Morphofunctional Features of Lymphoid Tissue of the Stomach in Some Wild Bird Species

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Abstract. The article presents the results of histological studies of topography, microstructure and functional features of the stomach lymphoid tissue in birds. The research was carried out on 12 species of wild birds belonging to 6 orders. Classical methods of histological specimens staining with hematoxylin and eosin, according to Weigert, Van Gieson, and argentum nitrate impregnation according to Kelemen were used when performing the work. It has been confirmed that the birds' stomach has glandular (proventriculus), muscular (ventriculus, gizzard) and pyloric parts, which are not equally expressed in separate species. In all parts of the stomach, lymphoid tissue is represented by local clusters, which in birds of the orders Galliiformes (P. colchicus, B. bonasia, P. crictatus); Anseriformes (B. canadensis); Passeriformes (P. pica, C. cornix); Gruidiformes (G. chloropus, F. atra); Ciconiiformes (C. ciconia) and Columbiformes (C. livia) are located in the tunica mucosa and submucosa, and in B. canadensis, F. atra, G. chloropus also in the tunica muscularis and tunica serosa. In L. lagopus and G. glandarius, lymphoid tissue has been found only in the proventriculus and its intermediate zone. Structural levels of lymphoid tissue (diffuse form, prenodules, primary and secondary lymphoid nodules) are unequally expressed in the birds’ stomach. In B. bonasia, B. canadensis, P. pica, C. cornix, C. ciconia, C. livia, only a diffuse form appears in all its parts. This form of lymphoid tissue is registered in the ventriculus of other birds species and proventriculus in L. lagopus and G. glandarius. All levels of the lymphoid tissue structural organization are detected in the proventriculus in P. crictatus, G. chloropus and in the proventriculus and pyloric part of the stomach in P. colchicus and F. atra. The content of lymphoid tissue is different in parts of the birds' stomach. Most of all it is contained in the intermediate zone of the proventriculus and the pyloric part of the stomach. Diffuse form is the most common among the levels of lymphoid tissue structural organization. The research results make it possible to more fully assess the morphofunctional status of wild birds and, accordingly, to find out the state of their immune system.

Keywords: digestive system, microstructure, immune formations, lymphoid nodules, lymphoid cells
INTRODUCTION

It is known that lymphoid tissue forms the functional basis of peripheral organs of hemo- and lymphopoiesis and lymphoid (immune) formations localized in the wall of tubular organs. In them, when exposed to antigens, lymphocytes differentiate into effector cells that determine specific immunity [1].

Despite the significant role of stomach lymphoid tissue in maintaining homeostasis, its structure and, accordingly, functional features in wild birds have been insufficiently studied and require significant clarification. This concerns the topography, features of the location, area and content of separate levels of its structural organization. Information about structure of the birds’ stomach and its parts in accordance with the requirements of the modern anatomical nomenclature of these animals demands significant clarifications.

The avian stomach is an enlarged part of the digestive tube and is located between the esophagus and intestines [1]. The general structure of the stomach is typical for all species of birds, but due to the fact that the range of their feed specialization is very wide, it has some specific features. In this regard, it is conventionally divided into three groups. The first group includes the stomach of carnivorous birds, the main function of which is to preserve food. The stomach of granivorous birds belongs to the second group. Its main function is the mechanical processing of the feed. The third group of stomachs is characteristic of herbivorous birds, the main function of which is the storage and mechanical processing of feed [2].

Until recently, it was believed that the avian stomach consists of two parts: proventriculus and ventriculus [3]. According to the modern International anatomical nomenclature of birds [4], three parts are distinguished in it: proventriculus, ventriculus and pyloric, not equally expressed in certain species. The proventriculus is an extension of the esophagus, spindle-shaped and consists of an apex, a body and an intermediate zone (isthmus) [5]. It connects to the esophagus by a narrower apex directed cranially, and with ventriculus by a short intermediate zone located caudally [6]. The ventriculus has a disc-like shape and passes into the pyloric part, from which the duodenum begins. The pyloric part is well developed in wild birds. The transition zone of the proventriculus to the ventriculus is named after acad. V.G. Kasyanenko National University of Life and Environmental Sciences of Ukraine. Its highest content is found in the sphincters area of intermediate zone and pyloric part, which regulate the food flow to ventriculus and the duodenum, respectively [9]. According to separate authors [2; 11], large lymphoid tissue clusters are concentrated in transition zone of the proventriculus to the ventriculus.

The purpose of this study was to establish the morphofunctional features of stomach lymphoid tissue in wild birds.

MATERIALS AND METHODS

The studies were carried out during 2015-2020 in the scientific laboratory of immunomorphology, Department of anatomy, histology and pathomorphology of animals named after acad. V.G. Kasyanenko National University of Life and Environmental Sciences of Ukraine, Kyiv. Material for research was selected from 12 species of mature wild birds belonging to 6 orders (Table 1). The material was selected from the funds of the I.I. Shmalhausen Institute of Zoology of the National Academy of Sciences of Ukraine. It was stored in containers with a 10% formalin aqueous solution. The birds were clinically healthy and showed no signs of disease. 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) [12] and the Decree of the First National Congress on Bioethics (Kyiv, 2001) [13].

| Order     | Species                               | Number |
|-----------|---------------------------------------|--------|
| Galliformes | Phasianus colchicus Linnaeus, 1758     | 5      |
|           | Bonasa bonasia (Linnaeus, 1758)       | 3      |
|           | Lagopus lagopus (Linnaeus, 1758)      | 3      |
|           | Pavo crictatus Linnaeus, 1758         | 3      |

Table 1. Bird species which histological material was used in the study

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Birds dissection and stomach preparation were performed according to existing guidelines [14]. Parts of the stomach were cut lengthwise with sharp-pointed scissors and carefully freed from the contents. The cuticle was also removed in the gizzard. From each part of the stomach with a sharp-pointed scalpel, pieces of their wall 1 cm long and 1 cm wide were cut out, which were labeled. The collected material was washed in running water for 24 hours. After that, it was dehydrated in alcohols of increasing concentration (50%; 60%; 70%; 80%; 96%; absolute alcohol) in each alcohol the material was kept for 2-3 hours. Then it was cleared and embedded in paraffin according to the generally accepted technique. The paraffin-embedded material was placed on wooden blocks. From the obtained blocks, with the help of a sled microtome MPS-2, histological sections with a thickness of 5–10 μm were made and placed on slides. Sections were stained with Karatsi hematoxylin and eosin – to establish the general microstructure of the stomach and its lymphoid tissue, according to Weigert – to detect elastic fibers, according to van Giezon – to identify collagen fibers and impregnated with silver nitrate according to Kelemen – for registration of reticular fibers. Stained and impregnated sections were immersed into Canadian balm and examined with an Olympus light microscope. Individual histopreparations and their fragments were photographed with a Nicon Coolpix S3100 camera. To compare the area and levels of structural organization of lymphoid tissue in parts of the stomach were used morphometric methods.

RESULTS AND DISCUSSION

General microstructure of the avian stomach

The avian stomach has three parts: glandular proventriculus, muscular ventriculus (gizzard) and pyloric. Each of these parts is formed by the tunica mucosa, tela submucosa, tunica muscularis and tunica serosa. Its mural organization have been similarly described in many bird species such as the Japanese quail [1], the Coot bird [15], the pigeon [11], the common moorhen [10].

The proventriculus tunica mucosa forms low longitudinal folds and is represented by the epithelium, lamina propria and lamina muscularis mucosae. The lamina epithelialis mucosae is a simple columnar and glandular. The lamina propria is formed by loose fibrous connective tissue. It is penetrated by numerous simple, weakly branched glands. The lamina muscularis mucosae is well developed and is represented by bundles of longitudinally oriented smooth muscle cells. The submucosa, like the lamina propria, is formed by loose fibrous connective tissue. It contains lobules of deep glands. Their excretory ducts open on the tunica mucosa surface with papillae. The structure of the deep glands lobules of the in birds of the studied species has its own species features. There are no deep glands in the area of the intermediate zone. In birds of certain species, the epithelium of the intermediate zone is covered with a delicate cuticle. The tunica muscularis of this zone is formed by three layers of smooth muscle cells: internal oblique, middle circular and external longitudinal (the latter is poorly developed in many bird species). Between the outer and middle layers of the tunica muscularis, layers of loose fibrous connective tissue with blood and lymphatic vessels and nerve plexuses are revealed. The tunica serosa is formed by loose fibrous connective tissue, which is externally covered with mesothelium. The gizzard is disc-shaped. On its lateral surfaces, tendon mirrors are visible, and in the cranial and caudal regions – the blind sacs of the same name. The intermediate zone of the proventriculus opens into the cranial blind sac. On the right lateral surface of the gizzard is the pyloric part, from which the duodenum begins. Simple cuboidal epithelium lined the surface of the tunica mucosa. The lamina propria contained simple tubular glands. Known, they produce a secret that forms a cuticle on the surface of the stomach mucosa, which performs a protective function. Its density varies in different species of birds. The lamina muscularis mucosae is absent in the tunica mucosa. The submucosa is represented by dense fibrous connective tissue. The tunica muscularis is best developed. It is formed by massive bundles of smooth muscle cells that form muscles. The pyloric part of the stomach is similar in structure to the gizzard, but its tunica muscularis is poorly developed, but lamina muscularis mucosae appears. It is formed by fragmented bundles of smooth muscle cells.

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Morphofunctional features of the avian stomach lymphoid tissue

The results of carried studies confirm the data of other authors [15] that in most bird species the stomach lymphoid tissue is associated with its tunica mucosa and tela submucosa. Thus, this tissue was found in all parts of the birds’ stomach of the orders Galliformes (P. colchicus, B. bonasia, P. crictatus) Anseriformes (B. canadensis) Passeriformes (P. pica, C. cornix) Gruiformes (G. chloropus, F. atra) Ciconiiformes (C. ciconia) and Columbiformes (C. livia). In L. lagopus and G. glandarius, lymphoid tissue was recorded only in the proventriculus and its intermediate zone, and it was not detected in other parts of it. In birds of the orders Anseriformes and Gruiformes, single clusters of this tissue are also contained in the tunica muscularis and tunica serosa. In this regard, it is proposed to distinguish the lymphoid tissue of the mucosa, muscularis and serosa based on the location in the stomach.

Lymphoid tissue in the lamina propria of the tunica mucosa of the birds stomach is located under the surface glands and between them, and in the submucosa of proventriculus – in the lobules of deep glands and between them (Fig. 1a, b) (except B. bonasia, L. lagopus, P. pica, G. glandarius, C. cornix and C. ciconia). Local clusters of lymphoid tissue in the submucosa are detected mainly around the blood vessels in the intermediate zone, the gizzard and pyloric parts of the stomach. In some species of birds, they pass along the blood vessels into the tunica muscularis [6].

Figure 1. Proventriculus of C. livia (a) and F. atra (b): 1 – lamina propria mucosae; 2 – lymphoid tissue; 3 – lobules of the deep glands; 4 – tunica muscularis. Van–Gieson, ×100; H&E staining, ×90

Lymphoid cells in the areas of lymphoid tissue localization infiltrate the lamina epithelialis mucosae and the glands epithelium, and are also occasionally detected in the lumens of latter. Similar processes in the stomach of various bird species have been observed by other researchers [16]. In the stomach tunica muscularis, lymphoid tissue clusters occur as its continuation from the tunica mucosa. In birds of the Anseriformes orders, they are located around the blood vessels in all its parts, in Gruiformes, and in some species of Galliformes (P.colchicus) – in the gizzard and pyloric parts of the stomach. In F. atra, lymphoid tissue is found in the tunica muscularis of the proventriculus and its intermediate zone.

In the subserosa of the stomach tunica serosa, lymphoid tissue is detected mainly in birds of the Anseriformes and Gruiformes orders. Thus, in the subserosa of the proventriculus and the intermediate zone of G. chloropus and F. atra, clusters of diffuse lymphoid tissue and primary and secondary lymphoid nodules are revealed. The presence of lymphoid tissue in the tunica muscularis and tunica serosa of the stomach of certain bird species is confirmed by the data of other authors [16] and indicates that their digestive system experience a greater antigenic effect, which is associated with their feed specialization.

In all parts of the birds’ stomach, lymphoid tissue is represented by unequal levels of structural organization. Thus, in the proventriculus, gizzard and pyloric part of the stomach of B. bonasia (Fig. 2a), B. canadensis, P. pica, C. cornix (Fig. 2b), C. ciconia and C. livia, only its diffuse form is revealed. In L. lagopus and G. glandarius, the lymphoid tissue of the proventriculus and its intermediate zone is also represented by a diffuse form, while in other parts of the stomach it is not observed. P.colchicus (Fig. 3a) and F. atra lymphoid tissue of the proventriculus, its intermediate zone and the pyloric part of the stomach is represented by both diffuse lymphoid tissue and lymphoid nodules. In G. chloropus, lymphoid nodules are found only in the proventriculus and its intermediate zone. In P.cricatus (Fig. 3b), lymphoid nodules are found only in the proventriculus.

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To establish the development of lymphoid tissue in the tunica mucosa and submucosa of various parts of the birds’ stomach, we determined the area that it occupies in them (Table 2). Most of all it is contained in the intermediate zone and the pyloric part of the stomach in most birds. In the intermediate zone, the largest area of this tissue is observed in B. canadensis, somewhat smaller in P. colchicus, and the smallest in L. lagopus, P. pica, C. ciconia and C. livia. In the pyloric part of the stomach, the largest area of lymphoid tissue is recorded in P. colchicus and the smallest in P. pica and C. livia, while it is absent in L. lagopus and G. glandarius. In the proventriculus and gizzard (ventriculus), lymphoid tissue occupies a much smaller area. In the proventriculus, it is the largest in F. atra, slightly smaller in P. colchicus and G. chloropus, and the smallest – in L. lagopus, G. glandarius, C. ciconia and C. livia. 

In the gizzard, the largest area of lymphoid tissue is recorded in P. colchicus, somewhat smaller in P. crictatus and G. chloropus, the smallest in C. ciconia and C. livia, and in L. lagopus and G. glandarius it is not observed. According to the obtained results, this is due to the morphological and functional characteristics of separate parts of the stomach, and in fact, with the time the contents remain in them and, accordingly, the duration of the effect of its antigens on the tunica mucosa. So, it is known [3] that food in the proventriculus does not linger for a long time. It is impregnated with the secretion of the glands, which contains bactericidal substances, and enters its intermediate zone. In the intermediate zone, the contents are retained for a longer time, which is due to the presence between it and the gizzard of the annular sphincter, which regulates the flow of contents into the gizzard [8].

Figure 2. The proventriculus of B. bonasia (a) and C. cornix (b): 1 – lamina propria mucosae; 2 – diffuse lymphoid tissue; 3 – lobules of the deep glands. H&E staining, ×100; ×90

Figure 3. The proventriculus of P. colchicus (a) and P. crictatus (b): 1 – lamina propria mucosae; 2 – lymphoid nodules; 3 – lobules of a deep glands. H&E staining, ×90; Weigert staining, ×100
Table 2. The area of lymphoid tissue in the stomach mucosa of birds (M±m, %)

| Species of birds | Glandular part | Intermediate zone | Muscular part | Pyloric part |
|------------------|----------------|-------------------|--------------|-------------|
| P. colchicus     | 6.14±0.06      | 17.66±0.12        | 7.75±0.30    | 31.22±0.25  |
| B. bonasia      | 3.29±0.33      | 5.65±0.16         | 2.10±0.06    | 6.57±0.25   |
| L. lagopus       | 1.25±0.05      | 0.89±0.05         | –            | –           |
| P. cristatus     | 2.15±0.04      | 10.10±0.16        | 5.78±0.09    | 25.60±0.17  |
| B. canadensis    | 3.62±0.04      | 28.76±0.16        | 3.98±1.24    | 13.60±0.15  |
| P. pica          | 3.12±0.14      | 1.87±0.06         | 1.13±0.06    | 1.05±0.05   |
| G. glandarius    | 1.36±0.05      | 8.17±0.48         | –            | –           |
| C. cornix        | 3.98±0.30      | 7.46±0.51         | 1.62±0.20    | 3.10±0.41   |
| G. chloropus     | 5.01±0.07      | 3.01±0.1          | 4.92±0.16    | 7.92±0.24   |
| F. atra          | 7.23±0.21      | 7.44±0.19         | 1.65±0.13    | 6.32±0.19   |
| C. ciconia       | 1.05±0.04      | 1.75±0.03         | 0.88±0.02    | 3.54±0.06   |
| C. livia         | 1.10±0.07      | 1.50±0.22         | 0.58±0.12    | 1.14±0.14   |

In the gizzard, as noted above, the area of the lymphoid tissue is insignificant, which is primarily due to its morphological features. It is known that the secretion of the glands of this part of the stomach contains keratin-like substances. It thickens on the surface of the tunica mucosa, forming the cuticle. The latter prevents the impact of antigens on it, which has also been reported by other researchers [17-19]. In the pyloric part of the stomach, due to the presence of a sphincter between it and the duodenum, its contents are retained for a longer time. Accordingly, the period of exposure to its antigens on the tunica mucosa is much longer.

As noted above, all levels of the structural organization of the lymphoid tissue are not detected in all parts of the birds’ stomach. In some species, only diffuse lymphoid tissue is recorded in the stomach, and in some parts of it there is no lymphoid tissue at all. That is, the morphofunctional maturity of the birds’ stomach lymphoid tissue and its separate parts is not the same. It is possible that such a presence of lymphoid tissue forms in the birds’ stomach, or its absence in some of its parts, is their specific feature. For a more exact answer to this question, it is necessary to conduct research on the development of the birds’ stomach lymphoid tissue in their ontogenesis.

In most bird species, in all parts of the stomach, only the first level of the structural organization of lymphoid tissue is recorded – its diffuse form (Table 3, 4). In birds, in which all levels of the structural organization of this tissue are found, diffuse lymphoid tissue occupies the largest area, primary and secondary lymphoid nodules occupy a smaller area, and perinodules – the smallest one. The latter were not observed in some parts of the stomach. Secondary lymphoid nodules in the lymphoid tissue of the proventriculus are most detected in F. atra, somewhat less in G. chloropus, and the least in P. cristatus and P. colchicus. In the intermediate zone, there are more of them in F. atra, somewhat less in G. chloropus, and least of all in P. colchicus. Their highest content in the pyloric part is recorded in F. atra, and the lowest – in P. colchicus. In the gizzard, the structural level of lymphoid tissue is represented only in a diffuse form, other levels of the structural organization of the lymphoid tissue are not found.

Table 3. The content of lymphoid tissue components in the proventriculus and in the intermediate zone (M±m, %)

| Species of birds | Glandular part | Intermediate zone |
|------------------|----------------|-------------------|
|                  | Diffuse lymphoid tissue | Pre-nodules | Primary nodules | Secondary nodules | Diffuse lymphoid tissue | Pre-nodules | Primary nodules | Secondary nodules |
| P. colchicus     | 79.03±0.26 | 1.67±0.15 | 4.71±0.25 | 14.59±0.19 | 88.83±0.20 | – | 0.94±0.16 | 10.23±0.30 |
| B. bonasia      | 100            | –          | –          | –          | 100            | –          | –          | –          |
| L. lagopus       | 100            | –          | –          | –          | 100            | –          | –          | –          |
| P. cristatus     | 87.52±0.76 | 1.56±0.26 | 5.56±0.70 | 5.36±0.63 | 100            | –          | –          | –          |
| B. canadensis    | 100            | –          | –          | –          | 100            | –          | –          | –          |
| P. pica          | 100            | –          | –          | –          | 100            | –          | –          | –          |

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The lymphoid tissue in the stomach wall of the studied species birds is unevenly located. In terrestrial species, it is contained mainly in the tunica mucosa and tela submucosa, and in waterfowl – also in the tunica muscularis and tunica serosa. The lymphoid tissue is unevenly located in certain parts of the stomach. It occupies the largest area in the wall of the intermediate zone of the proventriculus and in pyloric part, and in the gizzard – the smallest. The lymphoid tissue of the stomach not in all species of the studied birds has all levels of structural organization, which is an indicator of its morphological and functional maturity. To clarify this issue, it is necessary to conduct research on the development of lymphoid tissue in the ontogenesis of certain bird species.

The obtained data on the morphofunctional features of the lymphoid tissue of the birds stomach are recommended to be used in the work of scientists studying the organs of the immune system, and in educational work.

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Ключові слова: апарат травлення, мікроструктура, імунні утворення, лімфоїдні вузли, лімфоїдні клітини

Морфофункціональні особливості лімфоїдної тканини шлунка у окремих видів диких птахів

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Анотація. У статті наведені результати гістологічних досліджень топографії, мікроструктури та функціональних особливостей лімфоїдної тканини шлунка птахів. Дослідження проведено на 12 видах диких птахів, які належать до 6 рядів. При виконанні роботи використовували класичні методи пофарбування гістопрепаратів гематоксиліном та еозином, а також методи гістохімії.

Ключові слова: апарат травлення, мікроструктура, імунні утворення, лімфоїдні вузли, лімфоїдні клітини

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