Applied Biological Research 28(3): 342-350; (2026)
DOI: 10.48165/abr.2026.28.01.35

HISTO-MORPHOMETRY AND HISTOCHEMISTRY OF THE OESOPHAGUS OF INDIGENOUS POULTRY OF POONCH REGION OF JAMMU AND KASHMIR (INDIA)

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(Received 14 February, 2026; Accepted 3 August, 2026)

ABSTRACT

Oesophagus is an important part of avian digestive tract and no information is available on structure of oesophagus of indigenous poultry of Poonch region of Jammu and Kashmir. Hence, a study was conducted with the aim to characterize the gross, histomorphometry and histochemical composition of the oesophagus of indigenous poultry of Poonch region, thereby establishing baseline anatomical data for this local poultry population. Histologically, tunica mucosa was thrown into folds. Lamina epithelialis was composed of non-keratinized stratified squamous epithelium. Lamina propria contained mucous-secreting simple tubule-acinar glands. Each acinus was surrounded by myoepithelial cells. Glandular cells had foamy cytoplasm and were full of secretions. A few glands were associated with lymphoid follicles. Collagen fibers and reticular fibers formed basket like arrangement around each lobe and lobule of glands. Lamina muscularis was thick and folded supported by central collagenous core. Tunica muscularis consisted of inner circular (three prominent layers) and outer longitudinal layers. At certain locations, oblique muscles were also observed. Between muscle layers, parasympathetic ganglions were also present. Thickness of tunica mucosa, lamina epithelialis, tunica muscularis, tunica serosa/adventitia and greater diameter of glands was significantly ($p<0.05$) higher at cervical part of oesophagus. Histochemically, muscles were strongly positive for proteins and epithelium showed moderate reaction whereas glands showed weak reaction. The basement membrane was strongly positive for PAS, glands showed intense reaction and epithelium exhibited moderate reaction for neutral mucopolysaccharides. Only the superficial layer of lamina epithelialis and glands showed strong reaction for lipids. However, basal layer of lamina epithelialis and glands were moderately positive for glycogen.

Keywords: Gland, histochemical, histology, oesophagus, Poonch, poultry

INTRODUCTION

Jammu & Kashmir state falls in the Greater Himalayan mountain range which has considerable impact on its agro-climatic conditions. Poonch region is situated at high elevation and is significantly cooler than the rest of India. Poonch region's native fowl can endure temperatures as low as -40°C, and their rearing ensures the survival of local inhabitants. This local bird of Poonch region weighs about 2.1-2.5 kg (cock) and 1.6-1.8 kg (hen). Females are combless whereas males present red coloured comb. In mammals and birds, oesophagus is a crucial component of digestive tract through which food travels to the stomach or gizzard. Depending on the type of feeding and dietary patterns, digestive tracts of different species of birds show distinct traits (Zhu, 2015). The oesophagus is lengthy and extremely distensible due to the existence of longitudinal folds, which allows it to accommodate enormous amounts of food (Hamdi *et al.*, 2013). The oesophagus of birds is located on the right side of neck (Getty, 1975), whereas that of mammals is located on the left side. It is divided into two parts *viz*., cervical and thoracic parts, and is situated between oropharynx and glandular portion of stomach (Getty, 1975). Mammalian oesophagus has three parts i.e. cervical, thoracic and abdominal parts (Mule, 1991). Unlike mammals, oesophagus of birds do not have lower and upper sphincters, and their diameters vary depending on the sort of nourishment they receive (Nickel *et al*., 1977; Banks, 1993).

There are two competing theories about the structure of mucosal surface of avian oesophagus; i) the apertures of mucosal glands is covered by stratified squamous keratinized epithelium (Sisson and Grossman, 1986), and ii) the mucosal glands were present and the growth of epithelium is not keratinized (Nasrin *et al*., 2012). Literature is available on the histomorphology of oesophagus in partridge (Rossi *et al*., 2005), sea gull birds (Ali, 2014), emu (Madhu *et al*., 2015), wild bronze turkey (Yovchev and Penchev, 2019), kingfisher and hoopoe (AbdElnaeem *et al*., 2019), *Gallinula chloropus* (Abdellatif *et al*., 2022), ring-necked parakeet and black-shouldered kite (Rajab *et al*., 2022), geese (Khalaf *et al*., 2022). Micrometrical studies had been done on the oesophagus of pigeon and barn owl (Al-Juboory *et al*., 2015), wild bronze turkey (Yovchev *et al*., 2017), domestic pigeon (Kausar *et al*., 2019), ring-necked parakeet and black-shouldered kite (Rajab *et al*., 2022). Data is also available on the histochemistry of oesophagus in Grey-backed Shrike (Zhu, 2015), wild bronze turkey (Yovchev and Penchev, 2019), ring-necked parakeet and black-shouldered kite (Rajab *et al*., 2022). The proventriculus of local poultry of Poonch region has previously been studied (Sasan *et al*., 2023), however, no information is available on the histomorphometry and histochemistry of oesophagus of this unique bird of Poonch which prompted to undertaken this study. The study was aimed to generate information on gross, histomorphology, micrometry and histochemistry of oesophagus which is expected to unveil the distribution and localization of various histochemical components in the bird.

MATERIALS AND METHODS

Sample collection

The carcasses of six indigenous poultry breed of Poonch region (20 weeks age) were obtained from the Division of Animal Genetics and Breeding, F.V.Sc & A.H., SKUAST-Jammu (India). Immediately after collection, the digestive tracts were thoroughly washed and the oesophageal tissue samples were obtained. Then the samples were preserved for 48 h in 10% neutral buffered formalin.

Sample processing for light microscopy

After proper fixation, the tissue samples were washed, dehydrated, and cleared by standard tissue processing schedule (Luna, 1968). The tissue samples were embedded in paraffin wax (58-60°C). Tissue sections of 5 μm thickness were obtained from these blocks on clean glass slides with the help of rotary microtome (Yorco, Lipshaw type). The sections were subjected to various histological and histochemical analysis which included hematoxylin and eosin stain for routine histomorphology, Von Gieson and Verhoeff's stain for collagen and elastic fibres, Gomori stain for reticular fibers, periodic acid-Schiff's stain for neutral mucopolysaccharides, Best Carmine method for glycogen, Sudan black B for lipids and Ayoub-Shklar method for keratin (Luna, 1968). Bromphenol blue stain was used for basic proteins analysis (Pearse, 1972). The stained sections were analysed using an optical microscope (Magnus microscope, MX21i-TrLED). Digital histological photographs were captured with the help microscopic camera attached to the microscope using MagVision software.

Micrometrical parameters recorded

Micrometry was performed on hematoxylin and eosin stained sections by microscopic image analysis software (MagVision). Micrometrical parameters recorded in cervical and thoracic part of oesophagus included thickness of tunica mucosa, tunica submucosa, tunica muscularis, tunica adventitia, lamina epithelialis mucosae, lamina propria and lamina muscularis mucosae; greater and shorter diameter of oesophageal glands, height and width of oesophegeal folds and thickness of tunica mucosa, lamina epithelialis mucosae, lamina propria and lamina muscularis mucosae at oesophageal folds. All the micrometric observations were recorded in ten randomly selected fields of the stained tissue section.

Statistical analysis

The micrometrical results were presented as mean ± standard error. The data was subjected to standard statistical analysis (Snedecor and Cochran, 1994). One-way analysis of variance was used to determine the level of significant difference in mean values.

RESULTS AND DISCUSSION

The oesophagus of local poultry of Poonch region consisted of cervical and thoracic parts. Cervical part extended from oro-pharynx to crop whereas thoracic part was present beyond the crop to its opening into the proventriculus.

Histomorphology

Histologically, cervical and thoracic parts of oesophagus consisted of four tunics, namely tunica mucosa, tunica submucosa, tunica muscularis, and tunica adventitia (Fig. 1) as seen in oesophagi of geese (Shehan, 2012) and pheasants (Parisa *et al*., 2019). However, these results were in contradiction with findings of Al-Kinany (2017), AbdElnaeem *et al*. (2019) in kingfisher and Zhu (2015) in grey-backed shrike who described just three tunics without tunica submucosa.

Tunica mucosa was thrown into numerous longitudinal folds as earlier observed in emu (Madhu *et al*., 2015), wild bronze turkey (Yovchev and Penchev, 2019) and Myna (Thaker and Haba, 2022). These folds were more prominent in cervical part than in thoracic part. These mucosal folds facilitate the passage of food by increasing the inner surface of oesophagus (Banks, 1993). McGuire and Beerman (2012) have suggested that these folds provide flexibility to the oesophagus by enabling it to expand on entry of a bolus.

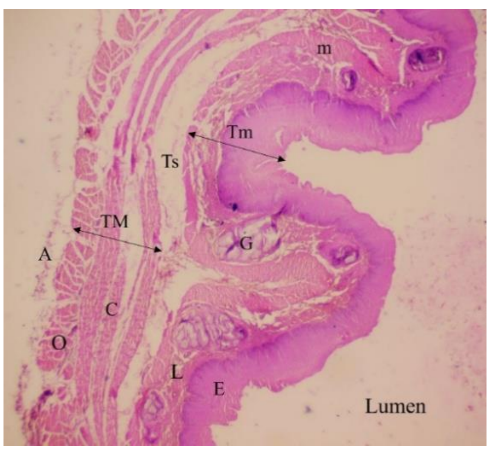

Fig. 1: Photomicrograph of cervical part of oesophagus showing tunica mucosa (Tm) having three parts namely lamina epithelialis (E), lamina propria (L) and lamina muscularis mucosae (m), tunica submucosa (Ts), tunica muscularis (TM) having inner circular (C) and outer longitudinal (O) layer, tunica adventitia (A). Lamina propria presented oesophageal glands (G). x40 H&E stain

In present study, the mucosal folds were lined with non-keratinized stratified squamous epithelium and centrally supported by core of connective tissue constituting the lamina propria similar to the findings of Rajab *et al.* (2022) in parakeet. However, they recorded keratinized stratified squamous epithelium in parakeet.

Tunica mucosae comprised of lamina epithelialis mucosae, lamina propria, and lamina muscularis mucosae (Fig. 1). Lamina epithelialis mucosae composed of thick non-keratinized stratified squamous (Fig. 2), consistent with the findings of Shina *et al*. (2005) in pigeon, Sagsöz and Liman (2009) in Japanese quail, Shehan (2012) in geese, Al-Juboory *et al*. (2015) in barn owl, AbdElnaeem *et al*. (2019) in kingfisher and Rajab *et al.* (2022) in kite. In contrast, keratinized stratified squamous epithelium was observed by Dellmann and Eurell (1998) in domestic fowl, Ali (2014) in sea gull birds, Yovchev and Penchev

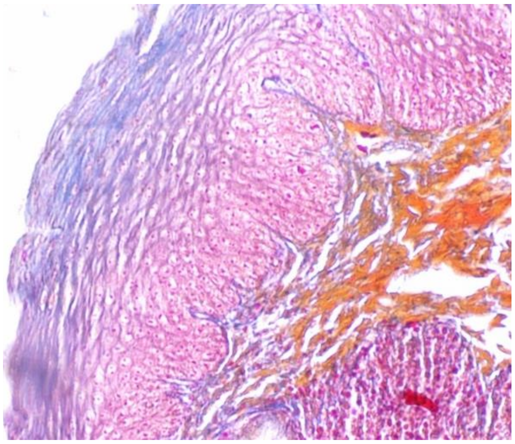

Fig. 2: Photomicrograph of cervical part of oesophagus showing stratified squamous epithelium which was non-keratinized. x 400 Ayoub-Shklar method for keratin

Fig. 3: Photomicrograph of thoracic part of oesophagus showing ducts of oesophageal glands (G) perforating (arrow) the overlying stratified squamous epithelium to reach the lumen. Glands were associated with lymphoid follicle (L). x100 H&E stain

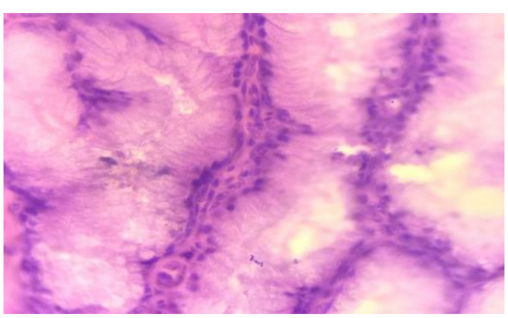

Fig. 4: Photomicrograph of oesophageal glands showing columnar cells with basally located flat nuclei. x1000 H&E stain

(2019) in wild bronze turkey and Rajab *et al*. (2022) in parakeet. The epithelial cells were more basophilic towards the basal layer and progressively became more acidophilic towards the apical surface of epithelium (Fig. 1).

The lamina propria consisted of dense, irregular connective tissue and mucous-producing simple tubulo-alveolar glands (Fig. 1) throughout the cervical and thoracic parts of oesophagus, consistent with the findings of Madhu *et al*. (2015) in emu. In contrast, Yovchev and Penchev (2019) in wild bronze turkey found only loose connective tissue in the lamina propria. The glands discharge their secretion into the lumen by ducts that perforate the overlying stratified squamous epithelium to reach the lumen (Fig. 3) which was in line with the findings of Rossi *et al.* (2005) in partridge. Glands were more numerous in cervical part than in thoracic part, corroborating with the findings of Zhu (2015) in shrike.

Mucosal glands were reported within the tunica submucosa of the oesophagus in myna (Thaker and Haba, 2022). Functionally, these glands contribute to the lubrication of the mucosal surface (George *et al.*, 1998). The secretory nature of oesophageal glands also varied among avian species. The glands were seromucous in rock dove, parakeet, and collared dove and mucous in the oesophagus of house sparrow, kestrel, and linnet (Rajabi and Nabipour, 2009).

The glandular epithelium was composed of simple columnar with basally located flat nuclei, consistent with the observations of Zhu (2015) in the grey-backed shrike. Glandular cells exhibited secretory activities and had foamy cytoplasm. The mucous cells were distinguished by their poorly stained cytoplasm and densely stained basal nuclei. Few of the glands were associated with lymphoid follicles (Fig. 3), suggesting a possible relationship between the glandular and immune components of the oesophageal mucosa. Similarly, Madhu *et al.* (2015) observed both diffuse and aggregation of lymphocytes in the lamina propria of oesophagus of emu bird. Collagen and reticular fibres formed basket-like arrangement around each lobe and lobule of the glands (Fig. 5 and 6). Each glandular unit was surrounded by myoepithelial cells.

In present study, the lamina muscularis mucosae were well-developed and organized longitudinally enforced by collagen fibres for providing support (Fig. 5). Lamina muscularis mucosae extended into the oesophageal folds (Fig. 1). This was supported by the findings of Sagsoz and Liman (2009) in Japanese quail and Rajab *et al*. (2022) in

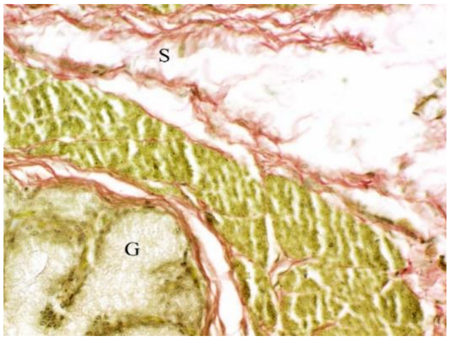

Fig. 5: Photomicrograph showing presence of collagen fibers (red colour) around oesophageal glands (G). Collagen fibers were also present in tunica submucosa (S). Lamina muscularis mucosae was also supported by collagen fibres. x400 Von Gieson & Verhoeff's stain

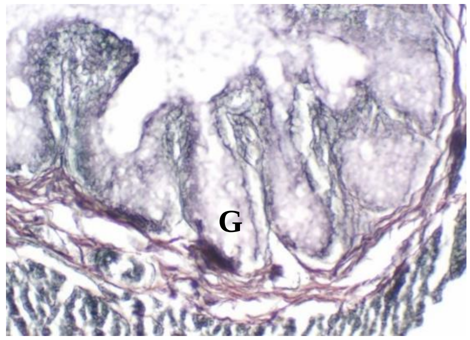

Fig. 6: Photomicrograph showing the presence of reticular fibers (black colour) around the oesophageal glands (G). Magnification x400 Gomori stain

parakeet. The well-developed lamina muscularis mucosae of avian oesophagus reportedly predispose to the production of a large number of longitudinal mucosal folds (Banks, 1993). Lamina muscularis of partridge oesophagus comprise of three layers of smooth muscle: an inner longitudinal, a middle circular, and an outer longitudinal (Rossi *et al.*, 2005). Lamina muscularis mucosae was absent in *Acridotheres tristis* (Thaker and Haba, 2022).

In present study, tunica submucosa was poorly developed with loose connective tissue layer containing vessels and nerves, as also reported by Dellmann and Eurell (1998) in domestic poultry and Rajabi *et al*. (2022) in parakeet and kite. However, Sagsöz and Liman (2009) did not report tunica submucosa in Japanese quail. Zaher *et al*. (2012) have reported that both cervical and thoracic portions of oesophagus have simple branched mucous glands that are dispersed throughout the submucosa.

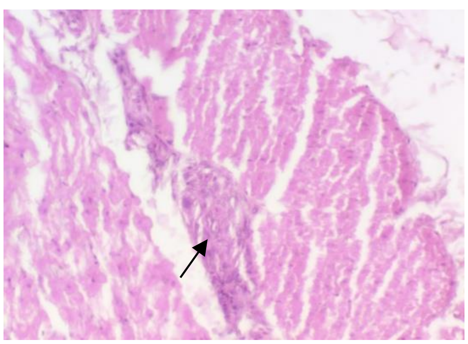

Fig. 7: Photomicrograph showing presence of nerve plexus (arrow) between muscle layers. x400 H&E stain

Tunica muscularis consisted of smooth muscle fibers arranged in two layers, inner circular and outer longitudinal layer (Fig. 1). Similar arrangement was observed in domestic fowl (Dellmann and Eurell, 1998), wild bronze turkey (Yovchev and Penchev, 2019) and *Gallinula chloropus* (Abdellatif *et al.*, 2022). Inner circular muscle layer had three prominent layers with nerve plexus located in between (Fig. 7). This is in agreement with the findings of Madhu *et al.* (2015) in emu. Thaker and Haba (2022) have reported three layered tunica muscularis in *Acridotheres tristis*. Inner and outer layers were arranged in circular manner, while middle layer was longitudinally arranged. Outer layer was tunica adventitia which consisted of loose connective tissue and was highly vascular (Fig. 1).

Micrometry

The micrometrical data is presented in Table 1. In present study, tunica mucosa was significantly ($p<0.05$) thicker in cervical part (513.58 ± 23.62 µm) than in thoracic part (383.13 ± 9.61 µm). The mean thickness observed was lower than that reported in *Gallinula chloropus* (609 ± 75 µm) by Abdellatif *et al*. (2022). Lower values have been reported in common wood pigeon, parakeet, and kite. Al-Juboory *et al.* (2015) reported a mucosal thickness of 79.22 µm in common wood pigeon, while Rajab *et al.* (2022) noted values of 123.5 ± 9.3 µm and 108.2 ± 8.97 µm in parakeet and kite, respectively. These interspecific differences may reflect variations in dietary habits and feeding behaviour. Lamina epithelialis mucosae was significantly ($p < 0.05$) thicker in cervical part (264.56 ± 26.09 µm) than in thoracic part (175.17 ± 10.81 µm). The thickness of lamina epithelialis mucosae in indigenous poultry other species such as of Poonch region was much higher than that reported in

Table 1: Different micrometrical parameters of oesophagus of local poultry of Poonch region

Parameters	Cervical part of oesophagus (µm)		Thoracic part of oesophagus (µm)	
	Mean	Standard error	Mean	Standard error
Thickness of tunica mucosa	513.58[a]	23.62	383.13[b]	9.61
Thickness of lamina epithelialis	264.56[a]	26.09	175.17[b]	10.81
Thickness of lamina propria	157.12	12.71	137.99	5.15
Thickness of lamina muscularis mucosae	91.78	17.56	83.00	4.02
Thickness of tunica submucosa	130.87	18.05	161.62	4.84
Thickness of tunica muscularis	433.43[a]	16.64	323.98[b]	53.47
Thickness of inner muscle layer	247.33	12.86	191.97	32.47
Thickness of outer muscle layer	142.82	13.75	116.53	21.28
Thickness of tunica serosa/adventitia	130.68[a]	15.05	86.40[b]	5.03
Greater diameter of gland	353.95[a]	16.55	262.01[b]	18.65
Shorter diameter of gland	182.74	13.09	203.37	14.90

Mean value with same superscript within row do not differ significantly ($p > 0.05$)

several other species such as rock dove (60-100 µm), collared dove (70-110 µm), parakeet (90-100 µm), kestrel (80-150 µm), house sparrow (50-80 µm) and Linnet (40-50 µm) (Rajabi and Nabipour, 2009). The comparatively greater epithelial thickness in the present study may be associated with increased protection of the oesophageal mucosa against mechanical abrasion during the passage of ingesta. Lamina propria and lamina muscularis mucosae were also thicker at cervical part than at thoracic part, however, these regional differences were significantly non-significant ($p > 0.05$).

The thickness of tunica submucosa did not show any significant difference ($p > 0.05$). However, the values were considerably higher than those reported in common wood pigeon (55.92 µm), barn owl (123.26 µm) (Al-Juboory *et al*., 2015), wild bronze turkey of 49 days age (35.0 ± 8 µm) (Yovchev *et al.*, 2017), but lower than the values in parakeet (245.1 ± 22.5 µm) (Rajab *et al*., 2022).

Tunica muscularis was significantly ($p < 0.05$) thicker at cervical part of oesophagus (433.43 ± 16.64 µm). However, it was much lesser than the values reported by Abdellatif *et al*. (2022) in *Gallinula chloropus* (1039 ± 198 µm). In parakeet and kite, the thickness of tunica muscularis was 281.3 ± 10.2 µm and 394.4 ± 21.05 µm, respectively (Rajab *et al*., 2022). In both cervical and thoracic regions, inner circular layer was consistently thicker than outer longitudinal layer probably owing to presence of three circular bands in inner layer.

Thickness of tunica adventitia was 130.68 ± 15.05 µm at cervical part which was significantly ($p < 0.05$) thicker than at thoracic part (86.40 ± 5.03 µm). Thickness at cervical part was close to the values (122 ± 56 µm) observed by Abdellatif *et al*. (2022) in *Gallinula chloropus*. In parakeet, the mean thickness of tunica adventitia was 62.4 ± 3.9 µm (Rajab *et al*., 2022).

The greater and shorter diameter of gland was 353.95 ± 16.54 µm and 182.74 ± 13.09 µm, respectively at cervical part and 262.01 ± 18.65 µm and 203.37 ± 14.90 µm, respectively at thoracic part. In parakeet, the mean length and width of glands was 207.1 ± 26.2 µm and 231.1 ± 11.3 µm, respectively. The same in kite was 106.2 ± 8.66 µm and 135.7 ± 8.19 µm, respectively (Rajab *et al*., 2022).

In present study, the oesophageal folds in the cervical region were relatively broad, with a mean width of 1203.47 ± 19.07 µm exceeding their mean height of 866.63 ± 22.88 µm. However, at thoracic part, folds were inconspicuous. The morphology and dimensions of oesophageal folds varied considerably among avian species. Al-Juboory *et al*. (2015) observed finger-like oesophageal fold in common wood pigeon with height of 699 µm and width of 349.5 µm whereas in barn owl, folds were leaf-like with height of 745.6 µm and width of 279.6 µm. Rajab *et al.* (2022) reported considerably smaller folds in the parakeet, with mean length and width of 323.9 ± 31.2 µm and 241.1 ± 13.8 µm, respectively, while those in the kite folds measured 728.9 ± 37.19 µm in length and 418.1 ± 23.18 µm in width. The marked interspecific variation in the size and configuration of oesophageal folds may reflect differences in oesophageal distensibility and functional adaptations associated with feeding habits and the passage of ingesta.

Histochemistry

Histochemical distribution of neutral mucopolysaccharides, basic proteins, glycogen, and lipids was recorded in different layers of cervical and thoracic parts of oesophagus. Histochemically, basement membrane exhibited a strong PAS reaction, whereas glands showed intense reaction which indicated the presence of neutral mucopolysaccharide (Fig. 8). These results are consistent with the observations of Zhu (2015) in grey-backed Shrike and Yovchev and Penchev (2019) in wild bronze turkey. However, the lining epithelium exhibited moderate reaction for neutral mucopolysaccharides whereas lamina propria exhibited weak PAS reaction which was in line with the findings of Yovchev and Penchev (2019) in wild broze turkey. Species-related differences in histochemical characteristics of oesophageal secretions were also reported. In Kestrel and Rose-ringed Parakeet, the secretions showed poor positive reaction with PAS; however, Rajabi and Nabipour (2009) did not record PAS positivity in the secretion of Linnet and Collared dove. The variation in PAS reactivity among species may reflect differences in the composition and functional properties of oesophageal mucus.

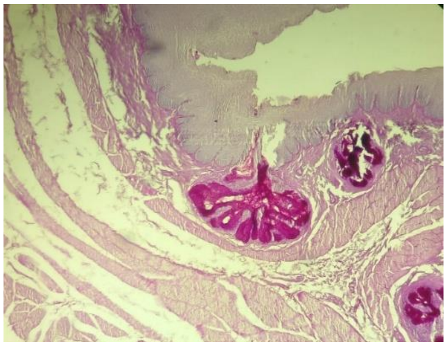

Fig. 8: Photomicrograph showing strong reaction of basement membrane and intense reaction of oesophageal glands for PAS. x100

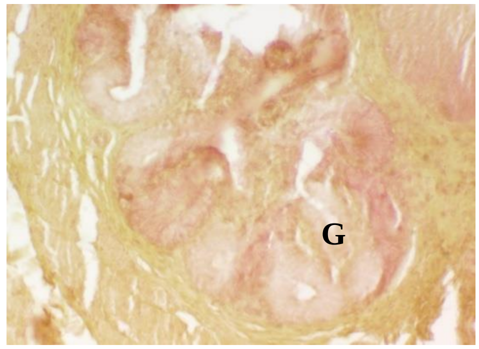

Fig. 9: Photomicrograph showing moderate reaction of of oesophageal glands (G) for glycogen. x400 Best Carmine stain

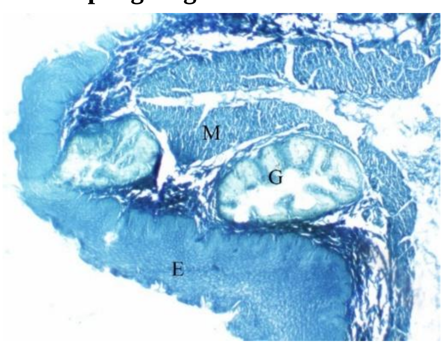

Fig. 10: Photomicrograph of oesophagus. Muscles (M) were strongly positive for proteins and epithelium (E) showed moderate to strong reaction whereas glands (G) showed weak reaction mainly towards basal part. x100 Bromphenol Blue stain

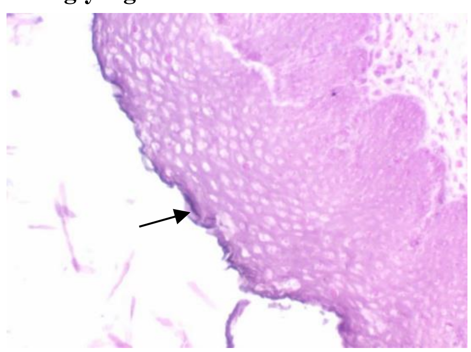

Fig. 11: Photomicrograph of oesophagus. Only the superficial layer of lamina epithelialis showed strong reaction for lipids. x400 Sudan Black B stain

The basal layer of lamina epithelialis and glands exhibited moderate positive reaction to glycogen (Fig. 9). The muscle layers exhibited strong positivity for proteins, while epithelium showed moderate to strong reactivity, and glands showed weak reaction (mainly towards basal part) (Fig. 10). Hamdi *et al*. (2013) observed exaggerated amount of proteinic element in the cytoplasm of stratified squamous epithelium of oesophagus of black-winged kite. Only superficial layer of lamina epithelialis and glands showed strong reaction for lipids (Fig. 11). The superficial layer of lamina epithelialis mucosae was negative for keratin indicating non-keratinized stratified squamous epithelium (Fig. 2).

Conclusion: The oesophagus of indigenous poultry of Poonch region exhibits well-developed histo-morphological and histochemical specializations suited for effective ingestion and transport of feed. The cervical oesophagus showed significantly greater development of mucosal, epithelial, muscular layers and glandular size compared to the thoracic part, highlighting regional functional differentiation. Histochemical reactions confirm the presence of protein-rich musculature and muco-polysaccharide-rich glands and epithelium, facilitating lubrication and protection. Overall, these findings provided baseline structural and functional data on the oesophagus of indigenous poultry, contributing valuable information for comparative anatomy and avian physiology.

Ethical statement: Ethical approval was not required for the present study, as the tissue samples were collected from Division of Animal Genetics & Breeding. No live animals were handled or subjected to any experimental procedures.

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