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Original Paper

Morphology of the Gastrointestinal Tract and its Lymphoid Tissue in the Common Blackbird (*Turdus merula*)

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The paper presents results of a microscopic study of the wall of the esophagus, stomach, and intestine and their lymphoid tissue in birds of the blackbird species. The material was selected from 10 adult blackbirds. It was embedded into paraffin according to the conventional method. The results of the study revealed that the general structure of the tunics of the esophagus, stomach, and intestine in the blackbird is similar to other birds, but exhibits certain characteristics. In the mucosa and submucosa of these organs, lymphoid tissue is noted, which is represented by different levels of structural organization. "Lymphocyte tracks" in the form of small diffuse lymphoid tissue cords are observed in the esophagus. Small accumulations of diffuse lymphoid tissue are also found in the muscular stomach. Solitary lymphoid nodules in the glandular stomach were found not only in the mucosa but also in the subserosa of the serosa. The largest accumulations of lymphoid tissue are observed in the esophageal and cecal tonsils and aggregated lymphoid nodules of the intestines, which are areas of significant penetration of antigens into the body of birds. In the places where they are located, infiltration of surface epithelium and crypts by lymphoid cells is observed. The smallest area of these immunocompetent structures is occupied by diffuse lymphoid tissue and much larger – by lymphoid nodules. The results of the research allowed to assess the morphofunctional status of lymphoid formations in the organs of the digestive canal of the blackbird and, accordingly, to determine the state of their immune system.

Keywords: birds, esophagus, stomach, intestine, lymphoid nodules

1 Introduction

Birds are an integral part of wildlife and occupy an important link in food chains. One of the most abundant birds in Ukraine is the order Passeriformes, which includes the family Turdidae. The blackbird (*Turdus merula*) is a bright representative of this family. It is a small songbird with black feathers in males and dark brown in females. Blackbirds of this species consume berries, insects, slugs, and earthworms. As for the latter, blackbirds can deftly pull earthworms out from the ground and swallow without tearing them into pieces (Evans et al., 2010). Organic substances (feed) enter the body of birds through the digestive organs and are used as raw materials for the synthesis of new macromolecules, or they are oxidized, as a result of which the energy necessary for their vital activity is generated. The digestive system of birds is represented by a digestive tube that begins with the oropharynx and ends with the cloaca. It also includes digestive glands located outside the digestive canal – the liver and pancreas. In the digestive tube, the digestive canal (tract) is distinguished, which is formed by the esophagus, stomach, and intestine. All these organs are involved in ensuring the digestion process and have a close relationship with all the apparatuses and systems of the bird's body (Kharchenko & Lykova, 2014).

Along with water and feed, genetically foreign agents (microorganisms, pathogenic bacteria, etc.) enter the bird's body. In response to this, immunocompetent structures (tonsils, solitary and aggregated lymphoid nodules, etc.) are formed in the walls of the digestive canal, which are attributed to the composition of the peripheral immune system (Burns, 1982). They are

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always located on the paths of possible penetration of antigens into the body. The basis of immunocompetent structures is formed by lymphoid tissue, which contains numerous cells of the lymphoid lineage located in mesh-like structures

of reticular tissue. Effector cells of T and B cells are able to recognize and neutralize antigens and rid the body of them. Lymphoid tissue is associated with the mucosa of the digestive canal and its development consists of several levels of structural organization. Among them are diffuse and prenodular forms and lymphoid nodules with and without lighter staining areas (Cooper & Mahroze, 2004). Some researchers explain the significant development of this tissue in the digestive organs of birds by the absence of tonsils of the lymphoepithelial pharyngeal ring (Pirogov-Waldeyer ring), which is typical for mammals since food is not retained in the oral cavity, and the absence of lymph nodes (excluding waterfowl) (Kovtun et al., 2018).

The digestive organs and their lymphoid formations in birds of certain trophic groups were studied most fully by Kharchenko and co-authors (2001, 2003, 2018). They revealed a general pattern of localization of immunocompetent structures. The largest number of them is located in the areas of the transition from one part of the digestive tube to another (at the borders between the esophagus and glandular stomach and the small intestine and rectum). There is evidence that in birds of certain species, lymphoid formations (pyloric tonsils) are also localized in the area of the exit of the muscular stomach and at the beginning of the duodenum (Nagy & Olah, 2007). The development of such formations depends on bird species, age, and dietary specialization. For example, in birds (great white and gray herons) whose primary diet is fish, the wall of the digestive organs is more intensively infiltrated by lymphoid elements than in species (purple heron) with fewer fish in their diet (Kots & Byrka, 2005). Lymphoid formations of the digestive organs in young birds have the best development, and after the onset of puberty, their number decreases, and the processes of involution occur, which are characteristic of all immune organs (Haley, 2017).

Along with literature data, the morphology of the digestive canal of the blackbird, and their features of microstructure, including lymphoid formations, are almost not studied. In this regard, conducting such research is relevant.

2 Material and methods

The study was conducted at the Academician V. G. Kasianenko Department of Anatomy, Histology and Pathomorphology of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine, Kyiv. The research material was collected from 10 adult blackbirds (*Turdus merula*), whose habitat was the forests within Kyiv (Ukraine). The material was obtained from the funds of the Department, which was stored in containers with a 12% formalin solution. The birds were clinically healthy and showed no signs of disease. Their average body weight was 97.20 ±3.16 g.

All interventions and slaughter of the birds were performed in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1986) and the Decree of the First National Congress on Bioethics (Kyiv, 2001) (Reznikov, 2003).

The histological and morphometric methods were used in the study. During the histological examinations, samples (size 1 × 1 × 0.5 cm) of digestive organs were collected from clearly defined areas: the cranial and caudal parts of the esophagus, the transition of the esophagus to the stomach, the glandular and muscular stomach, the intermediate zone that binds these two portions of the stomach, from the walls of the duodenum, jejunum, ileum, ceca, and rectum. Fixed samples were washed in running water to remove formalin, after which they were dehydrated with ethanol of ascending concentrations (70%, 80%, 96%, 100%). Paraffin, a compacted medium, was used to compact the dehydrated samples. Before compacting, they were soaked in two portions of chloroform, and then transferred to a chloroform-paraffin mixture and kept in a thermostat at a temperature of +37 °C. Subsequently, the samples were placed in two portions of melted paraffin, designated as paraffin No1 and paraffin No2, at a temperature of +54–55 °C, and a new portion of melted paraffin was poured into boxes previously moistened with glycerin. The obtained blocks were glued to bars, from which serial sections of a given thickness (5–8 µm) were made using a sliding microtome. The obtained histological sections stained with Carracci's hematoxylin and an aqueous eosin solution were used for an overview study and establishing the general micromorphology of the esophagus, stomach, and intestines, as well as conducting morphometry. The collagen fibers were identified according to Mallory's and Van Gieson's methods, and elastic fibers in fibrous connective tissue – according to Weigert's method. Stained sections were embedded into Canada balsam.

The obtained histological preparations were examined using Olympus CX43 and MBS-2 microscopes. The micromorphological features of the digestive organs and their lymphoid formations in the blackbird (*Turdus merula*) were studied. The morphometric microscopic examinations of the esophagus, stomach, and intestines were conducted using an eyepiece micrometer (eyepiece ruler), which is part of the Olympus CX43 microscope kit, and the point-counting method using a binocular microscope and a measuring grid.

The results of the conducted research were recorded in protocols, and their digital data were statistically processed using the StatSoft Statistica 13.1 (2016) software, taking into account the specifics of statistical methods in biological research. Data are presented in tables as *x* ±standard deviation. Individual histological preparations were photographed using a digital camera mounted in a Primo Star microscope (Carl Zeiss, Germany) and connected to a personal computer.

3 Results and discussion

The conducted studies showed that the general structure of the esophagus, stomach, and intestines in the blackbird (*Turdus merula*) is similar to other birds (Hassouna, 2001; Lykova & Kharchenko, 2021), but exhibits certain characteristics. Their wall is formed by the mucosa (epithelium, lamina propria, and muscular layer), submucosa, muscularis, and adventitia. In the thoracic-abdominal cavity, the adventitia is replaced by the serosa.

3.1 Micromorphology of the esophagus

The esophageal mucosa and submucosa of the blackbird form well-defined longitudinal folds (from 6 to 10), which are directed into the lumen of the organ and partially close it (Figure 1a, b).

The folds are mainly leaf-shaped, finger-shaped, less commonly wedge-shaped, and of varying sizes. Therefore, in this study, the folds were divided into three groups: large, medium, and small. The highest indices of height and width have large folds, slightly lower – medium folds, and the lowest – small folds. Accordingly, the total thickness of the esophageal wall in the area of the folds is greater than between the folds. Morphometric parameters of the esophageal structures are presented in table 1.

Some authors (Kots et al., 2002; Kharchenko & Lykova, 2014) claim that one of the factors, which determines the shape and parameters of the mucosal folds in the esophagus of birds, is the size of food objects and foraging activity.

The surface of the esophageal mucosa of the blackbird is lined with stratified weakly keratinized squamous epithelium, the keratinization of which is more pronounced in the cranial (cervical) part. The cells of the epithelial basal layer have a cylindrical shape and an oval nucleus. Above them are visible cells of polygonal shape, which are located in several layers. Destroyed nuclei are found in some flat-shaped cells that form the superficial layer of the epithelium. According to some authors, the structure of esophageal surface epithelium in birds depends on the type of dietary specialization. Therefore, it can be non-keratinized, partially keratinized, or keratinized (Ibrahim, 1992; Kharchenko & Byrka, 2003; El-naseery et al., 2021).

The lamina propria of the mucosa is a layer of loose connective tissue with a small number of cells. The thin collagen and elastic fibers, vessels, and small complexes of esophageal glands and their excretory ducts, densely arranged in chains, are revealed in the lamina propria of the mucosa. The glands occupy a significant area

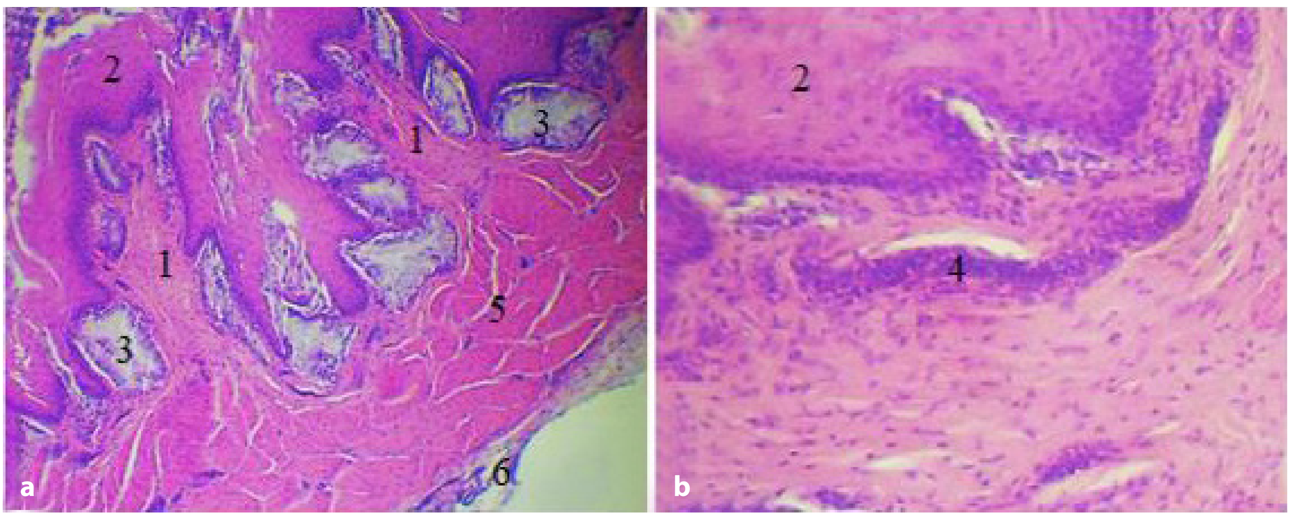

Figure 1 The cranial part of the esophagus in the blackbird (*Turdus merula*)
1 – mucosal folds; 2 – epithelium; 3 – esophageal glands; 4 – a cord of diffuse lymphoid tissue; 5 – muscularis; 6 – adventitia. Carracci's hematoxylin and eosin, ×63 (a); ×100 (b)

Table 1 Morphometric parameters of the esophagus in the blackbird (*Turdus merula*), $n = 10$ (x ±SD)

Parameters	x ±standard deviation
Total wall thickness (µm) – in the area of the folds – between the folds	 1,160.28 ±49.80 791.85 ±33.71
The height of the mucosal folds (µm) – large – medium – small	 654.38 ±21.76 450.92 ±12.26 280.44 ±10.42
The width of the mucosal folds (µm) – large – medium – small	 372.09 ±8.42 260.28 ±9.50 175.96 ±6.74
The area of the tunics (%) – mucosa along with the submucosa – muscularis – adventitia (serosa)	 50.47 ±1.65 44.80 ±1.77 4.73 ±0.41
The area of the esophageal glands in the mucosa (%)	39.61 ±3.21
The area occupied by lymphoid tissue in the esophageal tonsil (%)	27.47 ±2.59
Types of lymphoid tissue of the esophageal tonsil (%) – diffuse lymphoid tissue – nodules	 77.30 ±4.77 22.70 ±4.77

parameters were not defined

of this tunic. They are embedded in surface epithelium and produce mucous secretions. The secretions are released through excretory ducts on the surface of the mucosa. Each gland consists of secretory units that lie within the central cavity. The mucous cells of the glands have a conical shape with a wide base, their nuclei are flattened, located on the periphery, and inclusions of light blue mucus are visible in the cytoplasm. As is known, mucus does not contain enzymes, has an antibacterial effect, facilitates feed passage, and protects the esophageal mucosa against mechanical damage (Linden et al., 2008). In some areas of the mucosa, mainly under the epithelium, near the glands, and with a concentration around the blood vessels, there are cords formed by small diffuse lymphocyte clusters ("lymphocyte tracks"), which are components of immunocompetent structures. The epithelial layer is infiltrated by lymphocytes, where such clusters are located. Individual lymphocytes are also observed between glandular cells of the glands.

According to some authors (Kharchenko, 2004; Khomych et al., 2020), lymphocytes in the composition of gland secretions migrate into the esophageal lumen and are involved in the formation of protective mechanisms of the immune system of the digestive organs. The lymphoid nodules in the lamina propria of the esophageal mucosa in the blackbird were not detected. Ambiguous literature data on their presence in other bird species. They are absent in the esophagus of the finch and European bee-eater, while in the pheasant, quail, and rook, the lymphoid nodules that remain in a close morpho-functional relationship with the glands were revealed. Their occurrence is related to the dietary specialization of these birds. Along with plant foods, more bacteria, microorganisms, and toxins, products of their vital activity, enter the body of such birds (Kovtun & Kharchenko, 2005). The muscular layer of the esophageal mucosa in the blackbird is poorly developed, sometimes intermittent, represented by longitudinally oriented isolated groups of bundles of smooth muscle cells. The contraction of the latter facilitates feed passage from the esophageal lumen into the stomach. The submucosa is poorly developed and formed by loose connective tissue with a large number of fibrous structures, vessels, and nerve ganglia. It separates the muscular layer from the muscularis and creates the mobility of the mucosa during the formation of its irregular folds.

Smooth muscle tissue forms a well-defined middle tunic (muscularis) of the esophagus in the blackbird, which consists of three layers of smooth muscle cells. The circularly arranged bundles of cells form the middle layer, and longitudinally arranged – the inner and outer layers. The portions of loose connective tissue with vessels and nerve ganglia are found between the layers. The inner layer deepens into individual large folds and is involved in their formation. It should be noted that the outer layer of the muscularis is poorly developed and may be absent in some areas.

In the cranial part, the esophagus of the blackbird is covered with the adventitia, which occupies the smallest area. The adventitia is formed by loose connective tissue with blood vessels, nerve ganglia, and plexuses, and in the caudal (lower) part – by the serosa, which further expands to the stomach and other subsequent organs of the digestive canal. The feature of the serosa lies in the fact that it is covered with one layer of squamous epithelial cells.

In the caudal part of the esophagus, near the glandular part of the stomach (gastroesophageal junction), stratified squamous epithelium changes to simple columnar epithelium, the esophageal glands disappear, and deep proventricular glands grouped into lobules appear in the submucosa. These glands are peculiar to the glandular stomach. The thickened mucosa and submucosa of this area are rich in lymphoid reticular tissue, which forms the esophageal tonsil. This esophageal tonsil is composed of small clusters of diffuse lymphoid tissue and lymphoid nodules (with and without lighter staining areas) that limit crypt-like formations. Herewith, the diffuse form occupies a much larger area in lymphoid tissue than lymphoid nodules. The latter are locally found between the secretory areas of the esophageal glands, as well as near the blood vessels, and are mostly round-shaped. According to the authors, in such lymphoid nodules with lighter staining areas, lymphocytes under the influence of antigens are transformed into effector cells capable of producing antibodies that provide specific immunity (Coico & Thorbecke, 1985).

3.2 Micromorphology of the stomach

The stomach of the blackbird (*Turdus merula*), like most birds, has two well-developed portions (glandular and muscular), which differ not only in structure but also in function. The glandular stomach resembles the single-chamber stomach of mammals. In the glandular stomach, the food bolus is moistened with digestive juice enriched with enzymes. The muscular stomach has well-developed smooth muscles, which ensure the crushing and grinding of food and compensate for the absence of teeth in birds (Ahmed et al., 2011; Zhu et al., 2013). Unlike certain bird species (herons) that have a pyloric sac (Kots & Byrka, 2005), the pyloric part of the stomach is not developed in the blackbird. The morphometric parameters of the glandular and muscular stomach structures are shown in table 2.

The glandular stomach (proventriculus) of the blackbird is a thick-walled tube, the thickness of which is greater in the area of the folds than between the folds. The surface of the mucosa is uneven due to the presence of low longitudinal folds and gastric pits. It is covered with simple columnar epithelium of the intestinal type. In the blackbird, the structure of the glandular stomach is similar to other bird species (Ogunkoya & Cook, 2009; Batah et al., 2012) and forms a large "glandular field" that produces mucous-like secretions. The mucus layer protects the mucosa from mechanical damage and prevents stomach self-digestion by gastric juice (Abumandour, 2013). The lamina propria of the mucosa is formed by loose connective and reticular tissues and contains numerous simple tubular glands that produce mucus (Figure 2a). The excretory ducts of the glands open into shallow gastric pits. The presence of such glands in different bird species is also reported by other researchers (Jassem et al., 2016). As a rule, locally between the superficial tubular glands, clusters of diffusely located lymphocytes and single lymphoid nodules with oval and round shapes are observed at the base of the folds (Figure 2b, c). Some of them have light centers. The bundles of smooth muscle cells, located longitudinally, form a thin, weakly pronounced muscular layer of the mucosa. The latter is a continuation of the esophageal muscular layer.

The submucosa has a considerable thickness and contains deep proventricular glands, which are structurally complex and grouped into lobules. They are often referred to as the "glandular apparatus" (Kadhim, 2011; Sayrafi & Aghagolzadeh, 2020). The lobules of deep proventricular glands are located in one or two rows and have mostly elongated-oval, less commonly polygonal, or irregular shapes. In birds of various species, lobules can be conical, oval, pear-shaped, and elliptical in shape (Kharchenko, 2005). Each deep proventricular gland consists of a large number of vesicular glands that open through isolated ducts into the central excretory duct. The central excretory duct is directed to the surface of the mucosa. Lobules of deep proventricular glands are limited by layers of fibrous connective tissue, which are infiltrated by lymphocytes in some areas. Collagen and elastic fibers and blood vessels are found in the layers of this tissue. Some authors claim that they may contain single smooth muscle cells extending from

Table 2 Morphometric parameters of the stomach in the blackbird (*Turdus merula*), *n* = 10 (*x* ±SD)

Parameters	Portions of the stomach	
	glandular	muscular
Total wall thickness (µm)		
– in the area of the folds	1,871.49 ±20.84	–
– between the folds	1,624.04 ±40.46	–
– in the area of the side walls	–	3,911.62 ±57.61
– in the area of blind sacs	–	1,063.14 ±70.48
The area of the tunics (%)		
– mucosa along with the submucosa	73.59 ±0.96	18.30 ±1.25
– muscularis	22.18 ±0.64	77.54 ±1.64
– serosa	4.23 ±0.44	4.16 ±0.45
The number of lobules of deep proventricular glands (pcs)	44.50 ±0.75	–
The diameter of the lobules of the deep proventricular glands (µm)	788.19 ±52.10	–

parameters were not defined

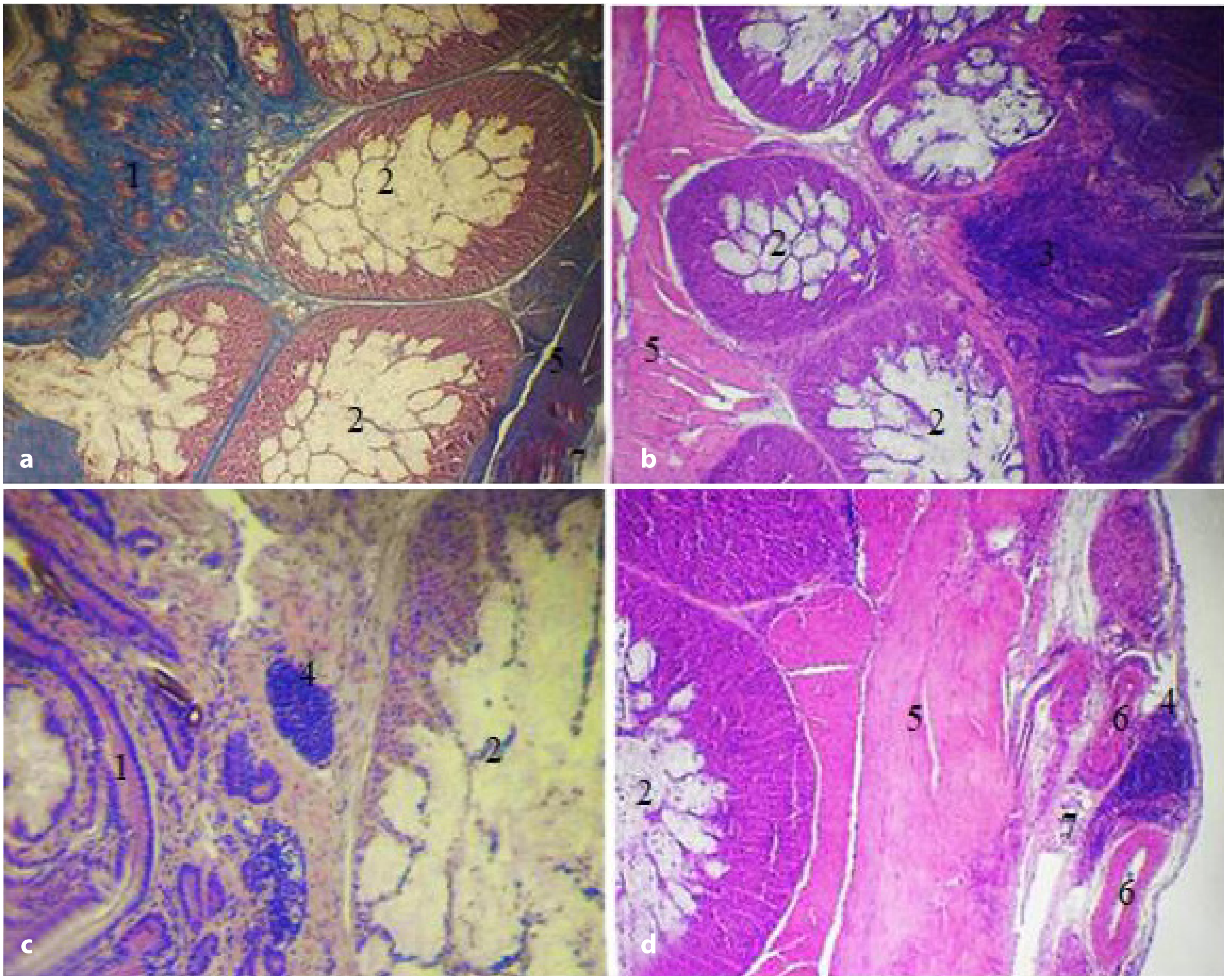

Figure 2 The glandular stomach of the blackbird (*Turdus merula*)
1 – superficial tubular glands; 2 – lobules of deep proventricular glands; 3 – diffuse lymphoid tissue; 4 – lymphoid nodules; 5 – muscularis; 6 – blood vessels; 7 – serosa. Carracci's hematoxylin and eosin, ×63 (b); ×90 (c, d). Staining according to Mallory's method, ×90 (a)

the muscular layer of the mucosa (Nasrin et al., 2012). It is possible that the contraction of these cells contributes to the release of secretions from deep proventricular glands.

Deep proventricular glands produce gastric juice containing enzymes, which penetrate the food bolus and provide chemical processing. Many authors report that the cells of these glands are of the same type and combine the morphological and functional features of mammalian chief and parietal cells (Zhang et al., 2016). It is considered that chlorides are formed at the apical pole of such cells and pepsinogen is formed at the basal pole. In addition to these enzymes, other proteolytic enzymes such as gastricsin and gelatinase are present in gastric juice (Sayrafi & Aghagolzadeh, 2020).

The smooth muscle tissue forms the muscularis of the glandular stomach in the blackbird. It consists of the middle circular layer (the most developed) and the outer and inner longitudinal layers. Besides, the inner longitudinal layer is the least developed. Some authors claim that in some bird species, the muscularis of the glandular stomach can be two-layered: inner longitudinal and outer circular (Catroxo et al., 1997), or three-layered: inner oblique, middle circular, and outer longitudinal (Jeurissen et al. al., 1989; Kadhim et al., 2011). The structure of the serosa is similar to the caudal part of the esophagus. However, in this study, single triangular-shaped lymphoid nodules located between large blood vessels were revealed in the subserosa of this tunic (Figure 2d).

In the transition zone (isthmus), which connects the glandular and muscular stomach, surface epithelium is covered with a thin cuticle, the number of deep proventricular glands decreases and they disappear. Correspondingly, the submucosa also thins. Simple tubular glands, which are characteristic of the muscular

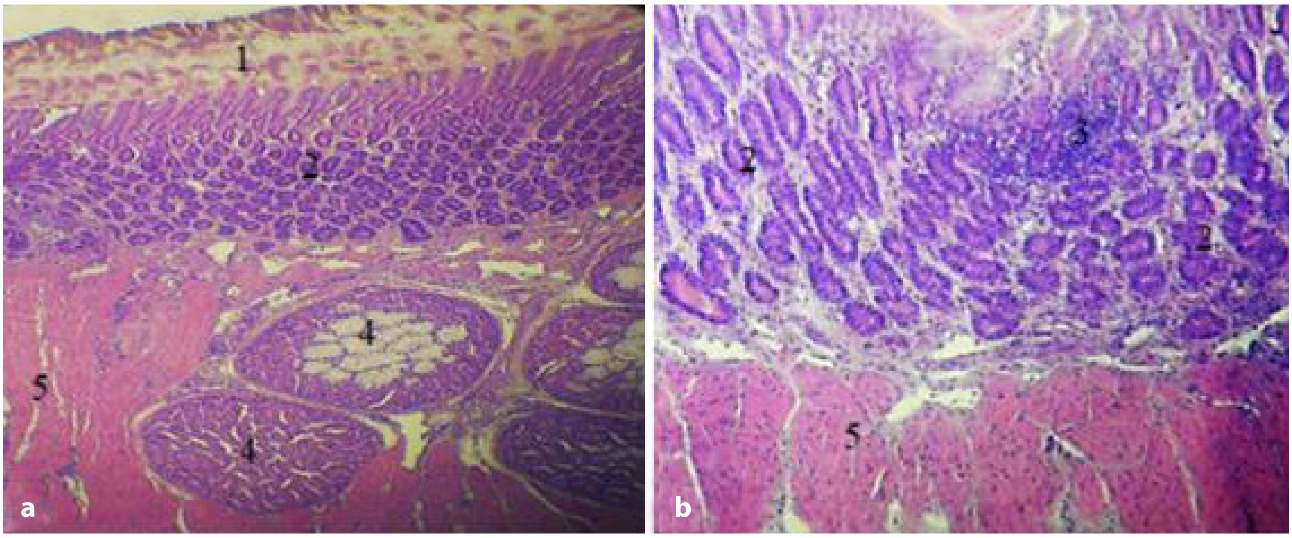

Figure 3 The intermediate zone (a) and the muscular part of the stomach (b) of the blackbird (*Turdus merula*)
1 – cuticle; 2 – tubular glands; 3 – diffuse lymphoid tissue; 4 – lobules of the deep proventricular glands; 5 – muscularis. Carracci's hematoxylin and eosin, ×63 (a); ×100 (b)

stomach, are observed in the lamina propria of the mucosa of this area (Figure 3a).

The main mass of the muscle stomach (ventriculus) of the blackbird, as in other bird species (Demirbag et al., 2015), consists of the high-mass muscles (main and intermediate). They are better developed in the area of the side walls and more poorly – in the area of the blind sacs, which affects the wall thickness. The mucosa of the muscular stomach is covered by simple cuboidal epithelium, the cells of which have the same height and width. Under the epithelial layer, tubular glands are observed in the lamina propria of this tunic. These glands produce keratin-like secretions, which perform a protective function. On the histological preparations, they resemble a yellowish strip that covers the epithelial layer. The thickness of the keratin-like film (cuticle) in different bird species is not the same and depends on age and dietary specialization (Demirbag et al., 2015). The tubular glands have the form of columns and lie perpendicular to the surface. There are isolated clusters of lymphoid cells between them, but the lymphoid nodules are not detected (Figure 3b). Some authors report that single lymphoid nodules can be observed in the mucosa of this part of the stomach in waterfowl (Khomych et al., 2021). Between the lamina propria of the mucosa and muscularis, dense fibrous connective tissue, in which fibrocytes are visible, forms a poorly developed submucosa.

The high-mass muscles form a thick muscularis, which is well-defined on the histological preparations. The muscularis is highly developed and occupies a much larger area than other tunics. The muscularis is formed by the bundles of smooth muscle cells that form the inner circular and outer longitudinal layers, which are most pronounced in the area of the blind sacs. The inner layer is more massive, and the bundles of smooth muscle cells have the form of right triangles, which are limited by layers of loose connective tissue. In the outer layer of this tunic, the bundles of these cells are located longitudinally.

3.3 Micromorphology of the intestine

The intestine of the blackbird (*Turdus merula*), as in other bird species, is a cylindrical tube that is folded in the form of loops in the thoracic-abdominal cavity. The composition, quality, and quantity of feed affect the size of the intestine (Kharchenko, 2001). The intestine is represented by the small and large intestines, in which digestion processes occur, and in the large intestine, besides, the formation and excretion of feces. The small intestine, starting from the muscular stomach, is represented by the duodenum, jejunum, and ileum. Besides, the jejunum is the longest. The large intestine includes two ceca and a rectum, which leads to the cloaca. Unlike mammals, birds do not have a colon. A feature of the ceca in the blackbird is that they resemble very short appendages, while in waterfowl (Dyshliuk et al., 2022), they reach considerable length. The morphometric parameters of intestinal structures are shown in tables 3 and 4.

Along the entire small intestine of the blackbird, the mucosa forms well-defined outgrowths – villi, which are formed by the epithelium and lamina propria. The villi of the small intestine are tall and thin, leaf-shaped and finger-shaped. Their highest height indices are detected in the jejunum, and their highest width indices – in the ileum.

The mucosa along with the submucosa of the small intestine occupies a significant area and forms low

Table 3 Morphometric parameters of the intestine in the blackbird (*Turdus merula*), n = 10 (x ±SD)

Parameters	Small intestine			Large intestine	
	duodenum	jejunum	ileum	cecum	rectum
The total wall thickness between the villi (µm)	476.58 ±15.32	361.10 ±8.43	377.59 ±15.94	515.07 ±11.79	329.94 ±13.79
The villi (µm) – height – width	 630.55 ±44.44 53.16 ±5.51	 654.38 ±21.15 49.49 ±4.29	 608.55 ±13.79 54.99 ±4.59	 460.08 ±15.93 98.98 ±6.13	 498.57 ±26.35 64.15 ±5.36
The area of the tunics (%) – mucosa along with the submucosa – muscularis – serosa	 84.17 ±1.11 12.42 ±0.88 3.41 ±0.48	 87.12 ±1.04 9.86 ±0.95 3.02 ±0.30	 89.67 ±0.44 7.55 ±0.31 2.78 ±0.32	 91.45 ±0.37 6.28 ±0.42 2.27 ±0.30	 83.65 ±1.17 12.47 ±0.93 3.88 ±0.61

parameters were not defined

Table 4 Morphometric parameters of lymphoid formations of the intestine in the blackbird (*Turdus merula*), n = 10 (x ±SD) (%)

Lymphoid formations	The area occupied by lymphoid tissue in the mucosa and submucosa	Types of lymphoid tissue	
		diffuse lymphoid tissue	nodules
Peyer's patch of the jejunum	65.66 ±2.71	40.60 ±3.82	59.40 ±3.82
Peyer's patch of the ileum	68.21 ±3.41	37.98 ±1.81	62.02 ±1.81
Cecal tonsil	80.47 ±3.28	27.96 ±4.13	72.04 ±4.13

parameters were not defined

folds. The mucosa, and accordingly the villi, are covered with simple columnar epithelium, at the apical pole of the cells of which microvilli forming a border are visible. The border epithelial cells are the most numerous. They have a cylindrical shape and an oval-elongated nucleus, which is located in the area of the basal pole. The borderless and light goblet cells are located in small numbers between the border cells. The goblet cells are characterized by a slightly expanded shape at the apical pole and pale cytoplasm due to significant inclusions of the mucus. The nucleus of goblet cells is located near their basal pole.

Intestinal glands (crypts) are located in the lamina propria of the mucosa, which is formed by loose connective tissue with singly located smooth muscle cells and numerous vessels of the microcirculation. They are located at different depths and, similar to villi, increase the absorption surface of the intestine. Crypts are the result of the epithelium deepening into the lamina propria. Lymphoid nodules and diffuse lymphoid tissue are noted in the thickness of the villi and the area of the crypts along the small intestine (Figs 4a, b, c, d). Also, in the jejunum and ileum, there are small groups of nodules, which form Peyer's patches (Figure 4e). Peyer's patches are visible visually above the surface of the mucosa without the use of additional techniques. Some lymphoid nodules do not have a well-defined connective tissue capsule, so their periphery is not clearly defined. The epithelium of the villi and crypts in the areas of nodules and Peyer's patches is infiltrated by cells of the lymphoid line, as a result of which a "lymphoepithelium" is formed. Lymphocytes are also observed in the lumen of some crypts. According to some authors, the small intestine of the rook and pheasant is more intensively infiltrated by elements of lymphoid tissue than that of the European bee-eater and finch, which is due to their different types of nutrition (Kovtun & Kharchenko, 2005). Plasma cells of this tissue in the small intestine produce a significant amount of immunoglobulin A, which plays an important role in regulating the absorption of a wide range of soluble antigens (chemical substances, bacterial bioproducts, etc.) (Logvinova et al., 2020). The muscular layer of the mucosa of the small intestine is represented by a longitudinal layer of smooth muscle cells. The loose fibrous connective tissue forms a thin, poorly developed submucosa, which consists of vessels, collagen and elastic fibers.

Along the entire length of the small intestine, the muscularis is formed by two layers of smooth muscle tissue: the inner circular layer that is the best developed, and the outer longitudinal layer. Between the layers of the muscularis, there are well-defined layers of loose fibrous connective tissue with blood vessels. An intermuscular nerve plexus, which is part of the enteric nervous system, is found in some areas near the blood vessels. This plexus is most pronounced in the ileum. Lymphoid elements in the muscularis of the duodenum and jejunum are not detected. In the ileum,

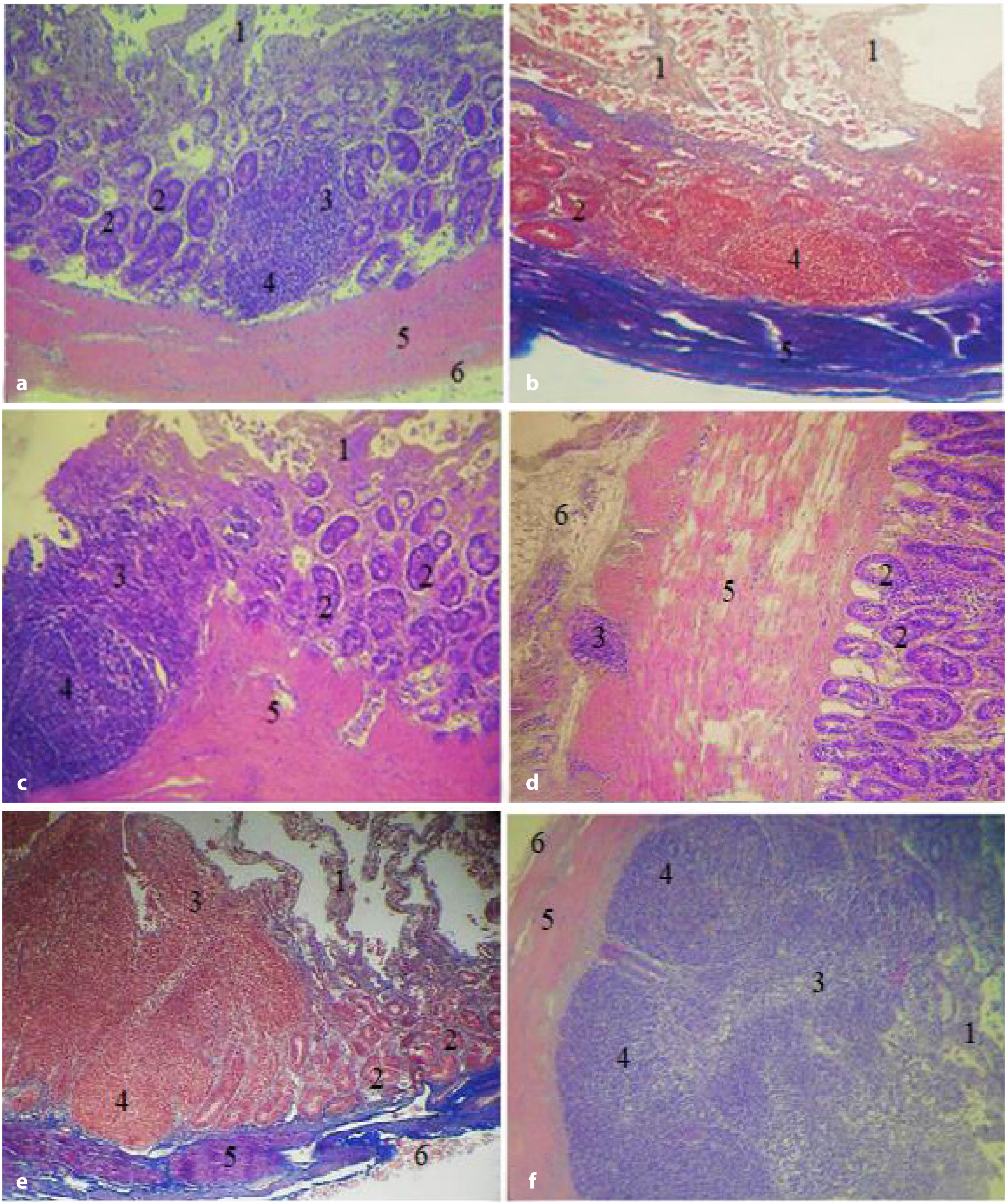

Figure 4 Lymphoid tissue of the duodenum (a), jejunum (b), and ileum (c, d). Peyer's patch of the ileum (e) and cecal tonsil (f) of the blackbird (*Turdus merula*)
1 – villi; 2 – intestinal glands (crypts); 3 – diffuse lymphoid tissue; 4 – lymphoid nodules; 5 – muscularis; 6 – serosa. Carracci's hematoxylin and eosin, ×63 (a, c, d, f). Staining according to Mallory's method ×63 (b, e)

some insignificant diffuse fields of lymphoid cells were observed in the outer layer of the muscularis. There is evidence that in waterfowl, individual lymphoid nodules may also be found in the muscularis of the small intestine (Logvinova et al., 2020; Dyshliuk et al., 2022).

The general structure of the tunics of the large intestine in the blackbird is similar to that of the small intestine, but it has certain features. The mucosa is more folded and contains fewer crypts. In the cecum, the height of the villi is lower, and the width is much greater than in the small intestine. The presence of the villi in the large intestine of different bird species was also reported by other authors (Georgescu et al., 2007). Goblet cells in surface epithelium and crypts are much more numerous, as mucus is involved in the formation of feces. The thickening of the mucosa is observed in the cecum compared with the rectum. This is due to the well-developed lymphoid tissue in the cecum, which forms cecal tonsils (Figure 4f). Some authors explain its intensive development by the presence in the cecum of bacteria, which process urea (Kobayashi et al., 2019).

In cecal tonsils, lymphoid tissue occupies the entire area of the lamina propria and submucosa of the mucosa and is represented by diffusely located lymphocytes and lymphoid nodules. The latter are mostly round and pear-shaped, less commonly elongated-oval. Secondary lymphoid nodules (with lighter staining areas) predominate over primary ones. Such accumulations of lymphoid tissue are located at the base of the villi and between the crypts. The latter due to the significant content of lymphoid tissue are poorly visible on histological preparations. The villi and crypts are infiltrated by lymphocytes. The muscularis of the ceca is significantly thinned. Some authors (Mazurkevych & Khomych, 2017) claim that cecal tonsils are not found in all birds. They are absent in waterfowl. Instead, these birds have cecal diverticula, in which lymphoid nodules are contained not only in the mucosa, but also in the muscularis. This study didn't reveal the elements of lymphoid tissue in the rectum of the blackbird.

4 Conclusions

The mucosa of the digestive canal of the blackbird (*Turdus merula*) forms longitudinal folds, which are most pronounced in the esophagus. The mucosa occupies a significant area in the glandular stomach and intestine and is slightly smaller in the esophagus. This tunic is poorly developed in the muscular stomach. The mucosa is associated with lymphoid tissue, the amount of which increases from the esophagus to the intestine. In the esophagus, "lymphocyte tracks" located mainly under the epithelium and around the esophageal glands are noted. In the areas of their localization, the infiltration of surface and glandular epithelium by lymphoid cells is observed. Single lymphoid nodules are noted in the glandular stomach between the superficial glands of the mucosa and in the subserosa. The lymphoid tissue of the muscular stomach is poorly developed and is represented by small clusters of lymphoid cells, which are mainly located between the tubular glands of the lamina propria. Lymphoid tissue is most pronounced in considerable immunocompetent structures (esophageal tonsil, cecal tonsils, and intestinal Peyer's patches). In such structures, it is represented by diffuse lymphoid tissue and lymphoid nodules and is located in the lamina propria of the mucosa and submucosa.

Further research should be directed to the study of the morphological features of the digestive canal and its lymphoid formations in other bird species of the order Passeriformes.

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