

A Comparative Histomorphological and Histochemical Study of the Proventriculus between Iraqi Male Guinea fowls

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Abstract

The current study aimed to describe and compare the histomorphological and histochemical structure of proventriculus in local Guinea fowls and Geese. In this study twenty four, healthy birds are used, obtained from local supplier at Baghdad province. Studied birds are divided into two equal groups (12) from each species and each one include (6 male and 6 female), each group was used for histological and histochemical approaches. After euthanasia, suitable size of specimens of its wall fixed in (10%) neutral buffered formalin for histological staining and others fixed in Bouin's solution for histochemical stains then processed in routine histological procedure, using the Hematoxylin-Eosin, Masson Trichrome, Periodic Acid Schiff (PAS) and Combined PAS-Alcian Blue (pH 2.5) stains.

The results showed the general histological structure of proventriculus in studied birds were similar, both proventriculus wall composed of mucosa, submucosa, muscularis and serosa, but with some morphometrical differences in its wall. Mucosa lined by simple columnar cells presents a numerous folds and superficial simple tubular glands. The thick submucosa predominantly constituted by voluminous and numerous deep submucosal glands, a compound tubular glands which organized in pyramidal or conical shape structures (adenomere). A thick tunica muscularis of smooth muscle oriented in an inner longitudinal layer and an outer circular layer. Serosa structured from loose connective tissue coated by mesothelium.

The histochemical findings in both studied birds proventriculus showed the surface lining columnar cells of mucosal folds luminal borders of mucosal glands, positively reacted with (PAS) stain, while the submucosal glandular epithelium showed negative reaction, cells lining the luminal borders of submucosal glands and their ducts gave a positive reaction apically. On applying PAS-AB (pH 2.5) stain, surface cells of mucosal folds, tubular mucosal glands and cells lining the luminal adenomers, collecting and excretory ducts of submucosal glands gives a positive reaction for PAS part of this technique, While the secretory cells of submucosal glands revealed negative reaction for this stain.

Introduction

Several studies were conducted to explore how the foods custom have influenced on the morphological characteristics and thereafter on physiological activities of digestive organs in birds (Caviedes-Vidal and Karasov, 2001). One of the very important organs composing the digestive system is the stomach. The stomach of the birds shows distinct two parts, the upper part is the glandular one which is the proventriculus, while the second one is the gizzard or the muscular stomach, the two parts are varied morphologically (size and shape) and functionally between species and within it, birds stomach is affected by the quantity and the quality. The proventriculus being large and distended in carnivores but often smaller in graminivores birds (Duke, 1997; Starck, 1999). Also it varied in its shape between avian species, In the black-winged kite appeared large, have a thin wall compared with seed eating and no present of the proventricular-ventricular isthmus, the two part formed one large pear-shaped structure (Hamdi *et al.*, 2013). Histologically, the wall of Moorhan proventriculus was consisted of a typical tubular organ layers Jassim *et al.*, (2016). In Rock pigeon and Egyptian laughing dove the wall of proventriculus is very thick and constructed of inner mucosa, submucosa, muscularis and serosa. The mucosa is thrown into folds, each fold was covered by a simple columnar epithelium which supported by a core of lamina propria (Madkour and Mohamed, 2018).

Submucosal glands represents the major portion composing of thickness of its wall. Simple single glands collected to brew lobules each of which converged into proventricular cavity near to surface. The cavities gathered to form a common duct which command to the surface during the apex of a tiny papilla (Nasrin *et al.*, 2012). In previous study on Blue and Yellow macaws proventriculus which were herbivores birds, its wall was structured by proventricular glands. The mucosa lined by simple cells of prismatic or cylindrical type, muscularis mucosa showed a spares longitudinally organized fibers interspersed among the connective tissue present between the glands lobes. Submucosa observed as a thin layer of loose connective tissue. Muscular layer constructed by a circular arrange smooth muscle fibers Rodrigues *et al.* (2012). There were no local studies on the histological, histomorphological structure and comparison of proventriculus between domestic Guinea fowls and Geese. Correspondingly, the current study designed to do so that using local Guinea fowls and Geese.

Materials and Methods

Twenty four domestic of Guinea fowls (*Numidia meleagris*) and Geese (*Anser anser*) were applied to conduct the present work. All birds were obtained from local suppliers in specific markets in Baghdad province. Studied birds were housed at animal house in a suitable cages of the Veterinary Medicine College/ university of Baghdad. These birds were fed and giving them water before the euthanasia and dissecting them, the studied birds were separative into two equal groups, each contains twelve (6 male and 6 female Guinea fowls) and (6 male and 6 female of Geese) for histomorphological and histochemical processes. These birds were euthanized by an (intravenous) injection of sodium pentobarbitone (80 mg kg^{-1}) as reported by (Mitchell and Smith, 1999), then dissected by making a mid-line incision in abdominal wall of each bird to view the coelomic viscera. The proventriculus was emptied from its contents then gently washing with normal saline. Suitable specimens from proventriculus of each bird were fixed in

10% neutral buffered formalin, dehydrated in a series of graded ethanol alcohol (70, 80, 90 and 100%) each for (two) hours, cleared in xylene for (1 hour), embedded in paraffin wax then blocked, sectioned to (6 μ m) thickness and stained with routine stain, Mayer's Hematoxylin and Eosin for general structure identification, Masson Trichrom stain for collagen and smooth muscle fibers. For conduct the histochemical processes, some specimens fixed in Bouin's fixative, then sectioned at (6 μ m) thickness and stained with PAS stain to illustrate the neutral mucin and basement membrane of the epithelium lining the proventriculus, while the combination of PAS-Alcian Blue pH (2.5) used for specific of neutral and acidic mucin (Luna, 1968; Bancroft and Stevens, 2010). The sections were imaged by Olympus microscope, using Dino-eye camera. The micromorphometric measurements which include (thickness of all tunicae of proventricular wall) were done using oculometer. The Statistical Analysis System- SAS (2012) program was used to detect the influence of various factors in study parameters. (T-test) used to significant comparison between means in this study.

This work aimed to investigate the histomorphological and histochemical of the proventriculus in domesticated guinea fowl and goose and comparison between them.

Results and Discussion

The histological examination of the proventricular wall of guinea fowl and geese was similar and showed the presence of four tunicae, namely; Mucosa, Submucosa, Muscularis and Serosa (Fig.1). These tunicae which structured the wall of this organ was similarly committed to paper in the proventriculus of chick (Nasrin, 2012), in Coot bird (AL-Aredhi., 2013) and in Moorhen *Gallinulachloropus* (Jassim et al., 2016), but the proventriculus wall of Black-tailed Crake the tunica submucosa was not detected (Zhu, 2015).

Tunica Mucosa

The tunica mucosa in both studied birds was similar and characterized by large longitudinal folds, finger like and others leaf like structures with different heights in both species, lined by simple columnar cells with a faint acidophilic cytoplasm and oval nuclei (Fig.1,2). This finding was similar to that observed in rock pigeon and Egyptian laughing dove by Madkour and Mohamed (2018), but disagree with Jassim *et al.* (2016) on common morhan in which the mucosa lined by simple columnar epithelium with goblet cells. The attached lamina propria was represented by loose connective tissue contained blood and lymphatic vessels, abundant lymphatic infiltration, lymphatic nodule, and represents the core of the mucosal folds (Fig.3), this finding was coincidence with previously reported in quail by Ahmed *et al.* (2011), in duck and pigeon by Hassan and Moussa (2012), in Rock pigeon and Egyptian laughing dove by Madkour and Mohamed (2018), indicating of some participation in the immune response Zaheret *et al.* (2012). Also possessed a superficial glands which were a simple branched tubular mucous glands that opened at the bases of the mucosal folds into common lumen of proventriculus by their ducts and lined with simple columnar cells (Fig.3). Similar finding found by Ogukoya and Cook (2009) in Australian passerines, Zaheret *et al.* (2012) in *Coturnix coturnix*, Zhu *et al.* (2015) in Yellow-billed grosbeak, Al-tae (2017) in Brown Falcon *Falco berigora*. Underlying the lamina propria was diffused smooth muscle fibers of

muscularis mucosa which invaginated under mucosa to the submucosa and surrounded the deep proventricular glands which located in submucosa beneath the muscularis mucosa, this finding was parallel with Rodrigues *et al.* (2012) in Blue and Yellow macaws. There was no significant differences in the tunica mucosa thickness, width of mucosal folds, but significantly differ in Mucosal folds length at ($P \leq 0.05$) between two studied birds (Table 1).

Tunica Submucosa

In both studied birds most thickness of proventricular wall was occupied by variable size (small and large) and shaped (conical, pear to round, oval and elongated) of numerous lobule called adenomere that represents the submucosal (deep) glands which arranged in two rows surrounded by perilobular fibrous connective tissue (Fig.4,5), as observed in other avian species as common wood pigeon by AL-Juboory *et al.* (2016), in Rock pigeon and Egyptian laughing dove by Madkour and Mohamed (2018), but differ from that observed in barn owl *Tyto alba* in which the deep glands were arranged in one row AL-Juboory *et al.* (2016), also disagree with King and Mclelland (1984) in chickens and in black-winged by Hamdi *et al.* (2013) pointed to the absence of these glands in submucosa which appeared as a thin loose connective tissue housing fine blood vessels and nerve endings also disagree with Zhu (2015) who reported the submucosa in the proventriculus wall of Black-tailed Crack was absent. Each lobule was composed of glandular tubules which radiating to the periphery of adenomere and lined by intensely eosinophilic simple cuboidal cells with round to oval shape nuclei and the cells were situated at the basal portions were separated apically from each other by a narrow spaces gives a serrated appearance (Fig. 6). Similar histological structure was found in black-winged Kite by Hamdi *et al.* (2013). All these cells were rested on the basement membrane that rested on a fine connective tissue which diffused among the secretory units, each tubule was opened by one duct into central lobule cavity then to the main excretory duct to the proventriculus lumen (Fig.7). The lining epithelium of central cavity and the excretory duct were tall simple columnar cells (Fig.7, 8). This finding was coincidence with Batahet *et al.* (2012) in *Fulica atra*. There was no significant differences between two studied birds in thickness of Submucosa, Length of Submucosal glands, but significantly differ in Diameter of Submucosal glands at ($P \leq 0.05$) (Table 1).

Tunica Muscularis

The muscularis of proventriculus in both studied birds was consisted of a thin inner longitudinally and thick outer circularly arranged layers, thin connective tissue observed between these layers rich in blood vessels (Fig.1B,7A).

Similarly to the current findings by Abumandour (2013) and Madkour and Mohamed (2018) whom observed that muscularis constructed by two layers of smooth muscle fibers in falcon and Rock pigeon and Egyptian laughing dove proventriculus respectively, but disagree with Denbow (2000) on parrot that the outer longitudinal layer was absent, also disagree with Rodrigues *et al.* (2012) on Blue and Yellow macaws; the muscular layer was composed of only circular layer of smooth muscle fibers. There was no significant differences in the tunica Muscularis thickness at ($P \leq 0.05$) between two studied birds (Table 1).

Tunica Serosa

The Serosa in two studied birds was made up of loose connective tissue rich in blood and nerve vessels, adipocytes and covered by mesothelium (Fig.1B,7A), similarly reported by Batahet *et al.* (2012) in *Fulica atrabird* and by Jassim *et al.* (2016) on Moorhen *Gallinula chloropus*. There was no significant difference in the tunica Serosa thickness at ($P \leq 0.05$) between two studied birds (Table 1).

Histochemical Findings

In both studied birds, the surface lining columnar cells of the mucosal folds possessed fine mucin granules (at the supra nuclear area) in these cells which were positively reacted with periodic acid Schiff (PAS) stain, also the luminal borders of the mucosal glands (tubular mucosal glands) were revealed positive reaction for the same technique (Fig.3B) indicating the existence of neutral type of mucin, as observed in Japanese quail by Ahmed *et al.* (2011), Zaher *et al.* (2012), Hamdi *et al.* (2013) in black-winged Kite and Abumandour (2013) in domestic fowl. The submucosal glandular epithelium showed negative reaction toward this procedure, while the cells that lined the luminal borders of the submucosal glands and their ducts gave a positive reaction on the apical borders of these cells (Fig. 9). Similar finding reported in *Numidameleagris* by Selvan *et al.* (2008), while AL-Samawy (2015) reported that the secretory cells of submucosal glands give a negative reaction, while the cells lined the ducts of submucosal glands give a positive reaction in their apical ends towards the same stain.

On employing PAS-AB (PH 2.5) stain, the surface lining epithelial cells of mucosal folds and the tubular mucosal glands give a positive reaction for PAS part of this technique (Fig.10), indicating the presence of neutral mucopolysaccharides, disagree with the finding of Hamdi *et al.* (2013) in black-winged kite and Selvan *et al.* The presence of both types of mucins (neutral and acidic) will aid to protect the mucosal surface from the hydrochloric acid effect and form a mucosal barrier (Hamdi *et al.*, 2013). The secretory cells of the submucosal glands revealed negative reaction for the same stain, while the columnar epithelial cells lined the luminal adenomers, collecting and excretory ducts of submucosal glands showed PAS part positive reaction with this stain.

Table 1: Comparison between Guinea Fowl and Goose

Parameters	Mean \pm SE		T-test and Sig.
	Guinea Fowl	Goose	
Thickness of Mucosa	956.00 \pm 16.91	904.00 \pm 50.65	123.15 NS
Length of Mucosal Folds	484.00 \pm 9.27	446.00 \pm 20.39	31.667 *
Width of Mucosal Folds	107.00 \pm 2.89	102.00 \pm 2.00	8.120 NS
Thickness of Submucosa	2180.00 \pm 51.47	2320.00 \pm 84.55	228.28 NS
Length of Submucosal glands	1468.00 \pm 138.25	1670.00 \pm 87.41	377.19 NS

Diameter of Submucosal glands	920.00 ± 37.41	1216.00 ± 73.86	190.94 *
Thickness of Muscularis	456.00 ± 6.78	454.00 ± 15.68	39.405 NS
Thickness of Serosa	48.00 ± 2.00	44.00 ± 2.45	7.292 NS
* (P≤0.05), NS: Non-Significant.			

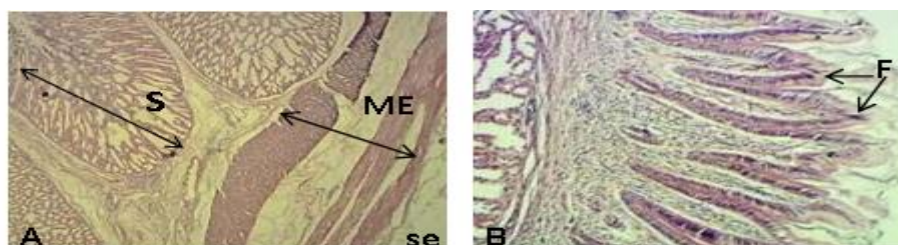


Fig.1- photomicrograph of the proventricular wall showed: Mucosa (M), Submucosa (S), Muscularis externa (Me), Serosa (Se), Mucosal Folds (F). A - 4X H&E stain in male Guinea fowl, B - 10X H&E Stain in Male Goose.

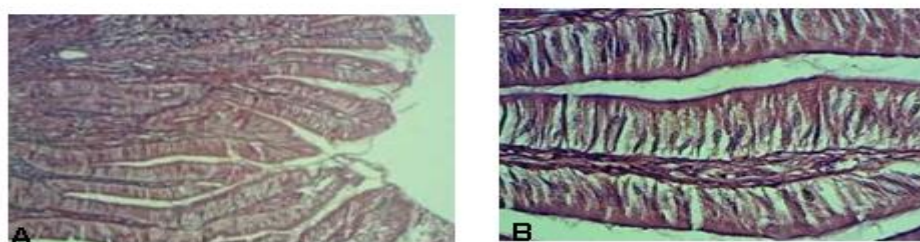


Fig.2: photomicrograph of the proventriculus in Male Fowl showed: Mucosal Folds (F), Epithelium; Simple columnar epithelium (E). 40X H&E Stain B - 40X H&E Stain.

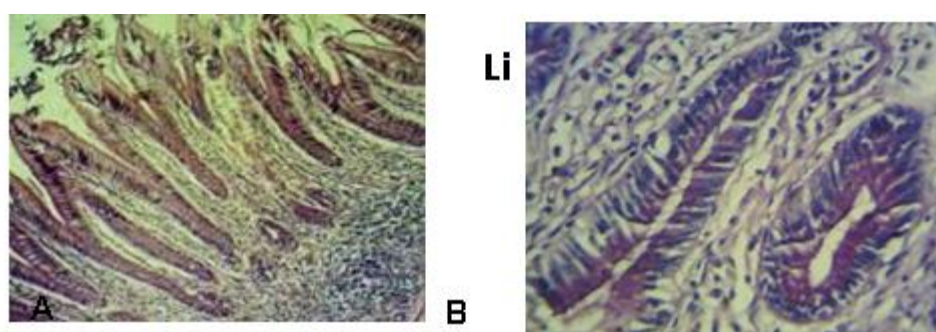


Fig.3: photomicrograph of the proventriculus in Male Goose showed: Mucosal Folds (F), Mucosal glands (black arrow), Lamina propria, Lymph Node (L), Lymphatic infiltration (Li). A - 10X H&E Stain. B - 10X H&E Stain.

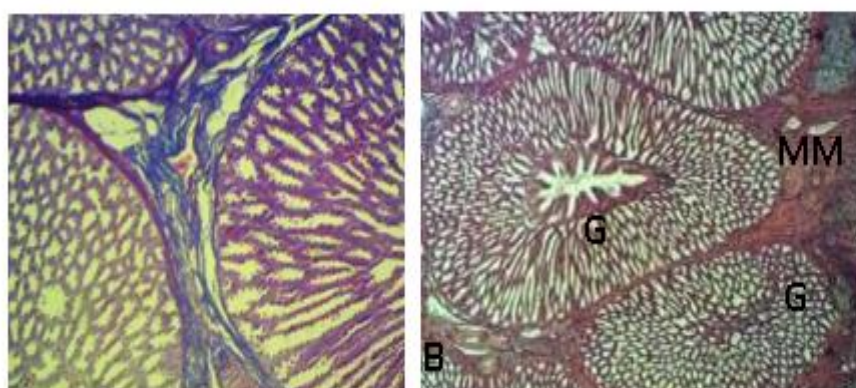


Fig.5: photomicrograph of the proventriculus showed: Different shapes and size of Sub mucosal glands(G), perlobular connective tissue(black arrow), Muscularis mucosa(Mm), A -10X Masson Trichrom, B - 4X H&E Stain in Male Guinea Fowl.

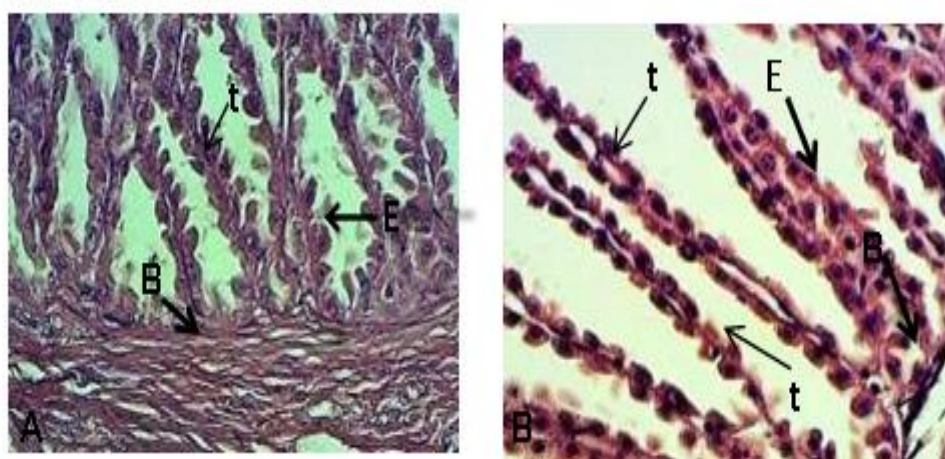


Fig: 6 photomicrograph of the proventriculus showed: Glandular tubule of (t), simple cuboidal epithelium(E), Basement membrane(B), 40x H&E stain in male guinea fowl.



Fig.7: photomicrograph of the proventriculus showed: Muscularis externa (ME), Serosa (S), Central cavity of submucosal glands (C), Main duct of excretory duct (M), Mucosal Folds (F). 4X H&E stain in Male Guinea Fowl, 4X H&E in Male Goose

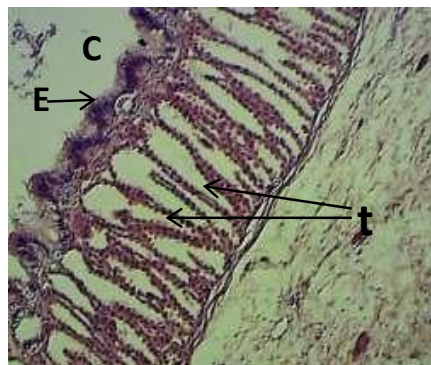


Fig.8: photomicrograph of the proventriculus in Goose showed:

Central cavity of submucosal glands (C), Epithelium (E), Glandular tubules (t), 10X H&E Stain.

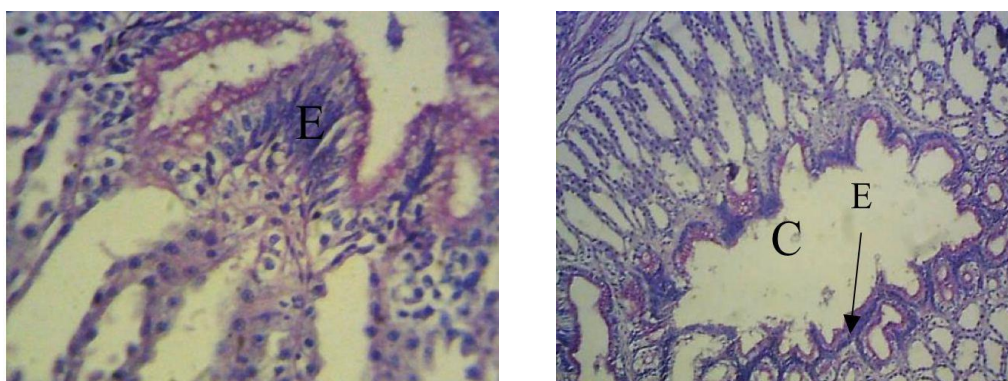


Fig.9: photomicrograph of the Proventriculus showed: Epithelium (E), Central cavity of submucosal glands (C) A- 40X PAS stain in Male Guinea Fowl.

B-: 10X PAS Stain in Male Goose.



Fig.10: photomicrograph of the proventriculus showed

Mucosal Folds(F), Epithelium (E), mucosal gland (black arrow)10X PAS-AB in Guinea fowl.

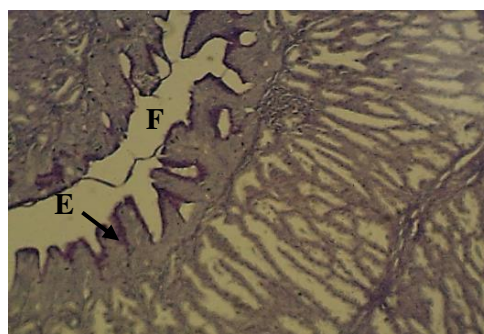


Fig.11: photomicrograph of the proventriculus

inMale showed: Mucosal Folds (F Epithelium(E), Combined PAS-AB stain 4 X

Conclusions:

This study concluded that generally the histological structure of proventriculus in studied birds were similar, similar to that of many birds, and both proventriculus wall composed of four tunicae, but with some morphometrical differences in its wall. The histochemical findings in both studied birds proventriculus showed the surface lining cells of mucosal folds, tubular mucosal glands and cells lining the luminal borders of submucosal glands and their ducts secrete a neutral mucin.

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