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Original Article

Histochemical and scanning electron microscopy of proventriculus in turkey

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ABSTRACT

Objective: The objective of this research was to study the histological structures of proventriculus by light and scanning electron microscope and to investigate the localization of glucagon immunoreactive cells within the turkey proventriculus.
Materials and methods: Ten adult healthy turkeys were used in this study. The specimens were fixed in 10% buffered neutral formalin. The tissue samples were studied through routine histological and immunohistochemical techniques. Other samples were used for scanning microscope.
Results: This study confirmed that the turkey proventriculus was formed from four tunics; tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. The lamina epithelialis was simple columnar and gave positive reaction in Periodic Acid Schiff (PAS) stain. The lamina propria contained simple tubular glands lined with columnar cells with lymphocyte infiltration. The submucosa contained proventricular glands formed with many round lobules. Each lobule was consisted of secretory tubules radiating from the central lumen of the lobule. The lining epithelium of the central lumen was columnar epithelium. The epithelium of the secretory tubules was cuboidal. Scanning electron microscopic observations showed the mucous membrane was consisted of many macroscopic papillae that formed from many folds which separated by furrows. Immunohistochemical observations revealed that glucagon immunoreactive cells were mainly determined inside the proventricular glands than within the surface epithelium mainly in the basal portion of the lobules and in the epithelium of central lumen of gland lobules.
Conclusion: The structure of proventriculus structure in turkey has some variations as compared to other species of birds, and this may be attributed to the diet and its nutritious behavior.

KEYWORDS

Histology; Glucagon; Immunoreactive cells; Proventriculus; SEM; Turkey

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INTRODUCTION

Poultry is a good source of protein in human diets. The turkey is reared in many parts in the world, and industrialized farming of turkey has made its meat cheaper. Like other birds, stomach of turkey has two chambers; the proventriculus or glandular stomach in which the gastric juices are secreted, and the gizzard or muscular stomach which accomplishes a mechanical function (Jassem et al., 2016).

The proventriculus is eliptical in shape, which is extended along with esophagus cranially. The gizzard is round and flat-shaped with a very thick wall and extends caudally to the proventriculus. In the avian stomach, the endocrine cells have been studied by light microscopy (Martínez et al., 1993), ultrastructurally (Martínez et al., 1993; Ogunkoya and Cook 2009) and immunohistochemically (Duritis et al., 2013). These cells are situated to the deep gland of proventriculus that secrete both pepsinogen and hydrochloric acid, and called as oxyntico-peptic cell (Zhang and Wang, 2018).

Glucagons produced in many organs as in the endocrine portion of pancreas and in the nervous systems and perform many biological actions in which several act as neurotransmitters (Kieffer and Habener, 1999). Glucagon is antagonist to insulin which stimulates each the proteolysis and lipolysis, on the 13th day of incubation immunoreactive cells are situated within the glandular portion of the stomach, however within birds getting old, inside the pyloric region of stomach as nicely (Rawdon and Andrew, 1999). Alison (1990) reported that the appearance of glucagon-immunoreactive cells begins at 13th day of incubation. The aim of this research was to study the histological structure of proventriculus by light and scanning electron microscope. Another object was to investigate localization of glucagon immunoreactive cells in turkey proventriculus to illustrate its possible functions.

MATERIALS AND METHODS

This study has been conducted on the turkeys according to the international ethical standard to minimize pain. The research protocol was approved by the research ethics committee of the Faculty of Veterinary Medicine, Zagazig University (No. Zu-IACUC/2/F/40/2018).

Tissue preparation for light microscopically studies: This study has been conducted on ten adult turkeys of both sexes from farms in Sharkia governorate. The specimens were immediately removed after slaughter, gently cleaned with normal saline to remove any debris then fixed in 10% buffered neutral formalin, for histological processing (Bancroft and Gamble, 2008). The slides stained by Harris's Hematoxylin and Eosin (H&E) stain. Van Gieson stain: for collagen and muscle fibers. Periodic Acid Schiff (PAS) technique for mucopolysaccharides.

Tissue preparation for immunohistochemistry (Bancroft and Gamble, 2008): The avidin-biotin peroxidase complex (ABC) method was utilised for 5 µm thick sections. All staining procedure was carried out according to manufacturer protocol of Thermo Scientific. Sections were deparaffinized and rehydrate tissue section through a graded series of ethanol. To minimize the endogenous peroxidase action, incubate slide in hydrogen peroxidase for 15 min then in wash in buffer. Slides were blocked for 20 min with a blocking buffer. Apply primary antibody (Glucagon. Catalog number: [Ab1 rabbit polyclonal antibody. RP1422R7] Lab Vision Company) then wash with buffer. Apply Biotinylated goat Anti-glucagon and incubate over night at room temperature and wash four times in phosphate buffer. Apply Streptavidin peroxidase and incubate over night at room temperature then rinse four times in buffer then incubate with the chromogenic substrate diamino-benzidine. Counterstain using hematoxylin and coverslip. All stained sections were examined with a standard light microscope and photographs were taken at Histology and Cytology department, Faculty of Veterinary Medicine, Zagazig University.

Tissue preparation for scanning electron microscopy (Bozzola and Russel, 1999): Specimens for scanning electron microscope were fixed in 2.5% glutaraldehyde in phosphate buffer, PH 7.3, then specimens were washed several times in phosphate buffer, 10 min each and post fixed in 1% buffered osmium tetroxide for 2 h at 4°C, specimens were again washed in phosphate buffer, PH 7.3 (three times) and then in distilled water (2 times), dehydrated in ascending grades of alcohol and critically point dried. They were then sputter coated with gold, and photographed JEOL JSM 5200 scanning electron microscope in Faculty of Medicine, Tanta University.

RESULTS

In turkey, the proventriculus was formed with four tunics; tunica mucosa, submucosa, muscularis and tunica serosa. The mucosa contains many folds. At the junction between proventriculus and gizzard, the heights of

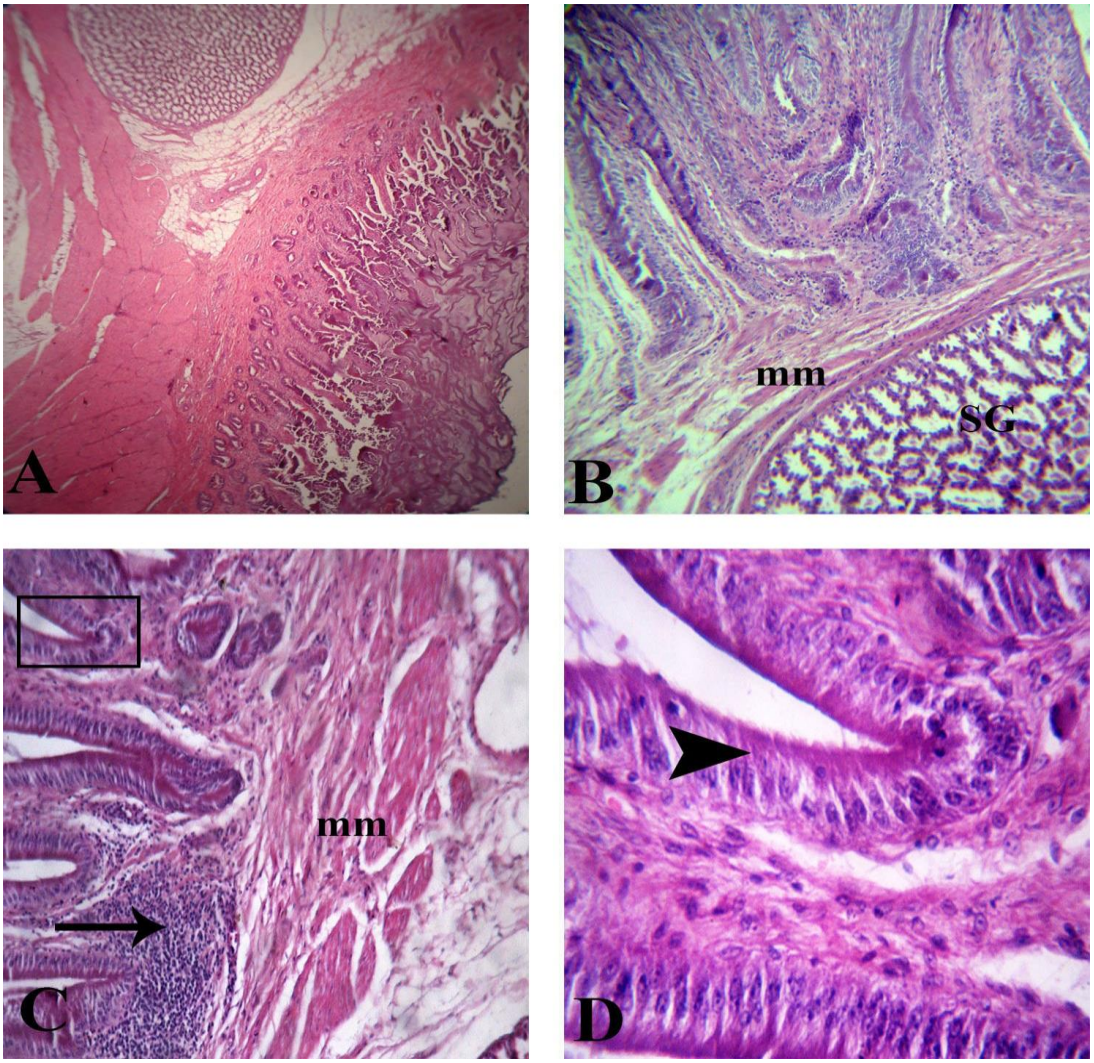

Figure 1. Photomicrograph of turkey proventriculus (A) showing the junction between the gizzard and proventriculus. (B) showing the mucosa and submucosal gland (SG). (C) showing the lamina propria contained the simple tubular glands and consisted of loose connective tissue with lymphocytic infilteration (arrow). (D) The magnification of the rectangle in image (1C) showed the tubular glands lined with columnar cells (arrow head), Muscularis mucosa (mm). Stain: H&E.x100 (A,B,C), x400 (D).

proventricular folds become decreased and the fibers of muscularis mucosa joined together. Tunica mucosa formed with three layers. Lamina epithelialis contained simple columnar cells. The lamina propria contained simple tubular glands lined with columnar cells with lymphocyte infiltration. Muscularis mucosa consisted with circular layer of smooth muscle fibers (**Figure 1**).

With Van Gieson stain, the lamina propria revealed the presence of mainly collagen fibers. The lamina epithelialis gave positive reaction to PAS stain. The submucosa consisted of connective tissue which contained the submucosal (proventricular) glands that constituted the thickest part of the proventriculus, which consisted with several round formed lobules. Each lobule consisted with secretory tubules radiating from the central lumen of the lobule. The lining epithelium of the central duct (lumen) of the gland was positively reacted to PAS stain. The central lumen might be crowded with secretory tubules. These gland lobules were surrounded by connective tissue capsule which was positively reacted with PAS stain (**Figure 2**).

The lining epithelium of the central duct (lumen) of the gland was columnar epithelium. The epithelium of the secretory tubules was cuboidal in shape and had serrated appearance (oxynticopeptic cells). The muscularis of the turkey proventriculus contained two layers of smooth muscle fibers. Thin, tunica serosa contained loose connective tissue covered with mesothelium (**Figure 3**).

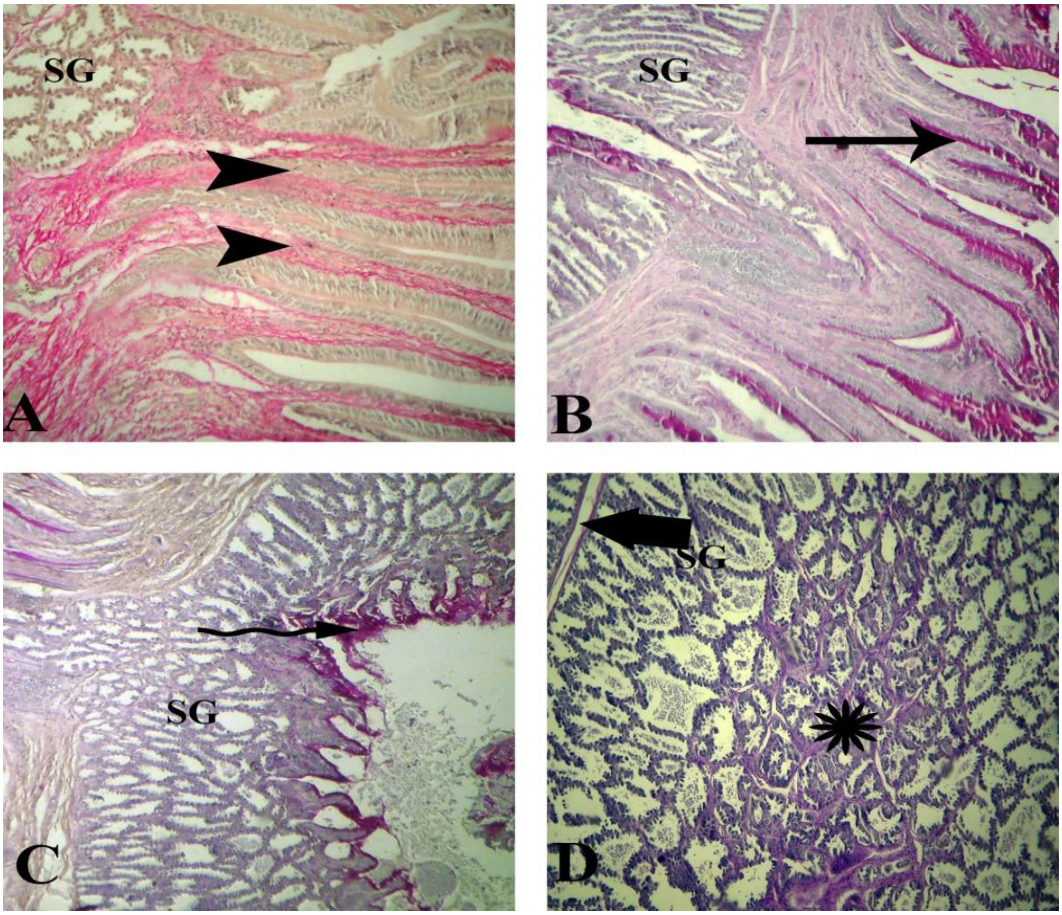

Figure 2. Photomicrograph of turkey proventriculus. Submucosal gland (SG). (A) showing the lamina propria contained collagen fibers (arrow heads). (B) showing lamina epithelialis gave positive reaction to PAS stain (arrow). (C) showing the lining epithelium of the central duct (lumen) of the gland was positively reacted to PAS stain (zigzag arrow). (D) showing Submucosal glands surrounded by connective tissue capsule which is positively reacted with PAS stain (thick arrow) and the central lumen was crowded with secretory tubules (star). Stain: Van Gieson stain in (A). and PAS stain in (B,C,D). x100 in (A,B,C,D).

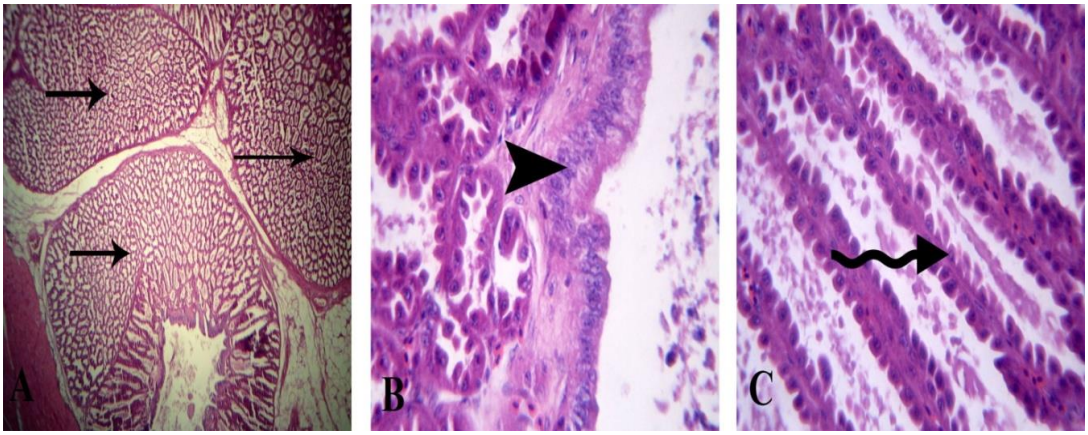

Figure 3. Photomicrograph of turkey proventriculus (A) showing round formed lobule consisted (arrows) of secretory tubules radiating from the central lumen of the lobule. (B) the lining epithelium of the central duct of the gland was columnar epithelium (arrow head). (C) showing the epithelium of the secretory tubules was cuboidal in shape and had serrated appearance (zigzag arrow). Stain: H&E. x100 (A), x400 (B,C).

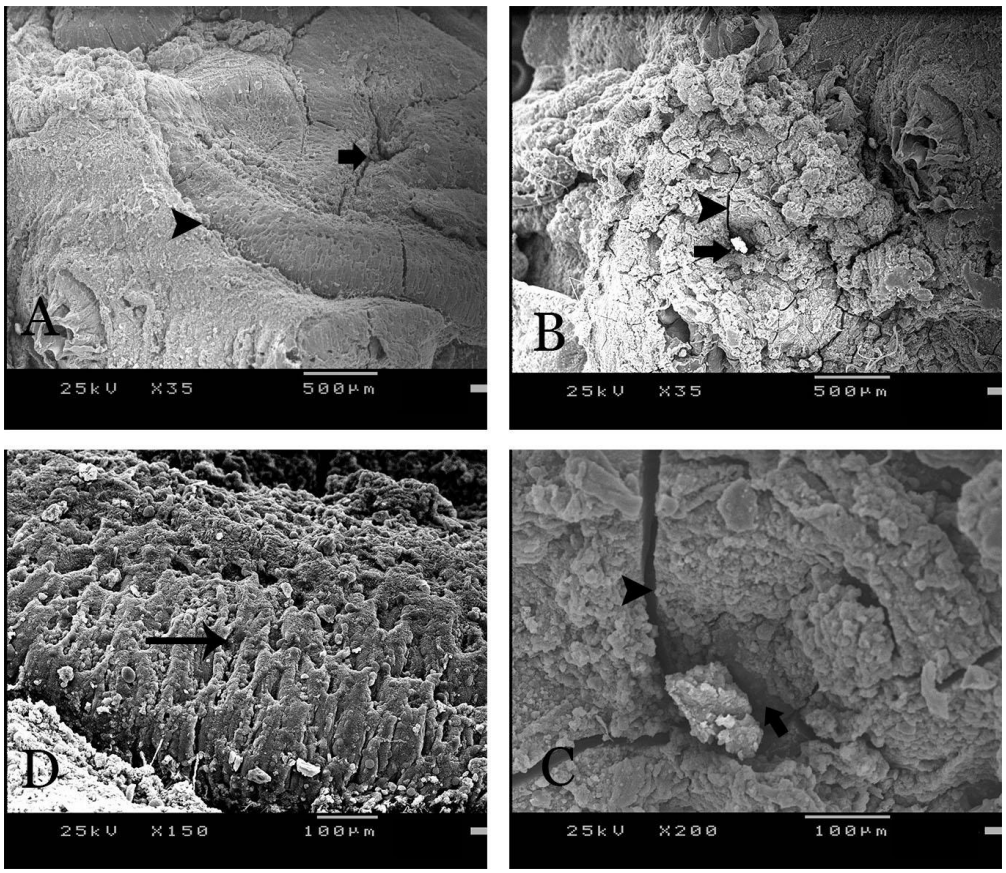

Figure 4. Scanning electron micrograph of turkey proventriculus. (A) showing the mucosal papillae and the proventricular opening (thick arrow) separated by furrows (arrow head). (B, C) showing the proventricular opening (thick arrow) and separated by longitudinal furrows (arrow head). The mucosal folds surrounding the opening is round in shape. (D) showing the mucosal folds were elongated (arrow).

Scanning electron microscopic observations showed the mucous membrane become consisted of many macroscopic papillae. These papillae were formed with many folds (plicae) which were separated via furrows. These folds were branched and surrounded the openings of the proventricular glands (**Figure 4**).

In the present work, the expression of antiglucagon was particularly observed in proventricular glands than in surface epithelium. Immunoreactive cells were detected within the basal portions of the folds and sulci of superficial epithelium with moderate reaction. The immunoreactive cells were distributed particularly within the basal portion of the lobules of proventricular gland and inside the epithelium of central lumen of gland lobules with strong reaction (**Figure 5**).

DISCUSSION

The structure of the bird stomach showed variations that depended upon the alimentary habits of the bird (McLelland, 1979). Davis (2007) revealed that gut morphology showed variations among different animal species depending on phylogenesis, size of animal, food and unique environmental pressures. The avian digestive system showed many organs that have greater inter-organ cooperation as compared to those found in mammals. The structures of proventriculus in several bird species have been reported previously; for example, guinea fowl (Selvan et al., 2008), broilers (Ogunkoya and Cook, 2009; Nasrin et al., 2012; Zhang and Wang, 2018), black tailed crake (Zhu, 2015) and yellow tailed crake (Zhu et al., 2013). The mucosa contains macroscopic papillae and microscopic folds; this confirms the previous studies in Greater Adjutant stork (Deka et al., 2017a) and Pati duck (Deka et al., 2017b). The shape and arrangement of proventriculus folds differed according to avian species as constituted by foliaceous vilosities (Fieri, 1984), branched (Lima and Sasso, 1985) or anastomotic (Rocha, 1991). The observations in the present work are similar to the findings of the previous reports on turkey (El-Zoghby, 2000), guinea fowl (Selvan et al., 2008), quail (Attia, 2008) and broilers (Nasrin et al., 2012), in which superficial

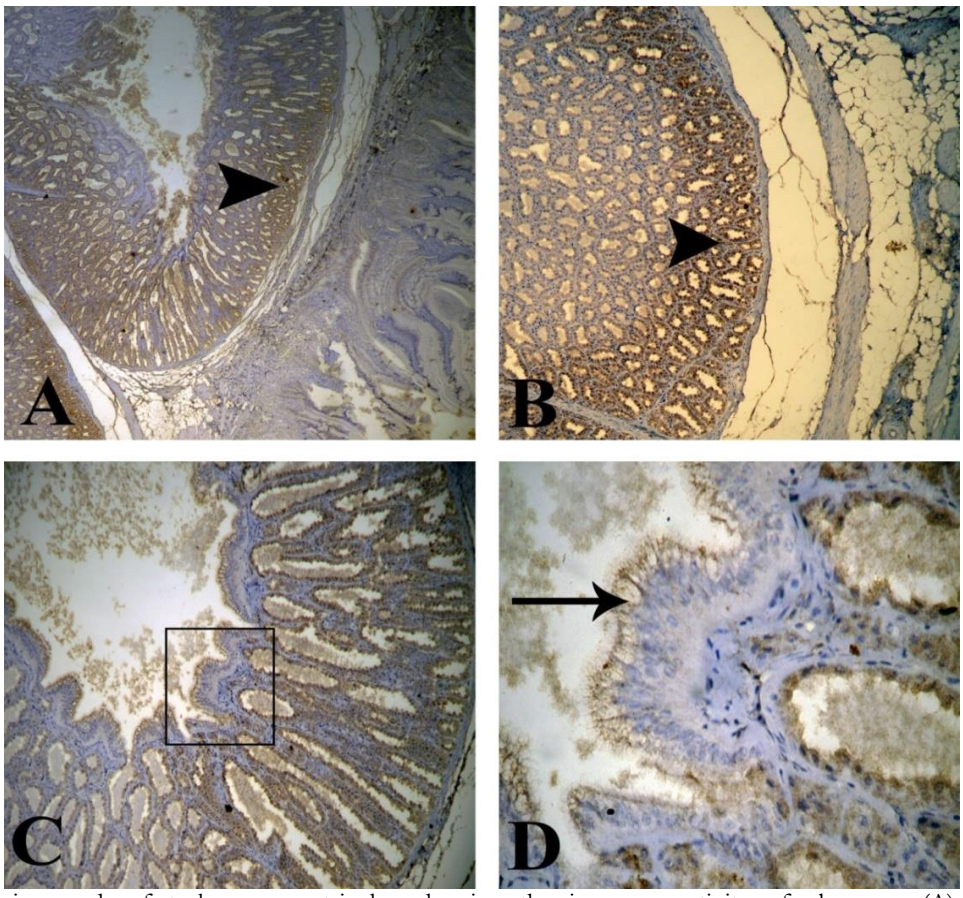

Figure 5. Photomicrograph of turkey proventriculus showing the immunoreactivity of glucagon. (A) showing gucagon immunoreactive cells are mainly observed in the proventricular glands (arrow head) than in the surface epithelium, the expression was detected in superficial epithelium with moderate reaction. (B) the expression was distributed mainly in the basal region of proventricular gland. (C) the expression was observed in the epithelium of gland lobules with strong reaction. (D) The magnification of the rectangle in image (4C) showed the expression (arrow). Immunoperoxidase staining. x100 (A,B,C), x400 (D)

epithelium of the tunica mucosa contains columnar cells, while in other species; it is prismatic type (Catroxo et al., 1997). Selvan et al. (2008) reported that the glandular epithelium of the submucosa has a negative reaction for mucins.

The results of the present study supported some previous studies which showed positive reaction to PAS stain in the surface epithelium. This reaction has protective function against the effect of HCL on mucosa in ducks (Prasad and Krishna1990; Shyla et al., 1993; Selvan et al., 2008). The variation in the shape of the surface epithelium in the domestic chicken might be due to species variation (Ogunkoya and Cook, 2009). In turkey proventriculus, the lamina propria contains the simple tubular glands with columnar cells and consists of loose connective tissue with lymphocyte infiltration. The current findings are in consistent with previous reports in ostrich (Bezuidenhout and van Aswergen, 1990; Catroxo et al., 1997; Ogunkoya and Cook, 2009; Nasrin et al., 2012; Deka et al., 2017a). The localization of these glands in the proventriculus differed among birds (Vial et al., 1977).

Submucosal glands constitute the thickest part of the proventriculus which consists of several round shaped lobules, as described in other birds like quail (Attia, 2008; Ogunkoya and Cook, 2009). These gland lobules are surrounded by connective tissue capsule which is positively reacted with PAS stain, as described previously (Rocha, 1991; Catroxo et al., 1997). Each lobule is consisting of secretory tubules radiating from the central lumen of the lobule. The epithelium of the secretory tubules are cuboidal in shape and has serrated appearance, as reported by Yamada et al. (1985) and Attia (2008) in quail and Nasrin et al. (2012) in broilers. The previous studies mentioned that the gland lobules of proventriculus contained Oxynticopeptic cell, the

proventriculus cells give hydrochloric acid and proteolytic enzymes (Randall and Reece, 1996). Catroxo et al. (1997) showed that endocrine cells with endocrine characteristics were observed between the secretory tubules of deep proventricular glands. The current study revealed an endocrine cell as detected by Castaldo and Lucini (1991) and Mendes et al. (2009). Ziswiler (1990) mentioned that the layer of mucosa indicated that the surface of the gut was increased.

In turkey proventriculus, the muscularis mucosa was present, as reported by Rocha (1991) and Catroxo et al. (1997) in the proventriculus of *Speotyto cunicularia*. While in quail, Attia (2008) mentioned that no muscularis mucosa was present. In the present study, muscularis of the turkey proventriculus contains two layers; this confirms the results of previous study in quail (Attia, 2008). However, Catroxo et al. (1997) revealed that the tunica musculosa contained three layers. The serosa was consisted of connective tissue, with many blood vessels and nerves, lined by mesothelium. This finding was similar to reports of Rocha (1991) and Attia (2008). The results of the present work are in consistent with previous studies revealed by scanning electron microscope of the mucous membrane of turkey proventriculus; which was consisted of many folds separated by furrows. These folds branched and surrounded the openings of the proventricular glands. These results are in agreement with the findings of El-Ghazali (2013). The number of the folds from the openings of the proventricular glands differed (Ogunkoya and Cook, 2009).

Polak et al. (1974) could not demonstrate the immunoreactive cells in the proventriculus of the quail and chicken. In contrast, Okamoto and Yamada (1981) confirmed the occurrence of endocrine cells in this region. Immunohistochemical studies revealed glucagon immunoreactive cells in chicken proventriculus (Timson et al., 1979). In the present study, the immunoreactive cells were distributed mainly in the basal portion of the lobules of proventricular gland and in the epithelium of central lumen of gland lobules with strong reaction and in the basal part of the folds and sulci of superficial epithelium with moderate reaction, while Yamada et al. (1985) mentioned that glucagon-immunoreactive cells were scattered primarily in the middle zone of the lobules. Glucagon immunoreactive cells were detected only in the deep proventricular glands, some cells in the superficial glands of the proventriculus and in the mucosa of ventricular side wall in ostrich stomach (Duritis et al., 2013; Bezuidenhout and Van Aswegen, 1990). Kirkegaard et al. (1982) found that glucagon and other related peptides had an important function as they have powerful inhibitors of gastric acid secretion and this fact explained the abundance of these cells in the proventriculus.

CONCLUSION

The histological structures of turkey proventriculus are examined in this study. The results reveal that the proventriculus in turkey has some similarities and some variations to other species of birds, and this may be attributed to its diet and its nutritious behavior.

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Nothing to disclose.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS' CONTRIBUTION

The samples were collected, research was carried out and the manuscript was wrote and revised by the author.

REFERENCES

1. Alison BC. The ontogeny and distribution of glucagon-and pancreatic polypeptide-immunoreactive cells in the gastrointestinal tract of the chicken. Anatomia Embryologia. 1990; 182:605–610. https://doi.org/10.1007/BF00186467
2. Attia HF. Some histological studies on the proventriculus of the quail during pre and post hatching periods. Minufiya Veterinary Journal. 2008; 5(2):441–453
3. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. 6th ed., Churchill, Livingstone, New York, London. 2008; p 83–92.
4. Bozzola J, Russel L. Electron microscopy. Principles and techniques for biologists. 2nd Edn., Boston, Toronto, London, Singapore: Jones & Artlett Publishers; 2 Sub Edn. 1998; p. 148–201.
5. Bezuidenhout AJ, Van Aswergen GA. A light microscopic and immunocytochemical study of the gastro-intestinal tract of the ostrich *(Struthio camelus)*. Onderstespoort Journal of Veterinary Research.1990; 57:37–48.

6. Deka A, Rajkhowa J, Das BJ, Das S, Devchoudhury KB. Gross and histomorphological observation of stomach of greater adjutant stork *(Leptoptilos dubius)*. Journal of Entomology and Zoology Studies. 2017a; 5(3):939–941.
7. Deka A, Sarma K, Rajkhowa J, Sarma M, Ahmed J. Macro and micro anatomical studies on oesophagus, proventriculus and gizzard of pati duck *(Anas platyrhynchos domesticus)* of Assam. International Journal of Chemical Studies. 2017b; 5(2):443–445.
8. Catroxo, MHB, Lima MAI, Cappellaro CEMPDM. Histological aspects of the stomach proventriculus and gizzard) of the red-capped cardinal (*Paroaria gularis gularis*, Linnaeus, 1766). Revista chilena de anatomía. 1997; 15(1):19-27. https://doi.org/10.4067/S0716-98681997000100003
9. Castaldo L, Lucini C. An immunohistochemical study on the endocrine cells in the gastrointestinal tract of domestic duck. European Journal of Basic and Applied Histochemistry. 1991; 35(2):131–143
10. Davis S. Endozoochory in the subtropical thicket: Comparing effects of species with different digestive systems on seed fate. MSc Thesis, Faculty of Science, Nelson Mandela Metropolitan University. 2007.
11. Duritis I, Mugurevics A, Mancevica L. The distribution of gastrin, somatostatin and glucagon immunoreactive (IR) cells in ostrich stomach during the pre- and post-hatching period. Anatomia Histologia Embryologia. 2013; 42:362–368. https://doi.org/10.1111/ahe.12023
12. El-Ghazali HM. Some morphological studies on the stomach of some birds. PhD Thesis, Zagazig University, Egypt. 2013.
13. El-Zoghby IA. Histological and histochemical studies on the digestive tract of the turkeys at different ages. PhD Thesis, Benha University, Egypt. 2000.
14. Fieri WJ. Aspectos anatômicos e histológicos do tubo digestivo da codorna Nothura maculosa maculosa, (Temminck, 1815). São Paulo. 1984; p. 109.
15. Jassem ES, Hussein AJ, Sawad AA. Anatomical, histological and histochemical study of proventriculus of common moorhen *(Gallinula Chloropus)*. Basrah Journal of veterinary Research. 2016; 14(6):73–82.
16. Kieffer TJ, Habener JF. The glucagon-like peptides. Endocrine Reviews. 1999; 20:876–913. https://doi.org/10.1210/edrv.20.6.0385
17. Kirkegaard P, Moody AJ, Holst JJ, Loud FB, Skov Olsen P, Christianesen J. Glicentin inhibits gastric acid secretion in the rat. Nature. 1982; 297:156–157. https://doi.org/10.1038/297156a0
18. Lima MAI, Sasso WS. Histochemical detection of glycoproteins in the gastric epithelia of Columba livia. Acta Histochemica. 1985; 76:145–150. https://doi.org/10.1016/S0065-1281(85)80051-9
19. Martínez A, López J, Sesma P. Development of the diffuse endocrine system in the chicken proventriculus. Cell Tissue Research. 1993; 271:107–113. https://doi.org/10.1007/BF00297548
20. McLelland J. Digestive system. In: King AS Mc Llelland Edn., Form and function in birds. London, Academic Press. 1979; p. 637.
21. Mendes RM, do Nascimento AA, Dos Santos CM, Cardoso TR, Pinheiro NL, Sales A. An immunocytochemical study of the endocrine cells in the stomach and duodenum of Zonotrichia capensis subtorquata *(Passeriformes, Emberizidae)*. Acta Histochemica. 2009; 111(1):83–92. https://doi.org/10.1016/j.acthis.2008.02.003
22. Nasrin M, Siddiqi MNH, Masum MA, Wares MA. Gross and histological studies of digestive tract of broilers during postnatal growth and development. Journal of the Bangladesh Agricultural University. 2012; 10(1):69–77. https://doi.org/10.3329/jbau.v10i1.12096
23. Ogunkoya YO, Cook RD. Histomorphology of the proventriculus of three species of australian passerines: *Lichmera indistincta*, *Zosterops lateralis* and *Poephila guttata*, Anatomia Histologia Embryologia. 2009; 38:246–253. https://doi.org/10.1111/j.1439-0264.2009.00930.x
24. Okamoto T, Yamada J. Light and electron microscopic studies on the endocrine cells in the duck proventriculus. Japanese Journal of Veterinary Science. 1981; 43:863–870. https://doi.org/10.1292/jvms1939.43.863
25. Polak JM, Pearse AGE, Adams C, Garaud JC. Immunohistochemical and ultrastructural studies on the endocrine polypeptide (APUD) cells of the avian gastrointestinal tract. Experientia, 1974; 30:564–567. https://doi.org/10.1007/BF01926354
26. Prasad RV, Krishna K. Histology and histochemistry of proventriculus of domestic duck. Mysore Journal of Agricultural Sciences.1990; 24:506–511.
27. Randall CJ, Reece RL. Color atlas of avian histopathology. New South Wales, Australia.1996.
28. Rawdon BB, Andrew A. Gut endocrine cells in birds: an overview, with particular reference to the chemistry of gut peptides and the distribution, ontogeny, embryonic origin and differentiation of the endocrine cells. Progress in Histochemistry and Cytochemistry. 1999; 34:3–82. https://doi.org/10.1016/S0079-6336(99)80004-7
29. Rocha SO. Aspectos morfológicos (histológicos) do tubo digestivo da coruja-buraqueira Speotyto cunicularia, (Molina, 1782). São Paulo. 1991; p. 73.
30. Selvan SP, Ushakumary S, Ramesh G. Studies on the histochemistry of the proventriculus and gizzard of post-hatch guinea fowl (*Numida meleagris*). International Journal of Poultry Science. 2008; 7(11):1112–1116. https://doi.org/10.3923/ijps.2008.1112.1116

31. Shyla P, Ommer PA, Lucy P. Structure and postnatal development of the proventriculus in the duck. Indian Journal of Poultry Science. 1993; 27:10–14.
32. Timson CM, Polak JM, Wharton J, Ghatei MA, Bloom SR, Usellini L, Capella C, Solcia E, Brown MR, Pearce AGE. Bombesin-like immunoreactivity in the avian gut and its localization to a distinct cell type. Histochemistry. 1979; 61:213–221. https://doi.org/10.1007/BF00496533
33. Vial JD, Garrido J, Dabike M, Koenig G. Muscle proteins and the changes in shape of avian oxynticopeptic cells in relation to secretion. Anatomical Record. 1977; 194:293–310. https://doi.org/10.1002/ar.1091940211
34. Yamada J, Kitamura N, Yamashita T. The relative frequency and topographical distribuion of somatostatin-, GRP-, APP-, Glucagon-, 5-HT and neurotensin-immunoreactive cells in the proventriculus of seven species of birds. Archivum. Histologicum. Japonicu. 1985; 48(3):305–314. https://doi.org/10.1679/aohc.48.305
35. Zhang H, Wang J. Morphology of the proventriculus of African ostrich. Indian Journal of Animal Research. 2018; 52(1):37–40.
36. Zhu L, Wang JJ, Sh XD, Hu J, Chen JG. Histological observation of the stomach of the Yellow-billed Grosbeak. International Journal of Morphology. 2013; 31:512–515. https://doi.org/10.4067/S0717-95022013000200025
37. Zhu L. Histological and histochemical study on the stomach proventriculus and gizzard) of black-tailed crake *(Porzana bicolor)*. Pakistan Journal of Zoology. 2015; 47(3):607–616.
38. Ziswiler V. Specialization in extremely unbalanced food: possibilities and limits of its investigation exlusively by functional morphology. Netherlands Journal of Zoology.1990; 40:299–311. https://doi.org/10.1163/156854289X00318

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