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Comparative Histological Studies on the Stomach of Two Avian Species (*Lonchura oryzivora and Upupa epops*)

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Abstract

Java Sparrow (Lonchura oryzivora) and Hoopoe (Upupa epops) were different avian species in their lifestyle. Java Sparrow feeds on seeds particularly rice whereas Hoopoe feeds on insects. The present investigation was designed to characterize the histological construction of the stomach in the two species. Seven Java Sparrows and seven Hoopoes were carefully dissected. The stomach was separated from each bird and fixed in 10% buffered formalin. Several procedures were done to prepare the histological sections and stained with different stains to illustrate the stomach structure by using light microscope. The stomach of the two species was constructed from two parts; periventricular and ventricular (glandular and muscular). The wall of each part in the two species was histologically characterized by four layers; serosal l. (the outermost layer), muscularis l., submucosal l. and mucosal l. (the innermost one). Submucosal layer was characterized by greenish color with Masson's stain due to its contents of connective tissue. In proventricular part of the stomach, the submoucosal layer was occupied with deep glands. These deep glands consisted of circular lobules in Lonchura oryzivora and oval lobules in Upupa epops. These glands contained neutral "mucopolysaccharides" in the two species. Also, many superficial glands were composed of compound tubules. The mucosal secretions were acid and neutral "mucopolysaccharides in Lonchura oryzivora whereas they were acid "mucopolysaccharides" in Upupa epops. In the two species, the mucosal layer of ventriculus were characterized by tubular glands with mixture of acid and neutral muco-secretions. Keratinous layer was observed in the lining of the ventricular part and termed as cutica gastrica. The thickness of cutica gastrica in Lonchura oryzivora was greater than that in Upupa epops. The observed results in this work were indicated that the nature of the bird's living and the type of its feeding may lead to some differences in the histological structure of its stomach.

Key Words: Bird, Stomach, Histological, Lonchura oryzivora, Upupa epops.

I. INTRODUCTION

In most birds, the typical structure of the digestive system was similar. However, there were certain variations related to bird lifestyle and feeding habit. Avian stomach is considered as essential organ of the GIT. It is worth mentioned that, the histology of the avian stomach was described by many investigators such as **Ahmed *et al.*, (2011), Zhu *et al.*, (2013), Jassem *et al.*, (2016), Beheiry (2018), and AL-Taai &**

Hasan (2020). Many authors such as **Al- Saffar & Eyhab (2014), Jassem *et al.*, (2016), Taki-El-Deen (2017), Saran *et al.*, (2019)** stated that the Avian's stomach has two elements; glandular (proventricular element) which filled with glandular juices and muscular (ventricular element or gizzard) which has mechanical role in food digestion.

The histological structure of gastric wall is characterized by four layer. The exterior one is serosal layer and followed by muscularis, submucosa and mucosa **(Said *et al.*, 2012 and Al- Abdul-Ridha *et al.*, 2019).** The muscle fibers (longitudinal and circular) were arranged in different pattern in the muscularis layer **(Saran & Meshram, 2021)**. Submucosa was characterized by connective tissue and deep proventricular glands. These glands were composed of glandular lobules that arranged radically and muco-secretions were produced from their lobules **(Beheiry, 2018 and Abdul-Ridha *et al.*, 2019).** The mucosal lining was formed from columnar or cubic epithelia **(Selvan *et al.*, (2008)**. **Taki-El-Deen (2017)** found that the ventricular wall was characterized by the same four layers that were found in proventriculus. **Hamdi *et al.*, 2013** stated that the mucosal layer characterized by tubular glands. Their secretions were acid and neutral in Japanese quail **(Ahmed *et al.*, 2011)**. In some bird, such as pheasant, the ventricular lumen was in contact with special keratinous layer that lined the mucosa **(Parisa *et al.*, 2019).** Whereas, this layer was absent in *Coturnix coturnix* **(Zaher *et al.*, (2012)**. **Ahmed *et al.*, (2011)** mentioned the protection role of the keratinous layer in the ventricular part of the stomach.

Lonchura oryzivora (Java Sparrow or Java rice Sparrow) feeds on seeds (mainly rice). *Upupa epops* (Hoopoe) feeds mainly on insects. Therefore, this study focuses on the histological structures of the stomach in the two species and clarify the similarities and differences between them for supporting the understanding about their feeding habits.

II. MATERIAL AND METHODS

The experimental animals

Seven adult birds of Java Sparrow and Hoopoe were obtained from ornamental shops and Abo-Rawash Constituency respectively. All birds were in good health. They represent two different orders in phylum aves. The systematic position according to Linnaeus (1758) main food of each bird are illustrated in the following table:

	Hoopoe	Java Sparrow
Order	Passeriformes	Bucerotiformes
Family	Upupidae	Estrildidae
Genus	*Upupa*	*Lonchura*
Species	*epops*	*orizyvora*
Main food	insects	seeds

Histological preparations

After dissection of birds, samples of proventriculus and ventriculus of each bird were fixed in 10 % concentration of neutral formalin and kept for one day. After that, all specimens were passed into ascending concentrations of ethanol for dehydration process. The terpineol was used in clearing process and the paraffin wax was used in embedding process. Sections thickness were prepared from 4 to 6 µm for the staining by H&E to demonstrate the usual histological structure (Bancroft and Gamble, 2002). Masson's trichrome stain was used to clarify the connective tissue (Mahoney, 1973) and Alcian PAS was used to

demonstrate the "mucopolysaccharides" contents (Hotchkiss, 1948).

III. RESULTS

The stomach of Java Sparrow, *Lonchura oryzivora*

Histologically, the stomach wall was composed of four layers; serosa (the outermost layer), muscularis, submucosa and the innermost layer which was called the mucosa. The serosa was characterized by squamous epithelium. There was a thin musculature in the cardiac portion. It was formed from two types of muscle fibers; the outer one was circular and the inner one was longitudinal (Fig.1). Thin connective tissue was observed in the submucosal layer. It was stained with green color by Masson's stain (Fig.2). Two types of gastric glands were found in mucosal layer. They were differentiated into deep and superficial (. The deep glands were constructed from circular lobes that separated from each other by a septum of connective tissue (Fig.1). Also, each lobe was lined with cubic cells. Superficial glands were tubular and composed of columnar cells (Fig. 2). The muco-secretions of deep glands were neutral whereas they were acid and neutral in the superficial glands (Fig.3&4). The ventricular wall was consisted of the four layers. Serosa was made up of simple squamous epithelia. The muscularis was differentiated into thick circular fiber layer (outward) and longitudinal fiber layer (inward) (Fig.5). The Mucosal layer was characterized by many tubular glands that made up of columnar epithelia (Fig.6). Secretions of these glands was acid and neutral "mucopolysaccharides". Thick keratinous layer was found in contact with ventricular lumen (Fig.5). This layer that termed as cutica gastrica was characterized by acid and neutral secretions (Fig.7).

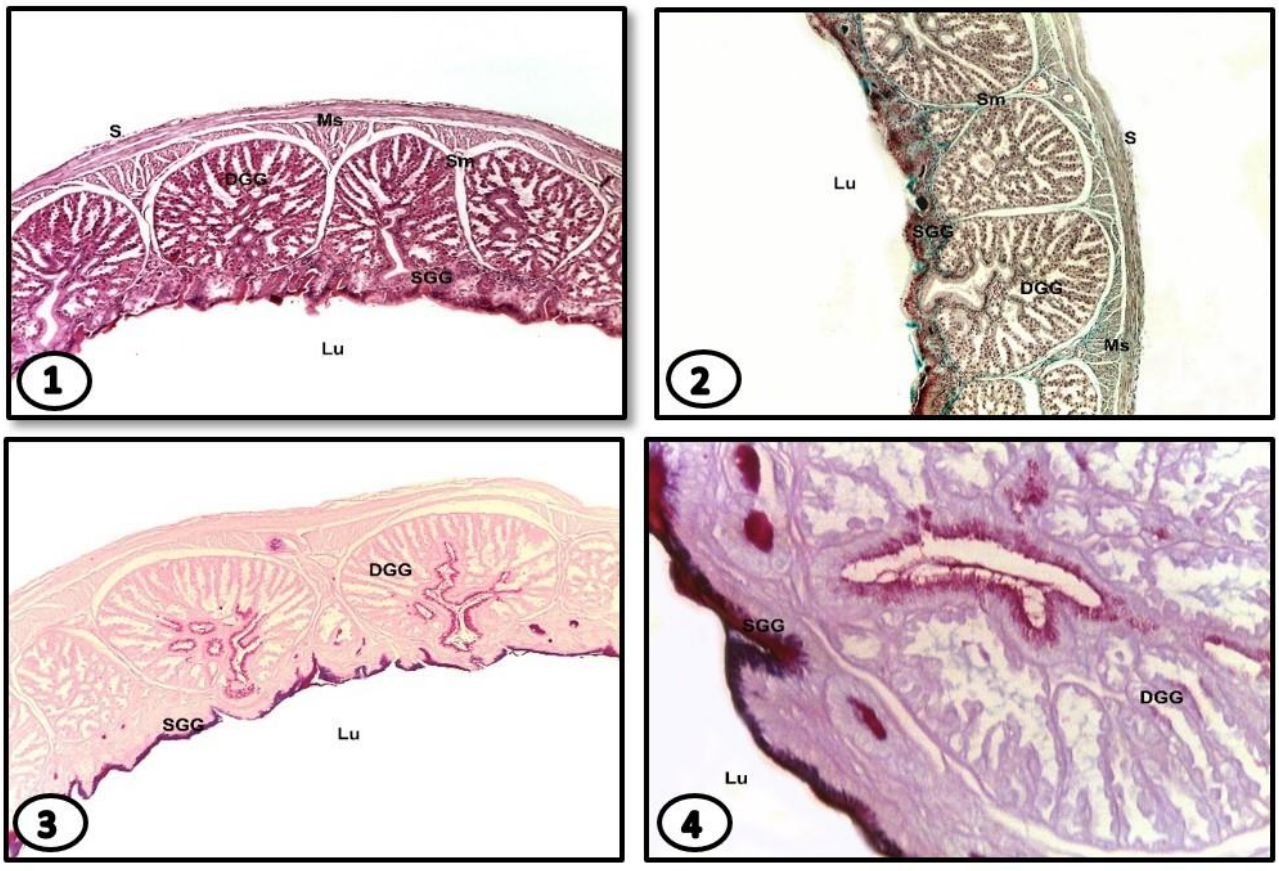

Fig.(1): Light photomicrograph of *Lonchura oryzivora*'s proventriculus showing: Serosa (S), Muscularis (Ms), Submucosa (Sm), Deep Gastric Gland (DGG), Superficial Gastric Gland (SGG) and Lumen (Lu). (H&E. stain X. 100).

Fig.(2): Light photomicrograph of a transverse section of *Lonchura oryzivora*'s proventriculus showing: Serosa (S), Muscularis (Ms), Submucosa (Sm), Deep Gastric Gland (DGG), Superficial Gastric Gland (SGG) and Lumen (Lu). (Masson's trichrome stain X.100).

Fig.(3): Light photomicrograph of *Lonchura oryzivora*'s proventriculus showing: Deep Gastric Gland (DGG), Superficial Gastric Gland (SGG) and Lumen (Lu). (Alcian PAS. stain X.100).

Fig.(4): Light photomicrograph of *Lonchura oryzivora*'s proventriculus showing: Deep Gastric Gland (DGG), Superficial Gastric Gland (SGG) and Lumen (Lu). (Alcian PAS. stain X.400).

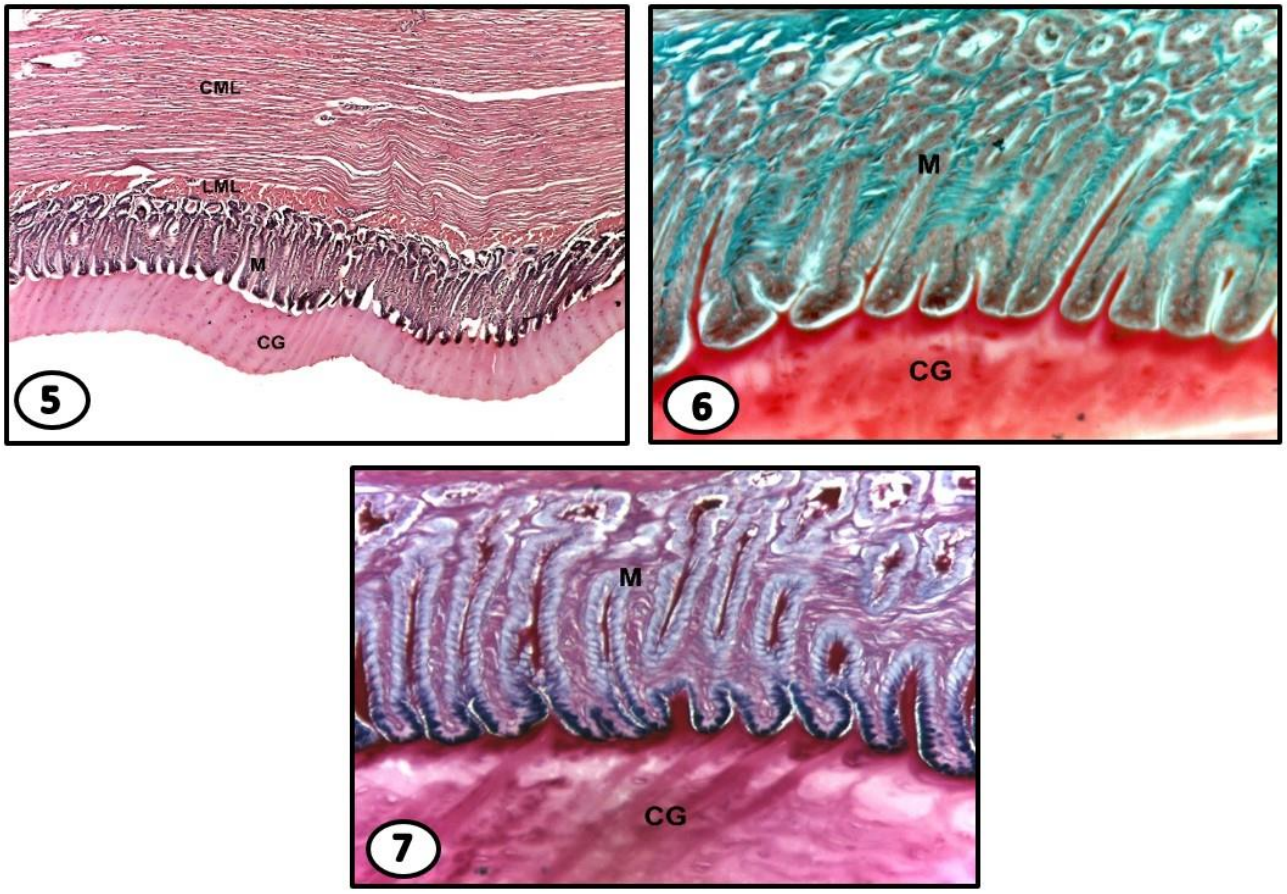

Fig.(5): Light photomicrograph of *Lonchura oryzivora*'s ventriculus showing: Circular Muscle Layer (CML), Longitudinal Muscle Layer (LML), Mucosa (M) and Cutica Gastrica (CG). (H&E. stain X. 100).

Fig.(6): Light photomicrograph of a transverse section of *Lonchura oryzivora*'s ventriculus showing: Mucosa (M) and Cutica Gastrica (CG). (Masson's trichrome stain X.400).

Fig.(7): Light photomicrograph of *Lonchura oryzivora*'s ventriculus showing: Mucosa (M) and Cutica Gastrica (CG). (Alcian PAS. stain X.400).

The stomach of Hoopoe, *Upupa epops*

Histologically, the proventricular wall was constructed from serosa, musculature layer, submucosa and mucosal layer. The squamous epithelium was well observed in the serosal layer. Thin outer circular and thin inner longitudinal layers were found in the muscularis (Fig.8). The submucosa was represented by connective tissue which identified by its green color with Masson's stain. Well represented deep gastric glands were distributed in submucosal layer (Figs. 8&9). The glandular lobules had oval appearance (Figs.8&10). Superficial gastric glands were found in the mucosal layer and formed from many tubules arranged in folds (Fig.10). The muco-secretions of deep gastric glands were neutral whereas, the superficial gastric glands had acid muco-secretions (Fig.11). The ventricular wall lined externally by serosal layer that characterized by simple squamous epithelia. The musculature consisted of thick outer circular fibers and thick inner longitudinal fibers (Fig.12). The submucosa was characterized by connective tissue that colored green with Masson's stain (Fig.13). The mucosal layer characterized by many tubular glands that made up of columnar epithelia. The nature of these glands was acid and neutral "mucopolysaccharides". Thin keratinized layer (cutica gastrica) was found in contact with lumen. This layer had acid and neutral secretions (Fig.14).

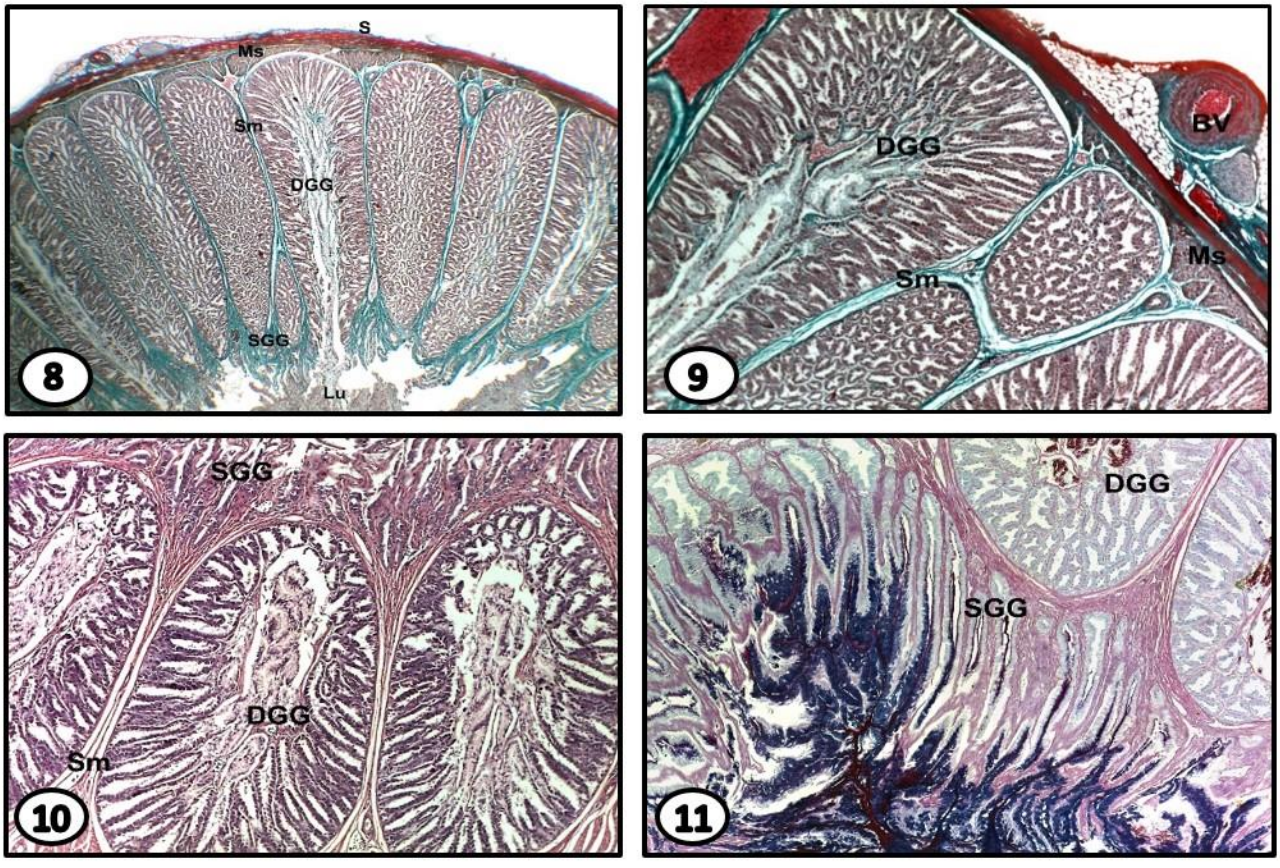

Fig.(8): Light photomicrograph of *Upupa epops*'s proventriculus showing: Serosa (S), Muscularis (Ms), Mucosa (M), Submucosa (Sm), Deep Gastric Gland (DGG), Superficial Gastric Gland (SGG) and Lumen (Lu). (Masson's trichrome stain X.40).

Fig.(9): Light photomicrograph of *Upupa epops*'s proventriculus showing: Blood Vessel (BV), Muscularis

(Ms), Submucosa (Sm) and Deep Gastric Gland. (Masson's trichrome stain X.100).

Fig.(10): Light photomicrograph of *Upupa epops*'s proventriculus showing: Submucosa (Sm), Deep Gastric Gland and Superficial Gastric Gland (SGG). (H&E. stain X. 100).

Fig.(11): Light photomicrograph of a transverse section of *Upupa epops*'s proventriculus showing: Deep Gastric Gland (DGG) and Superficial Gastric Gland (SGG). (Alcian PAS. stain X.400).

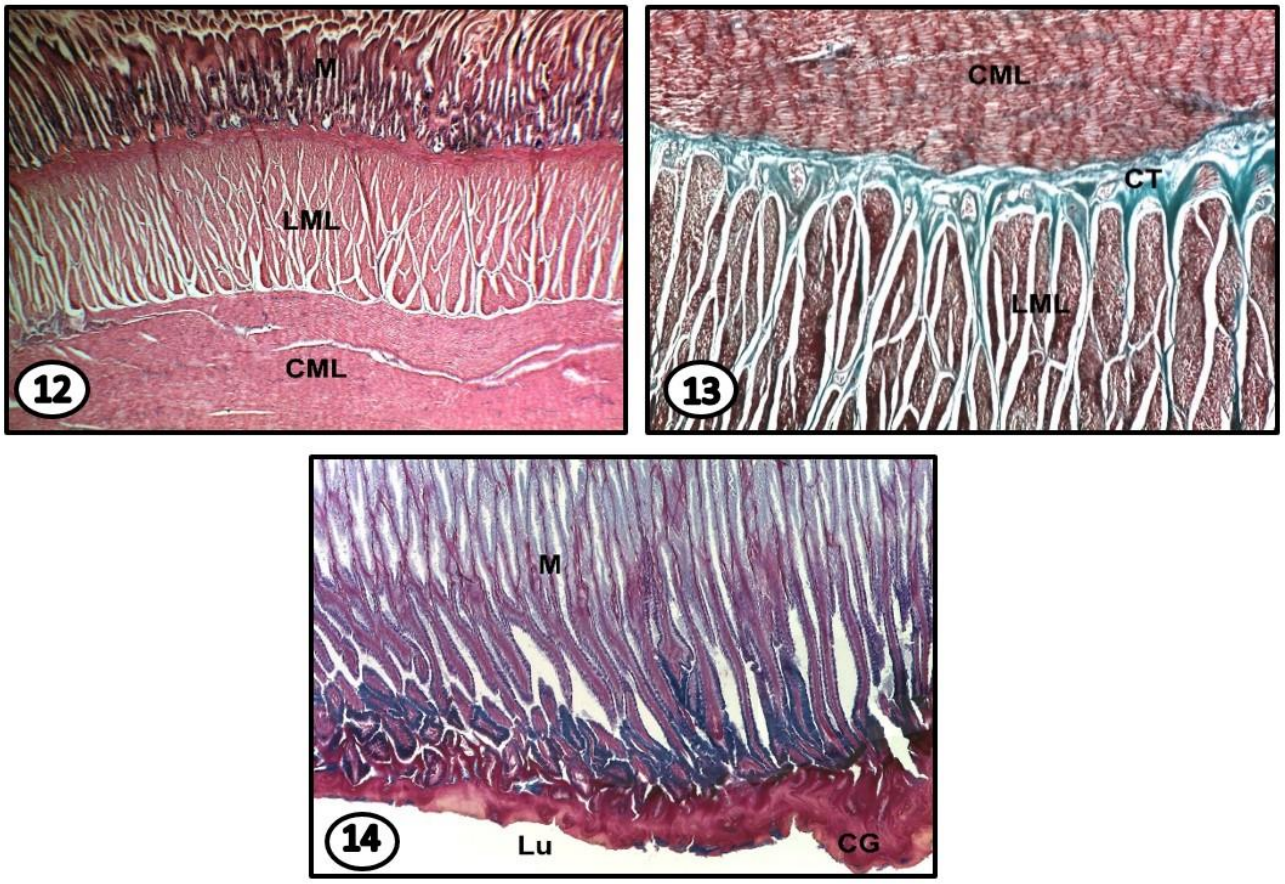

Fig.(12): Light photomicrograph of *Upupa epops*'s ventriculus showing: Circular Muscle Layer (CML), Longitudinal Muscle Layer (LML) and Mucosa (M). (H&E. stain X. 40).

Fig.(13): Light photomicrograph of a transverse section of *Upupa epops*'s ventriculus showing: Circular Muscle Layer (CML), Connective Tissue (CT) and Longitudinal Muscle Layer (LML). (Masson's trichrome stain X.100).

Fig.(14): Light photomicrograph of *Upupa epops*'s ventriculus showing: Mucosa (M), Cutica Gastrica (CG) and Lumen (Lu). (Alcian PAS. stain X.100).

IV. DISCUSSION

Histologically, the stomach wall in *Lonchura oryzivora*'s & *Upupa epops* was composed of four layers; serosa (the outermost layer), muscularis, submucosa and the innermost layer which was termed as the mucosa. These results were supporting the observations obtained by **Saffar *et al.*, (2015)** and **Saran & Meshram (2021)**. Whereas, **Zhu *et al.*, (2013)** found only three layers in "Yellow billed grosbeak" *viz* the

submucosal layer was not recorded in the wall structure.

Serosal layer had connective tissue that rich in vasculature and nerves. This result was found by **Rocha & Lima (1998)** in burrowing owl, **Ahmed *et al.*, (2011)** in Japanese quail, Beheiry (2018) in Turkey, **Kausar *et al.*, (2019)** in Domestic Pigeon and **Saran & Meshram (2021)** in Guinea fowl. Muscularis Layer in *Lonchura oryzivora* & *Upupa epops* composed of exterior circular and interior longitudinal muscle fibers. Same finding was noted by **Saran & Meshram (2021)** in Guinea fowl. Whereas, conversely arrangement of the two layers *viz*, exterior longitudinal and interior circular muscle fibers was observed by **Kausar *et al.*, (2019)**, **Parisa *et al.*, (2019)**. Furthermore, only single layer was observed by **Rodrigues *et al.*, (2012)** in " macaw".

The submucosal layer was signified by connective tissue that stained with green color with Masson's stain. Also, **Jassem *et al.*, (2016)** and **Saran & Meshram (2021)** were described the same structure in submucosa of Common moorhen and Guinea fowl respectively. Deep proventricular glands were observed inside submucosa of the two species under the current investigation. Same results were observed by **Beheiry (2018)**. The glandular lobules were oval in *Upupa epops* and circular in *Lonchura oryzivora*. Whereas, **Abdul-Ridha *et al.*, (2019)** found that each glandular lobule has pear-shaped structure. The glandular lobules in *Lonchura oryzivora* & *Upupa epops* were arranged in radial columns and all lobules were leading to a central cavity or lumen. Similar results were shown in Turkey by **Beheiry (2018)** and pheasant by **Parisa *et al.*, (2019*)*** respectively. Secretions of deep proventricular glands of both birds under investigation were neutral "mucopolysaccharides". Similar result was recorded by **Beheiry (2018)**.

Superficial proventricular glands of both birds under investigation were consisted of compound tubules. The mucosal folds were formed from columnar cells with basal nuclei. This finding was noted in Guinea fowl by **Selvan *et al.*, (2008)** and in adult starling bird by **AL-Taai & Hasan (2020)**. Whereas, **Batah *et al.*, (2012)** and **Al-Saffar & Al-Samawy (2015)** were found that the lining was constructed from simple cubic cells. The nature of mucosal secretions was acid "mucopolysaccharides" in *Upupa epops*. But they were acid in addition to neutral "mucopolysaccharides" in *Lonchura oryzivora*. **Ahmed *et al.*, (2011)** and **Zhu (2015)** were recorded acid and neutral "mucopolysaccharide" secretions in Japanese quail and Grey-Backed Shrike respectively. **Mohammadpour & Sobhani (2016)** stated the protection role of gastric secretions against any dangerous outcome from mechanical action of hard particles inside stomach, microorganisms and enzymes.

The ventricular wall in *Lonchura oryzivora* & *Upupa epops* was consisted of the same four layers which found in proventricular wall. Serosal layer was made up of simple squamous epithelia. The ventricular musculature was differentiated into two layers *viz*, circular fibers (outward) and longitudinal fibers (inward). This result agreed with **Abdul-Ridha *et al.*, (2019)** in domestic pigeon and domestic quail. **Taki-El-Deen (2017)** noted that the circular fibers were located internally and the longitudinal fibers were located externally in Duck & Whimbrel. Whereas, the muscular layer in pheasant was composed of internal oblique fibers and external circular fibers **(Parisa *et al.*, 2019)**.

The ventricular mucosal layer in *Lonchura oryzivora* & *Upupa epops* was characterized by many tubular glands made up of columnar epithelia. Similar result was found in *Elanus caeruleus* **(Hamdi *et al.*, 2013)**. Secretions of these glands in both *Lonchura oryzivora* & *Upupa epops* were acid and neutral "mucopolysaccharides". This result was noted by **Ahmed *et al.*, (2011**) in Japanese quail. keratinous layer was found in contact with ventricular lumen in both *Upupa epops* and *Lonchura oryzivora* and termed as

cutica gastrica. This layer was observed by **AL-Saffar& Eyhab (2014)** in mallard and by **Parisa *et al.*, (2019)** in pheasant. Whereas, this layer was lacked in "Scope Owl" (**Zaher *et al.*, 2012)**. Cutica gastrica characterized by acid and neutral secretions in both *Lonchura oryzivora*'s *& Upupa epops*. Whereas, it has neutral mucin contents in pheasant **Parisa *et al.*, (2019). Ahmed *et al.*, (2011)** recorded the protection role of cutica gastrica against the mechanical contractions & lumen contents during digestion.

In conclusion, the histological basic structures in the avian 's stomach such as the main wall layers and gastric glands were clearly observed in *Lonchura oryzivora*'s *& Upupa epops*. However, some differences were found in each species. For instance, the shape of proventricular deep gastric glands was circular in *Lonchura oryzivora*'s and oval in *Upupa epops*. The nature of superficial proventricular glands was neutral in *Lonchura oryzivora*'s and acid in *Upupa epops*. The outer muscle layer of the ventricular part was thick in *Lonchura oryzivora*'s whereas, both layers (outer and inner) were thick in *Upupa epops*. Also, the cutica gastrica in *Lonchura oryzivora*'s was thicker than that in *Upupa epops*. Therefore, the present study revealed that the variations of food nature and habitat of the two species led to some differences in the histological structures of the stomach.

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