Seasonal Variations in the Digestive Tract of the Little Owl, Athene noctua : Anatomical, Histological, and Scanning Electron Microscopical Studies

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3 authors, including:

Fatma Mahmoud
Assiut University

Mayada Youssef
Aswan University

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Seasonal Variations in the Digestive Tract of the Little Owl, *Athene noctua*: Anatomical, Histological, and Scanning Electron Microscopical Studies

Nahed A. Shawki, Fatma A. Mahmoud and Mayada Y. Mohamed*
Department of Zoology, Faculty of Sciences, Assiut University, Assiut, Egypt

Abstract

The digestive tract of the little owl, *Athene noctua* (Strigiformes: Strigidae), is described in two different seasons. The digestive tract of this bird follows the basic model for that of a predatory bird. The cervical esophagus is not expanded to form a crop. The internal surface of the esophagus forms numerous longitudinal folds provided with numerous mucous glands. These longitudinal folds increase in number and vary in depth posteriorly. The folds of the proventriculus are composed of simple branched tubular glands. The ventriculus is lined by a thin layer of koilin. The number of goblet cells gradually increases from the duodenum to the rectum, and the lymphatic tissue diffuses within the lamina propria. The esophageal glands secrete acid mucopolysaccharides, while the gastric glands of the stomach, the goblet cells, and crypts of Lieberkühn secrete acid mucopolysaccharides. Proteins were observed in the different histological structures of the digestive tract. Morphometric and histometric studies showed differences between summer and winter in the esophagus and glandular stomach (especially in winter), but no seasonal differences were seen in the muscular stomach, or small and large intestines.

Key words: digestive tract, histomorphometric, little owl, seasonal

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Introduction

Evolution has led the digestive tract of birds to feature unique anatomical and histological structures, quite different from those in other animal orders. It has evolved to take advantage of the physical and chemical characteristics of a wide variety of food types (Klasing, 1999). Avian digestive systems have a number of features that distinguish them from those of mammals, one of these being adaptation to a feeding strategy that allows for maximum ingestion of food in a short time (Cummins, 1996). Most animals face seasonal fluctuations in environmental conditions, including variations in food availability and composition. In this sense, it is expected that animals that do not migrate should exhibit reversible (seasonal) phenotypic adjustments in their physiology to face environmental variations. The morphological and physiological responses of the digestive tract are good examples of how phenotypic flexibility allows organisms to cope with dietary switching (Starck, 1999; Naya et al., 2007; Karasov et al., 2011). It is known that feeding habits, diet quality, and quantity of food influence the size of the organs within the avian digestive system as well as their morphology, histology, and content (Chikihan & De Speroni, 1996).

The little owl, *Athene noctua* (Strigiformes: Strigidae), is a territorial bird whose territory is at a maximum in the courtship season in late spring (February–March). The home range of this bird varies with the type of habitat and time of year (Staggenborg et al., 2017). Both parents are involved in hunting to feed their chicks for about 7 weeks (Lewis, 2013). Food quality and quantity requirements increase during egg incubation and to feed chicks within the winter season (Amininasab et al., 2017). Food resources may influence the body condition of individuals in ways that affect their performance in the breeding season (Harrison et al., 2011). The aim of this study was to investigate how seasonal changes in food type and availability affect the digestive tract of the little owl by examining its morphology and histological structure, as well as organ histochemistry.

Materials and Methods

Ten healthy adult little owls were trapped alive in the summer (5) and winter (5) seasons ($n = 10$; Fig. 1a). Prior to dissection, the owls were euthanized according to the protocols of the Ethical Committee of the Faculty of Pharmacy, University of Assiut.

The body length of the little owl, measured from the tip of the beak to the end of the rump, was measured with a tape measure. For gross anatomy, a longitudinal incision was made along the mid-ventral surface of the abdomen, and topographic photographs of the digestive system within the body of the animal were taken. The position and relationships of the parts of the digestive tract were examined *in situ*. The length of the digestive tract was measured with a tape measure after being removed from the body cavity. After being removed from the body cavity, the digestive tract was cleaned using saline solution and fixed in

*Corresponding author: Mayada Y. Mohamed, E-mail: hany_tlb@yahoo.com
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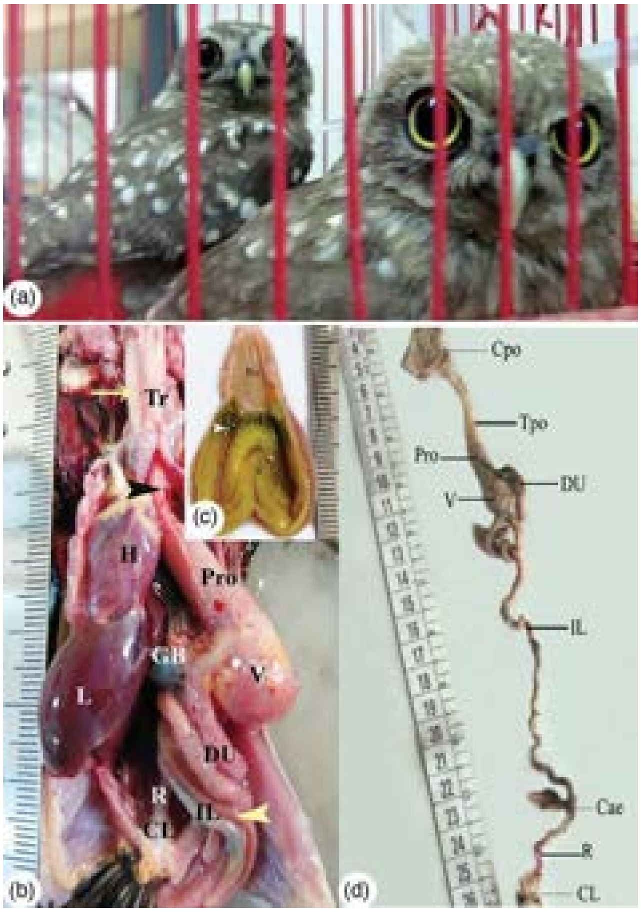

Fig. 1. (**a**) The little owl *A. noctua*, (**b**) the dissected little owl showing the digestive tract and associated organs, trachea (Tr), heart (H), liver (L), gall bladder (GB), cervical esophagus (yellow arrow), thoracic esophagus (black arrowhead), proventriculus (Pro), ventriculus (V), ileum (IL), rectum (R), cloaca (CL), and ceca (yellow arrowhead), (**c**) the stomach of the little owl showing proventriculus (Pro), ventriculus (V), and koilin membrane (arrowhead), (**d**) an isolated digestive tract of the little owl showing the cervical portion of the eosphagus (Cpo), the thoracic portion (Tpo), proventriculus (Pro), ventriculus (V), duodenum (DU), ileum (IL), intestinal ceca (Cae), rectum (R), and cloaca (CL).

10% neutral buffered formalin. The lengths of the digestive tract and its segments were measured, with measurements for each season were kept separately.

For scanning electron microscopy (SEM) investigations of the digestive tract, fine sections were prepared, and then directly fixed in 5% glutaraldehyde in cacodylate buffer for at least 48 h. The specimens were mounted, sputter-coated with gold, and examined under a Jeol SEM (JSM-5400 LV) at 15 kV (Yamauchi et al., 1990; Maneewan & Yamauchi, 2003).

For the histological study, the contents of the digestive tract were first rinsed out using saline solution, and then small pieces of the various segments were fixed in neutral formalin solution. After fixation, the sections were dehydrated, cleared with methyl benzoate, embedded in paraffin wax, and then transversely sectioned to a thickness of 6 μm. Sections were differentially double-stained with hematoxylin–eosin and Masson's trichrome stain (Drury & Wallington, 1980).

For the histochemical detection of mucopolysaccharides and proteins, the following staining procedures were employed: periodic acid–Schiff (PAS) stain and Alcian blue (pH 2.5) stains for neutral and acidic mucosubstances, respectively; and mercuric bromophenol blue (MBB) stain for proteins (Bancroft & Stevens, 1996).

For histomorphometric analysis in the two different seasons, the thickness of tissue layers in the esophagus, proventriculus, ventriculus, duodenum, ileum, ceca, rectum, and cloaca were measured with light microscope model Olympus BX43 (software: cell sense ver1.18) attached with camera model Olympus DP74, and the results were recorded as mean ± SE for each digestive tract part.

Statistical analysis was done using Statistical Package for Social Science system version 23. The significance of differences in means between seasons was determined using the Student *t*-test, with significance accepted at $p < 0.05$.

Results

The digestive tract of the little owl consists of the esophagus, proventriculus, ventriculus (gizzard), small intestine (duodenum, jejunum, and ileum), large intestine (paired elongated ceca and rectum), and cloaca or colorectum, which opens to the outside by the cloacal opening (vent) (Figs. 1b, 1d, 2a–2c).

Histologically, the wall of the digestive tract is made up of a four-layered functional tunica: mucosa, submucosa, musculosa, and the outermost serosa. The muscularis mucosa is present in some parts of the digestive tract and absent in others.

In summer, the average length of the little owl body was 17.5 ± 1.48 cm, and the average length of the digestive tract was 33. 5 ± 5.196 cm. In winter, body and digestive tract lengths were 17.3 ± 0.68 and 35.67 ± 1.97 cm, respectively (Fig. 2d).

The Esophagus

The esophagus lies dorsal to the trachea in the anterior region of the neck and runs posteriorly along its right side. The esophagus is divided into the considerably larger cervical part and the smaller thoracic part. None of the specimens investigated showed any evidence of a crop-like dilation (Figs. 1b, 1d, 2a, 2b).

The SEM investigation of the interior surface of both regions of the esophagus showed the presence of closely adjacent longitudinal folds, with detached cells, as well as numerous orifices of the esophageal glands (Figs. 3a, 3b, 5a, 5b).

Histologically, the esophageal folds appeared as leaf-like structures lined by a non-keratinized stratified squamous epithelium and incubate compound tubulo-alveolar glands (esophageal glands). The lamina propria consisted of dense collagenous connective tissue containing the esophageal glands, and lymph and blood vessels. The muscularis mucosa is located between the esophageal glands, the mucosa, and submucosa. The muscularis externa consisted of inner longitudinal and outer circular muscle fibers separated by connective tissue. The serosa is composed of a thin layer of fibrous connective tissue containing blood vessels and nerve endings (Figs. 3c–3f, 5d–5g).

Histochemically, the esophageal glands are composed of mucoid alveoli. These glands exhibited a strong PAS reaction and marked Alcianophilia at pH 2.5 (Figs. 4a–4c; Table 1). Moreover, the esophagus exhibited the presence of an exaggerated amount of protein elements after staining with MBB, with the protein appearing to fill the cytoplasm of the stratified squamous epithelium, the muscular layer, and the lamina propria. On the other hand, the esophageal glands showed a negative response to MBB (Fig. 4d; Table 1).

Morphometrically, in summer, the average length of the esophagus is 4.58 ± 0.66 cm, while in winter it was 5.17 ± 0.68 cm (Fig. 2d). The histomorphometric study of the layers of

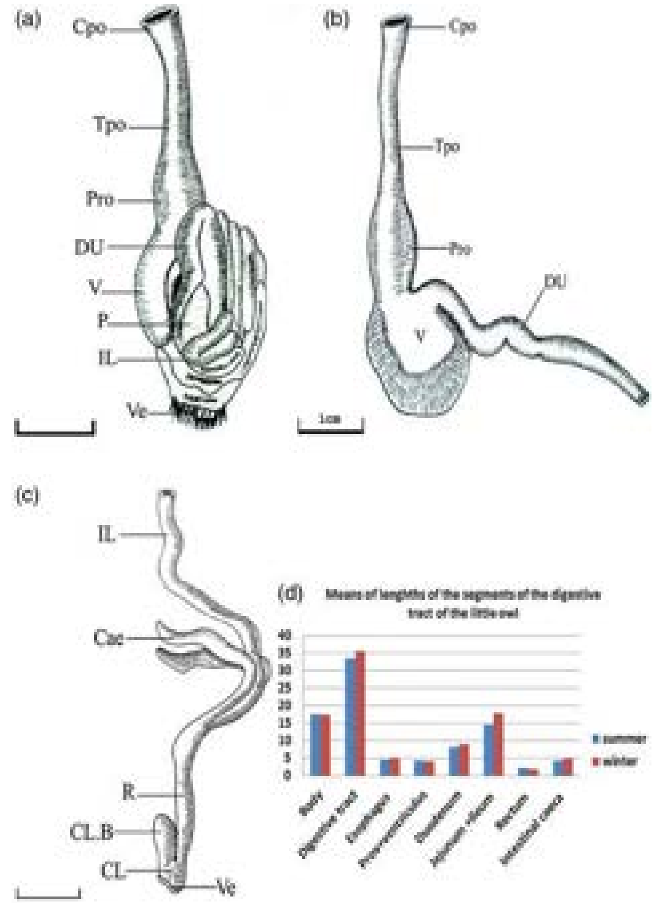

Fig. 2. (**a**) Anatomical drawing of an isolated digestive tract of the little owl showing the cervical portion of the esophagus (Cpo), the thoracic portion of esophagus (Tpo), proventriculus (Pro), ventriculus (V), duodenum (DU), pancrease (P), the spiral coils of the ileum (IL), and vent (Ve), (**b**) anatomical drawing of the digestive tract, cervical portion of the esophagus (Cpo), the thoracic portion of the esophagus (Tpo), proventriculus (Pro), ventriculus (V), and duodenum (DU), (**c**) anatomical drawing of the digestive tract of the little owl showing ileum (IL), the elongated ceca (Cae), rectum (R), cloaca (CL), cloaca bursa (CL.B), and vent (Ve), (**d**) comparison between lengths (cm) of body, digestive tract, esophagus, prov. + ventriculus, duodenum, jejunum + ileum, rectum, and intestinal ceca of the little owl in summer and winter.

the cervical region of the esophagus showed that the average thickness of the muscularis was 480.72 ± 140.25 μm in summer and 770.77 ± 180.62 μm in winter. The average thickness of the mucosa (with esophageal glands) was 540.4 ± 38.78 μm in summer and 855.38 ± 44.99 μm in winter. The average thickness of the epithelium was 115.61 ± 38.04 μm in summer and 171.69 ± 28.04 μm in winter. The thicknesses of the muscularis, mucosa, and epithelium layers were significantly different in summer compared to winter ($T = -3.11$, $p = 0.011$; $T = -12.99$, $p = 0.001$; and $T = -2.907$, $p = 0.016$, respectively; Fig. 4e).

In the thoracic region of the esophagus, the average thickness of the muscularis was 537.21 ± 283.84 μm in summer and 644.78 ± 135.75 μm in winter. The average thickness of the mucosa (with esophageal glands) was 779.8 ± 140.93 μm in summer and 738.73 ± 200.29 μm in winter. The average thickness of the epithelium in summer was 123.76 ± 38.04 μm and in winter 132.21 ± 28.04 μm. The thicknesses of the muscularis, mucosa, and epithelium layers were not significantly different in summer compared to winter ($T = -0.837$, $p = 0.43$; $T = 0.411$, $p = 0.69$; and $T = -0.378$, $p = 0.71$, respectively; Fig. 5c).

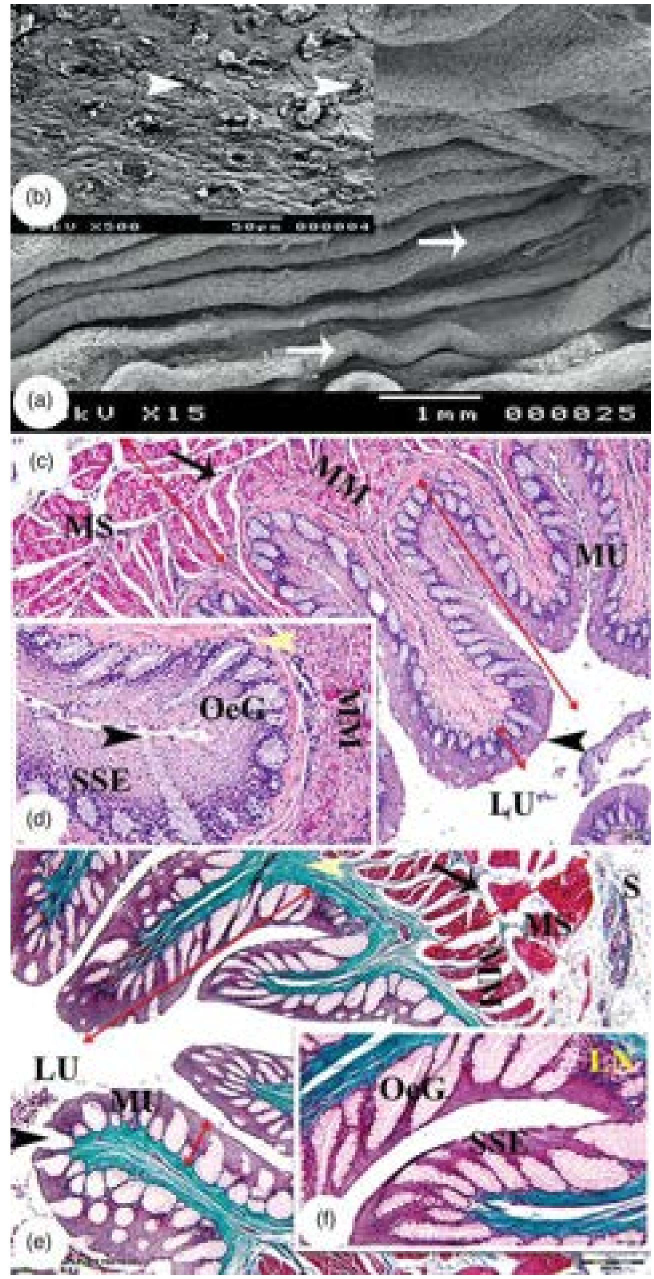

Fig. 3. (**a**) Scanning electron micrograph of the cervical esophagus of the little owl showing the closely adjacent longitudinal folds (arrow), (**b**) high magnification of figure (**a**) showing the orifices of the esophageal glands with secretions (arrowheads), (**c**) transverse section of the cervical esophagus in the summer season showing the tunicae: mucosa (MU) with esophageal glands with numerous openings (arrowhead) into the lumen of the esophagus (LU), submucosa (arrow), musculosa (MS), and muscularis mucosa (MM) (H&E stain), (**d**) high magnification of figure (**c**) showing the numerous esophageal glands (OeG) with the openings (black arrowhead), non-keratinized stratified squamous epithelium (SSE), muscularis mucosa (MM), and the lamina propria (yellow arrowhead), (**e**) transverse section of the cervical esophagus in the winter season showing the tunicae: mucosa (MU) with esophageal glands with numerous openings (arrowhead) into the lumen of the esophagus (LU), submucosa (arrow), musculosa (MS), muscularis mucosa (MM), lamina propria (yellow arrowhead), and serosa (S) (trichrome stain), (**f**) high magnification of figure (**e**), showing the numerous esophageal glands (OeG), non-keratinized stratified squamous epithelium (SSE), and the lymph nodules (LN).

The Stomach

The stomach is formed of two distinct parts, the glandular portion (pars glandularis), which is posterior to the esophagus, and the

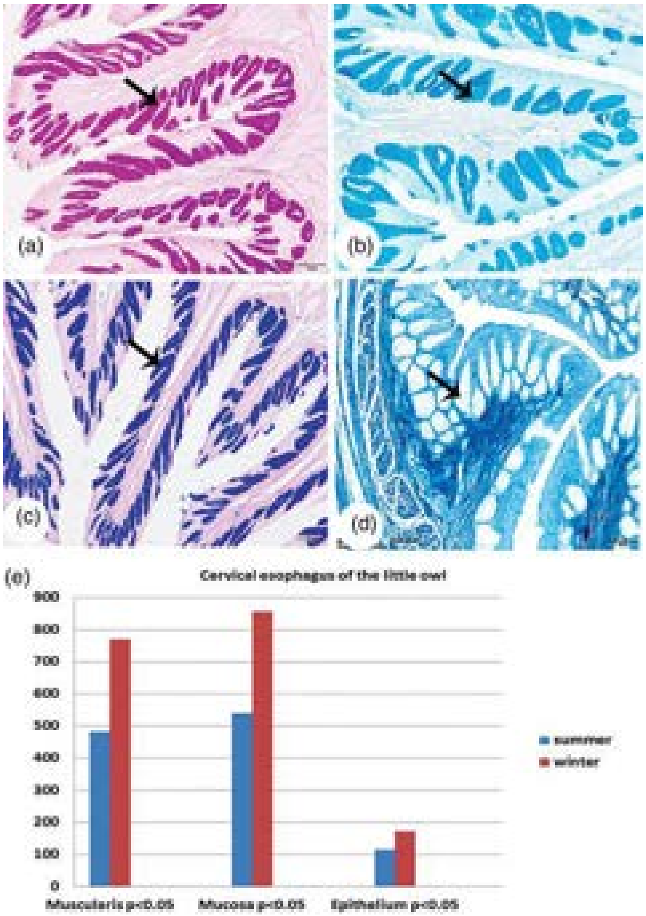

Fig. 4. (**a**) Transverse section of the esophagus of the little owl, showing the carbohydrate contents in the esophageal glands (arrow) (PAS-positive stain), (**b**) transverse section of the esophagus, showing the contents of acid mucopolysaccharides in the esophageal glands (arrow) (Alcian blue stain), (**c**) transverse section of the esophagus showing acid and neutral mucopolysaccharides in the esophageal glands (arrow) (PAS–Alcian blue stain), (**d**) transverse section of the esophagus showing the protein contents and the negative reaction of the esophageal gland (arrow) (Bromophenol blue stain), (**e**) a comparison between means of thickness (μm) of muscular layer, mucosa, and the epithelium forming the mucosa of the cervical region of the esophagus of the little owl in summer and winter showing significant differences ($p < 0.05$).

muscular portion or gizzard (pars muscularis), which is located posterior to the proventriculus. Since a proventricular–ventricular isthmus was not present, the two parts formed one large pear-shaped organ (Figs. 1b–1d, 2a, 2b). The ventriculus (gizzard) is lined by koilin, a cuticle layer that appears green or yellow in color (Fig. 1c).

SEM investigation of the interior surface of the proventriculus indicated the presence of papilla-like structures forming the proventricular glands with dorsally located ducts (Fig. 6a), while the investigation of the gizzard mucosa exposed a continuous layer of the gastric tubular glands (Fig. 7). All the glands appear to be virtually identical, with koilin membrane on its surface in patches (Figs. 8a, 8b).

Histologically, the glandular stomach wall is formed of the serosa, musculosa, submucosa, muscularis mucosa, and mucosa. The serosa is made up of a layer consisting of loose connective tissue. The musculosa consisted of a thick layer of circular muscle fibers and an interior layer of longitudinal muscle fibers. The submucosa is a thin loose areolar connective tissue containing a number of fine blood capillaries and nerve endings (Figs. 6b–6e).

The mucosal layer is formed of dense connective tissue that extended to hold two types of gastric glands: the deep and superficial gastric glands. The deep gastric glands are of a compound-branched alveolar type lined with simple cuboidal epithelial cells. The superficial glands are of a simple tubular type and appear in the form of numerous folds of mucosal epithelium. Within the proventricular gland capsule, septa of thin connective tissue are present (Figs. 6b–6e).

The muscularis mucosa extends to the lamina propria and is composed of smooth muscle fibers manifesting a network appearance surrounding the bodies of the deep gastric glands which composed of branched tubular glands consisting of cuboid epithelial cells. All of the branched tubular glands open into the main unique tubule also lined by cuboid cells, which leads to the proventricular luminal surface (Figs. 6b–6e).

The mucous membrane of the ventriculus is thrown into well-developed narrow and deep folds, lined with a thin tough keratin-like layer known as the cutica gastrica (koilin). The mucosa of the ventriculus of the stomach has compound tubular epithelial cells with basal nuclei these glands secrete the koilin membrane as vertical rods and horizontal layers. In the lamina, propria are the simple tubular glands whose glandular cells are similar to those of the superficial lining epithelium. The submucosa is a thin layer of loose connective tissue containing a number of blood vessels. The musculosa consists of smooth muscle fibers, an inner thick circular muscle layer, an outer thin longitudinal muscle layer, and oblique muscle fibers between them (Figs. 8c, 8d, 9a).

Histochemically, the ductular cells in the proventriculus and the glandular tubules of the ventriculus showed a strongly positive PAS reaction (Figs. 7a, 9b; Table 1) and strong reaction (blue color) with Alcian blue at pH 2.5. In addition, the cells of the surface epithelium of the tubular glands and the epithelium of the crypts exhibited a strong PAS reaction. The secretory materials within the lumen of the glandular tubules showed a moderate reaction with Alcian blue at pH 2.5 (Figs. 7b, 9c; Table 1).

The ductular cells of the proventriculus gave a purple color with the Alcian blue–PAS stain (Fig. 7c), while the tubular glands in the ventriculus showed a strong affinity to Alcian blue and were negative to PAS. The secretory materials within the lumen of the glandular tubules exhibited a moderate reaction with Alcian blue and PAS reaction (purple color) (Fig. 9d; Table 1).

The ductular cells of the proventriculus also showed a moderate reaction with MBB, while the cytoplasm of the simple cuboidal epithelial cells that lined the deep gastric gland, the muscularis, and the connective tissue of the lamina propria stained deeply (Fig. 7d; Table 1). In the ventriculus, the cells of the tubular glands and crypts showed a strong response to MBB (Fig. 9e; Table 1).

Morphometrically, the average length of the stomach was 4.25 ± 0.42 cm in summer and 4.12 ± 0.52 cm in winter (Fig. 2d).

The histomorphometric study of the layers of the proventriculus showed that the average thickness of the muscularis was 263.03 ± 60.49 μm in summer and 512.81 ± 25.97 μm in winter, the average length of the deep gastric gland was 421.95 ± 33.35 μm in summer and 347.73 ± 44.47 μm in winter, the average length of the superficial gland was 403.89 ± 27.37 μm in summer and 152.44 ± 64.66 μm in winter, and the average thickness of the epithelium was 17.51 ± 4.82 μm in summer and 26 ± 7.18 μm in winter.

The thicknesses and lengths of the layers of the muscularis, superficial gland, and epithelium differed significantly in summer compared to winter ($T = -9.29$, $p = 0.000$; $T = 4.71$, $p = 0.005$; and

Table 1. Histochemical Reaction.

Histochemical reaction				
Glands	PAS	PAS+AB (pH 2.5)	AB (pH2.5)	MBB
The esophageal glands	+	+	+	-
Ductular cells of proventriculus	+	+	+	±
the tubular glands of ventriculus	+	+	+	+
the goblet cells of the small intestine	+	+	+	-
the goblet cells of the rectum	+	+	+	-
the goblet cells of intestinal caeca	+	+	+	-

PAS, periodic acid–Schiff reagent; AB, Alcian blue at PH 2.5; MBB, mercuric bromophenol blue; +, strong positive reaction; −, negative reaction; ±, moderate reaction.

$T = 7.42$, $p = 0.001$, respectively), but did not differ in the length of the deep gastric gland ($T = 0.000$, $p = 1.0$) (Fig. 7e).

The histomorphometric study of the layers of the anterior portion of the ventriculus showed that the average length of the tubular gastric gland was 216.94 ± 150.50 μm in summer and 359.51 ± 92.29 μm in winter, the average thickness of the koilin membrane was 498.89 ± 174.95 μm in summer and 374.79 ± 136.33 μm in winter, and the average thickness of the epithelium was 17.25 ± 3.17 μm in summer and 21.77 ± 4.72 μm in winter. The differences in lengths and thicknesses between the tubular gastric gland, koilin, and epithelium were not significant in summer compared to winter ($T = -1.978$, $p = 0.08$; $T = 1.37$, $p = 0.21$; and $T = -1.95$, $p = 0.08$, respectively) (Fig. 10a).

In the posterior portion of the ventriculus, the average length of the tubular gastric gland was 315.54 ± 61.48 μm in summer and 359.7 ± 83.5 μm in winter, the average thickness of the koilin membrane was 401.25 ± 184.78 μm in summer and 441.47 ± 145.15 μm in winter, and the average thickness of the epithelium was 14.21 ± 4.04 μm in summer and 24.68 ± 4.41 μm in winter. The differences in height and thickness between layers in the tubular gastric gland and the koilin membrane were not significant in summer compared to winter ($T = -1.04$, $p = 0.32$; $T = -0.42$, $p = 0.68$), but the difference in the thickness of the epithelial layers was significant in summer compared to winter ($T = -4.29$, $p = 0.002$) (Fig. 10b).

The Small Intestine

The small intestine consists of the duodenum, jejunum, and ileum (Figs. 1b, 1d, 2a–2c). The duodenum is a relatively long "U"-shaped loop (Fig. 2a), while the jejunum and ileum are formed of cone-shaped spirals of coils (Fig. 2a). There are no sharp separations between the duodenum and ileum other than slight differences in their diameter (Figs. 1b, 1d, 2a, 2b); in color, the duodenum is yellow, while the ileum is dark green (Fig. 1b).

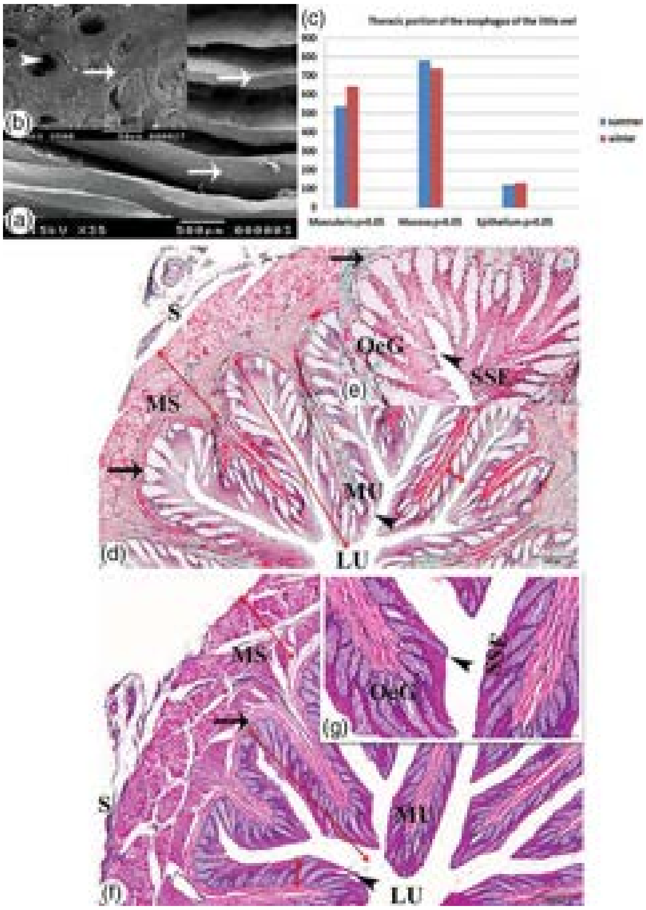

Fig. 5. (**a**) Scanning electron micrograph of the thoracic portion of the esophagus showing the spaced out longitudinal folds (arrows), (**b**) high magnification of figure (**a**), showing numerous openings of the esophageal glands (arrowhead) and the detached epithelium (arrow), (**c**) a comparison between means of thickness (μm) of muscular, mucosa layers, and the epithelium of the thoracic region of the esophagus in summer and winter showing no significant differences ($p > 0.05$). (**d**) Transverse section of the thoracic esophagus in the summer season showing the tunicae: mucosa (MU), esophageal gland with opening (arrowhead), lamina propria (arrow), musculosa (MS), and serosa (S). Trichrome stain, (**e**) high magnification of figure (**d**) showing esophageal glands (OeG) with opening (arrowhead), non-keratinized stratified squamous epithelium (SSE), and lamina propria (arrow). (**f**) Transverse section of the thoracic esophagus in the winter season showing, the tunicae: mucosa (MU), esophageal gland with opening (arrowhead), lamina propria (arrow), musculosa (MS), and serosa (S). H&E stain, (**g**) high magnification of figure (**f**) showing esophageal glands (OeG) with opening (arrowhead), non-keratinized stratified squamous epithelium (SSE).

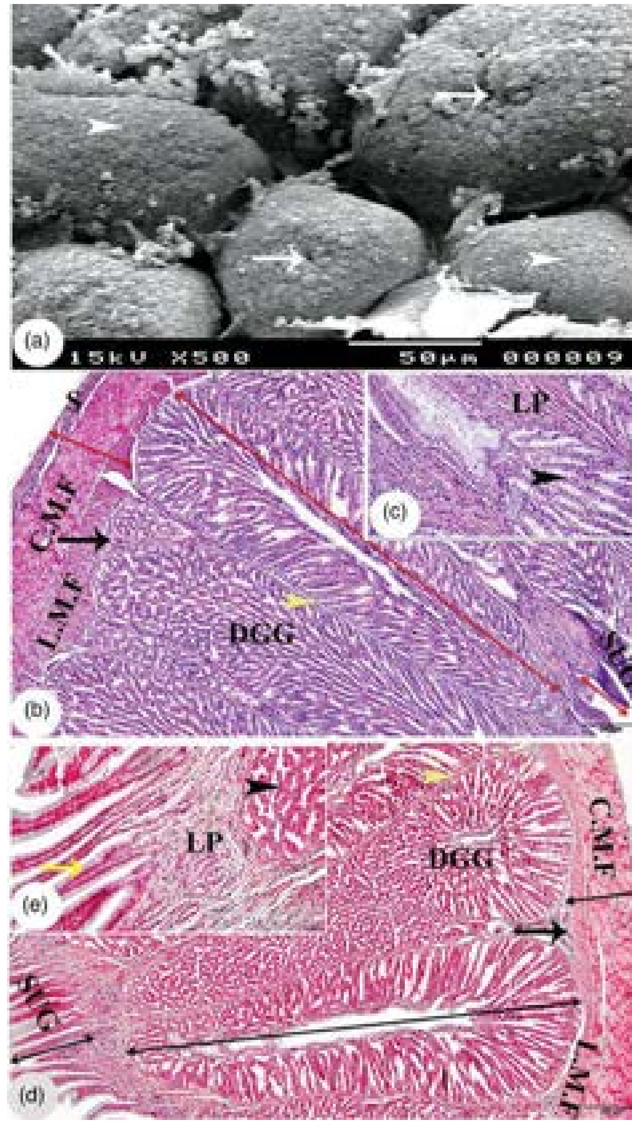

Fig. 6. (**a**) Scanning electron micrograph of the interior surface of the proventriculus of the little owl showing the papilla-like structures of the proventricular glands (arrowheads) and the openings of these glands (arrows), (**b**) transverse section of the proventriculus in the summer season showing tunicae: serosa (S), the circular muscle fibers (C.M.F), longitudinal muscle fibers (L.M.F), submucosa (arrow), the deep gastric gland (DGG), the gland capsule (yellow arrowhead), and superficial gland (SUG), H&E stain, (**c**) high magnification of figure (**b**) showing the branched tubular glands of the deep gastric gland (arrowhead), and the lamina propria (LP), (**d**) transverse section of the proventriculus in the winter season showing tunicae: serosa (S), the circular muscle fibers (C.M.F), longitudinal muscle fibers (L.M.F), submucosa (arrow), the deep gastric gland (DGG), the gland capsule (yellow arrowhead), and superficial gland (SUG), trichrome stain, (**e**) high magnification of figure (**d**) showing the branched tubular glands of the deep gastric gland (arrowhead), the lamina propria (LP), and the epithelium forming the superficial gland (yellow arrow).

SEM observations revealed that the duodenal villi were leaf-like in shape (Fig. 11a, 12), while the ileal villi were finger-like and a little compressed on both sides (Fig. 13a).

Histologically, the small intestine exhibited the usual elements of the tunica: mucosa, submucosa, musculosa, and serosa. The mucosa of the intestine is thrown into simple longitudinal villi. These are deep, narrow, and numerous in the duodenum forming a zigzag shape in the transverse section (Figs. 11b, 11c), while in the ileum, the villi are elongated finger-like and less numerous (Figs. 13b, 13c). The epithelial lining of both villi and crypts is represented by a single layer of tall columnar cells interspersed with goblet cells more numerous in the ileum than in the duodenum. The lamina propria consists of loosely packed connective tissue containing blood vessels and muscle fibers. The muscularis mucosa in the duodenum and ileum consists of longitudinal and circular muscle fibers, and the submucosa has a thin layer of loose connective tissue possessing many blood vessels. The tunica muscularis is constructed of a thin outer longitudinal layer and a thick inner circular layer. Between these muscle bundles lies a fine dispersed narrow connective tissue layer containing many large blood vessels. The tunica serosa is formed from a layer of simple squamous epithelium under which a thin layer of loose connective is present (Figs. 11b, 11c, 13b, 13c).

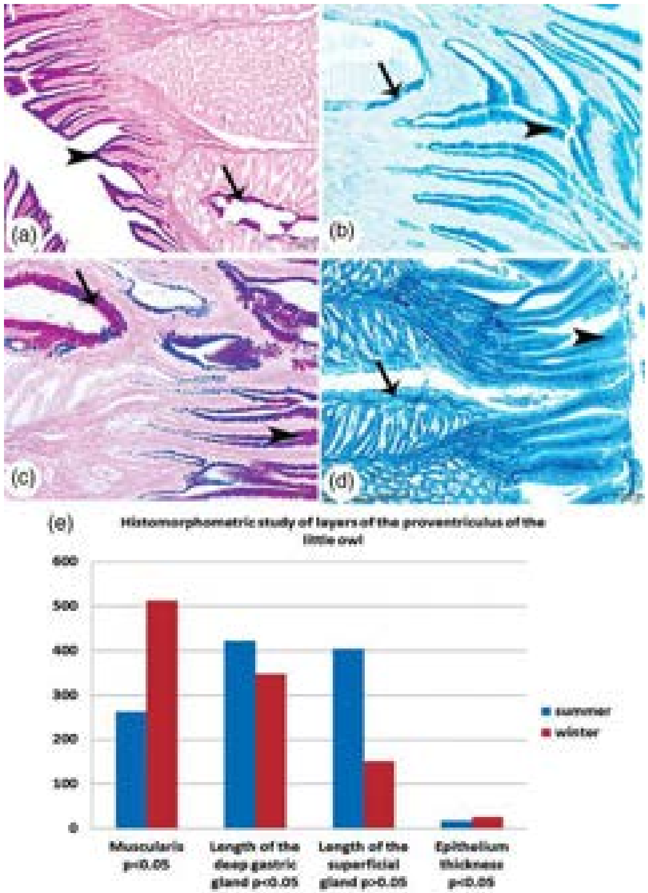

Fig. 7. (**a**) Transverse section of the proventriculus of the little owl showing the carbohydrate contents in the deep gastric glands (arrow) and the superficial glands (arrowhead) (PAS stain), (**b**) transverse section of the proventriculus, showing the acid mucopolysaccharides contents (Alcian blue stain), (**c**) transverse section of the proventriculus, showing acid and neutral mucopolysaccharides (PAS–Alcian blue), (**d**) transverse section of the proventriculus, showing the protein contents (bromophenol blue stain), (**e**) a comparison between the means of thickness (μm) of muscular layer, superficial gland, lumen of the deep gastric gland, and the epithelium forming the superficial glands of the little owl in summer and winter showing significant differences ($p < 0.05$) while the lumen of glands was not significant ($p > 0.05$).

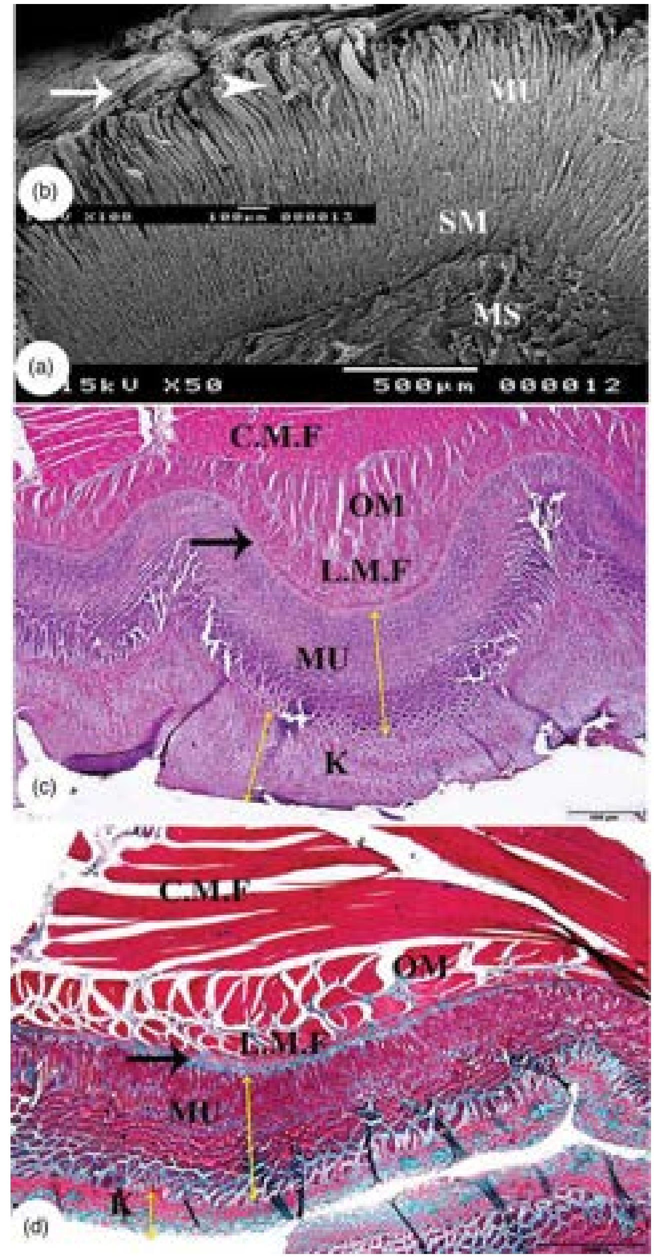

Fig. 8. (**a**) Scanning electron micrograph of the cross-section of the ventriculus of the little owl showing the muscularis (MS), submucosa (SM), and mucosa (MU), (**b**) high magnification of figure (**a**), showing the gastric tubular glands (arrowhead), and koilin membrane (arrow), (**c**) transverse section of the ventriculus of the little owl in the summer season showing the mucosa (MU), koilin membrane (K), submucosa (arrow), musculosa consisting of circular muscle fibers (C.M.F), longitudinal muscle fibers (L.M.F), and oblique muscle fibers (OM). H&E stain, (**d**) transverse section of the ventriculus of the little owl in the winter season showing the mucosa (MU), koilin membrane (K), submucosa (arrow), musculosa consisting of circular muscle fibers (C.M.F), longitudinal muscle fibers (L.M.F), and oblique muscle fibers (OM). Trichrome stain.

Histochemically, the villi in the small intestine, which possess goblet cells exhibited strong reactions to PAS (Figs. 12a, 14a; Table 1), Alcian blue (pH 2.5)–PAS (Figs. 12c, 14c; Table 1), and Alcian blue (pH 2.5) (Figs. 12b, 14b; Table 1), while showing negative reaction with MBB (Figs. 12d, 14d; Table 1).

Morphometrically, the average length of the duodenum was 8.17 ± 1.13 cm in summer and 9 ± 1.82 cm in winter (Fig. 2d), while the average length of the jejunum-ileum was 14.67 ± 2.34 cm in summer and 17.83 ± 2.73 cm in winter (Fig. 2d).

The histomorphometric study of layers in the duodenum showed that the average thickness of the muscularis was $94.04 \pm 10.15\ \mu$m in summer and $151.74 \pm 80.71\ \mu$m in winter, the average height of the mucosa (villi+ Lieberkühn) was $541.002 \pm 242.45\ \mu$m and $406.57 \pm 76.31\ \mu$m in winter, and the average thickness of the epithelium was $33.95 \pm 10.97\ \mu$m in summer and $29.72 \pm 4.21\ \mu$m in winter. The differences in thicknesses of the muscularis, mucosa, and epithelium layers were not significantly different in summer compared to winter ($T = -1.74$, $p = 0.14$; $T = 1.296$, $p = 0.24$; and $T = 0.884$, $p = 0.41$, respectively) (Fig. 12e).

The histomorphometric study of the layers of the ileum showed that the average thickness of the muscularis was $87.85 \pm 23.24\ \mu$m in summer and $69.82 \pm 11.71\ \mu$m in winter, the average height of the mucosa (villi+ Lieberkühn) was $474.59 \pm 190.91\ \mu$m in summer and $221.73 \pm 107.78\ \mu$m in winter, and the average thickness of the epithelium was $28.91 \pm 5.92\ \mu$m in summer and $22.74 \pm 2.61\ \mu$m in winter. The thicknesses of the

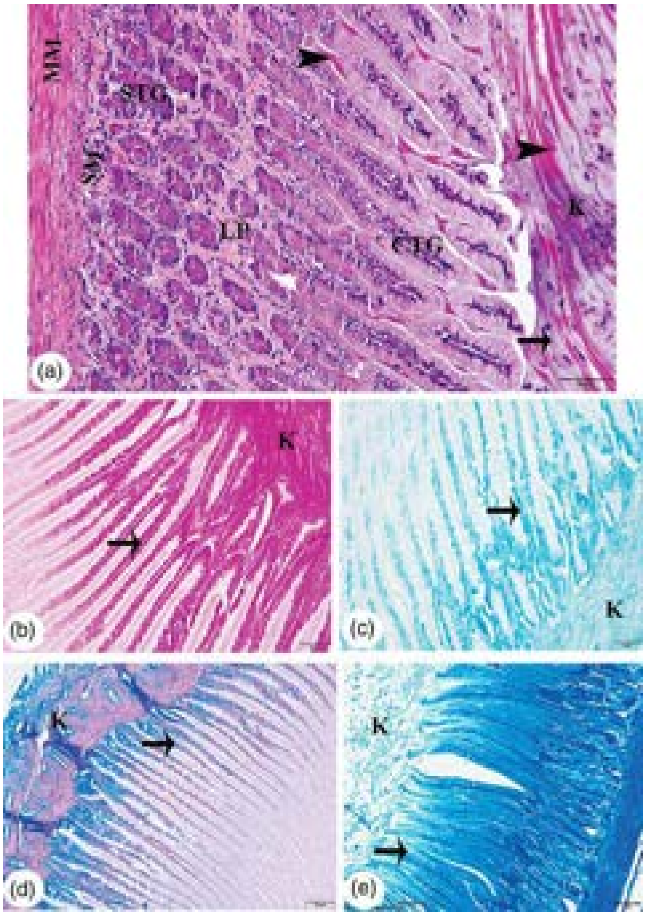

Fig. 9. (**a**) Transverse section of the ventriculus showing muscularis mucosa (MM), submucosa (SM), lamina propria (LP), simple tubular glands (STG), compound tubular glands (CTG), the horizontal matrix of koilin layer (arrow), the vertical rods of the koilin layer (arrowheads). H&E stain, (**b**) transverse section of the ventriculus of the little owl showing the concentration of carbohydrates in the compound tubular glands (arrow), and in the koilin membrane (K) (PAS-positive stain), (**c**) transverse section of the ventriculus showing concentration of acid mucopolysaccharides in the compound tubular glands (arrow) and in the koilin membrane (K) (Alcian blue stain), (d) transverse section of the ventriculus showing the mucopolysaccharide concentration in the surface epithelium of the compound tubular glands (arrow), and in the koilin membrane (K), (PAS–Alcian blue stain), and (e) transverse section of the ventriculus showing the protein content in the surface epithelium of the compound tubular glands (arrow), and in the koilin membrane (K) (bromophenol blue stain).

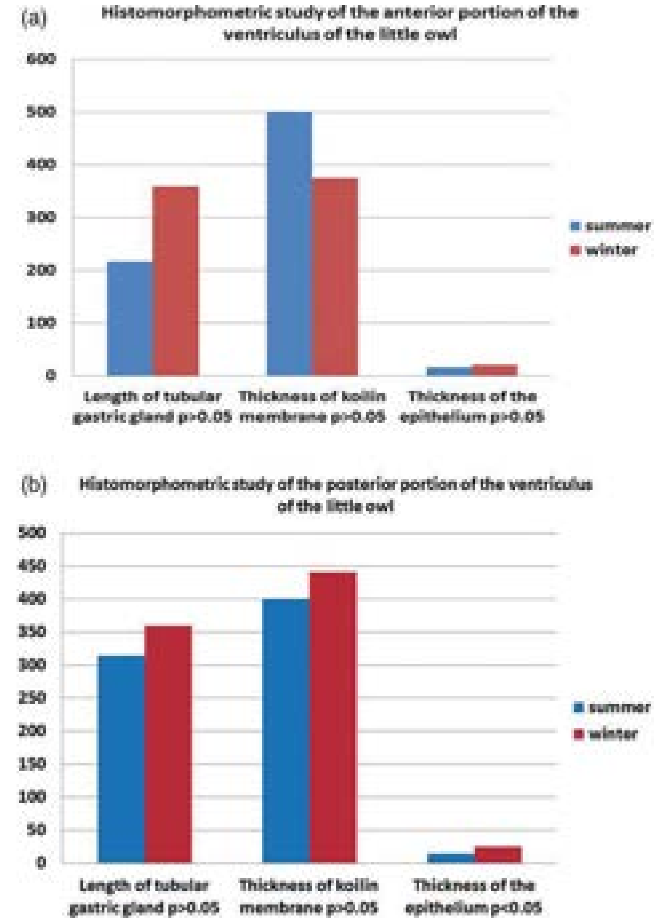

Fig. 10. (**a**) A comparison between means of thickness (μm) of the gastric gland, koilin membrane, and epithelium of the anterior portion of the gizzard of the little owl in summer and winter showing no significant differences ($p > 0.05$), (**b**) a comparison between means of the thickness (μm) of the gastric gland, koilin membrane, and epithelium of the posterior portion of the gizzard in summer and winter showing no significant differences ($p > 0.05$) except for the epithelium was significant ($p < 0.05$).

layers in the muscularis and epithelium were not differ significantly between summer and winter ($T = 1.697$, $p = 0.12$; $T = 2.334$, $p = 0.06$), but there was significant difference in height and thickness of the mucosa between summer and winter ($T = 2.825$, $p = 0.02$, respectively) (Fig. 14e).

The Large Intestine

The large intestine consists of the rectum, intestinal ceca, and cloaca. The rectum is a short straight tube initiating at the end of the small intestine and opening distally at the cloaca (Figs. 1b, 1d, 2c). SEM investigation indicated that the mucous membrane of the rectum internally is thrown into numerous shallow longitudinal folds (Fig. 15a).

Histologically, the wall of the rectum consists of tunicae: mucosa, submucosa, muscularis mucosa, muscularis, and serosa. Mucosa consists of numerous leaf-like villi and numerous rectal glands in the base of these villi. The surface epithelial lining of these villi consists of simple columnar cells. The columnar cells tend to be obscured by the large number of goblet cells. The lamina propria consists of loose connective tissue fibers and is infiltrated by large numbers of lymphocytes. The muscularis mucosa of the rectum consists of a thin layer of muscle that extends to the core of villus in the mucosal folds. The tunica muscularis consists of an inner circular layer, which is well developed, and a thin outer longitudinal layer (Figs. 15b, 15c).

Histochemically, the goblet cells in the surface mucous epithelium reacted strongly to PAS (Fig. 16a; Table 1). A strong bluish coloration was observed in the goblet cells, and a reddish coloration in their basal regions with Alcian blue (pH 2.5)–PAS (Fig. 16c; Table 1). A strong bluish coloration was observed in the goblet cells with Alcian blue (pH 2.5) (Fig. 16b; Table 1). The application of MBB indicated the absence of protein in the goblet cells and localized protein in the absorptive cells of the mucosal folds (Fig. 16d; Table 1).

Morphometrically, the average length of the rectum was 2.25 ± 0.99 cm in summer and 1.83 ± 0.93 cm in winter (Fig. 2d).

The histomorphometric study of the layers of the rectum showed that the average thickness of the muscularis was 266.86 ± 98.22 μm in summer and 332.82 ± 215.38 μm in winter, the

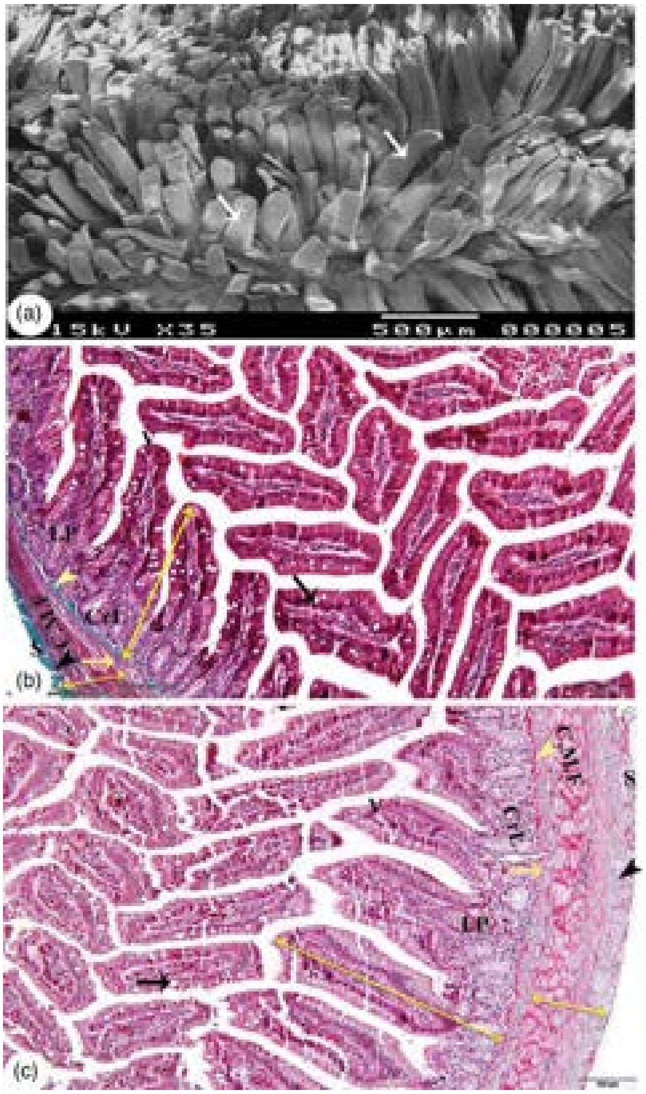

Fig. 11. (a) Scanning electron micrograph of the interior surface of the duodenum of the little owl showing the numerous leaf-like villi (arrows), (b) transverse section of the duodenum of the little owl in the summer season showing the serosa (S), longitudinal muscle fibers (black arrowhead), circular muscle fibers (C.M.F), muscularis mucosa (yellow arrow), submucosa (yellow arrowhead), crypts of leiberkühin (CrL), lamina propria (LP), villi (V), and goblet cells (black arrow) (trichrome stain), (c) transverse section of the duodenum showing high concentration of carbohydrates in the goblet cells (arrows) (PAS stain), (c) transverse section of the duodenum of the little owl in winter season showing the serosa (S), longitudinal muscle fibers (black arrowhead), circular muscle fibers (C.M.F), muscularis mucosa (yellow arrow), submucosa (yellow arrowhead), crypts of Lieberkühn (CrL), lamina propria (LP), villi (V), and goblet cells (black arrow) (trichrome stain).

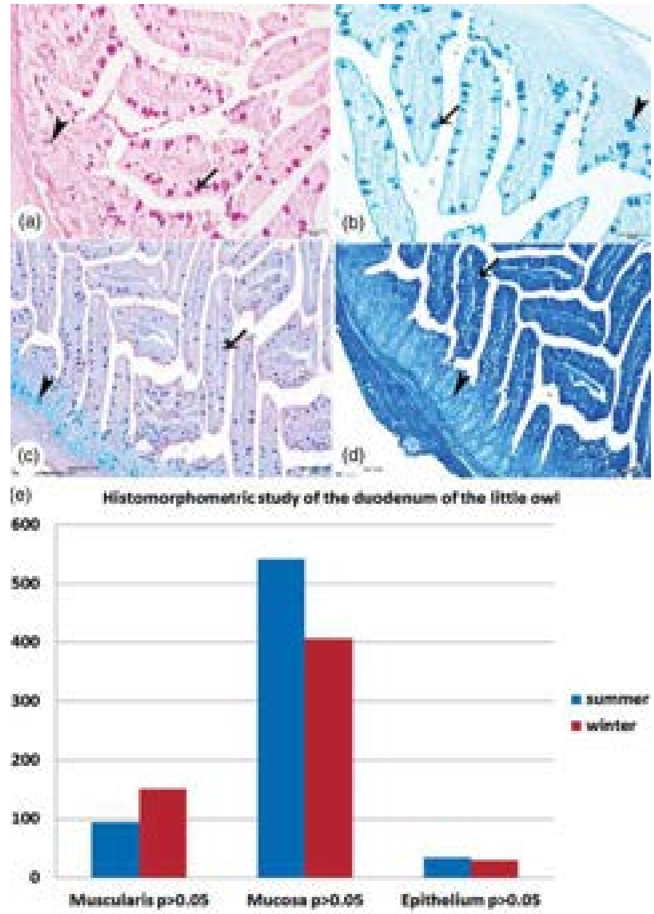

Fig. 12. (a) Transverse section of the duodenum of the little owl showing the carbohydrate content in the goblet cells in the villi (arrow), and in the crypts of Lieberkühn (arrowhead) (PAS stain), (b) transverse section of the duodenum showing high concentration of acid mucopolysaccharides in the goblet cells in the villi (arrow), and in the crypts of Lieberkühn (arrowhead) (Alcian blue stain), (c) transverse section of the duodenum showing acid and neutral mucopolysaccharides in the goblet cells in the villi (arrow), and in the crypts of Lieberkühn (arrowhead) (PAS–Alcian blue stain), (d) transverse section of the duodenum showing the negative reaction of bromophenol blue in the in the goblet cells in the villi (arrow), and in the crypts of Lieberkühn (arrowhead) (bromophenol blue stain). (e) A comparison between means of the thickness (µm) of muscular layer, mucosa, and epithelium of the duodenum in summer and winter showing no significant differences ($p > 0.05$).

average height of the mucosa (villi+ Lieberkühn) was $179.52 \pm 21.65\ \mu m$ in summer and $169.4 \pm 31.72\ \mu m$ in winter, and the average thickness of the epithelium was $35.93 \pm 7.27\ \mu m$ in summer and $31.02 \pm 8.37\ \mu m$ in winter. The differences in thicknesses in the mucosa and epithelium layers were not significant between summer and winter ($T = 0.425$, $p = 0.68$; $T = 1.31$, $p = 0.22$, respectively), but there were significant differences in the thickness of the muscularis between summer and winter ($T = 19.19$, $p = 0.000$, respectively) (Fig. 16e).

The large intestine has a pair of well-developed blind-ending ceca that project from the proximal part of rectum at its junction with the small intestine; the two ceca are elongated and approximately equal in length (Figs. 1b, 1d, 2c).

SEM observations of the interior surface of the distal part of the intestinal ceca showed plicae, well-developed folds (Fig. 17a).

Histologically, the distal part of the intestinal ceca mucous membrane is raised into simple flattened villi. The villi are lined by a simple columnar epithelium whose cells resemble those in the small intestine. The villi are incubated with goblet cells and crypts, and the muscle layer is thick. The muscularis mucosa is composed of a thin layer of longitudinal muscle fibers. Accordingly, the narrow and thin submucosa connective tissue layer merges into the lamina propria. The musculosa consists of two layers of unstriated muscle fibers: an outer longitudinal layer and an inner circular layer. The outer longitudinal muscle layer is considerably thinner than the circular muscle layer. The ceca are covered externally with a thin serosal layer, formed of simple squamous epithelial cells (Figs. 17b, 17c).

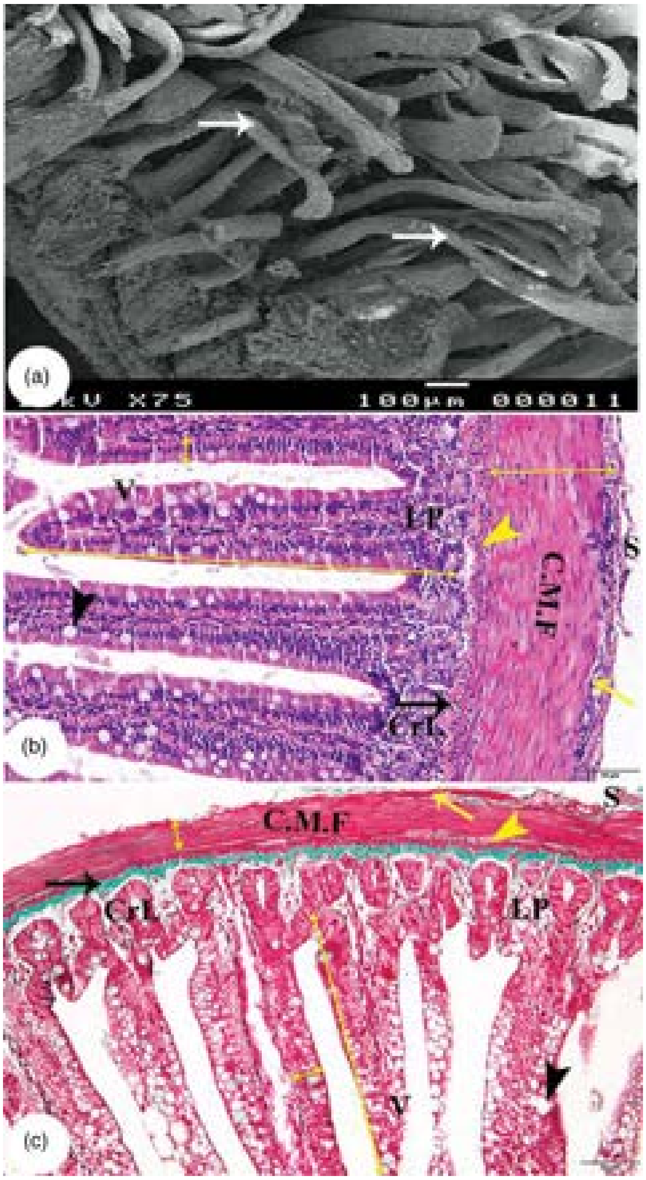

Fig. 13. (**a**) Scanning electron micrographs of interior surface of the ileum of the little owl showing finger-like villi (arrows), (**b**) transverse section of the ileum in the summer season showing the serosa (S), longitudinal muscle fibers (yellow arrow), circular muscle fibers (C.M.F), muscularis mucosa (yellow arrowhead), submucosa (black arrow), villi (V) and numerous goblet cells (black arrowheads), and lamina propria (LP) with crypts of Leiberkühn (CrL) (H&E stain), (**c**) transverse section of the ileum in the winter season showing the serosa (S), longitudinal muscle fibers (yellow arrow), circular muscle fibers (C.M.F), muscularis mucosa (yellow arrowhead), submucosa (black arrow), villi (V) and numerous goblet cells (black arrowheads), and lamina propria (LP) with crypts of Leiberkühn (CrL) (Trichrome stain).

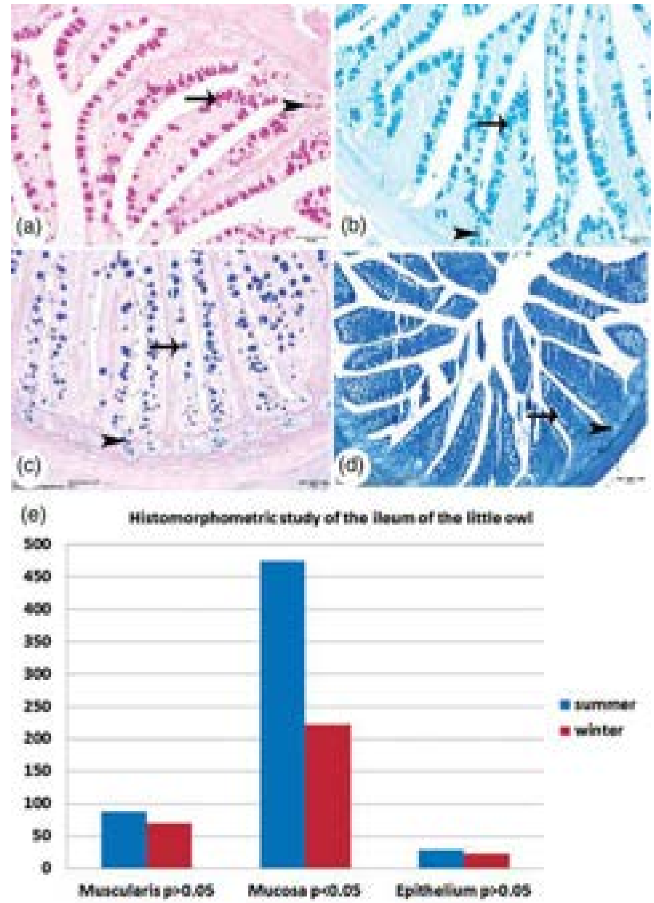

Fig. 14. (**a**) Transverse section of the ileum, showing the carbohydrate content in the goblet cells of the villi (arrow), and the crypts of Leiberkühn (arrowhead) (PAS stain), (**b**) transverse section of the ileum showing the acid mucopolysaccharides in the goblet cells of the villi (arrow), and the crypts of Leiberkühn (arrowhead) (Alcian blue stain), (**c**) transverse section of the ileum showing acid and neutral mucopolysaccharides in the goblet cells of the villi (arrow), and the crypts of Leiberkühn (arrowhead) (PAS–Alcian blue stain), (**d**) transverse section of the ileum showing the protein content and the negative reaction of the goblet cells in the villi (arrow), and the crypts of Leiberkühn (arrowhead) (bromophenol blue stain), (**e**) a comparison between means of the thickness (μm) of muscular layer, mucosa, and epithelium of the ileum of the little owl in summer and winter showing no significant differences ($p > 0.05$) except for the mucosa.

Histochemically, carbohydrates were seen to be localized in the goblet cells following a positive PAS reaction (Fig. 18a; Table 1). Acid and neutral mucopolysaccharides were demonstrated in the goblet cells by Alcian blue (pH 2.5)–PAS in the goblet cells (Fig. 18c; Table 1). The goblet cells gave a strong positive reaction (dark blue) with Alcian blue (pH 2.5) (Fig. 18b; Table 1) but reacted negatively with MBB. This indicated an absence of protein in the goblet cells; however, there was localized protein in the absorptive cells of the mucosal folds (Fig. 18d; Table 1).

Morphometrically, the average length of the intestinal ceca was 4.33 ± 0.26 cm in summer and 4.92 ± 0.92 cm in winter (Fig. 2d).

The histomorphometric study of layers of the intestinal ceca showed that the average thickness of the muscularis was 78.46 ± 31.68 μm in summer and 100.25 ± 29.54 μm in winter, the average height of the mucosa (villi+ Lieberkühn) was 261.84 ± 126.76 μm in summer and 254.197 ± 33.75 μm in winter, and the average thickness of the epithelium was 26.77 ± 2.64 μm in summer and 24.04 ± 2.29 μm in winter. The differences in thicknesses of the muscularis, mucosa, and epithelium layers were not significant between summer and winter ($T = -1.23$, $p = 0.25$; $T = 0.14$, $p = 0.89$; and $T = 1.91$, $p = 0.08$, respectively) (Fig. 18e).

Discussion

The digestive tract of the little owl, *A. noctua*, is typical for avian species, consisting of a double-ended open tube (as is also the case in mammals) beginning at the esophagus and ending at the vent. In sequential order, its main components are esophagus, proventriculus, ventriculus (gizzard), intestine, ceca, rectum, and cloaca.

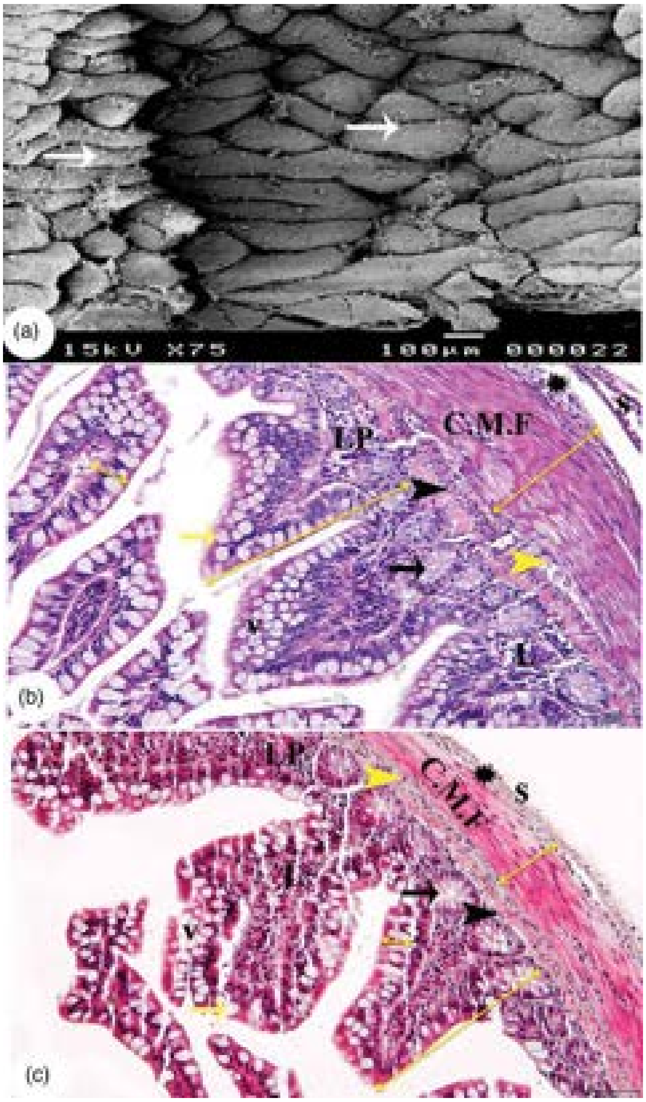

Fig. 15. (**a**) Scanning electron micrograph of the interior surface of the rectum of the little owl showing numerous well-developed longitudinal folds (arrows), (**b**) transverse section of the rectum of the little owl in the winter season showing the serosa (S), longitudinal muscle fibers (star shape), circular muscle fibers (C.M.F), muscularis mucosa (black arrowhead) extends into the villi (V), goblet cells (yellow arrow), submucosa (yellow arrowhead), rectal glands (black arrow), and lamina propria (LP) infiltrated by lymphocytes (L), H&E stain, (**c**) transverse section of the rectum of the little owl in the summer season showing the serosa (S), longitudinal muscle fibers (star shape), circular muscle fibers (C.M.F), muscularis mucosa (black arrowhead) extends into the villi (V), goblet cells (yellow arrow), submucosa (yellow arrowhead), rectal glands (black arrow), and lamina propria (LP) infiltrated by lymphocytes (L), trichrome stain.

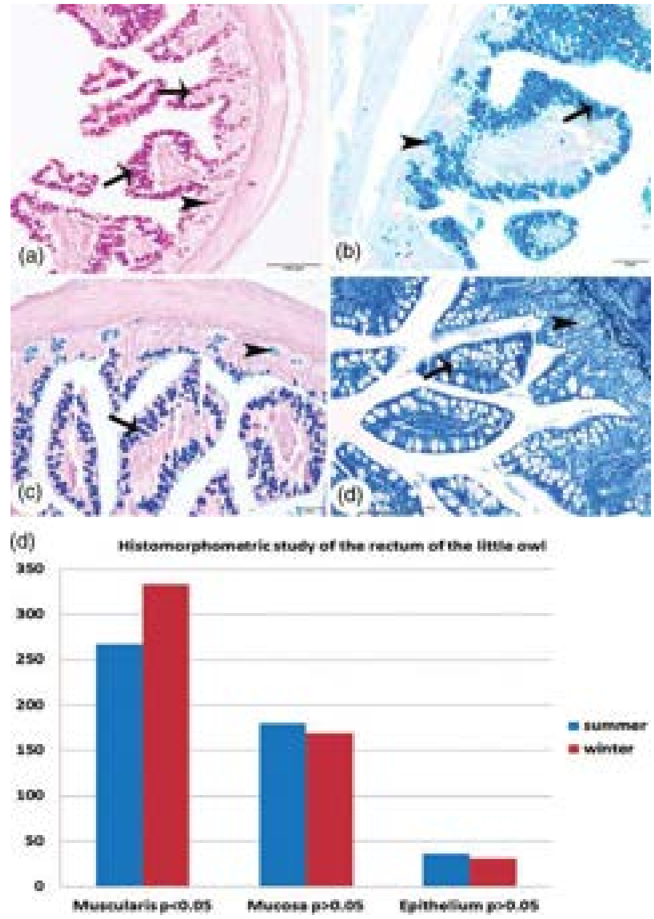

Fig. 16. (**a**) Transverse section of the rectum of the little owl, showing the carbohydrate content in the goblet cells of the villi (arrow), and in the rectal gland (arrowhead) (PAS stain), (**b**) transverse section of the rectum showing the acid mucopolysaccharides in the goblet cells of the villi (arrow), and in the rectal gland (arrowhead), (Alcian blue stain), (**c**) transverse section of the rectum showing acid and neutral mucopolysaccharides in the goblet cells goblet cells of the villi (arrow), and in the rectal gland (arrowhead) (PAS–Alcian blue stain), (**d**) transverse section of the rectum showing the protein content and the negative reaction of the goblet cells goblet cells of the villi (arrow), and in the rectal gland (arrowhead) (bromophenol blue stain), (**e**) a comparison between means of the thickness (μm) of muscular layer, mucosa, and epithelium forming the mucosa of the rectum in summer and winter showing no significant differences ($p > 0.05$) except for the muscularis was significant ($p < 0.05$).

Some of these structures may be vestigial or may have become lost in some species during evolution.

This study revealed that the little owl does not possess a crop and that its esophagus is composed of cervical and thoracic regions. The cervical region was found to be longer than the thoracic, a finding in accordance with that of Rajabi & Nabipour (2009) in Passeriformes and that has also been observed in other Strigiformes. The esophagus of these bird species lacks the capacity to dilate to form a crop, but instead, they have a fusiform enlargement proventriculus that is used for the storage of ingested food (Duke, 1997; Klaphake & Clancy, 2005).

The morphology results in this study reveal that the internal surface of the esophagus of the little owl is highly distensible owing to the longitudinal folds extending along its mucosal surface and because the esophagus is provided with a relatively thick layer of smooth muscle. The folds are coated with a nonkeratinized stratified squamous epithelium. These results for the macroscopic structure of the esophagus agree with those for other avian species (Ma, 2009; Sagsöz & Liman, 2009; Gelis, 2013; El-mansi et al., 2021). These authors concluded that there is a relationship between the histological structure of the esophagus and the type of nutrition and that the size of the esophageal folds is dependent on the size of the food that the birds swallow.

The esophagus is widest in species that swallow large pieces of food and also in those that store food along the whole length of the organ (Tivane, 2008). Furthermore, the esophagus of the little owl possesses a thick muscularis externa distributed as inner

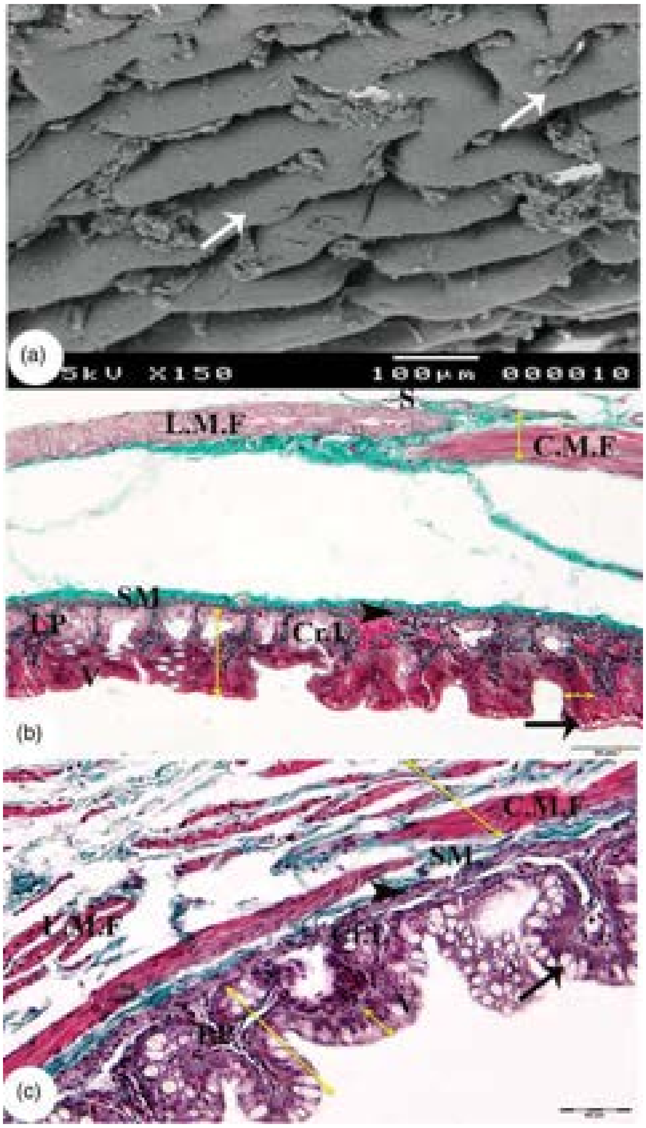

Fig. 17. (**a**) Scanning electron micrograph of the interior surface of the distal region of the intestinal ceca showing parallel and low folds of mucosa (arrows), (**b**) transverse section of the distal region of the intestinal ceca in the winter season showing the flattened villi (V) with numerous goblet cells (arrow), crypts of Leiberkühn (CrL) in the lamina propria (LP), submucosa (SM), and muscularis mucosa (arrowhead) (trichrome stain), (**c**) transverse section of the distal region of the intestinal ceca in summer season showing the flattened villi (V) with numerous goblet cells (arrow), crypts of Leiberkühn (CrL) in the lamina propria (LP), submucosa (SM), and muscularis mucosa (arrowhead) (trichrome stain).

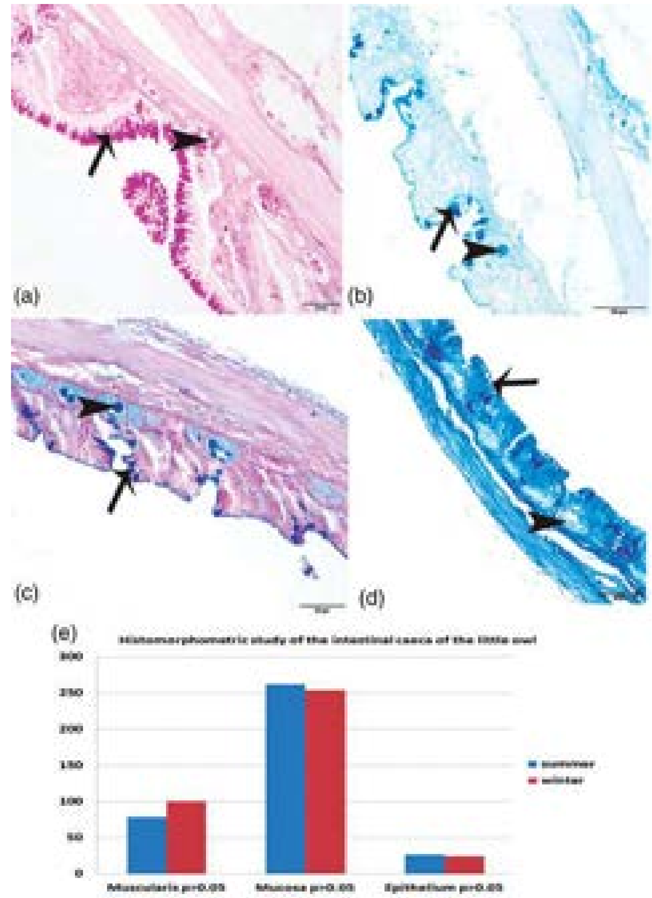

Fig. 18. (**a**) Transverse section of the intestinal ceca showing the carbohydrate content in the goblet cells of the villi (arrow) and the crypts of Leiberkühn (arrowhead) (PAS stain), (**b**) transverse section of the intestinal ceca showing acid mucopolysaccharides in the goblet cells of the villi (arrow) and the crypts of Leiberkühn (arrowhead) (Alcian blue stain), (**c**) transverse section of the intestinal ceca showing acid and neutral mucopolysaccharides in the goblet cells of the villi (arrow) and the crypts of Leiberkühn (arrowhead) (PAS–Alcian blue stain), (**d**) transverse section of the intestinal ceca showing protein content and the negative reaction of the goblet cells of the villi (arrow) and the crypts of Leiberkühn (arrowhead) (bromophenol blue stain), (**e**) a comparison between the thickness (μm) of muscular layer, mucosa, and epithelium of the intestinal ceca in summer and winter showing no significant differences ($p >$ 0.05).

longitudinal muscle bundles and outer circular ones, similar to that found in other avian species by AL-Juboury (2015).

In this study, histomorphometry results indicated that the thickness of muscularis, mucosa layer, and epithelium in the cervical region of the esophagus increased significantly in winter compared to summer, but no significant changes in the thoracic region were noted. It is known that feeding habits, diet quality, and quantity of food affect the histological structure of the digestive system (Zhu, 2015). In these Strigiformes species, including the little owl, there is no crop, but the esophagus replaces its functions, for example, by lubricating the food. The length of the cervical region of esophagus in the little owl allows the food time to mix with the esophageal secretions to facilitate transport to the stomach. The esophageal glands distributed in the mucosal layer secrete acid and neutral mucopolysaccharides.

An increase in the thickness of the mucosa of the esophagus results in an increase in the number of glands and thus an increase in the amount of secretion, especially in winter. During winter, the bird's diet varies in composition and quality, reflected in changes in the morphology and activities of the digestive tract to aid in the build-up of sufficient reserves required for breeding. This may explain the changes that occur in the esophagus region, especially during the breeding season (winter). Alshamy et al. (2018) pointed out that different growth rates are due to a complex interaction of physiological and environmental factors, including diet composition, diet form, and feeding strategy.

In this study, we observed that the stomach of the little owl is divided into a glandular portion and a muscular portion, without a proventricular–ventricular isthmus, producing one large pear-shaped organ. This is in agreement with the results of Hamdi et al. (2013), who studied the morphology of the stomach of the black-winged kite, *Elanus caeruleus*.

The proventriculus in the little owl is an elongated spindle-shaped organ. The same organ shape was observed in pigeon, dove, duck, fowl, and quail by Ibrahim (1992), in dove and owl by Mot (2010), and in falcon by Abumandour (2013). The ventriculus or muscular portion in the little owl is small in size and thin-walled.

The principal function of the proventriculus is the secretion of gastric juice and to assist the transport of food from the esophagus to the muscular stomach (Chikihan & De Speroni, 1996). The proventriculus exhibits variability in size between avian species, being relatively short in insectivorous birds because they ingest foods that are very rich in protein, but longer in granivorous species to allow for the more efficient digestion of food that contains less protein (Chikihan & De Speroni, 1996). Duke (1997) stated that the proventriculus appears quite large and distensible in carnivores that ingest large food items such as the black-winged kite, with the stomach being large in size and thin-walled compared to that of seed-eating avian species; the proventriculus in the little owl was observed to be small compared to that in granivorous species.

As little owls prey on small mammals, amphibians, and small birds, their diet is high in protein, for which efficient digestion is required. Digestion efficiency depends not only on the size of organ but also on the amount of gastric juice secreted from glands in the gut. In the little owl, the glandular portion or proventriculus is small, but it possesses numerous orifices of gastric glands, visible even to the naked eye, whose secretions are rich in neutral and acid mucopolysaccharides. In general, the gastric glands secrete hydrochloric acid and pepsinogen (Oliveira et al., 2008). Ziswiler (1967) speculated that sulfomucins present in the stomach of mammals might form complexes with pepsin, stabilizing or buffering enzymes. Altamirano et al. (1984) suggested that glycoproteins play an important role not only in the protection of the gastric epithelium but also in the initiation of the absorption process of some molecules. Zhu (2015) confirmed that the mucins in the surface epithelial cells and glandular lumina might form a resistant mucosal barrier to protect the epithelium from physical damage caused by the luminal contents or bacterial invasion.

The present study reveals that the proventriculus is influenced by seasonal changes. When the nutritional quality of food is high, a large number of nutrients are degraded into short-chain fatty acids; thus, more papillae can be formed and the surface area increases (Ding et al., 2018). This finding accords with the results of the present study, which showed that the thicknesses of the proventriculus layers were greater in winter than in summer.

In the little owl, the internal surface of the ventriculus folds to form well-developed narrow and deep folds. These folds are lined with a thin tough keratin-like layer known as the cutica gastrica (koilin). As previously mentioned, thick cutica gastrica are observed in granivorous species (Taki-El-Deen, 2017). This tough layer, in combination with grit taken in with food, allows the processing of hard food, helped by the contractions of the muscularis, resulting in the trituration of ingested grain.

In carnivorous species, such as owls and hawks, which produce pellets, undigested food does not pass through the small intestine and the colon but is instead egested through the beak (Kostuch & Duke, 1975; Fuller & Duke, 1978). The muscular stomach in these birds is considered a compensatory organ for the lack of a chewing system and plays an important role in the mechanical phase of digestion as a filter for undigested material such as bones, feathers, and hair. The koilin layer is the most notable structure in the muscular stomach, and it has been described in different species (Bacha & Bacha, 2003; Dellmann & Eurell, 2006; Hanafy et al., 2020). The koilin layer is a keratinoid substance, a polysaccharide-protein complex (McLelland, 1991). In the present study, the koilin layer in the little owl was shown to be thin, and the tubular glands were shown to be rich in neutral and acid mucopolysaccharides and proteins.

The present study showed increasing in the thickness of the epithelium of the tubular gastric glands in winter than in summer season contributed to an increase in the digestive area, therefore, an increase in the amount of digestive juices secreted by these glands.

Numerous previous studies have confirmed that there is a relationship between food content and length of intestines (Karasov & Martinez del Rio, 2007; Hanafy et al., 2021; Goodarzi et al., 2021). Birds whose diet has high fiber content have larger intestines and ceca than do those that eat seeds and fruits. The present study showed no differences at the morphological and histological level in the small or large intestine, while showed increasing in the thickness of mucosa (villi height) in summer than in winter, this may be an indication of greater cell proliferation in that segment.

Conclusions

Digestive organs are strongly influenced interspecifically and intraspecifically by diet quality and quantity. The morpho-histometric variations observed in this study in the digestive tract of the little owl indicate a degree of adaptability to food and environmental conditions. The little owl is non-migratory, so it does not face changes in environmental conditions. Morphological and histological modifications of the digestive tract of the little owl may be necessary to support increased energy requirements for thermoregulation needed in the winter breeding period.

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