

Histological Observation of the Stomach of the Yellow-billed Grosbeak

Observación Histológica del Estómago del Pepitero de Cola Negra

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SUMMARY: The stomachs of the yellow-billed grosbeaks were examined by means of light microscopy. The mucous membrane of the proventriculus presented many folds and sulci. The sulci were lined by simple columnar cells with basal nuclei and clear acidophilic cytoplasm. The proventriculus glands were constituted by many round or elliptical glandular lobules. The sulci and the collecting duct of compound tubular glands showed an intense positive reaction in PAS and AB stain. The gizzard was characterized by an internal abrasion-resistant lining cuticle and a thick muscular layer. The cuticle, gizzard glands and muscular layer in two sides were thicker than those in the cranial and caudal.

KEY WORDS: Yellow-billed Grosbeak; Proventriculus; Gizzard; Histology.

INTRODUCTION

The stomach structure of birds presents variations, which depend on the dietary habits of each species. The knowledge of the morphology of stomach is useful for surgical treatment of pathological conditions and management of birds in captivity. The stomach morphology of fowls, especially in the order of Galliformes, has been described (Selvan *et al.*, 2008; Kadhim *et al.*, 2011), but that of yellow-billed grosbeaks has not been reported. Yellow-billed grosbeak (*Eophona migratoria*) is a small-sized traditional caged bird, which is famous for its attractive sound in China. It belongs to the order Paridae some and the family Fringillidae and inhabits in woods and orchards (Mackinnon & Kweren, 2000). It feeds on the seeds and the berries of plants, while the diet consists of some insects in idiophase. The aim of the present study is to give a detailed description of the histological architecture of stomach in yellow-billed grosbeak.

MATERIAL AND METHOD

Six adult yellow-billed grosbeaks of either sex were used. They were purchased from pet stores and checked

for their health status before being euthanized with ethyl ether. The handle of the specimens was approved by The Animal Experimentation Ethics Committee of Qujing Normal University. For the histological study, fragments of the proventriculus and gizzard were immersed in neutral buffered formalin for 24 hours and then submitted to the dehydration process with alcohol and embedded in paraffin wax. Histological sections of 6-7 mm in thickness were obtained and stained with H-E, periodic acid-Schiff (PAS), Alcian-blue stain (AB) and combination of PAS and AB for identification of neutral and acid mucopolysaccharides. The sections were documented and captured with Olympus microscope. The photographs were analyzed and measured by the Motic Advance Images 3.2 software (China).

RESULT AND DISCUSSION

In all birds, the stomach is anatomically and histologically subdivided into two parts: proventriculus (glandular stomach) and gizzard (muscular stomach). The wall of the proventriculus and gizzard routinely consists

of four layers (Kadhim *et al.*) the mucous membrane (tunica mucosa), the submucosa (tela submucosa), the muscular layer (tunica muscularis) and the serosa (tunica serosa), but this paper has not identified the existence of submucous layer in proventriculus, which coincides to the findings of Guo-Tai (2009) in proventriculus of *Tetraogallus himalayensis*.

The mucous membrane of the proventriculus presented many folds and sulci like red jungle fowl, guinea fowl and red-capped cardinal (Catroxo *et al.*, 1997; Selvan *et al.*; Kadhim *et al.*) (Fig. 1A). The sulci were lined by simple columnar cells with basal nuclei and clear acidophilic cytoplasm. The cells were absent over the folds, while in red jungle fowl the folds were lined by a columnar epithelium (Kadhim *et al.*). The folds, most of which were the mucous papillas, were protruded from the mucous epithelium. The lamina propria consisted of loose connective tissues, with proventriculus glands and lymphocitary infiltration. The proventriculus glands were compound tubular glands, which took up most part of the gastric wall. They were constituted by many round or elliptical glandular lobules. The lobules were composed of numerous collecting tubules drained into one collecting cavity, which opened as a secretory duct in mucous membrane surface. The secretory duct and the smooth muscle fibers of two sides consisted of a mucous papilla in the luminal surface. The glandular cells were low columnar or orbicular-ovate, and the free end of cells did not contact with nearby cells, so the glandular epithelium was dentate.

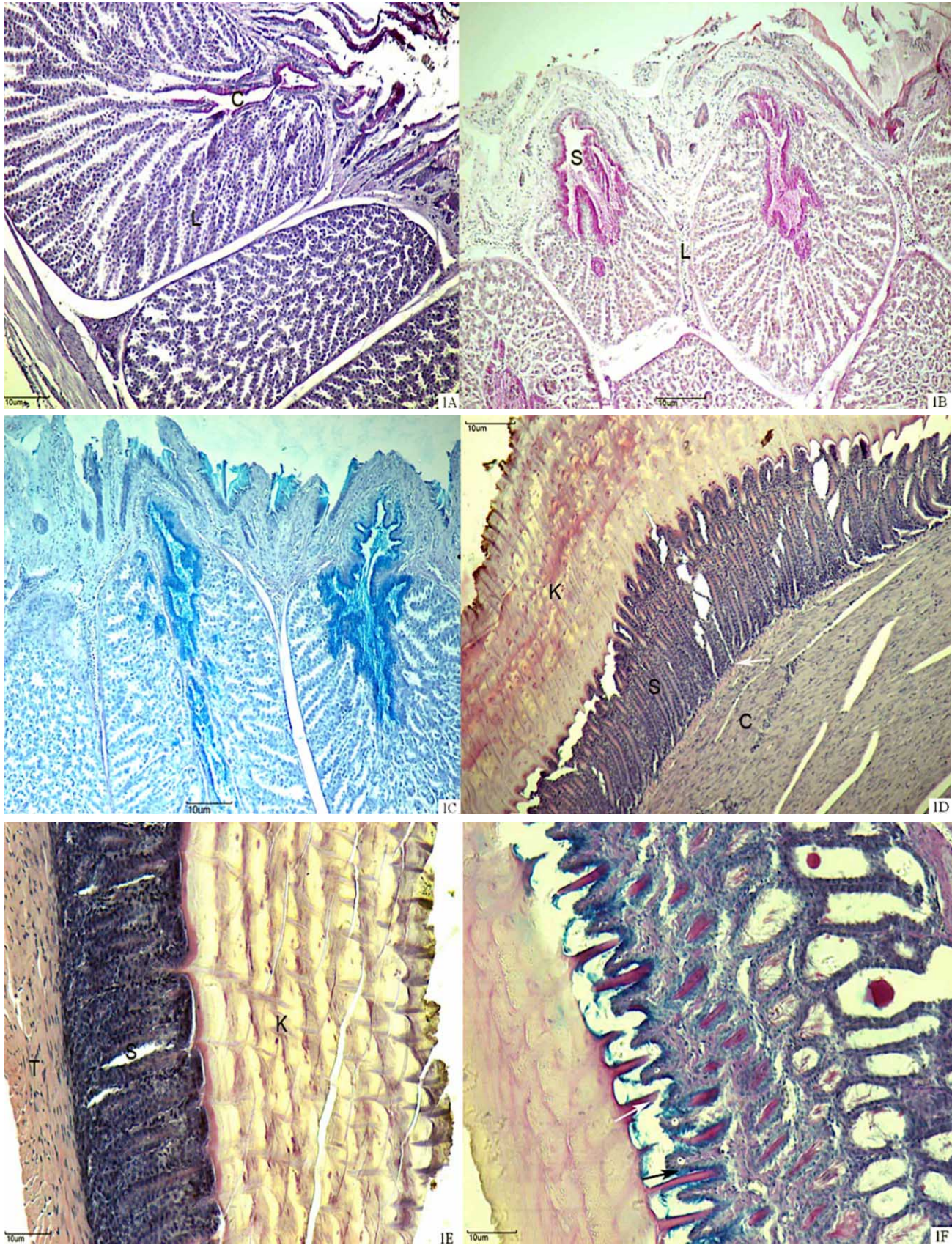
The lamina muscularis mucosae consisted of two layers of longitudinal muscle, divided by the proventriculus glands. The internal layer was located beneath and between mucous papilla, and the external layer was located between the proventriculus glands and the tunica muscularis. With many nodular lymphatic tissues in them, the septa of connective tissues separating the lobules were not well developed. The tunica muscularis consisted of thick interior circular muscle and thin exterior longitudinal muscle. The results were in according with the findings of Kadhim *et al.*, but in contrary with the findings of Rossi *et al.* (2005), and Guo-Tai, who stated that of the tunica muscularis was constituted by inner longitudinal muscle and outer circular muscle. The tunica externa consisted of connective tissues rich in blood vessels.

The mucous cells over the sulci showed a weak intense positive reaction in Pas stain (Fig. 1B), but an intense positive reaction in AB stain (Fig. 1C). The results were in according with the findings of Selvan *et al.*, in

guinea fowl. The neutral and acid mucin formed a resistant mucosal barrier to protect the mucosal epithelium from the acids and enzymes secreted by the glands. The collecting ducts and the lumen showed an intense positive reaction in Pas and AB stain (Figs. 1B and 1C). This revealed that the glands secreted acid and neutral mucin.

The gizzard possessed an internal lining cuticle called koilin (Fig. 1D). The koilin was PAS-positive, which coincided with the reports of Selvan *et al.*, and Kadhim *et al.*, Zhen (1995) stated that the koilin was the compensation of the absence of the teeth. The thickness of the koilin in two sides was 0.33-0.36 mm, but the thickness of the koilin in the cranial and caudal was only 0.25-0.27 mm (Fig. 1E). The koilin was laminated and its surface was dentated. The koilin consisted of two layers in the tunica mucosa: the horizontal matrix and the vertical rods. The horizontal matrix was between the koilin and the surface epithelium. The vertical rods were located in the lumina of the simple tubular glands. The horizontal matrix and the vertical rods were easy to discriminate in combined AB-PAS stain, as the horizontal koilin was AB-positive, while the vertical koilin was PAS-positive (Fig. 1F). These showed that the horizontal matrix were acid and neutral mucopolysaccharide, while the vertical rods was acid. This result was in contrary with the findings of Selvan *et al.*, who stated that the cells lining the surface were also PAS-positive.

The epithelium of the gizzard was lined by simple columnar cells. The surface of the epithelium showed many crypts, and the bases of the crypts were the open of the simple tubular glands. The thickness of simple tubular glands in two sides were thickest, which was 0.25-0.27 mm, especially the opposite areas beneath the folds, but the thickness of simple tubular glands in the cranial and caudal was only 0.1-0.14 mm. This paper has not identified the existence of the lamina muscularis mucosae in the gizzard. This result coincided with the findings of Catroxo *et al.*, and Kadhim *et al.* The submucous layer was a layer of dense connective tissue located between the simple tubular glands and the tunica muscularis. The muscular layer consisted of a variable thickness circular muscle, which attached to the submucous layer. The muscular layer of the *Tetraogallus himalayensis* was constituted by interior circular muscle and exterior longitudinal muscle (Guo-Tai). The muscular layer in two sides was 1.6-1.8 mm thick and was gradually thinning towards the cranial and caudal, while in the cranial and caudal of the gizzard, the muscular layer only consisted of tendinous connective tissues of 0.06 mm in thickness (Fig. 1E). This result was in according with the findings of Rossi *et al.*, who stated the gizzard of the partridge was biconvex.



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RESUMEN: Los estómagos del Pepitero de cola negra fueron examinados mediante microscopía óptica. La membrana mucosa del proventrículo presentó muchos pliegues y surcos. Los surcos estaban revestidos por células columnares simples con núcleos basales y un citoplasma claro acidófilo. Las glándulas proventriculares estaban constituidos por abundantes lóbulos glandulares redondos o elípticos. Los surcos y conductos colectores de las glándulas tubulares compuestas mostraron una reacción positiva e intensa a las tinciones de PAS y AB. La molleja estomacal se caracterizó por una cutícula de revestimiento resistente a la abrasión interna y una capa muscular gruesa. La cutícula, glándulas de la molleja y capa muscular en ambos lados eran más gruesas que en la craneal y caudal.

PALABRAS CLAVE: Pepitero de cola negra; Proventrículo; Molleja; Histología.

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Fig.1. A). A glandular lobule (L) was constituted by numerous secretory tubules drained into one collecting cavity (C). HE Stain. B). The secreted duct (S) of the proventricular glands showed a positive reaction in PAS stain, lymphocitary infiltration (L). C). The collecting duct of the gastric glands and the mucous cells over the sulci showed an intense positive reaction in AB stain. D). The surface of gizzard possessed a rough cuticle (K). Simple tubular gland (S), Circular muscle (C). The submucous layer was a layer of dense connective tissue (white arrow). HE stain. E). In the cranial and caudal of the gizzard, the circular muscle was absent and the muscular layer only consisted of tendinous connective tissues of 0.06 mm in thickness. The thickness of the simple tubular glands(S) and the cuticle (K) were thinnest. F). The horizontal matrix (black arrow) showed PAS positive reaction, but the horizontal matrix (white arrow) and the luminal of the simple tubular gland showed AB positive reaction in Combined AB-PAS stain.