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Gross Anatomical, Histological, Histochemical and Scanning Electron Microscopic Examination of Glandular Stomach of Chinese Goose (*Anser cygnoides*)

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ABSTRACT

Background: The digestive system is responsible for the intake, conversion and absorption of nutrients and the elimination of wastes from the body in order to provide the necessary energy for the continuation of vitality and functions. The aim of this study was to examine the glandular stomach of the Chinese goose (*Anser cygnoides*) by histological, histochemical and scanning electron microscopic methods.

Methods: The glandular stomachs of 12 (6 males and 6 females) healthy adult Chinese geese (*Anser cygnoides*) were used in the study. The tissues taken were subjected to routine histological procedures.

Result: Gross anatomically, it was seen that the Proventriculus was a fusiform or spindle-shaped organ, extending along the median plane between the esophagus and the muscular stomach. Scanning electron microscopic (SEM) observations revealed that the glandular stomach surface of Chinese goose (*Anser cygnoides*) contained many mucosal folds. Parallel grooves and proventricular gland openings were observed between these folds. Histologically, it was observed that the glandular stomach wall of Chinese goose (*Anser cygnoides*) consisted of four layers: tunica mucosa, submucosa, tunica muscularis and tunica serosa. The tunica mucosa was observed to have lamina epithelialis, lamina propria and lamina muscularis sublayers. Single layer prismatic cells secreting mucus were found in the lamina epithelialis layer. These cells showed local Alcian Blue (AB) pH:2,5 positive reaction. Lamina muscularis consisted of longitudinal smooth muscle fiber bundles. Compound, branched tubular glands were observed in the submucosal layer. Again, a regional AB positive reaction was observed in the lumen-facing parts of the tubular glands. Periodic acid Schiff (PAS) positive reaction was noted in the basal parts of the tubular glands. In Gordon-Sweet (GS) staining method, reticular threads easily distinguished in all areas where connective tissue present

Key words: Chinese goose, Glandular stomach, Histology, Scanning Electron Microscopy (SEM).

INTRODUCTION

As the name suggests, this Chinese breed has a history thought to date back to 3000 BC and constitutes one of the two ancestors of domestic geese (Önk and Kırmızıbayrak, 2019). Commonly found in Mongolia, southeastern China and Russia, Chinese goose (*Anser cygnoides*) is a small and remarkable breed characterized by the protrusions on their heads (Ran *et al.,* 2021). Chinese geese (*Anser cygnoides*) can have brown or gray/white feathers, the legs, beak and knob are orange in white geese, while the legs are orange and the beak and knob are black in brown geese. In male geese, the knob is larger than that in females, which allows for visual gender discrimination (Tilki and Saatçı, 2013).

The proventriculus, also known as the true stomach, is the glandular stomach where digestion primarily begins. Here, digestive enzymes such as hydrochloric acid and pepsin are added to the food. It precedes the ventriculus where the nutrients are being ground and therefore this organ is called the proventriculus (Gofur, 2020). The proventriculus, which is a fusiform or spindle-shaped organ, extending on the median plane between the esophagus and the muscular stomach, is about 5 cm long, 2 cm in diameter and its size varies from species to species. It is located between the two lobes of the liver and its ventral side is in contact with the liver (Karadağ and Nur, 2004).

No histological and electron microscopic studies on the glandular stomach of Chinese goose were found in the literature. The aim of present study was to investigate the Chinese goose (*Anser cygnoides*) glandular stomach by histological, histochemical and scanning electron microscopic methods.

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MATERIALS AND METHODS

In present study, 12 (6 males and 6 females) healthy adult Chinese geese (*Anser cygnoides*) were used. Approval was obtained Selcuk University Veterinary Faculty Experimental Animal Production and Research Center Ethics Committee (SÜVDAMEK).

Chinese Geese (*Anser cygnoides*) obtained from a private enterprise were injected with 10-12 mg/kg xylazine for premedication and 30-40 mg/kg ketamine (ketalar) intramuscularly for anesthesia. Tissue samples were then taken from the glandular stomach. Each tissue taken for light microscopic examination was fixed in 10% neutral buffered formaldehyde for 24 hours and then washed. Afterwards, the samples, which underwent routine histological tissue follow-up, were blocked in paraffin. The prepared paraffin blocks were cut at a thickness of 5 µm and examined under a Leica DM2500 model light microscope using the hematoxylin and eosin (H&E) (Luna, 1968), Alcian Blue (AB) pH:2,5, Periodic acid Schiff (PAS) and Gordon-Sweet staining method the photographs of the relevant parts were taken.

The tissues taken for examination with scanning electron microscopy were washed in phosphate buffer, re-fixed in 2.5% glutaraldehyde, dehydrated in acetone series and then dried (Kapakin, 2006). The tissues placed on metal plates were coated with gold-palladium at a thickness of 18-20 nm in a sputter coater and examined under a LEO 440 scanning electron microscope.

RESULTS AND DISCUSSION

Gross anatomical findings

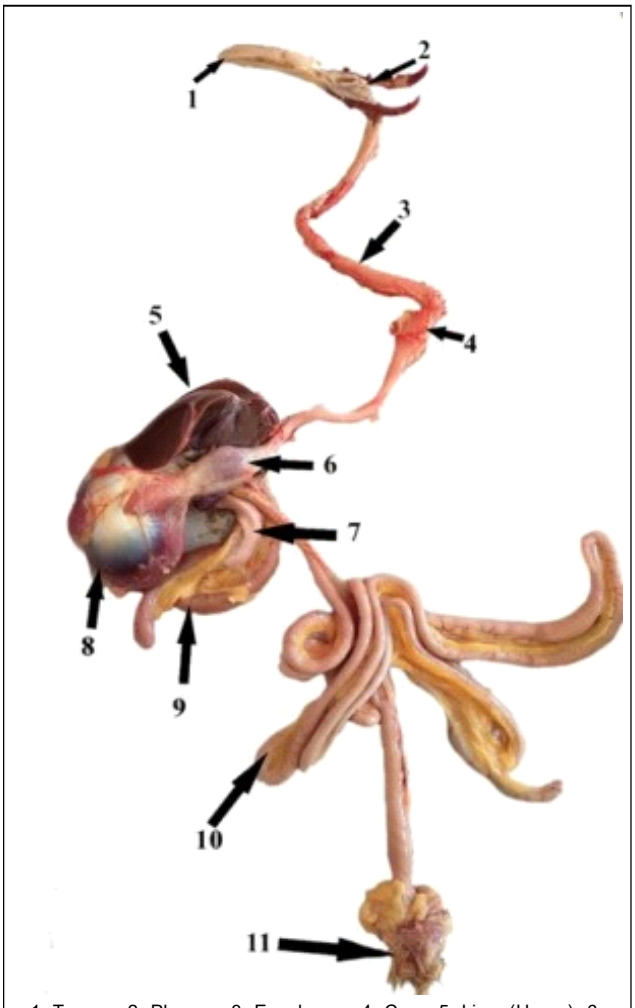
The stomach of Chinese geese (*Anser cygnoides*) consisted of two parts, the glandular stomach (proventriculus) and the muscular stomach (gizzard) located one after the other. The proventriculus, also known as the true stomach, preceding the ventricle or gizzard. The Proventriculus was a fusiform or spindle-shaped organ, extending on the median plane between the esophagus and the stomach. It was located between the two lobes of the liver and its ventral side was in contact with the liver (Fig 1).

Scanning microscopic observation

Scanning electron microscopic observations revealed that the glandular stomach surface of Chinese goose (*Anser cygnoides*) contained many mucosal folds (Fig 2a). Parallel grooves and proventricular gland openings were observed between these folds (Fig 2b, 2c, 2d). These folds were seen to surround the orifices of the proventricular glands in a parallel manner (Fig 2d).

Histological structure

The results of histological study showed that the glandular stomach wall of Chinese goose (*Anser cygnoides*) consisted of four layers: tunica mucosa, submucosa, tunica muscularis and tunica serosa (Fig 3). The tunica mucosa had lamina epithelialis, lamina propria and lamina muscularis sublayers (Fig 4a). Single layered prismatic cells secreting mucus were found in the lamina epithelialis layer (Fig 4b). Nodular and diffuse lymphoid tissues were observed in the lamina propria (Fig 4c). It was noted that the lamina muscularis consisted of longitudinal smooth muscle fiber bundles (Fig 4d). Compound, branched tubular proventricular glands were observed in the submucosa (Fig 5a). These glands were lined by single-layered cuboidal epithelial cells and were in the form of lobules that were clearly separated from each other and contained a central cavity. The central cavities were observed to have draining ducts extending radially into the connective tissue between the lobules (Fig 5b). Connective tissue and capillary blood vessels (with erythrocytes) were also observed between these glands (Fig 5c). This layer constituted the largest part of the proventriculus. Circular smooth muscle fibers were observed in the tunica



1: Tongue, 2: Pharynx, 3: Esophagus, 4: Crop, 5: Liver (Hepar), 6: Proventriculus (Glandular stomach), 7: Pancreas, 8: Ventriculus (Muscular stomach), 9: Small intestines, 10: Large intestines, 11: Cloaca.

Fig 1: Digestive system organs of Chinese goose (*Anser cygnoides*).

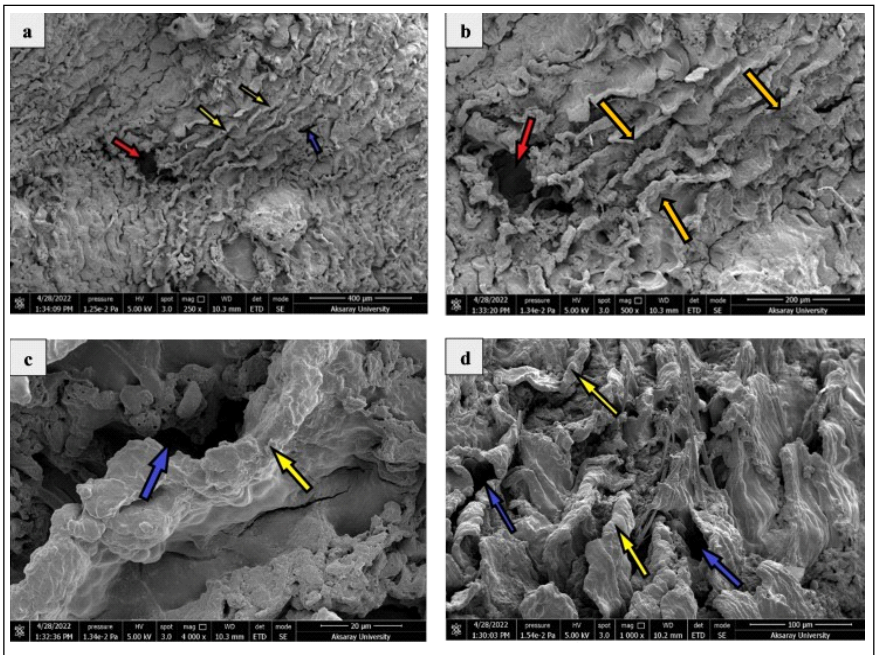
muscularis layer. The tunica serosa layer was observed at the outermost layer (Fig 6).

Histochemistry

While no reaction was observed in Chinese goose (*Anser cygnoides*) glandular stomach in the Alcian Blue pH: 1.0 staining method, a locally positive reaction was observed in the lumen-facing parts of the lamina epithelialis, tubular glands and release their secretions into the lumen in the submucosa in the Alcian Blue pH:2.5 staining method (Fig 7a, 7b, 7c). However, a very intense AB positive reaction was observed in the lumen facing parts of the lamina epithelialis in the transitional region from the glandular stomach to the muscular stomach (not reaching the muscular stomach yet) (Fig 7d). In PAS staining method, a positive reaction was observed in the basal parts of the tubular glands (Fig 8). In Gordon Sweeth staining method, reticular threads easily distinguished in all areas where connective tissue present (Fig 9).

Altaai and Al-Taai (2020) in the adult starling, Hussein *et al*. (2020) at brown falcon, Taher *et al*. (2020) in common moorhen, F.J. Al-Saffar and Al-Samawy, (2015) in green-headed mallard, Abumandour (2014) in Eurasian hobby, Al-Saffar and Eyhab (2014) in pallid scops owl, Hassan and Moussa (2012) in domestic pigeons, Selvan *et al*. (2008) in guinea fowl, reported that the stomach was located between the esophagus and the intestine and consisted of two parts: the proventriculus (glandular stomach) and the ventricle (muscular stomach, gizzard). In present study, when examined anatomically, it was seen that the stomach of Chinese geese (*Anser cygnoides*) consisted of two parts: the glandular stomach (proventriculus) and the muscular stomach (gizzard) located one after the other.

In scanning electron microscopic observations of turkeys, Beheiry (2018) stated that the proventriculus mucous membrane consisted of many mucosal folds separated by grooves and these folds surrounded the openings of the proventricular glands on the surface. Abdel Maksoud *et al*. (2022) reported that in the capped crow, the proventricular glands consisted of variably sized openings, which appeared as bulging tubes with regular circular outlines and are surrounded by anastomosed microscopic folds. A similar pattern of anastomosed microscopic folds has been reported in the burrowing owl (Rocha and de Lima, 1998). Different arrangements of these folds have been reported as branched in the turkey (Beheiry, 2018) and as rosette-shaped concentrically arranged folds in the pigeon and laughing dove (Madkour and Mohamed, 2019). In present study, scanning electron microscopic observations revealed that the glandular stomach surface of Chinese goose (*Anser cygnoides*) contained many mucosal folds. Between these folds, parallel grooves and proventricular gland openings were observed on the

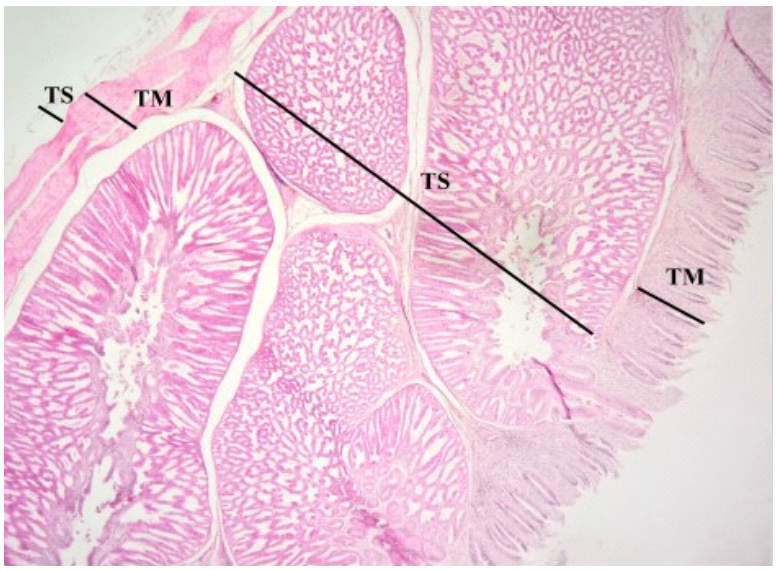


a) Mucosal folds (yellow arrows), proventricular opening (red arrow), groove between folds (blue arrow). Scanning electron microscope (SEM) image. b) Proventricular opening (red arrow), mucosal folds (yellow arrows). Scanning electron microscope (SEM) image. c) Mucosal folds (yellow arrow), inter-fold groove (blue arrow). Scanning electron microscope (SEM) image. d) Longitudinal view of mucosal folds (yellow arrows) and grooves (blue arrows) on the glandular stomach surface. Scanning electron microscope (SEM) image.

Fig 2: Glandular stomach wall of Chinese goose (*Anser cygnoides*).

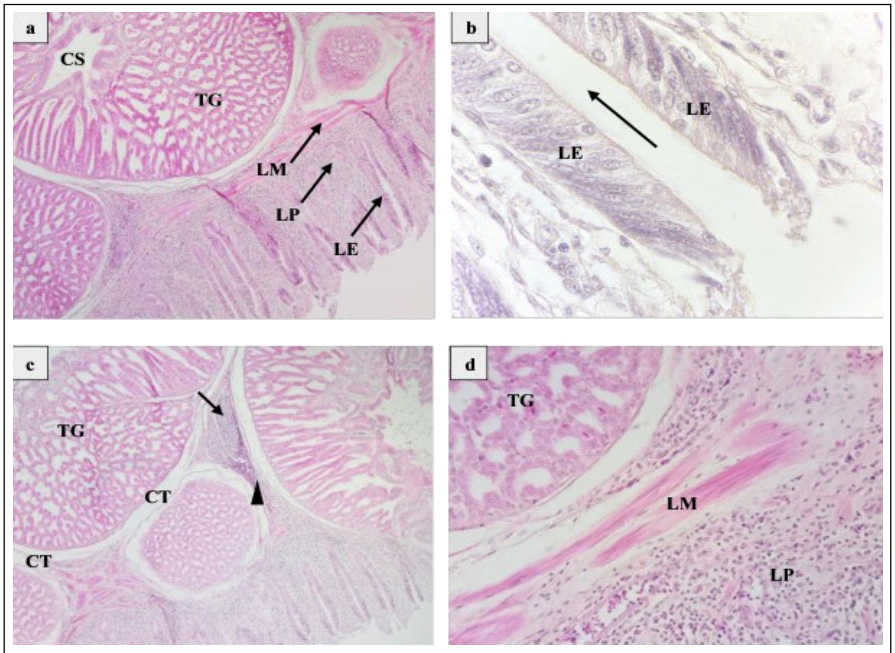
surface. It was seen that these folds surrounded the proventricular gland openings in a parallel manner.

In the studies by Selvan *et al.* (2008) in guinea fowl, Hassan and Moussa (2012) in domestic pigeons, Abumandour (2014) in Eurasian hobby, Al-Saffar and Eyhab (2014) in pallid scops owl, Al-Saffar and Al-Samawy (2015) in green-headed mallard, Altaai and Al-Taai (2020) in the adult starling, Hussein *et al.* (2020) in brown hawk, Taher *et al.* (2020) in common moorhen, Saran and Meshram (2020) in Guinea Fowl, Sasan *et al.* (2023) in Poonchi



TM: Tunica mucosa, TS: Tunica Submucosa, TM: Tunica Muscularis, TS: Tunica Serosa. Hematoxylin Eosin (HE) staining, X4.

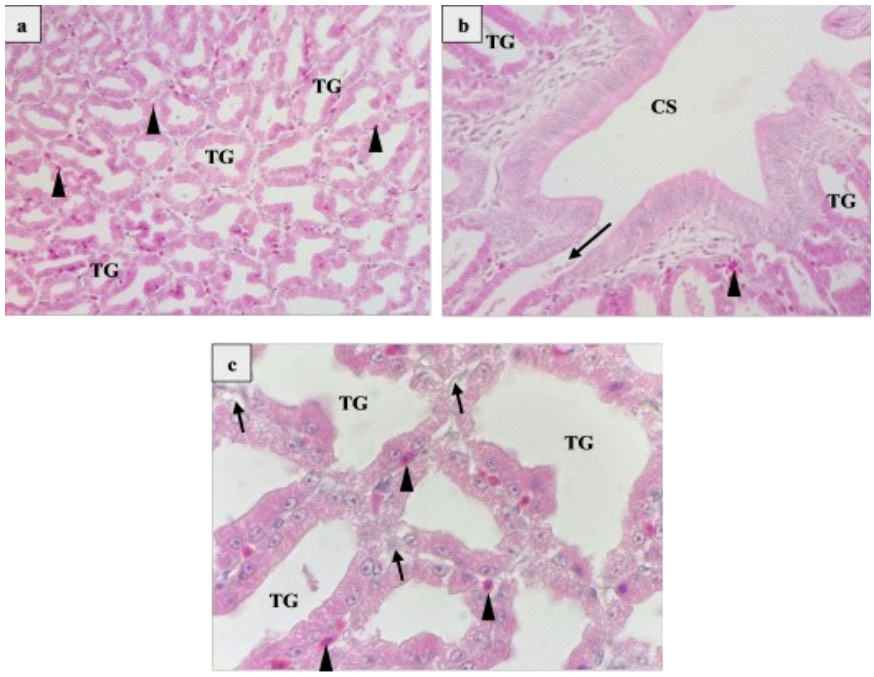
Fig 3: Light microscopic image of Chinese goose (*Anser cygnoides*) glandular stomach.



a) LE: Lamina epithelialis, LP: Lamina propria, LM: Lamina muscularis, TG: Tubular glands, Central space (CS). Hematoxylin Eosin (HE) staining, X10. b) Lamina Epithelialis (LE), Sulcus (arrow). Hematoxylin Eosin (HE) staining, X100. c) Nodular lymphoid tissue (arrow), Tubular lymphoid tissue (arrowhead), TG: Tubular glands, CT: Connective tissue. Hematoxylin Eosin (HE) staining, X10. d) LM: Lamina muscularis, TG: Tubular glands, LP: Lamina propria. Hematoxylin Eosin (HE) staining, X40.

Fig 4: Light microscopic image of Chinese goose (*Anser cygnoides*) glandular stomach.

birds, it was reported that the glandular stomach wall consisted of tunica mucosa, submucosa, tunica muscularis and tunica serosa layers. In contrast, Zhu *et al.* (2013) stated that the glandular stomach wall of Japanese grosbeak was composed of three layers: tunica mucosa, tunica muscularis and tunica serosa. In present study on the stomach of Chinese geese (*Anser cygnoides*), the results of histological finding showed that the glandular



a) Tubular glands (TG) in the submucosa, capillary blood vessels (arrowheads). Hematoxylin Eosin (HE) staining, X40. b) Central space (CS) between tubular glands in the submucosa, TG: Tubular glands, draining duct (arrow), capillary blood vessels (arrowhead). Hematoxylin Eosin (HE) staining, X40. c) TG: Tubular glands, connective tissue (arrows), capillary blood vessels (arrowhead). Hematoxylin Eosin (HE) staining, X100.

Fig 5: Light microscopic image of Chinese goose (*Anser cygnoides*) glandular stomach.



TG: Tubular glands, TM: Tunica muscularis, TS: Tunica serosa. Hematoxylin Eosin (HE) staining, X10.

Fig 6: Light microscopic image of Chinese goose (*Anser cygnoides*) glandular stomach.

stomach wall of Chinese geese (*Anser cygnoides*) consisted of four layers: tunica mucosa, submucosa, tunica muscularis and tunica serosa.

Abdel Maksoud *et al.* (2022) in hooded crows, Altaai and Al-Taai (2020) in adult starlings and Koçak and Özaydin (2019) in poultry reported that the glandular gastric mucosa has lamina epithelialis, lamina propria and lamina muscularis sublayers. In present study, it was observed that the tunica mucosa had lamina epithelialis, lamina propria and lamina muscularis sublayers.

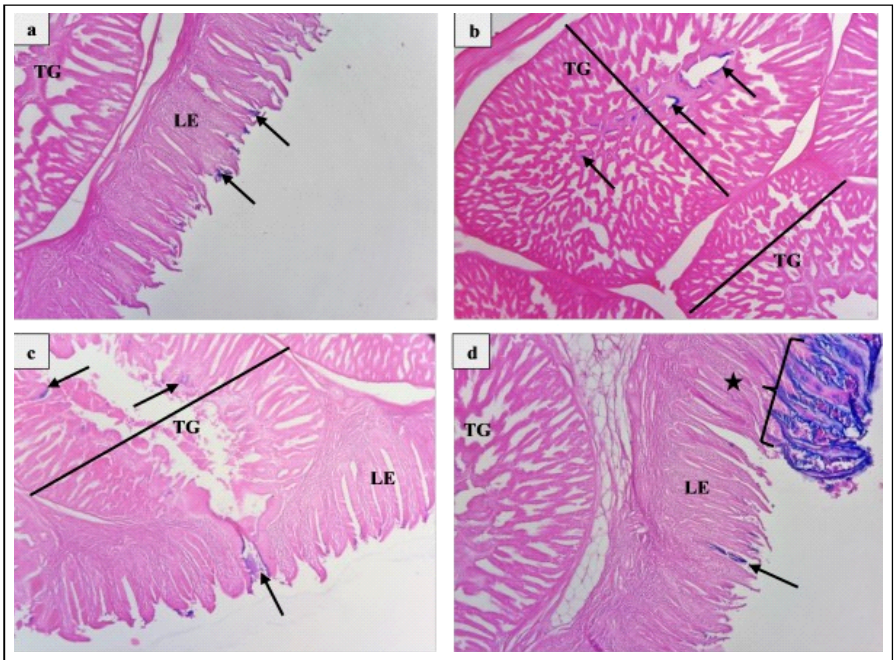
Abdel Maksoud *et al.* (2022) in the capped crow and Özer and Girgin (2016) in poultry reported the presence of single-layered columnar cells with mucus secretion in the glandular stomach lamina epithelialis. In present study, single-layer prismatic cells that secreting mucus were found in the lamina epithelialis layer.

Eidaroos *et al.* (2008) observed a thin cuticle covering the proventricular mucosal surface of some birds (geese, balady ducks, white chickens, quails and ostrich). No such cuticle was found in present study.

Abdel Maksoud *et al.* (2022) in the hooded crow and Koçak and Özaydin (2019) in poultry stated that the glandular gastric mucosa contained numerous nodular or diffuse lymphoid tissues in the lamina propria. Regarding the intraepithelial lymphocytes and lymphatic aggregations, which are particularly noticeable at the bases of mucosal folds and in the lamina propria, Salem (1997) reported that their presence may provide an immunological defense function to the avian proventriculus. Moreover, Zaher *et al.* (2012) attributed the presence of nodular and diffuse lymphatic tissues in quails to the participation of the proventriculus in the immune response.

Jackwood (2003) in chickens and Abdel Maksoud *et al.* (2022) in the hooded crow stated that the tunica submucosa of the glandular stomach consisted of proventricular glands separated by connective tissue containing blood vessels. In present study, proventricular glands, connective tissue and blood vessels were observed in the tunica submucosa of the glandular stomach.

In the studies by Ogunkoya and Cook (2009) in the Australian passerine, Kadhim *et al.* (2011) in red junglefowl, Zhu *et al.* (2013) in Japanese grosbeak, Al-Saffar and Al-Samawy (2015) in green-headed mallard and Abdel Maksoud *et al.* (2022) in the hooded crow, it was reported that the proventricular glands in the submucosa constitute the largest part of the glandular stomach wall thickness. In present study, compound, branched tubular glands in the

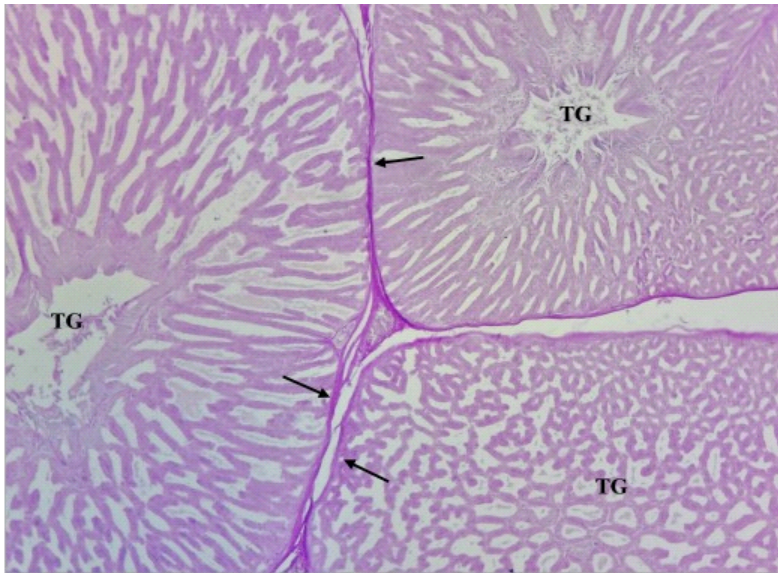


a) Tubular glands (TG), lamina epithelialis (LE), local positive reaction in the lamina epithelialis (arrows). Alcian Blue pH:2,5 staining method, X10. b) Tubular glands (TG), local positive reaction in tubular glands (arrows). Alcian Blue pH:2,5 staining method, X10. c) Tubular glands (TG), lamina epithelialis (LE), local positive reaction in tubular glands and areas where secretions are released into the lümen (arrows). Alcian Blue pH:2,5 staining method, X10. d) Tubular glands (TG), lamina epithelialis (LE), local positive reaction in the lamina epithelialis (arrow), very intense positive reaction in the lumen facing parts of the lamina epithelialis in the transitional region from the glandular stomach to the muscular stomach (star). Alcian Blue pH:2,5 staining method, X10.

Fig 7: Light microscopic image of Chinese goose (*Anser cygnoides*) glandular stomach.

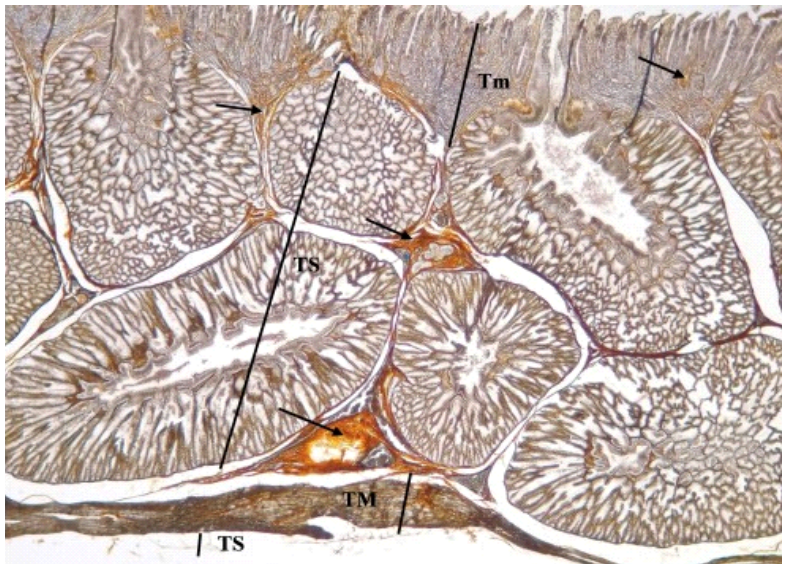
submucosa layer were observed and these glands consisted of single-layered cubic epithelial cells, constituted the largest part of the glandular stomach wall thickness.

Saffar and Al-Samawy (2015) in green-headed mallards, Sayrafi and Aghagolzadeh (2019) in starlings and Al- and Abdel Maksoud *et al*. (2022) in the hooded crow reported that the proventricular glands in the glandular stomach wall were separated from each other by a connective tissue to form lobules and each lobe is connected to the proventriculus lumen by secondary ducts. In present study, it was observed that the proventricular glands were surrounded by connective tissue and are in the form of lobules clearly separated from each other and contain a central cavity and the central cavities were connected to the lumen of the glandular stomach through draining ducts extending radially to the connective tissue between the lobules. These results were juxtaposed with those observed by Das *et al*. (2017), who noticed connective tissue septa rich in collagen and elastic fibers surrounding the glandular lobules and Sayrafi and Aghagolzadeh (2019),



Tubular glands (TG), positive reaction in the basal parts of the tubular glands (arrows). PAS staining method, X10

Fig 8: Light microscopic image of Chinese goose (*Anser cygnoides*) glandular stomach.



Tm: Tunica mucosa, TS: Tunica submucosa, TM: Tunica muscularis, TS: Tunica serosa, reticular threads (arrows). Gordon Sweeth staining method, X4.

Fig 9: Light microscopic image of Chinese goose (*Anser cygnoides*) glandular stomach.

who reported smooth muscle fibers derived from the lamina muscularis mucosa.

In the studies by Abumandour (2014) in eurasian hobby, Al-Saffar and Al-Samawy, (2015) in the green-headed mallard and Sayrafi and Aghagolzadeh (2019) in starlings, Saran and Meshram (2020) in Guinea Fowl, it was reported that the tunica muscularis layer of glandular stomach consisted of inner longitudinal and outer circular muscle layers. Kadhim *et al.* (2011) in the red junglefowl and Saran and Meshram (2021) in Guinea Fowl, stated that the tunica muscularis layer consisted of inner circular and outer longitudinal muscle layers. In present study, only circular smooth muscle fibers were observed in the tunica muscularis layer.

In the studies by Kadhim *et al.* (2011) in red junglefowls, Hassan and Moussa (2012) in domestic pigeons, Sayrafi and Aghagolzadeh (2019) in starlings and Hussein *et al.* (2020) in brown falcons, it was reported that the tunica serosa layer was the outermost. In present study, it was observed that tunica serosa layer was the outermost in the histological sections of the glandular stomach. The tunica serosa consisted of loose connective tissue covered by a layer of mesothelial cells. Rossi *et al.* (2005) in partridges reported the presence of some smooth muscle fibers this tunic. In addition, ganglionated nerves were seen in the tunica serosa of Australian songbirds (Ogunkoya and Cook, 2009) and starlings (Sayrafi and Aghagolzadeh, 2019).

Ahmed *et al.* (2011) in the Japanese quail, Hamdi *et al.* (2013) in the black-winged kite and Abdel Maksoud *et al.* (2022) in hooded crow observed positive PAS and positive AB (neutral and acidic mucopolysaccharides, respectively) reactions in the proventriculus mucosa. However, Shyla and Lucy (1992) observed only a PAS-positive reaction in the surface epithelium covering the duck proventriculus. In present study, no PAS-positive reaction was observed in the lumen-facing parts of the lamina epithelialis. However, a localized AB-positive reaction was observed in the lumen-facing parts.

Zaher *et al.* (2012) in *Coturnix coturnix*, Hamdi *et al.* (2013) in black-winged kite, Udoumoh and Ikejiobi (2017) in African pied crow (*Corvus albums*) and Abdel Maksoud *et al.* (2022) in hooded crow reported that supra-nuclear apices of submucosal glands showed intense PAS stain and negative AB stain reaction. In present study, PAS positive reaction was observed in the basal parts of submucosal glands, while AB positive reaction was observed in the apical parts of the cells lining the central cavity of tubular glands.

CONCLUSION

The proventriculus of the Chinese goose (*Anser cygnoides*) was observed to be a fusiform or spindle-shaped organ extending between the esophagus and the stomach on the median line. Scanning electron microscopic (SEM) observations revealed that the glandular stomach surface of Chinese goose (*Anser cygnoides*) contains many mucosal folds. Parallel grooves and proventricular gland openings were observed on the surface between these folds. These folds were seen to surround the orifices of the proventricular glands in a parallel manner. It was observed that the glandular stomach wall consisted of four layers: tunica mucosa, submucosa, tunica muscularis and tunica serosa. It was observed that the tunica mucosa had lamina epithelialis, lamina propria and lamina muscularis sublayers. Single-layered prismatic cells with mucus secretion were observed in the lamina epithelialis layer and these cells showed regional AB positive reaction. Compound, branched tubular glands were observed in the submucosa layer. AB positive reaction was observed regionally in the luminal parts of the tubular glands. PAS positive reaction was noted in the basal parts of the tubular glands. In the Gordon Sweeth (GS) staining method, reticular threads were easily distinguished in all areas where the connective tissue was located. These histomorphological features of the proventriculus of the Chinese goose (*Anser cygnoides*) may explain its adaptation to different feeding habits.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

Approval was obtained with the decision dated 06/01/2022 and numbered 2021/143 of Selcuk University Veterinary Faculty Experimental Animal Production and Research Center Ethics Committee (SÜVDAMEK).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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