

HISTOLOGY AND HISTOCHEMISTRY OF THE CERVICAL OESOPHAGUS IN THE EIGHTEEN WEEKS- OLD SIRUVIDAI CHICKEN

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ABSTRACT

Siruvidai chicken is an indigenous ecotype and reared as backyard poultry in Ariyalur district of Tamil Nadu state. The oesophagus is a distensible and tube shaped organ situated on the right side of neck in the birds. The present study was conducted on six numbers of 18 weeks- old Siruvidai chicken. The tissues pieces from the cervical oesophagus were collected and fixed in 10 percent neutral buffered formalin and processed for histological and histochemical staining methods. The oesophagus was composed of four tunics viz., tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. The lamina epithelium was lined by stratified squamous epithelium with less keratinisation. The lamina propria contained mucous glands. The lamina muscularis was distinct. The submucosa was thin. The tunica muscularis has two layers. The tunica adventitia covered cervical oesophagus. The presence of carbohydrates in the oesophageal glands and proteins in the muscle cells of lamina muscularis and tunica muscularis layers were demonstrated by histochemical staining methods. Micrometric observations were recorded on the thickness of different layers and diameter of mucous gland of cervical oesophagus.

Keywords: Oesophagus, *Siruvidai* chicken, histology, histochemistry

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INTRODUCTION

The *Siruvidai* chicken is an indigenous ecotype reared as backyard poultry in Ariyalur district of Tamil Nadu state (Churchil *et al.*, 2023). Backyard native chicken rearing is an important livelihood activity for small scale farmers in Tamil

Nadu and contributes substantially to fulfil the household nutrition and income (Senthilkumar *et al.*, 2025).

The avian gastrointestinal tract has undergone a multitude of changes during evolution to become a unique anatomical and physiological structure when compared to other animal orders (Stacey, 2006). The oesophagus is a narrow, muscular, highly-distensible tube which extends from the glottis at the posterior end of the pharynx, through the neck and thorax to join with the glandular stomach in the abdominal cavity (Hodges, 1974). The present study was conducted to record the histological and histochemical details of the oesophagus in eighteen weeks - old Siruvidai chicken, due to paucity of scientific work on this species.

MATERIALS AND METHODS

Six apparently healthy eighteen weeks - old Siruvidai chicken were collected from Poultry Research Station, Madhavaram Milk Colony, Chennai-51 and brought to Department of Veterinary Anatomy, Madras Veterinary College, Chennai-07. For histological and histochemical studies, the tissue samples from the cervical part of the oesophagus were collected and fixed in 10 percent neutral buffered formalin. After fixation, the tissue materials were dehydrated in graded alcohols, cleared in xylene and were embedded in paraffin wax at 58-60°C. Sections of 5-6 µm were cut. The histological and histochemical techniques were employed (Bancroft and Stevens, 1996) for observation.

IAEC Approval

The birds used in the study were sacrificed after the approval of ethical committee. Reference Number is 21/SA/IAEC/2024.

RESULTS AND DISCUSSION

The oesophagus in eighteen weeks -old Siruvidai chicken was seen on the right side of neck. The cervical oesophagus was 15 cm long. It is in accordance with the observations of Nickel *et al.* (1977) in birds. The oesophageal wall was composed of four tunics viz., tunica mucosa, tunica submucosa, tunica muscularis and tunica adventitia in cervical oesophagus (Fig. 1).

The tunica mucosa was composed of three layers viz, lamina epithelium, lamina propria and lamina muscularis. The luminal border of mucosa was irregular in cervical oesophagus. The tunica mucosa showed five primary folds in the cervical oesophagus on an average (Fig. 1) which is similar to the findings of Aydin *et al.* (2022) in Partridges. In the cervical oesophagus, the primary mucosal folds were broad, short and composed of lamina epithelium, lamina propria, lamina muscularis and tunica submucosa. The secondary folds formed conical projection only with lamina epithelium (Fig. 2). It is in accordance with the observations of Mahdy and Mohammed (2024) in domestic pigeons.

The lamina epithelium was lined by less keratinized stratified squamous epithelium (Fig.1), concurrent to the

findings of Hodges (1974) in fowl and Ali *et al.* (2023) in Kestrel and pigeon. In contrast, the mucosa was formed by a non-keratinized stratified epithelium in homing pigeon (Kadhim and Mohamed, 2015), in Japanese quails (Kausar *et al.*, 2016), in kingfisher (AbdElnaeem *et al.*, 2019) and in the Partridges (Aydin *et al.*, 2022).

The base of the lamina epithelium was uneven and showed finger-like projections. The basal layer was lined by a single layer of darkly stained basophilic columnar cells with elongated nucleus situated towards the base of the cell. The cell layers above the basal layer showed densely packed cells with more eosinophilic granular cytoplasm, centrally placed vacuolated nuclei and a prominent nucleoli. Towards lumen, the cells boundaries were lost and the nuclei was pyknotic and in many cells the nuclei degenerated and the cells detached from the topmost layer of the lamina epithelium but the cornification was less (Fig. 1). It is in total agreement with the observations of Hodges (1974) in fowl. The basement membrane of lamina epithelium was positive to reticular stains. The mean thickness of the lamina epithelium was $542.32 \pm 44.24 \mu\text{m}$.

The lamina propria was thick and composed of loose connective tissue, oesophageal glands, blood and lymph vessels and nerve fibers. The loose connective tissue contained predominantly collagen fibers (Fig. 2), few reticular fibers (Fig. 3) and very less elastic fibers. It is in accordance with the observation of Hodges (1974) in fowl. In the present study, it was observed that the thickness of the lamina propria was $189.88 \pm 18.66 \mu\text{m}$ in the eighteen weeks-

old Siruvidai chicken. This layer was extending into the projections of the lamina epithelium. It provided better anchorage to the epithelium (Fig. 2).

The oesophageal glands were simple branched tubuloalveolar mucous glands (Fig. 1). The mucous glands were lined by a single layer of columnar cells and the nucleus were displaced towards the basal part of the cell and were flattened as observed by Banks (1981) in avian and Dellmann and Brown (1981) in mammals. These glands were extending between lamina muscularis and lamina epithelium (Fig. 2). It is in agreement with findings of Verma *et al.* (2000) in broiler chicken. The glands were surrounded by a thin layer of connective tissue fibers like a capsule. From this, thin strands of connective tissue septa extended in between the secretory units (Fig. 3) which is concurrent to the findings of Ali *et al.* (2023) in Kestrel and Hoopoe.

In the present study, it was observed that an average of fourteen number of oesophageal glands were present in one cross section of cervical oesophagus. The glands were round shaped with five to eight secretory units in each gland (Fig. 2) as reported by Mobini (2014) in broiler chicken. In the present study, it was observed that the diameter of the oesophageal glands were $312.60 \pm 9.9 \mu\text{m}$. Initial portions of the duct was lined by columnar cells, which gradually changed to stratified squamous epithelium that opened on the surface of lamina epithelium into the lumen (Fig. 5).

The oesophageal glands showed a positive reaction to Periodic Acid Schiff

(PAS) which indicated the presence of neutral mucins (Fig 4) as recorded by Aydin *et al.* (2022) in Partridge. In combined Alcian Blue – Periodic Acid Schiff reaction, the cellular cytoplasm of oesophageal glands predominantly stained bright blue which indicated the presence of acid mucins. However, neutral mucins were also noticed (Fig. 5). The glands showed negative staining for proteins in mercury- bromophenol blue method (Fig.6) as observed by Ali *et al.* (2023) in Kestrel and Hoopoe.

The lamina muscularis was distinct, thick and made up of smooth muscle fibers arranged longitudinally (Fig.1) as observed by Kadhim and Mohamed (2015) in homing pigeon. Its thickness particularly increased within the core of mucosal folds. The thickness of lamina muscularis was $127.78 \pm 15.68 \mu\text{m}$ in the present study. The lamina muscularis and tunica muscularis bundles were surrounded by reticular fibers (Fig. 3).

The submucosa was thin, distinct and composed of loose connective tissue (Fig. 2), blood vessels and nerves. However, Naser *et al.* (2024) reported that the submucosa was well-developed in ostrich which is similar to the present study. But in contrary, the submucosa was reported to be less distinct in turkey.

In Siruvidai chicken, tunica muscularis of cervical oesophagus was composed of two layers of smooth muscles fibers viz., inner circular and outer longitudinal layer (Fig.1). The inner circular layer was $249.11 \pm 7.41 \mu\text{m}$ in thickness and the outer longitudinal layer was 212.71 ± 8.07

μm in thickness. It is in agreement with the observations of Madhu *et al.* (2015) in emu. In contrary, Naser *et al.* (2024) observed that in turkey, the skeletal muscle fibers were present in addition to the smooth muscle cells. In the present study, it was also observed that the outer layer was uniform in thickness. In between the inner circular and outer longitudinal layer of muscle fibers, a thin layer of vascular connective tissue containing blood vessels and nerve bundles were noticed. It is in total agreement with the observations of Hodges (1974) in fowl.

Both lamina muscularis and tunica muscularis showed positive reaction to the presence of proteins by mercury-bromophenol blue method (Fig.6) as reported by Ali *et al.* (2023) in kestrel and hoopoe but not in rock pigeon.

The tunica adventitia formed the outermost layer. It consisted of connective tissue fibers, blood vessels, nerves and fat cells (Fig.1). It is in agreement with the findings of Naser *et al.* (2024) in ostrich and turkey.

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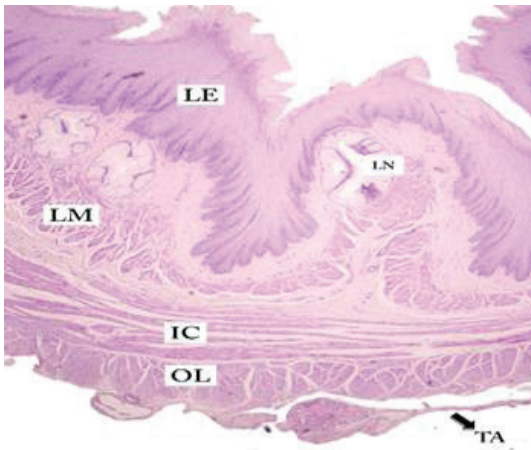


Fig 1.Photomicrograph of the cross section of the cervical oesophagus of an 18 weeks- old Siruvidai chicken LE-Lamina Epithelium , LM-Lamina Muscularis, LN-Lymphatic Nodule, IC-Inner circular layer, OL-Outer longitudinal layer, TA-Tunica Adventitia H&E X 40

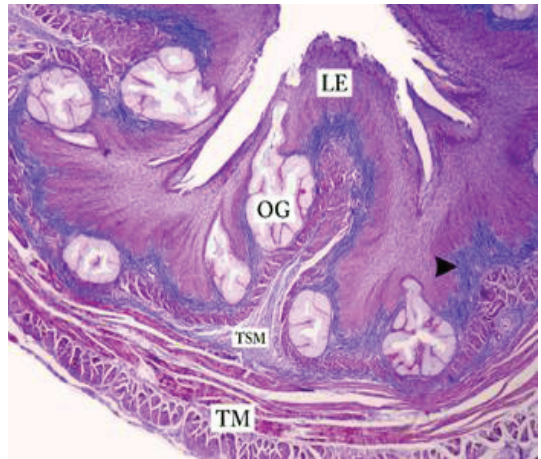


Fig 2.Photomicrograph of the cross section of the cervical oesophagus of an 18 weeks- old Siruvidai chicken showing collagen fibers LE-Lamina Epithelium, OG- Oesophageal Glands, TSM- Tunica Submucosa, TM-Tunica muscularis, Arrowhead- Collagen fibres. Masson's Trichrome X 40

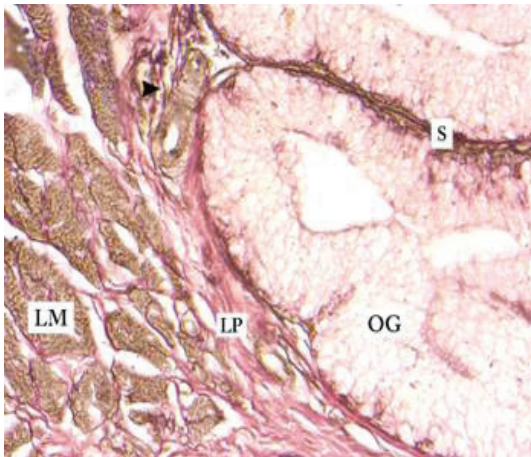


Fig 3. Photomicrograph of the cross section of the cervical oesophagus of an 18 weeks- old Siruvidai chicken showing reticular fibers LP-Lamina Propria, LM-Lamina Muscularis, OG- Oesophageal Glands , S – septa , Arrowhead- Reticular fibres Gomori's method X 400

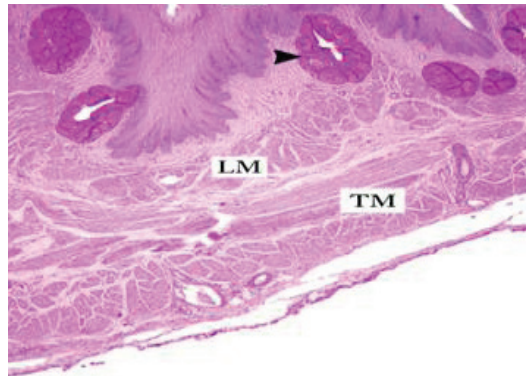


Fig 4.Photomicrograph of the cross section of the cervical oesophagus of an 18 weeks -old Siruvidai chicken showing neutral mucins LE-Lamina Epithelium, LM-Lamina Muscularis, TM- Tunica Muscularis, Arrowhead-Neutral mucins PAS X 40

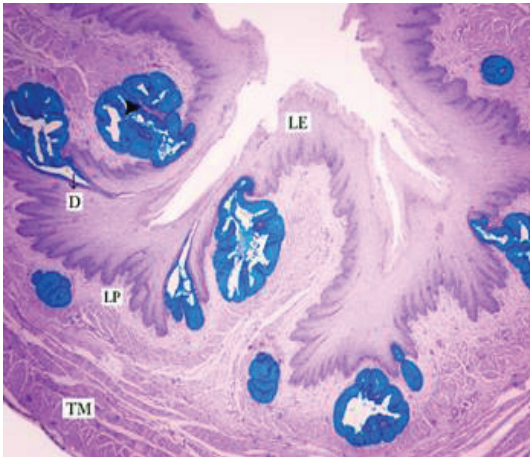


Fig 5. Photomicrograph of the cross section of the cervical oesophagus of an 18 weeks -old Siruvidai chicken showing acid and neutral mucins LE-Lamina Epithelium, D-Duct, LP-Lamina Propria, TM- Tunica Muscularis, Arrowhead-PAS positive material Combined PAS-AB X 40

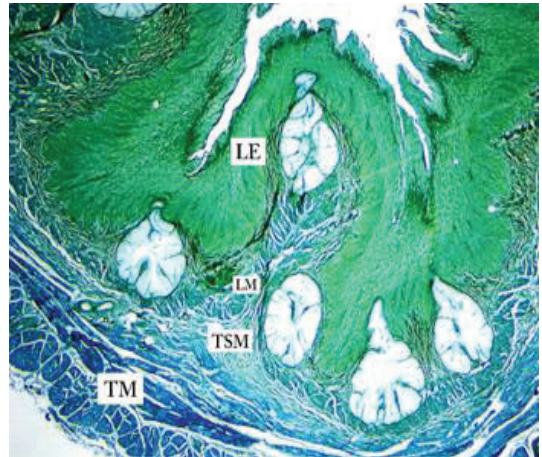


Fig 6. Photomicrograph of the cross section of the cervical oesophagus of an 18 weeks-old Siruvidai chicken showing proteins LE-Lamina Epithelium , LM-Lamina Muscularis, TSM- Tunica Submucosa, TM-Tunica Muscularis Mercury- Bromophenol Blue X 40

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