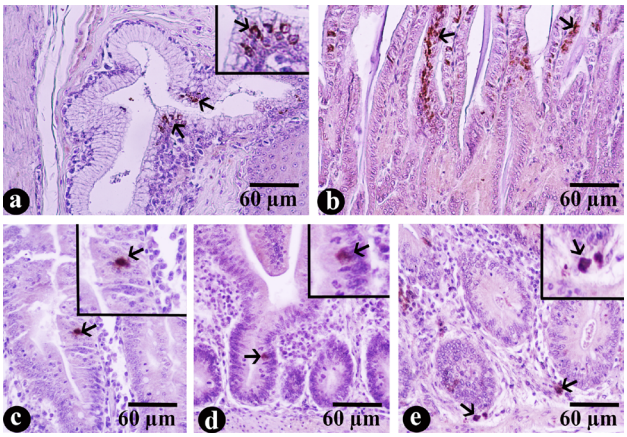

Fig. 3. Histological structure of gastrointestinal tissues of red legged partridge stained with anti-GIP antibodies; (a) proventriculus; (b) ventriculus; (c) duodenum; (d) jejenum and (e) ileum. Arrows show immunoperoxidase positive stained cells.

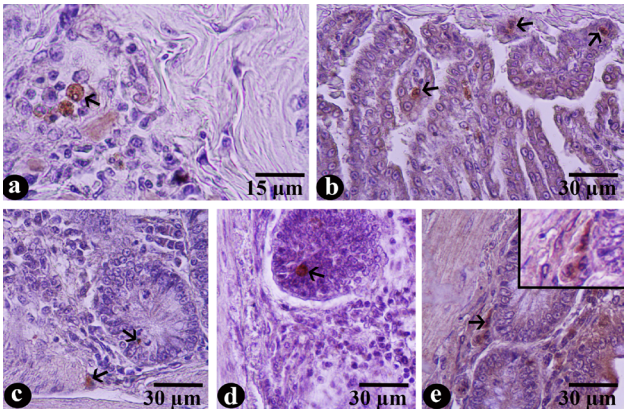

Fig. 4. Histological structure of gastrointestinal tissues of red legged partridge stained with anti-Grelin antibodies; (a) proventriculus; (b) ventriculus; (c) duodenum; (d) jejenum and (e) ileum. Arrows show immunoperoxidase positive stained cells.

Ghrelin-positive cells are located between the proventriculus and ventriculus lamina propria gland epithelial cells. In duodenum, jejunum and ileum, low-intensity ghrelin-positive cells are located between intestine crypts and in lamina propria. Ghrelin cell density is presented in Table I and Figure 4.

CCK positive cells are present in proventriculus and ventriculus, especially in the lamina propria layer. In duodenum, a small number of CCK positive cells are located in the villus lamina propria. In jejunum and ileum, low-intensity CCK positive cells are present between the epithelial cells of the intestinal villus surface. The CCK cell density is presented in Table I and Figure 5.

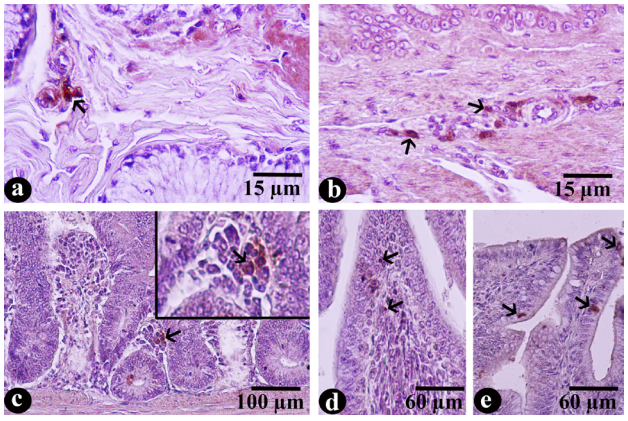

Fig. 5. Histological structure of gastrointestinal tissues of red legged partridge stained with anti-CCK antibodies; (a) proventriculus; (b) ventriculus; (c) duodenum; (d) jejenum and (e) ileum. Arrows show immunoperoxidase positive stained cells.

DISCUSSION

Food intake is controlled and regulated via complex systems including both central and peripheral sites of control, such as the gastrointestinal tract, liver, and brain. Many bioactive molecules were determined to be effective in the regulation of feeding behavior by the neural network in birds (Tachibana and Tsutsui, 2016). The neuroendocrine system cells that produce hormonal secretion undertake the most important function in the absorption and regulation of food in the digestive tract. The neuroendocrine cells are found in singularity or in groups between mucosa epithelium of gastrointesitinal tract (Tarakçı *et al*., 2008; Şimşek *et al*., 2011; Beheiry, 2018). We have determined the relative frequency and distribution of motilin, vasoactive intestinal polypeptide (VIP), gastrointestinal polypeptide (GIP), ghrelin and cholecystokinin (CCK) cells in the red legged partridges.

Yamamoto *et al*. (2008) reported that motilin-immunoreactive cells were rarely found in the small intestine in hens while Rawdon and Andrew (1981) reported that they were found little in the ventriculus and duodenum in chicks in the incubation period. In addition,

Mendes *et al*. (2009) found motilin-immunoreactive cells only in the study of some bird species' ventriculus. In the present study, motilin-immunoreactive cells have been observed in proventriculus, jejunum and ileum at medium density and in ventriculus and duodenum at low density in chukar patridges.

In this study, it has been determined that VIP-immunopositive cells are found rarely in ileum in chukar partridge and in low-intensity in the other digestive organs. In literature it was reported that the VIP-immunopositive cells were found rarely in duodenum, cecum and rectum and in jejunum and ileum at low density in chicks in the period of incubation (Rawdon, 1984), in the nervous structure of the intestinal wall in resident ducks (Castaldo and Lucini, 1991) and only in rectum in frogs (Ku *et al*., 2000).

It was reported that there were no anti-GIP cells in the gastrointestinal tract when Rawdon and Andrew (1981) studied chicks in incubation period (Rawdon and Andrew, 1981). However, Pirone *et al*. (2011) suggested that GIP-immuno positive cells of different shapes were found in the duodenum, jejunum and ileum. The number of GIP-immunopositive cells of the jejunal epithelium, both in the villi and crypts, seemed to be greater than in the duodenum and in the ileum. Some previous studies identified GIP immuno-positive cells in the mammalian intestine of various species including dog (Damholt *et al*., 1999), sheep (Bunnett and Harrison, 1986) and camel (Ali *et al*., 2007). In this study, the examination of GIP positive cell density in chukar patridge revealed that in the proventriculus they were located among the secretory epithelium cells in the lamina propria at low density and in ventriculus they were located among the surface epithelium cells at medium density. GIP positive cells were detected in duodenum, jejunum and ileum at low density.

Ghrelin regulates nutrient intake, energy, balance, body weight, gastric motility and acid secretion, endocrine pancreas function, and glucose metabolism (Kamegai *et al*., 2000; Nakazato *et al*., 2001). Previous studies reported that ghrelin-immunopositive cells were present in the entire gastrointestinal tract in in ostriches (Wang *et al*., 2009), in hens (Neglia *et al*., 2005), and most commonly in the proventriculus chickens (Wada *et al*., 2003). In the present study, it has been determined that Ghrelin immune positive cells are found at low density in the gastrointestinal tract in all of the materials used.

Castaldo and Lucini (1991) reported that anti-cholecystokinin (CCK) cells were present in the intestinal tract from the duodenum to the col-rectum and according to Rawdon and Andrew, they were present in the duodenum rarely, in the jejunum and ileum at low density in chicks (Rawdon and Andrew, 1999). It has also been reported that some reptiles such as turtle (Ku *et al*., 2001) and snake (Lee *et al*., 1999) have CCK-8 immunoreactive cells in the fundus and pylorus regions of gizaard and in the duodenum. Lee and Ku (2004) reported that CCK-8-positive cells were observed from pylorus to small intestine and showed the highest frequency in pylorus in the grass lizard. In ostrich, CCK is mainly produced in duodenum, whereas, in chicken, CCK production is concentrated at the transit from jejunum to ileum (Jønson *et al*., 2000). In all of the materials used in the present study anti-CCK cells were present in the entire small intestine and in addition in the preventriculus and ventriculus at low density in concurrence with previous reports (Rawdon and Andrew, 1981, 1999; Castaldo and Lucini, 1991).

CONCLUSION

In conclusion, the regional distribution and relative frequency of immunoreactive cells in the red legged partridges are essentially similar to those of other avian species (Saito *et al*., 2002; Ahmed *et al*., 2011; Pirone *et al*., 2011; Budipitojo *et al*., 2016). However, some characteristic differences observed in this species, concerning the relative frequency and distribution of these immunoreactive cells in the gastrointestinal tract, seem to be affected by feeding habits of different avian species. It is thought that the histochemical and immunohistochemical characteristics and distribution of endocrine cells obtained from this study might be a source for future histomorphological and physiological investigations.

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Statement conflict of interest

The authors have declared no conflict of interest.

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