RESEARCH ARTICLE

Histomorphological Organization of the Thoracic Oesophagus in *Siruvidai* Chicken

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Abstract

The present work represents the structural characteristics of the thoracic part of oesophagus in *Siruvidai* chicken (n=24). Tissue samples of the thoracic oesophagus were collected from four different age groups (2, 6, 12 & 18 weeks old, 6 each) and these samples were subjected to routine histological and histochemical staining to identify cellular architecture and secretory activity. The thoracic oesophageal wall was made up of four tunics. The tunica mucosa had three layers and was thrown into folds. Stratified squamous epithelium forms the lamina epithelial layer. Beneath this, the mucosa was supported by a well defined (space between words) lamina propria and lamina muscularis. Oesophageal mucous glands were present in the lamina proprial layer. The tunica submucosa was primarily composed of loose connective tissue. An inner thick circular and an outer thin longitudinal layer made the tunica muscularis layer. The outermost layer was tunica serosa. The presence of acid and neutral mucins were observed in the oesophageal glands and the proteins were observed in the lamina muscularis and tunica muscularis layers.

Keywords: Histochemistry, Histology, Mucous glands, *Siruvidai* chicken, Thoracic oesophagus.

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Introduction

Poultry industry is one of the leading industries as it is the fast-growing and adds in our economy (Kausar *et al*., 2016). According to 20th Livestock Census, the poultry population in India is 851.81 million. Tamil Nadu ranks first among all states with 120.81 million birds (DAHD, 2025). In Tamil Nadu, *Siruvidai* is an unregistered indigenous chicken ecotype mainly found in Ariyalur district. In recent years among poultry farmers, it has become increasingly popular because of its ability to adapt well to tropical climates, its bright plumage that helps protect it from predators, and its strong mothering instincts (Vasanthi *et al*., 2022; Churchil *et al*., 2023). In domestic fowl, the oesophagus is anatomically divided into a longer cervical part and a shorter thoracic part and functionally, the oesophagus lubricates food and carries it into the digestive system (Hodges, 1974). The present study provides the histological and histochemical details of the thoracic part of oesophagus in *Siruvidai* chicken, contributing to comparative avian anatomy.

Materials and Methods

The research work was carried out after obtaining ethical clearance from the Institutional Animal Ethics Committee (IAEC) under Reference No. 21/SA/IAEC/2024. A total of 24 healthy *Siruvidai* chickens representing four different age groups including 2 weeks, 6 weeks, 12 weeks and 18 weeks were obtained from the Poultry Research Station, Madhavaram Milk Colony, and Chennai (India). Six birds in each group irrespective of sex were utilized for this study in the Department of Veterinary Anatomy, Madras Veterinary College, and Chennai. The thoracic part of the oesophagus were carefully collected and fixed in 10 % neutral buffered formalin for further processing. The fixed tissue materials were dehydrated in graded alcohols, cleared in xylene and were embedded in paraffin wax at 58-60°C. These paraffin blocks were sectioned to 5-6 µm thickness. The slides were prepared and subjected to routine histological processing (Bancroft and Stevens, 1996), followed by specialized histochemical staining methods such as Periodic acid-Schiff's

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(PAS) reaction for neutral polysaccharides, Combined Alcian blue-PAS technique for acid and neutral mucins (Singh and Sulochana, 1996), Modification of Mowry's 1958 colloidal iron method for acid mucopolysaccharides (Luna, 1968) and Mercury - Bromophenol Blue method for proteins (Pearse, 1960) were done. Then detailed microscopic examinations of the prepared slides were carried out.

Results and Discussion

In *Siruvidai* chicken, the oesophagus was present on the right side of neck which was in accordance with the observations of Verma *et al*. (2000) in fowl. The four tunics of thoracic oesophageal wall were composed of tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa in all the studied age groups (Fig. 1, 2). The tunica mucosa consisted of three well-defined layers, namely the lamina epithelium, lamina propria, and lamina muscularis.

The tunica mucosa formed primary folds with small secondary projections. These folds made the luminal border of mucosa to be appeared in regular shape and had wider lumen. On an average, 5-6 primary folds were observed from 2-week-old birds to 18-week-old birds as age increased. Whereas, Aydin *et al.* (2022) in partridges, noticed six to eight folds in their thoracic parts. The primary folds were consisted of lamina epithelium, lamina propria, lamina muscularis and tunica submucosa (Fig. 1). These folds were tall, narrow, and deep, giving more capacity to expand than cervical part. It was similar to the findings of Aydin *et al.* (2022) in Partridges but differed from the findings of Kadhim and Mohamed (2015) in the homing pigeon, who described that the longitudinal folds were deeper in the cervical oesophagus than in the thoracic oesophagus. The secondary projections appeared as conical in shape that composed solely of lamina epithelium. In the thoracic oesophagus, the projection was shorter than the cervical oesophagus.

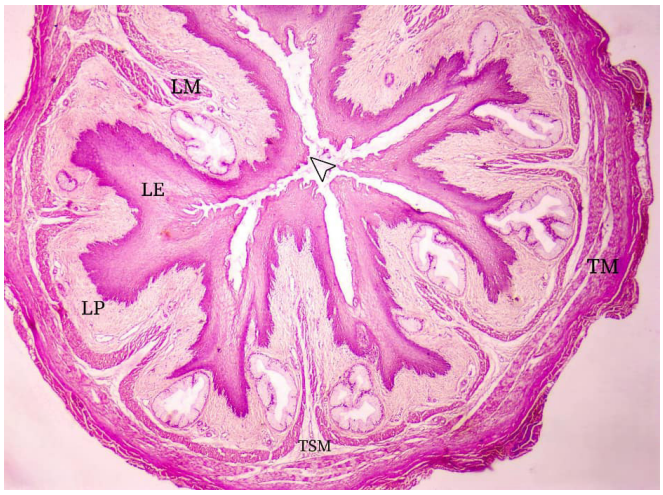


Fig. 1: Photomicrograph of the thoracic oesophagus in 6-week-old *Siruvidai* chicken showing the tunics of the oesophageal wall. LE-Lamina epithelium, LP-Lamina propira, LM-Lamina muscularis, TSM-Tunica submucosa, TM-Tunica muscularis, Arrowhead - Primary fold (H&E x 40).

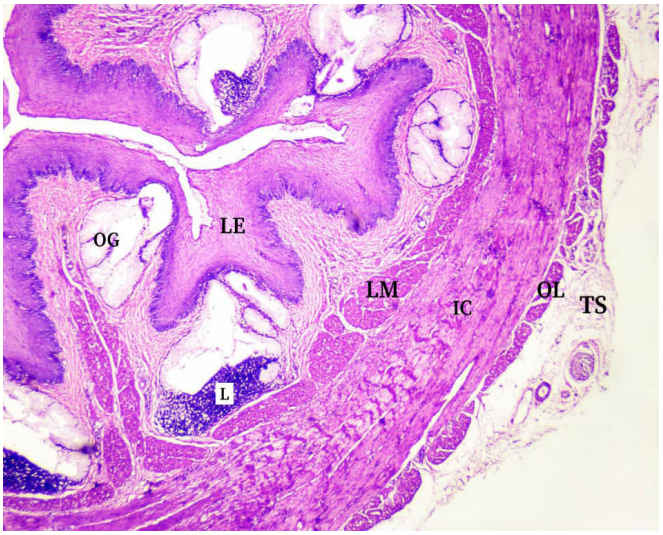


Fig. 2: Photomicrograph of the thoracic oesophagus in 12-week-old *Siruvidai* chicken showing all the tunics of oesophageal wall. LE-Lamina epithelium, OG- Oesophageal gland, L-Lymphocytic infiltration, LM-Lamina muscularis, IC-Inner circular layer, OL-Outer longitudinal layer, TS-Tunica serosa (H&E x 40).

In the present study, it was observed that the lamina epithelia was lined by stratified squamous epithelium with less keratinization (Fig. 3), which was concurrent to the findings of Hodges (1974) in fowl. This epithelial type providing resilience against mechanical stress on the oesophagus. In contrast, the mucosa was formed by a non-keratinized stratified epithelium in emu (Crole, 2009), in broilers (Nasrin *et al*., 2012), in homing pigeon (Kadhim and Mohamed, 2015) and in Japanese quails (Kausar *et al*., 2016). The epithelial base showed short finger-like down growths, which interdigitate with the lamina propria to provide better anchorage to the epithelium. The columnar cells in the basal layer were darkly stained (Fig. 3). The cells above the basal cells possessed eosinophilic granular cytoplasm, centrally placed vacuolated nuclei and a prominent nucleoli. There were pyknotic nuclei and they were degenerated in many cells, and from the topmost layer of the lamina epithelium, the cells got detached as they move towards lumen, which was in agreement with Hodges (1974) in the fowl and Ali *et al*. (2023) in cervical oesophagus of kestrel, pigeon and hoopoe. The sloughed cells seen in the lumen were in accordance with the findings of Hodges (1974) in the fowl.

Beneath this layer, the lamina propria was well demarcated and mainly comprised of loose connective tissue, fibroblasts, blood vessels, lymph vessels, lymphocytic infiltration and oesophageal glands (Fig. 2, 4), which was similar to the observations of Banks (1981) in avian.

Loose connective tissue predominantly consisted of more collagen (Fig. 5). In contrary, Mobini (2014) noted the absence of lymphatic tissue in the lamina propria of broiler chicken. As previously observed, this layer was extending into the projections of the lamina epithelium (Fig. 1).

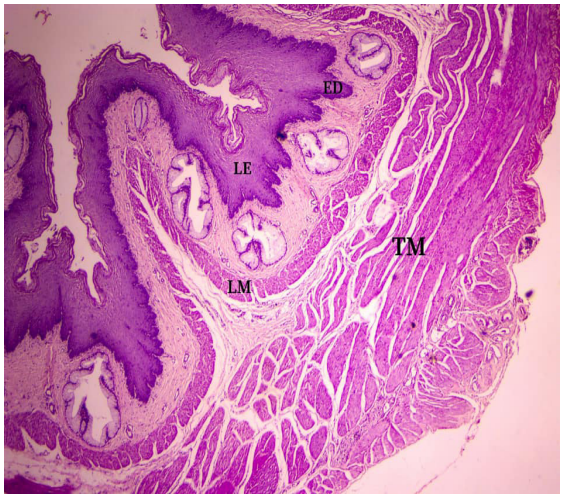


Fig. 3: Photomicrograph of the thoracic oesophagus in 18-week-old *Siruvidai* chicken showing the stratified squamous epithelial lining. LE-Lamina epithelium, ED- Epithelial downgrowth, LM-Lamina muscularis, TM-Tunica muscularis (H&E x 40).

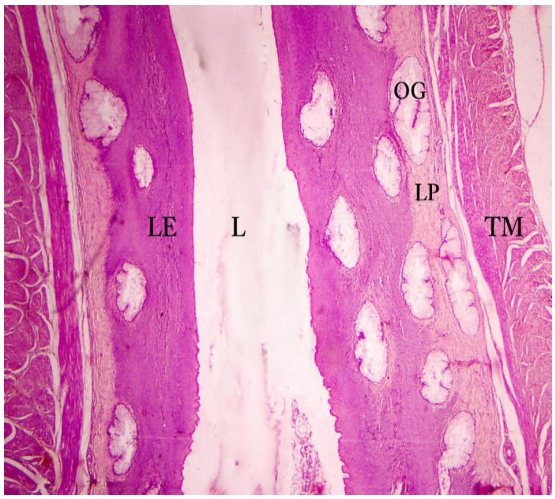


Fig. 4: Photomicrograph of the thoracic oesophagus in two week- old *Siruvidai* chicken showing the oesophageal glands in the lamina propria. LE-Lamina Epithelium, L-Lumen, OG- Oesophageal Gland, LP-Lamina Propria, TM-Tunica Muscularis (H&E x 40).

In *Siruvidai* chicken, the oesophageal glands were present at different levels - top, base and side of folds in the proprial layer. The oesophageal glands were simple branched tubuloalveolar mucous gland (Fig. 6) as mentioned by Banks (1981) in avian. In contrast to the present findings, it was reported in kestrel as simple branched alveolar type (Ali *et al.*, 2023) and in broilers as simple branched tubular mucous glands (Mobini, 2014). The oesophageal glands were small, numerous, less branched in the present findings. It had wide lumens with secretory material in some glands, which was consistent with the findings of Mobini (2014) in broiler chicken. It was observed that an average of 20 number of oesophageal glands were present in one cross section of thoracic oesophagus. The glands were round to cone shaped and made up of 2-5 glandular units (Fig. 1). In contrast, the glands possessed only one or two units in the fowl (Hodges, 1974), two to three units in partridge (Aydin *et al.*, 2022) and seven or more units in broiler chicken (Mobini, 2014). In 2-week-old birds, the oesophageal glands were less in number compared to other age groups.

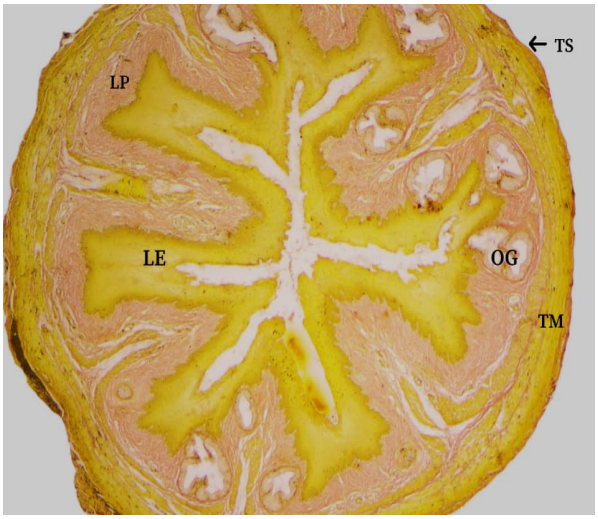


Fig. 5: Photomicrograph of the thoracic oesophagus in 6-week-old *Siruvidai* chicken showing the collagen fibres in lamina propria, tunica submucosa, intermuscular layer and tunica serosa. LE-Lamina epithelium, LP-Lamina propria, OG-Oesophageal gland, TM-Tunica muscularis, TS-Tunica serosa (H&E x 40).

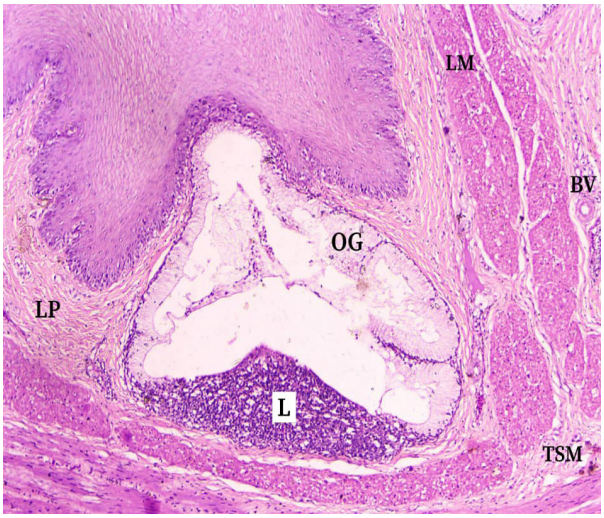


Fig. 6: Photomicrograph of the thoracic oesophagus in eighteen week-old *Siruvidai* chicken showing the oesophageal gland with lymphoid infiltration at its base. LP-Lamina propria, LM-Lamina muscularis, L-Lymphoid infiltration, BV-Blood vessels, TSM-Tunica submucosa, OG-Oesophageal ghland (H&E x 100).

The glands were lined by a single layer of tall columnar cell with elongated nuclei at the base of the cells (Fig. 7). It was in agreement with Banks (1981).The apical ends of these glands were in contact only with the lamina epithelium (Fig. 6) as reported by Verma *et al.* (2000) in broiler chicken.

The glands were enclosed by a thin capsule of connective tissue fibres. From this capsule, a delicate strands of connective tissue septa extended into the glands, between the secretory units (Fig. 8a), which was in accordance with the report made by Ali *et al.* (2023) in cervical oesophagus of kestrel and hoopoe.

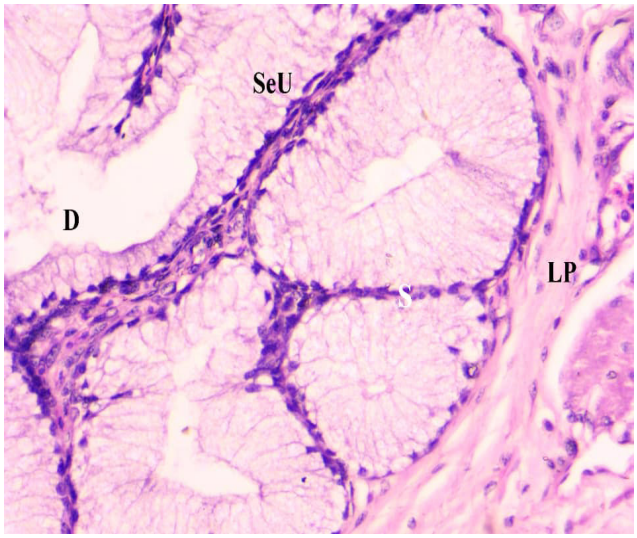


Fig. 7: Photomicrograph of the thoracic oesophagus in 12-week-old *Siruvidai* chicken showing the oesophageal gland lined by columnar epithelium. LP-Lamina propria, S-Septa, D-Duct, SeU- Secretary units (H&E x 400).

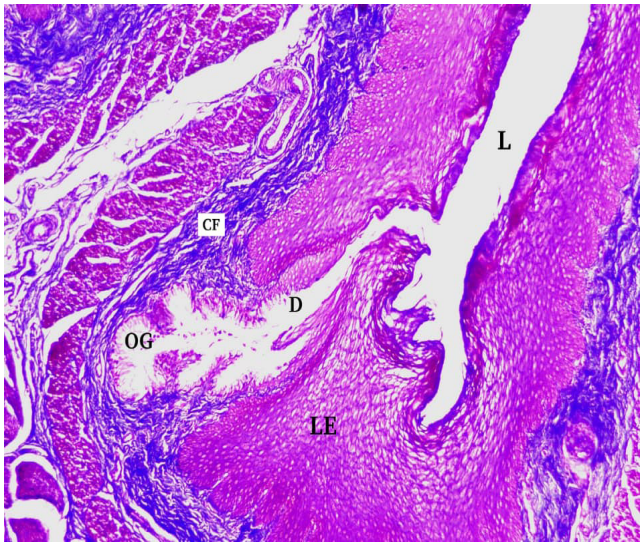


Fig. 8a: Photomicrograph of the thoracic oesophagus in 12-week-old *Siruvidai* chicken showing ductal epithelium of oesophageal gland opening into the lumen through duct and presence of collagen fibres around the gland. OG-Oesophageal gland, LE-Lamina epithelium, CF-Collagen fibres, L-Lumen, D-Duct (Masson's trichrome x 100).

The ductal epithelium of the oesophageal gland showed transition from columnar cells initially to stratified squamous cells on opening into the lumen (Fig. 8b). It was similar to the observations of Sagsoz and Liman (2009) in Japanese quail and Aydin *et al*. (2022) in partridges.

The lamina muscularis was arranged as a thick, distinct longitudinal smooth muscle layer (Fig. 6), which was in agreement with the findings of Hodges (1974) in fowl. The muscle bundles were surrounded by reticular fibres. Across all the studied age groups of the *Siruvidai* chicken, the lamina muscularis was continuous and well-developed. It extended deeply into the primary folds in the studied four age groups (Fig. 3).

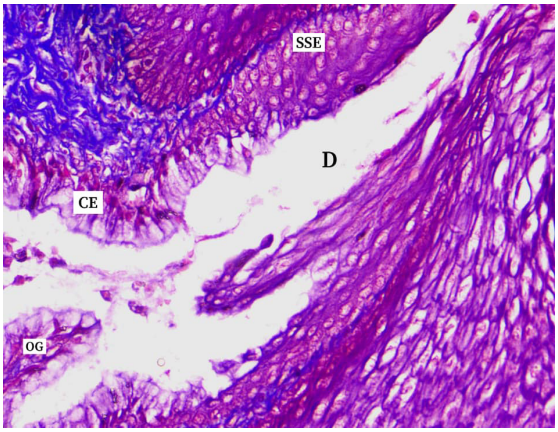


Fig. 8b: Photomicrograph of the thoracic oesophagus in twelve week-old *Siruvidai* chicken showing transition of ductal epithelium in the magnified oesophageal gland. OG-Oesophageal gland, D-Duct, CE-Columnar epithelium, SSE-Stratified squamous epithelium (Masson's trichrome x 400).

The submucosa was composed of loose connective tissue (Fig. 6), blood vessels and nerves that formed a thin, distinct layer, which provides structural support as reported by Mobini (2014) in broilers. As reported by Getty (1975), no glands were present in the submucosa of birds as in mammals, and this concurred with the present study. In 2-week-old birds, the submucosa was poorly developed in certain areas, whereas the submucosa was comparatively developed in 6-week, 12-week and 18-week-old birds.

The tunica muscularis in the present study on *Siruvidai* chicken was formed by an inner thick circular and an outer thin longitudinal layer of smooth muscle fibres (Fig. 2). It concurred with the observation of Hodges (1974) in the fowl and Crole (2009) in emu. On contrast to the present observation, it was mentioned that the skeletal muscle fibres were present in addition to the smooth muscle cells in turkey by Naser *et al*. (2024). This arrangement of muscle layers might be contributing to the coordinated peristaltic movement and efficient food transport. A thin layer of vascular connective tissue was noticed in between the inner circular and the outer longitudinal layer of muscle fibres and this was in total agreement with the observations of Hodges (1974) in fowl. The inner circular layer was thicker than the outer longitudinal layer (Fig. 2). In 2-week-old birds, the inner circular layer appeared as bundles and was thicker in diameter than the outer layer. The thickness of the tunica muscularis was found to be increased with age as similar to the observations in broiler chicken (Mobini, 2014).

In the thoracic oesophagus, the outermost tunica serosa consisted of connective tissue interspersed with blood vessels, nerves and was covered by a single layer of squamous cells (Fig. 2). This arrangement aligned with the description of Mobini (2014) in broiler chicken. It showed intense staining for collagen fibres (Fig. 7).

The histochemical techniques were carried out to understand the functional features of the thoracic oesophagus. The oesophageal glands were PAS-positive,

showing neutral mucins. Combined PAS-AB staining showed bright blue, indicating more acid mucins with mild neutral mucins (Fig. 9). The glands showed positive reactions, indicating strong histochemical activity for mucopolysaccharides.

Mowry's colloidal iron confirmed acid mucins in glands (Fig. 10). These glands are important for producing mucus, which protects the oesophageal lining and helps food move easily, which was concurrent with the observations of Aydin *et al*. (2022), who reported that mucins secreted by mucous-producing cells function as an antimicrobial effect, lubricity and physical barrier.

Muscularis layer stained uniform blue, showing proteins. The epithelium showed positive for proteins, while the oesophageal glands were unstained in mercury - bromophenol blue. This pattern was consistent with the observations of Ali *et al*. (2023) in kestrel and hoopoe.

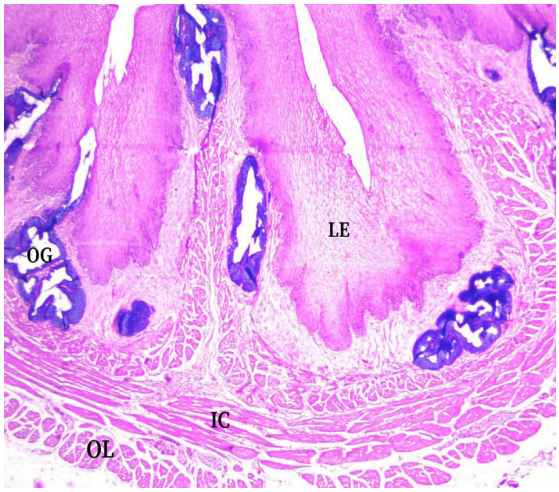


Fig. 9: Photomicrograph of the thoracic oesophagus in eighteen week-old *Siruvidai* chicken showing intense positive reaction for both the mucins in the oesophageal glands. LE-Lamina epithelium, OG-Oesophageal gland, IC-Inner circular layer, OL-Outer longitudinal layer (PAS-AB x 40).

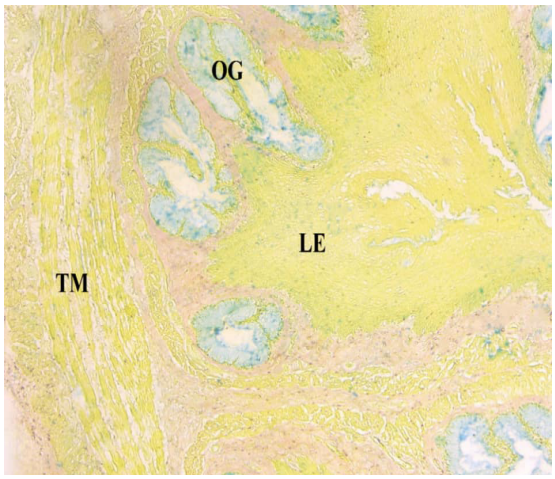


Fig. 10: Photomicrograph of the thoracic oesophagus in twelve week-old *Siruvidai* chicken showing the presence of acid mucins in the oesophageal glands. LE-Lamina epithelium, OG-Oesophageal gland, TM-Tunica muscularis (Mowry's Colloidal Iron x 40).

Conclusion

The histology and histochemistry of the thoracic oesophagus in the *Siruvidai* chicken revealed a well-organized mucosa lined by stratified squamous epithelium, mucous glands containing neutral and acidic mucins, and a distinct muscularis externa. The mucinous secretions observed in the glands play a key role in lubricating food for smooth passage towards the proventriculus. The detailed anatomical account of the thoracic oesophagus of this indigenous ecotype provides essential reference data and serves as a foundation for future histomorphological and clinicopathological studies across avian species.

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