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Histoarchitectural and Histoenzymatic Studies on Gizzard of Guinea Fowl (*Numida meleagris*)

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ABSTRACT

Background: The gizzard is a muscular stomach of the Gastrointestinal tract of bird that holds sparing crystals which aids as the mechanical multure component for food material to be ingested by birds. Its specialty is to grinding of ingested food material after secretion of HCL (Hydrochloric Acid) and pepsinogen enzymes in the proventriculus.

Methods: The twelve samples of gizzard of guinea fowl were fixed in 10% NBF (Neutral buffered formalin) and Bouin's fluid for histological while chilled acetone and chilled ethanol for histochemical studies. The fresh unfixed tissues were used for the cryostat section for the demonstration of different enzymes.

Result: The tubular glands were main feature of mucosal layer but sometime acinar glands also found as the replacement of tubular glands with lymphoid aggregation. The horizontal koilin was also noticed between long mucosal folds. Tunica submucosa was discovered in squeezed manner in comparison to the mucosa. The tunica muscularis has shown inner circular and outer longitudinal as the thick layer. It was intermingling with collagen and elastic fibers. Histochemically the gizzard was examined for glycogen, alkaline phosphatase, acid phosphatase and succinic dehydrogenase.

Key words: Gizzard, Guinea fowl (*Numida meleagris*), Histomorphology, Histochemistry, Non-glandular stomach.

INTRODUCTION

The ventriculus or gizzard, a muscular stomac, is the hind segment of stomach connecting proventriculus to the small intestine. Its specialty is to grind the ingested food material. The muscular thick wall of gizzard holds diminutive crystals which aids as the mechanical crushing component for food material to be digested by birds (Saran *et al*. 2019). The functional stricture of the gizzard is analogous to the teeth in other animals (Fritz *et al.* 2011). It characterizes with the great morphological and functional variability in same and even in different species. The histological and histochemical studies on the gizzard of the Guinea fowl (*Numida meleagris*) might be beneficial to enhance the scientific knowledge about its performance.

MATERIALS AND METHODS

The ventriculus of 12 guinea fowl birds, irrespective of sex were collected at meat shops and carried the same on ice to the laboratory. The study was carried out during the period of 2017 to 2018, in the Department of Veterinary Anatomy, College of Veterinary and Animal Science, Navania, Udaipur (RAJUVAS, Bikaner) Rajasthan.

The collected samples were fixed and processed for haematoxylin and eosin method for histological findings, Weigert's method for elastic and collagen fibers and Van-Gieson method for collagen fibers (Singh and Sulochana, 1996). Gomori's cobalt method was used to ascertain the activity of alkaline phosphatase and acid phosphatase as per (Luna, 1960, Bancroft and Cook, 1994 and Singh and Sulochana, 1996). McManus's method was used for glycogen activity. The Davenport's (1960) method was used for succinic dehydrogenase.

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RESULTS AND DISCUSSION

Histological studies

Gizzard was observed as the spheroid component with four tunics from inside to outside as the tunica mucosa, tunica sub-mucosa, tunica muscularis and tunica serosa. In the luminal surface of mucosal folds, a cuticle layer of koilin was evident. It was a keratinous hard membrane which works as the support system for the grinding of food material in ventriculus. In the region of lamina propria of mucosa, there were long tubular glands that were surrounded by by cuboidal as well as columnal epithelial cells. In between the long mucosal folds, the horizontal koilin was also noticed (Fig 1). Rarely, the tubular glands were replaced by acinar glands. These glands were placed adjoining to koilin membrane at abluminal end (Fig 2).

The substantial horizontal koilin layer was also observed as the surrounding connective tissue in between the acinar glands and lymphoid aggregation. The acinar glands and horizontal koilin were shown their prominancy in absence of lamina propria (Fig 3 and 4).

The tunica submucosa was present in squeezed manner rather than the mucosa, wherein the interwoven collagen fibers were seen. The tunica muscullariswas present as a thin inner circular and a thick outer longitudinal layer. It was intermingling with collagen and elastic fibers. The outermost tunica serosa has shown its existence as the limiting membrane which has bordering adipose connective tissue with blood vessels (Fig 5).

Histochemical studies

The PAS reaction was observed for glycogen at koilin layer, also at the mucosal folds and glandular epithelium (Fig 6). The activity for alkaline phosphatase was detected in the koilin layer and connective tissue septae (Fig 7).

The intensity of acid phosphatase activity was very mild in between the koilin layer and mucosal folds (Fig 8).

The succinic dehydrogenase activity was observed at the koilin layer and also at internal and external tunica muscularis (Fig 9).

The present study in respect of ventricle or gizzard has revealed the long mucosal folds along with the tubular glands in lamina propria. It was surrounded by the mixed epithelium after cuboidal and columnar cells that was in accordance to the reportings revealed by (Rodrigues *et al.* 2012) in Blue and Yellow macaws. The present studies also showed the mucosal low folds which were covered by simple columnar epithelium continuous with lamina propria located tubular mucous glands which was similar to the findings of (Mehra and Kumar, 2020) in Gizzard of the Broiler chicken. The horizontal koilin in between long mucosal folds was also detected with some traces of the koilin cuticle in the crypts of gastric glands. A few acinar glands in mucosal layer of ventriculus at the bordering abluminal end of koilin membrane. The observance of tubular glands in present studies is not being supported by available documentation as various accessible evidential records have identified the ventriculus or gizzard as the muscular stomach only. The encountered alteration might be attributed to the output of higher quantum of secretion required while maintaining softer and workable cuticlar layer of koilin for the grinding of ingested food material. Therefore, pertaining to gizzard and its histomorphological findings it is appropriate to be termed as Glandulo muscular stomach.

A thin interior circular and a thick exterior longitudinal muscle fiber layer were observed as reported by Zhu (2015) in Black-tailed crake (*Porzana bicolor*) and Al-Saffar and Al-Samawy (2015) and in Mallard (*Anas platyrhynchos*). The tunica serosa showed the connective tissue wherein blood vessels were embedded as reported by Al-Saffar and Al-Samawy (2015) in Mallard (*Anas platyrhynchos*).

The PAS activity for glycogen observed at koilin layer, mucosal folds and glandular epithelium were in concurrence

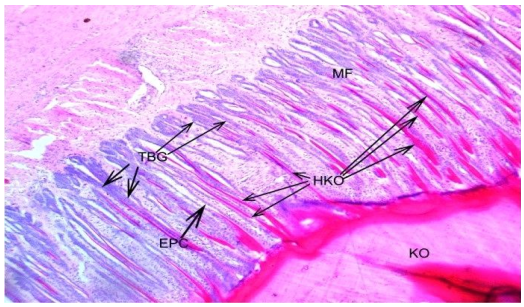

Fig 1: Photomicrograph showing the histological details of gizzard. KO- Koilin Membrane, HKO- Horizontal Koilin, EPC- Epithelial Cells and TBG- Tubular Glands.
Haematoxylin and Eosin 100X.

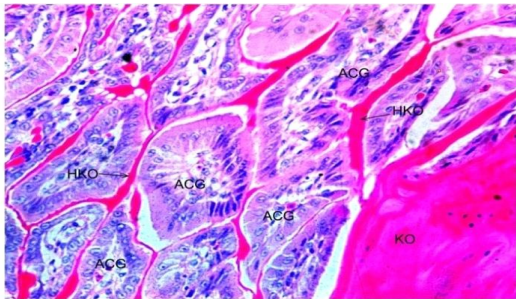

Fig 2: Photomicrograph showing the histological details of gizzard. KO- Koilin Membrane, HKO- Horizontal Koilin and ACG- Acinar Glands.
Haematoxylin and Eosin 400X.

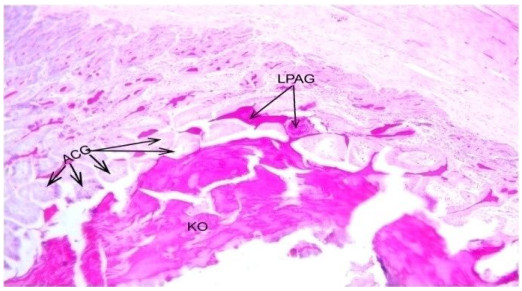

Fig 3: Photomicrograph showing the histological details of gizzard. KO- Koilin Membrane, ACG- Acinar Glands and LPAG- Lymphoid Aggregation.
Haematoxylin and Eosin 100X.

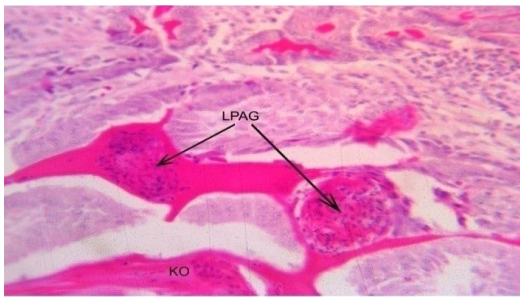

Fig 4: Photomicrograph showing the histological details of gizzard. KO- Koilin Membrane and LPAG- Lymphoid Aggregation.
Haematoxylin and Eosin 400X.

with as reported by Hamdi *et al.* (2013) in black winged kite (*Elanuscaeruleus*), black tailed crake (*Porzana bicolor*) (Zhu, 2015) and cattle egret (*Bubulcus ibis*) (Hussein and Rezk, 2016). These observations by different workers were completely in agreement with the present findings. Positive activity showing components were involved into the optimal function for which glycogens acted as the source of energy. The lymphocytic aggregation and the glycogen may show their presencetogether. However, the glycogen beyond limitation may results the infection which can be arrested by natural defense mechanism after lymphoid aggregation. Hence both were seen as the check and balance mechanism in maintaining normal function of tissue.

The present findings have revealed weak alkaline phosphatase activity at the koilin layer, connective tissue septae of submucosa, mucosal layer and tunica muscularis. Moreover, the very mild activity of acidic phosphatase was noticed in between the koilin layer and mucosal folds. The present findings were in complete consensus with Selvan *et al.* (2008) in post hatched Guinea fowl and Das *et al.* (2017) in Kadaknath fowl as they had negative activity. The lower of alkaline phosphatase and acidic phosphatase activity was there which exhibited the functional superiority of that organ component.

Higher activity of succinic dehydrogenase was observed in the koilin layer and the internal and external tunica muscularis. Contrary, Selvan *et al.* (2008) has noticed negative activity of succinic dehydrogenase in post hatched guinea fowl. The enzyme succinic dehydrogenase functions for mitochondrial cell respiration and thereby the energy generation. Even, the presence of this enzyme work to inhibit the cancerous growth by oxygen modulation. As the gizzard has stronger musculature and grinding koilin membrane it necessitate the oxygen modulation. Exhibition of intense activity of this enzyme at koilin layer and also at internal as well as external tunica muscularis might be considered as the natural but defense mechanism of the bird and the supportiveness towards conclusive findings unearthed by present studies.

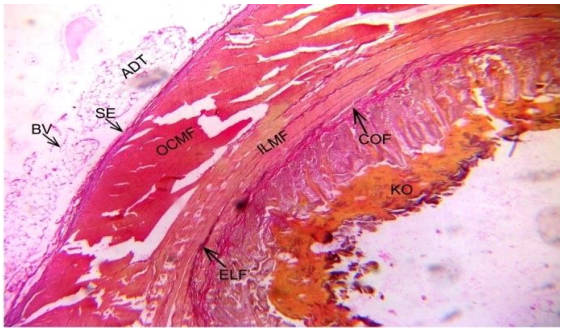

Fig 5: Photomicrograph showing the histological details of gizzard. KO- Koilin Membrane, COF- Collagen Fibers, ELF- Elastic Fibers, ILMF- Inner Longitudinal Muscles Fibers, OCMF- Outer Circular Muscles Fibers, ADT- Adipose Tissue, SE- Serosal Layer and BV- Blood Vessel.
Weigert's Stain 40X.

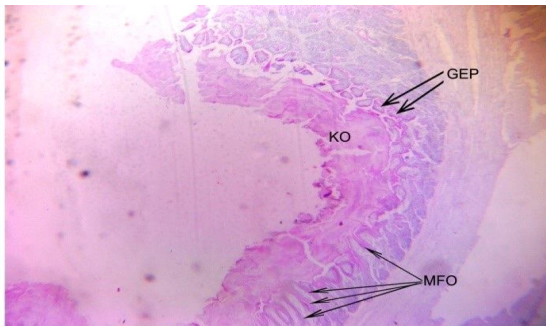

Fig 6: Photomicrograph showing the histological details of gizzard. KO- Koilin Membrane, MFO- Mucosal Folds and GEP- Glandular Epithelium.
PAS 40X.

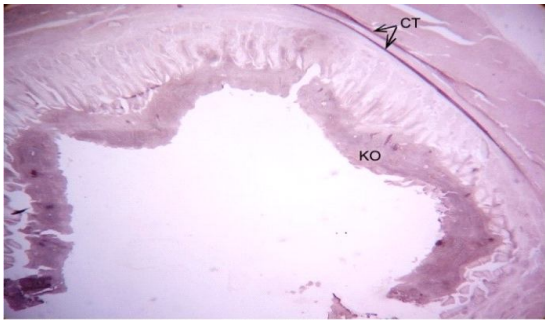

Fig 7: Photomicrograph showing the histological details of gizzard. KO- Koilin Membrane and CT- Connective Tissue.
Alkaline Phosphatase 40X.

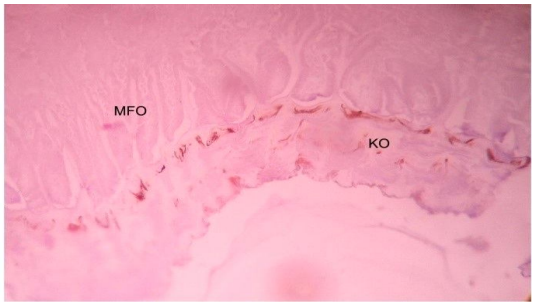

Fig 8: Photomicrograph showing the histological details of gizzard. KO- Koilin Membrane and MFO- Mucosal Folds.
Acidic Phosphatase 100X.

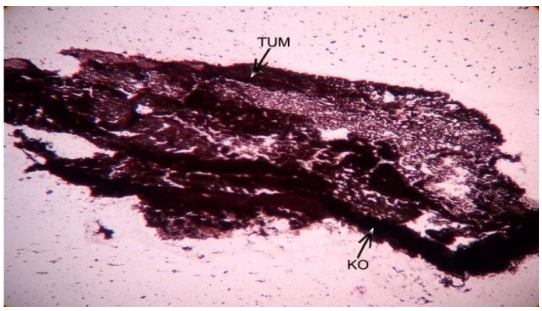

Fig 9: Photomicrograph showing the histological details of gizzard. KO- Koilin Membrane and TUM- Tunica Muscularis.
Succinic Dehydrogenase 40X.

CONCLUSION

The gizzard was a spheroid component with usual four tunics along with I a cuticle layer of koilin, the keratinous hard structure for the grinding of food material. It was appropriately recognized as glandulo muscular stomach after the presence of lamina propria located tubular mucous glands. The lesser intensity of alkaline and acidic phosphatase has exhibited the functional vigour of the organ. The intense activity of succinic dehydrogenase enzyme was observed at koilin and also at internal as well as external tunica muscularis.

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