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Morphological features of immune structures in the stomach of turkeys during the post-vaccination period

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Abstract. Up to 70% of antigenic material enters the birds' bodies via the digestive system; this material acts as an aetiological factor and leads to a range of diseases. Consequently, immune structures develop in their walls, the cells of which protect the body against agents carrying genetically foreign information. The aim of this study was to trace the development of immune formations in the stomach of turkeys under vaccination and to determine the timing of their morphofunctional maturity. For the study, 66 individuals of Big-6 turkeys were selected and divided into an experimental (vaccinated) and a control (unvaccinated) group. Material for histological examination was collected on days 1, 10, 20, 30, 40 and 50 of life. It was shown that in turkeys of both the experimental and control groups, the structural organisation of lymphoid tissue, which forms the basis of immune formations, develops in stages – from diffuse accumulations of lymphocytes to the formation of pre-nodules and lymphoid nodules. Thus, in day-old turkeys, local accumulations of diffusely arranged lymphocytes were observed in the glandular part of the stomach, maintaining close contact with epithelial cells of the surface epithelium and the epithelium of tubular glands. By day 10, pre-nodules with a denser arrangement of lymphocytes were recorded in both groups, while in the experimental group primary lymphoid nodules bounded by a capsule were also present. The latter were detected in the control group from day 20. Secondary lymphoid nodules with germinal centres in the glandular part of the stomach were observed in the experimental group from day 20, and in the control group from day 30, indicating the state of morphofunctional maturity of the immune formations. In the muscular part of the stomach, immune formations were poorly

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developed. In turkeys of both groups, isolated accumulations of lymphocytes were recorded from day 30, and in some individuals of the experimental group, clusters of secondary lymphoid nodules were detected in the deep layers of the muscular coat by day 50. The obtained results make it possible to clarify the mechanisms of specific immune response associated with T- and B-cells, which will contribute to the development of new effective strategies for combating poultry diseases

Keywords: histological studies; histotopography; diffuse lymphoid tissue; pre-nodules; lymphoid nodules; morphofunctional maturity; poultry

Introduction

Modern industrial poultry farming is characterised by high stocking densities, intensive rearing practices and significant epizootic risks, which places increased demands on the system for the prevention of infectious diseases. Under such conditions, vaccination remains one of the key tools for inducing specific immunity and ensuring the stability of poultry production. At the same time, the effectiveness of vaccination depends to a large extent on the morphofunctional state of the immune system's organs, in particular the immune structures associated with the mucous membranes of the digestive tract.

To prevent infectious diseases and combat stock losses on poultry farms, birds are vaccinated with live and inactivated vaccines to establish long-lasting immunity. As noted by C. Ceccopieri & J.P. Madej (2024), changes in the environment, the evolution of pathogens and the establishment of large-scale production are constantly altering the nature of infectious diseases affecting domestic poultry. Furthermore, the intensive breeding of various bird species to achieve desired production characteristics causes immunosuppressive stress. It is precisely the morphofunctional study of the avian immune system that will enable specialists to develop more modern protocols and devise new vaccination strategies in poultry farms.

R. Capotă *et al.* (2025) in their work classified the organs of the avian immune system into central organs, where blood cells are formed, and peripheral organs. They classified the red bone marrow and the thymus – where T-lymphocytes arise, mature and proliferate – as central organs, along with the cloacal sac, where B-lymphocytes undergo similar processes. The peripheral organs of the immune system include the spleen, lymph nodes (in waterfowl) and immune (lymphoid) structures of the digestive, respiratory and skin systems, and the Harderian gland. These organs serve as the site where antigens encounter immunocompetent cells, the site of antigen recognition and the development of cellular and humoral immune responses, the site of interaction between immunocompetent cells, their proliferation, antigen-dependent differentiation, and the site of accumulation of immune response products. G. Garagulya *et al*. (2022) believed that the purpose of humoral immunity is to rid the body primarily of antigenically foreign exogenous substances, whilst that of cellular immunity is the elimination of autoantigens, which may turn out to be the body's own mutated and denatured cells. According to E.C. Lavelle & R.W. Ward (2022) and C. Ceccopieri & J.P. Madej (2024), the digestive organs of birds, which are in contact with the external environment, are constantly exposed to antigenic influences, resulting in the development of immune structures associated with the mucous membranes (GALT – gut-associated lymphoid tissue) within their walls. According to research by S. Usenko (2023), such structures were also recorded in the muscular and serous membranes. Immune structures are represented by significant clusters (tonsils, Peyer's patches), isolated lymphoid nodules, local accumulations of diffuse lymphoid tissue, and intraepithelial lymphocytes, etc.

According to V.H. Mutlak & S.S. Faraj (2024), the absence of teeth and a soft palate, along with limited involvement in oral digestion, facilitates the rapid swallowing of water and food and their passage into the stomach. In most bird species, according to the findings of E. Khayoon & A. Rechag (2024) and Y.Y. Al-Seady (2025), the stomach is divided into glandular and muscular parts, the boundary between which is clearly marked by a constriction (isthmus). Microscopically, the stomach wall consists of mucous, muscular, and serous membranes, which differ in structure and function. In the glandular part, lobules of deep glands of the mucous membrane produce gastric juice responsible for digestion, whereas in the muscular part, a well-developed muscular coat enables compression and grinding of feed through muscle contractions. According to research by N.J. Monisha *et al.* (2024), isolated localised clusters of diffuse lymphoid tissue and lymphoid nodules of varying degrees of maturity were found in the gastric wall of birds. The microstructural features of the digestive organs and their lymphoid structures, particularly in the stomach, have been relatively well studied in gallinaceous birds and certain wild birds with different trophic specialisations, as noted in the works of S. Usenko (2023) and A. El-Mansi *et al*. (2025).

An analysis of scientific sources revealed that information on the microscopic structure of the immune structures of the turkey stomach in the specialist literature is limited, and the influence of vaccination on the timing of their morphofunctional maturity remains insufficiently studied. This necessitates the conduct of specialised morphological studies in this area. Thus, the aim of the study was to determine the morphofunctional state of the immune structures of the turkey stomach in relation to age following vaccination, which are directly responsible for the immunological reactivity of the birds' organism.

Literature Review

As noted by S. Qinghui *et al*. (2020), the stomach of most bird species consists of two parts: the glandular and muscular regions, connected by a narrow section – the isthmus – which, according to research by D. Yovchev (2022), develop most intensively in the first few days after hatching. A significant area of the mucous membrane of the glandular part of the stomach, according to F. Taki-El-Deen (2017), is occupied by lobules of deep glands that produce gastric juice. In carnivorous and insectivorous birds, these are single-lobed, whereas in omnivorous and herbivorous-granivorous birds, they consist of many lobules separated by connective tissue septa. Each lobule is formed by glands (tubules) which, when joined, form the central and secondary ducts. Several secondary ducts combine to form a primary duct that opens onto a protruding papilla of the mucous membrane. In the muscular part of the avian stomach, the muscular coat, composed of a powerful layer of smooth muscle tissue, ensures peristalsis, mixing, and movement of feed, while the cuticle covering the mucous membrane is relatively dense and protects it from damage by hard particles.

In the study by R. Matsumoto & Y. Hashimoto (2000), it was noted that on the 20th day of chicken incubation, diffuse clusters of lymphocytes were first detected in the mucous membrane of the glandular part of the stomach and were located in the lamina propria between the tubular glands, their excretory ducts and at the border with the surface epithelium. On the 7th day after hatching, CD3+ lymphocytes occupied the central part, whilst His-C1+ B-lymphocytes were located in the peripheral areas of the lymphoid clusters. Lymphoid nodules with pale centres in the lobules of the deep glands were recorded by the researchers during the third week of the postnatal period of avian ontogenesis. N.K. Singh & M.K. Sinha (2022) reported that in Gramparia chickens, the glandular part of the stomach contained lymphoid tissue, the cells of which were predominantly located near the papillae of the mucous membrane.

V. Khomych *et al.* (2021) conducted a study of the immune structures of the stomach in various species of sexually mature birds. The researchers demonstrated that the development of the structural levels of lymphoid tissue in the stomach and its parts varies. In the gastric mucosa of the hazel grouse, goose, magpie, crow, stork and pigeon, only the first structural level was detected – a diffuse form of lymphoid tissue. The latter was also present in the glandular part of the stomach of the partridge and jay, and in the muscular part of the stomach of most of the birds studied. All levels of structural organisation of lymphoid tissue were observed in the stomach of the goose and in the glandular and pyloric parts of the hen, guinea fowl, quail, turkey, pheasant and partridge. Its highest concentration was recorded in the intermediate zone (isthmus) and the pyloric part of the stomach.

At the same time, the study by N. Hamoda & A. Farag (2018) reported that the lamina propria of the mucous coat in the glandular and muscular parts of the pigeon's stomach contains diffuse lymphoid tissue, which is localised predominantly near and between the tubular glands. Similar foci of lymphoid tissue were detected in the mucosa at the junction of the stomach and duodenum, where pyloric tonsils formed, and lymphocytes migrated into the lower layers of the epithelium, forming lymphoepithelium. M.F. Kovtun & L.P. Kharchenko (2005) reported that in pheasants and quails, the wall of both parts of the stomach is moderately infiltrated with lymphoid cells. In pheasants, isolated oval and round nodules were found in the mucous coat of the glandular part of the stomach, predominantly located near the excretory ducts of the compound glands. The researchers observed significant lymphocyte infiltration and large lymphoid nodules in the wall of both parts of the stomach of the common rook, whilst in the golden oriole and the chaffinch, lymphoid elements predominated in the serous membrane and between the layers of the muscular coat.

As established by L.P. Kharchenko & I.A. Lykova (2013), the walls of both parts of the stomach in wading birds are moderately infiltrated with lymphoid cells. In the common redshank, ruff, red-breasted sandpiper, and dunlin, the researchers noted marked lymphocytic infiltration of the connective tissue layers between the lobules of deep glands in the glandular part of the stomach, whereas in the wood sandpiper, greenshank, and grey plover, lymphoid nodules were identified within the walls of these lobules. M.K.A. Maksoud *et al.* (2022) reported that in the hooded crow, the lamina propria of the mucous membrane of the glandular stomach consisted of connective tissue infiltrated with lymphocytes, with solitary lymphoid nodules located between tubular glands. The submucosal layer contained well-developed tubuloalveolar glands grouped into lobules, composed of oxynticopeptic and enteroendocrine cells.

S. Das *et al.* (2020) noted that in an Indian breed of chicken, accumulations of lymphocytes in the glandular part of the stomach were mainly located in three distinct regions of the mucous membrane: the lamina propria, connective tissue, and near the superficial glands. However, in day-old and 7-day-old birds, the distribution of lymphoid tissue was not clearly defined or noticeable. Well-organised lymphocytic aggregates were observed in 28-day-old and 112-day-old birds, predominantly in the lamina propria beneath the epithelium and near the superficial glands. In 112-day-old birds, a marked increase in the size and clustering of lymphoid tissue was observed, with clearly expressed aggregation of lymphoid nodules possessing well-defined germinal centres. These accumulations of lymphoid tissue were separated by layers of connective tissue.

Thus, the results of the cited studies indicate that immune formations in the stomach wall of birds are characterised by considerable species-specific, age-related, and topographical variability, and that their structural organisation is closely associated with the functional state of the digestive system and the characteristics of the immune response to antigenic load. The physiological processes associated with the development of immune structures in the stomach wall of birds are of significant scientific interest and highlight their importance in the establishment of the immune defence system. Understanding the influence of vaccination on the formation of such structures has practical significance and will enable the development of more advanced preventive and therapeutic strategies.

Materials and Methods

Histological studies of the stomach of turkeys were carried out during 2025 in the educational, scientific and production laboratory "Centre of Biomorphological Technologies" of the Department of Biomorphology of Vertebrates named after Acad. V.G. Kasyanenko of the National University of Life and Environmental Sciences of Ukraine. All procedures and euthanasia were performed in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (1986), Law of Ukraine No. 3447-IV (2006), Order of the State Committee of Veterinary Medicine of Ukraine No. 365 (2010), and in accordance with ARRIVE recommendations (n.d.). Approval for the study was obtained from the Bioethics Commission of the National University of Life and Environmental Sciences of Ukraine for the use of animals in scientific research (No. 045/2025, dated 26 June 2025).

A total of 66 female turkeys of the hybrid broiler breed Big-6 were selected for the study. All birds were clinically healthy and showed no signs of disease. At one day of age, they were divided into an experimental (vaccinated) and a control (unvaccinated) group. Vaccination of the birds was carried out in accordance with the vaccination prevention and treatment schedule. Turkeys of both groups were kept under the conditions of the poultry farm of LLC "Volodar" (Tetiiv, Ukraine).

The anatomical level of the study involved sampling from two parts of the stomach (glandular and muscular). For this purpose, samples were collected on days 1, 10, 20, 30, 40, and 50 of life. For comprehensive histological and morphometric studies, histological preparations were produced using standard techniques. Tissue samples from the glandular and muscular parts of the stomach were placed in histological cassettes and fixed in a 10-12% aqueous solution of neutral formalin. The formalin-fixed material was washed in running tap water for 24-48 hours. To remove water, samples were dehydrated in ethanol of increasing concentrations – 40°, 70°, 96°, and 100° – for one to three hours. After dehydration, the material became fragile and brittle; therefore, paraffin embedding was used for consolidation. Prior to embedding, the material was passed through a mixture of alcohol and chloroform, then through pure chloroform, followed by a chloroform-paraffin mixture (at 37°C), and finally paraffin (at 55-56°C). The material was then placed into special metal moulds, embedded in molten paraffin, and solid paraffin blocks were obtained. Histological sections 5-10 μm thick were prepared using a sliding microtome MPS-2 (MedTech-Price, Ukraine). To study the microscopic structure of the stomach, sections were stained with Karatsi haematoxylin and eosin, according to Weigert's method, and silver nitrate impregnation was performed according to Kelemen. The area of lymphoid tissue and its structural-functional levels were determined using the point-counting method with a binocular microscope and a measuring grid. Measurements of lymphoid nodules (round and oval) were performed using an eyepiece micrometer with an Olympus CX43 microscope (Labdepo, Japan).

The obtained results were recorded in protocols. Digital data from morphometric studies were processed on a personal computer using StatSoft Statistica 13.1 (2016) software. Statistical significance was assessed by calculating the arithmetic mean (M) and the standard error of the mean (m) using Student's t-test at three significance levels: $P < 0.05$, $P < 0.01$, and $P < 0.001$. Microphotography of selected histological sections of the glandular and muscular parts of the turkey stomach was performed using a Primo Star video camera (Carl Zeiss, Germany) integrated into the microscope and connected to a personal computer.

Results and Discussion

General microscopic structure of the stomach wall

Research findings have shown that the turkey's stomach begins at the caudal end of the oesophagus with a thickening of the digestive tract (the glandular portion), which is connected by a narrow isthmus to a substantial, thick-walled, disc-shaped expansion (the muscular portion). The glandular and muscular parts of this organ differed significantly in the microscopic structure of their walls, which is due to the different functional roles of each of these parts. At the junction of the stomach and duodenum, a third – the pyloric part – was faintly visible; this part is similar in microstructure to the muscular part but has a thinner wall. The mucous membrane of the glandular part of the stomach in turkeys from both study groups was highly developed and formed numerous small, uneven folds directed towards the lumen of the organ. Its inner layer consisted of simple columnar glandular epithelium, the cells of which were densely packed and secreted a mucous substance. A viscous secretion formed a protective film covering the entire inner surface of the glandular stomach (Fig. 1).

Figure 1. Mucous membrane of the glandular part of the stomach of a day-old turkey

Note: *1 – surface epithelium with mucous secretion; 2 – tubular glands of the lamina propria; 3 – muscularis mucosae; 4 – lobules of deep glands of the submucosa; 5 – layers of loose fibrous connective tissue. Stained with haematoxylin and eosin, ×80*

Source: *authors' own work*

The surface epithelium extended into the lamina propria, forming numerous gastric pits; mitoses were observed within the cells of these pits, indicating active intraepithelial replacement of the differentiated and secretory portions of the epithelial cells by younger cell generations. Similarly, A.A. Alsanosy *et al.* (2021), when studying the organs of the digestive tract in domestic chickens and common quails, established that the folds of the proventriculus varied in height; the researchers proposed referring to these as folds, and the intervening depressions as grooves. Short, simple tubular glands of the lamina propria of the mucous membrane extended from the base of the sulcus. As noted by A.M. Abdellatif *et al.* (2022) and D. Saran & B. Meshram (2021), the mucus covering the glandular portion of the avian stomach is enriched with acidic mucopolysaccharides possessing bactericidal properties, which play an important role in lubricating and protecting the surface layers of the mucous membrane from pathogenic microflora and toxins.

The lamina propria of the gastric mucosa is rich in fibrillar elements due to collagen and elastic fibres, which are most pronounced in birds aged 40 and 50 days. Its foundation is formed by loose fibrous connective tissue, which provides structural support to the surface epithelium, serves as a connection to the muscular layer, and contains a large number of cellular elements, well-defined blood vessels, and superficial tubular glands. The muscular plate is discontinuous in places and consists of bundles of smooth muscle cells arranged predominantly in a circular pattern. However, it is not present in all birds; in particular, according to D. Yovchev (2022), it is absent in the wild bronze turkey.

The largest area of the mucous membrane was occupied by complex, sac-like deep glands, which are grouped into lobules with a centrally located cavity. A complex of numerous glandular tubules (glandular tubes) in the form of columns radiated outwards from the centrally located cavity. The glands were formed by epithelium which, depending on the phase of secretion, was simple cuboidal or cylindrical and produced gastric juice. The latter was stained pink and was observed in the cavities of individual lobules. The lobules of the deep glands were bounded by layers of loose fibrous connective tissue containing predominantly small-calibre arteries and veins, and a well-developed elastic plexus that transitioned into intermuscular connective tissue. In the area of the gastric intermediate zone, the lobules gradually disappeared, and the submucosal layer became thinner. The muscular and serous layers of the glandular part of the stomach were relatively thin (Fig. 2).

Figure 2. Wall of the glandular part of the stomach of a turkey from the experimental group at 50 days

Note: *1 – lobule of a deep gland of the submucosa; 2 – muscular coat: 2a – inner longitudinal layer, 2b – middle circular layer, 2c – outer longitudinal layer; 3 – elastic fibres; 4 – serous membrane. Weigert staining, ×90*

Source: *authors' own work*

In the muscular coat, which is formed by smooth muscle tissue, three layers were clearly distinguished: the outer and inner longitudinal layers and the middle circular layer. The circular layer was considerably thicker than the longitudinal ones. The latter were represented by muscle bundles of various shapes with an oblique arrangement of myocytes. Between the layers of the muscular coat, accumulations of adipose cells within loose fibrous connective tissue, as well as nerve ganglia and blood vessels, were observed. Bundles and sheets of smooth muscle cells were bounded by elastic, collagen, and reticular fibres. The serous membrane consisted of a thin layer of loose fibrous connective tissue with well-developed vessels and nerve ganglia, covered by simple squamous epithelium. The mucous membrane of the muscular part of the stomach in turkeys of both studied groups was covered with a cuticle (a keratin-like layer) with a greenish tint, which was removed during the preparation of histological specimens (Fig. 3).

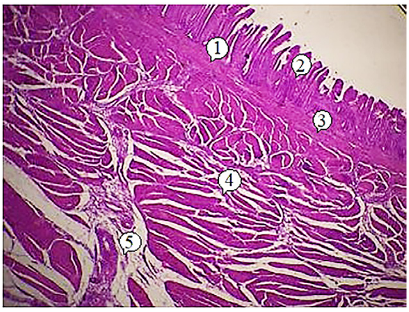

Figure 3. Muscular part of the stomach of a day-old turkey

Note: *1 – mucous membrane; 2 – tubular glands of the lamina propria; 3 – submucosa; 4 – bundles of smooth muscle cells of the muscular coat; 5 – loose fibrous connective tissue with blood vessels. Stained with haematoxylin and eosin, ×80*

Source: *authors' own work*

A.M. Abdellatif *et al*. (2022) divided the cuticular secretion of birds into two parts: a vertical component, which filled the gastric pits and glandular lumina, and a horizontal component, which spread across the surface of the gastric epithelium. According to research by J.L.E. Illanes Herrero (2023), the cuticle consists of specific keratin-like proteins and may take on a greenish colour due to bile being secreted from the duodenum into the stomach. In this process, the upper layer of the cuticle is worn away during the mechanical processing of feed, while the lower layer is renewed by new portions of secretion.

The surface layer of the mucous membrane of the muscular part of the stomach is formed by simple cuboidal epithelium, which invades the lamina propria and forms gastric pits. The lamina propria contains small blood vessels, loose fibrous connective tissue with prominent collagen and elastic fibres, and simple, actively secreting tubular glands. The latter were arranged in clusters, separated by layers of loose fibrous connective tissue rich in collagen fibres. At the base of the glands, they were slightly dilated, and mitotic figures were observed in their cells. In the lumen of the glands, a pink-coloured secretion was detected in the form of a narrow strip, which over time completely filled the lumens of the gastric pits and emerged from them onto the surface of the mucous membrane. Beneath the glands, dense, unorganised fibrous connective tissue formed the submucosal layer. The muscular layer was of considerable thickness and consisted of asymmetrically arranged intermuscular and lateral muscles, which had different orientations of smooth muscle cell bundles and ensured the function of compressing and grinding the feed. Between the bundles of smooth muscle cells, there were well-defined layers of loose fibrous connective tissue of slight thickness, within which blood vessels, nerve plexuses and ganglia were observed. The serous membrane had a structure similar to that of the glandular part of the stomach.

Histotopography and structure of the immune structures of the stomach

The immune structures of the glandular part of the turkey stomach are not visible macroscopically. They can only be detected by microscopic examination. With increasing age in turkeys of both study groups, the levels of structural and functional organisation of the lymphoid tissue, which forms the basis of the immune structures, developed sequentially, from the appearance of foci of diffuse lymphoid tissue to the formation of pre-nodules and lymphoid nodules, both with and without clear centres. In day-old turkeys, isolated localised, insignificant, diffusely distributed elements of lymphoid tissue without rarefactions or distinct borders were observed in the glandular portion of the stomach; these occupied a small area of the mucous membrane and were located in the region of the apices and bases of the folds of this membrane, beneath the superficial epithelial layer, between the columnar glands of the lamina propria, around blood vessels and near individual bundles of cells of the muscularis mucosae (Fig. 4).

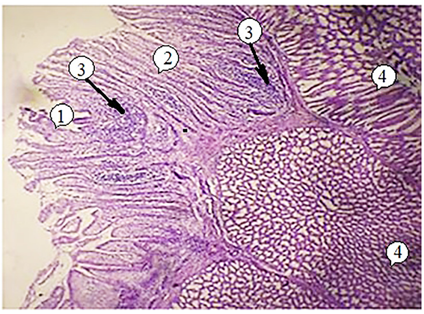

Figure 4. Diffuse lymphoid tissue in the glandular part of the stomach of a day-old turkey

Note: *1 – surface epithelium; 2 – tubular glands of the lamina propria; 3 – foci of diffuse lymphoid tissue; 4 – lobules of deep glands of the submucosa. Stained with haematoxylin and eosin, ×80*

Source: *authors' own work*

Diffusely distributed lymphocytes, some of which were undergoing mitosis, were also observed between the epithelial cells of the surface and glandular epithelia. Due to lymphocytic infiltration, the structure of the epithelium was disrupted and it became spongy. In areas where foci of diffuse lymphoid tissue were localised, thin elastic and more prominent collagen fibres were detected, and proliferation of reticular tissue with mesh-like reticular fibres was observed, between which lymphoid cells and macrophages were found. According to C. Casteleyn *et al.* (2010), reticular tissue creates a specific microenvironment for the differentiation of T- and B-lymphocytes into effector cells. In 10-day-old turkeys, an increase in the histogenesis of lymphoid tissue in the immune structures of the mucous membrane of the glandular part of the stomach was observed; its area increased by 121.83% (2.2-fold) and in the control group by 30.46% (1.3-fold, $P < 0.01$) compared with the corresponding figure at one day of age (Table 1). In turkeys of both groups, in addition to foci of diffusely arranged lymphocytes, pre-nodules with a dense arrangement of cellular elements were recorded, and in turkeys of the experimental group, primary lymphoid nodules were also observed (Figs. 5, 6). Reticular fibres in the pre-nodules formed a multicellular network; some of them at the periphery had a certain orientation, although they did not form a capsule. Diffuse lymphoid tissue and pre-nodules were detected mainly in the lamina propria of the mucous membrane and partly in the superficial regions of the submucosa at the boundary with the muscularis mucosae. Moreover, individual accumulations of lymphoid tissue from the two layers of the mucosa (the lamina propria and the submucosa) were interconnected.

Table 1. Area of the mucous membrane of the glandular part of the stomach in turkeys, %, $M \pm m$, $n = 6$

Group of birds	Area of the mucous membrane	
	without lymphoid tissue	with lymphoid tissue
	Day 1	
Hatching	98.03 ± 0.18	1.97 ± 0.17

Table 1. Continued

Group of birds	Area of the mucous membrane	
	without lymphoid tissue	with lymphoid tissue
	Day 10	
Experimental	95.63 ± 0.62	4.37 ± 0.62
Control	97.43 ± 0.18***	2.57 ± 0.18**
	Day 20	
Experimental	94.38 ± 0.28	5.62 ± 0.28*
Control	96.56 ± 0.34**	3.44 ± 0.34**
	Day 30	
Experimental	92.66 ± 0.30	7.34 ± 0.30
Control	93.37 ± 0.15*	6.63 ± 0.15
	Day 40	
Experimental	89.22 ± 0.41	10.78 ± 0.41
Control	91.45 ± 0.30**	8.55 ± 0.30**
	Day 50	
Experimental	87.68 ± 0.27*	12.32 ± 0.27*
Control	89.30 ± 0.36	10.70 ± 0.36

Note: ** – P < 0.05; ** – P < 0.01; *** – P < 0.001 compared with the corresponding value of the previous parameter*
Source: *authors' own work*

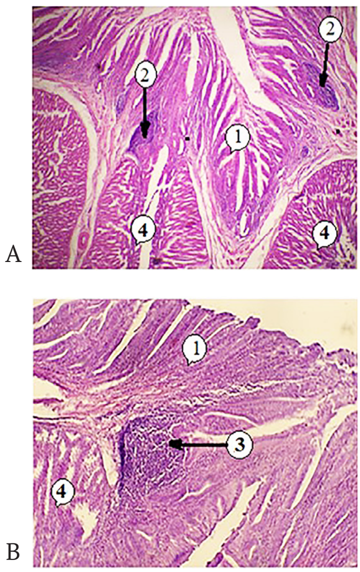

Figure 5. Glandular part of the stomach of 10-day-old turkeys of the control (A) and experimental (B) groups
Note: *1 – tubular glands of the lamina propria; 2 – foci of diffuse lymphoid tissue; 3 – pre-nodule; 4 – lobules of deep glands of the submucosa. Stained with haematoxylin and eosin, ×80 (A); ×90 (B)*
Source: *authors' own work*

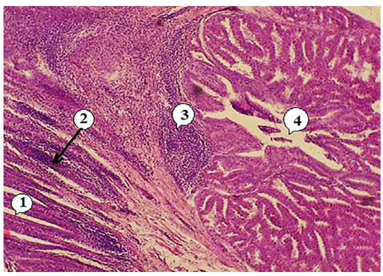

Figure 6. Primary lymphoid nodule in a lobule of a deep gland of the glandular part of the stomach of a 10-day-old turkey from the experimental group
Note: *1 – tubular glands of the lamina propria; 2 – foci of diffuse lymphoid tissue; 3 – primary lymphoid nodule; 4 – lobule of a deep gland of the submucosa. Stained with haematoxylin and eosin, ×90*
Source: *authors' own work*

In turkeys of the experimental group, primary lymphoid nodules were formed by a dense arrangement of cells and a clearly defined thin peripheral band forming a capsule. A reticular network structure with reticular fibres was observed in their central part, while at the periphery the fibres, together with collagen fibres, contributed to the capsule. Primary lymphoid nodules were recorded in the lamina propria of the mucous membrane and at the periphery of lobules of the deep glands that were in contact with the muscularis mucosae. Their lymphoid elements, a considerable proportion of which were in a state of proliferation, infiltrated the glandular units. Due to the localisation of primary lymphoid nodules, the contours of the lobules of the deep glands changed, and the glandular units themselves lacked clear boundaries.

In 20-day-old turkeys, the area of lymphoid tissue in the mucous membrane of the glandular part of the stomach increased by 28.60% (1.3-fold, $P < 0.05$) in the experimental group and by 33.85% (1.3-fold, $P < 0.01$) in the control group compared with the corresponding values of the previous group (Table 1). In turkeys of the experimental group, in addition to the first three levels of structural organisation of lymphoid tissue, secondary lymphoid nodules with pale (reactive) centrally located regions were recorded, in which mitotic divisions of lymphoid lineage cells were observed (Fig. 7).

A

B

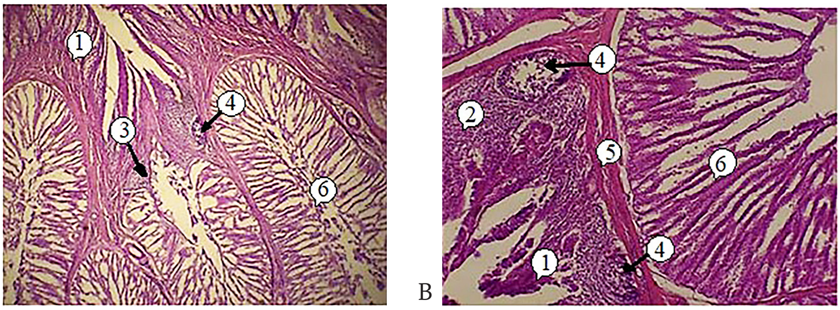

Figure 7. Glandular part of the stomach of 20-day-old turkeys of the experimental group (A, B)
Note: *1 – tubular glands of the lamina propria; 2 – foci of diffuse lymphoid tissue; 3 – primary lymphoid nodule; 4 – secondary lymphoid nodules; 5 – muscularis mucosae; 6 – lobules of deep glands of the submucosa. Stained with haematoxylin and eosin, ×80 (A); ×90 (B)*
Source: *authors' own work*

Secondary lymphoid nodules were located in the deep regions of the lamina propria and were adjacent to the muscularis mucosae. Some of them were surrounded by diffusely distributed lymphocytes. In the light centres of the nodules, reticular fibres were sparse and fragmented, whilst at their periphery they had a specific orientation (circular) and formed a sheath. Collagen fibres were also observed in the latter. In turkeys of the control group, in addition to local clusters of diffuse lymphoid tissue and pre-nodules, primary lymphoid nodules were detected, located in the lamina propria of the mucous membrane (Fig. 8).

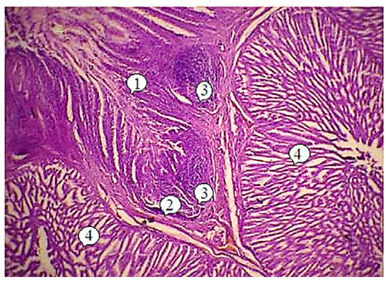

Figure 8. Glandular part of the stomach of a 20-day-old turkey from the control group
Note: *1 – tubular glands of the lamina propria; 2 – lamina propria of the mucous membrane; 3 – primary lymphoid nodules; 4 – lobules of deep glands of the submucosa. Stained with haematoxylin and eosin, ×80*
Source: *authors' own work*

V.T. Khomych & N.V. Dyshlyuk (2008) found that in unvaccinated Shever 579 crossbred chickens, the immune structures of the glandular part of the stomach reach full morphofunctional maturity on day 20, whereas in vaccinated chickens this occurs on day 15. This indicates that vaccinating day-old chicks against infectious diseases stimulates and accelerates the development of a fully-fledged immune response to antigens. N.J. Monisha *et al*. (2024) noted in their studies that in sexually mature broiler hens, the content of lymphoid nodules in the lamina propria of the gastric mucosa is greater than in males.

In 30-day-old turkeys, the area of lymphoid tissue in the mucous membrane continued to increase (by 30.60%, 1.3-fold in the experimental group and 95.64%, 1.9-fold in the control group) compared with the corresponding figures for the previous group (Table 1). In birds of the experimental group, isolated secondary lymphoid nodules were detected in individual deep lobules of the submucosal glands, which are located near the inner longitudinal layer of the muscular layer and in the interlobular stroma of fibrous connective tissue (Fig. 9).

Figure 9. Secondary lymphoid nodules in a lobule of a deep gland of the glandular part of the stomach of a 30-day-old turkey from the experimental group

Note: *1 – lobule of a deep gland; 2 – central cavity; 3 – glandular units; 4 – secondary lymphoid nodules. Stained with haematoxylin and eosin, ×100*

Source: *authors' own work*

In turkeys of the control group, secondary lymphoid nodules were formed on the basis of primary ones and were observed between the superficial glands of the lamina propria; they were not detected within the lobules of the deep glands. At 40 and 50 days of age, the area of lymphoid tissue accumulations in the mucous membrane of the glandular part of the stomach continued to increase in turkeys of the experimental group by 46.87% (1.5-fold) and 14.28% (1.1-fold, $P < 0.05$), respectively, and in the control group by 28.96% (1.3-fold, $P < 0.01$) and 25.15% (1.3-fold) (Table 1). In some histological preparations, clusters and chains of lymphoid nodules were observed in the lamina propria of the mucous membrane of the glandular part of the stomach (Fig. 10). Individual lymphoid nodules were also detected in the muscular coat of 50-day-old turkeys from the experimental group. As noted above, the total area of lymphoid tissue in the mucous membrane of the glandular part of the stomach increased with the age of the birds. Over the study period (from hatching to 50 days), its content increased by 525.38% (6.2-fold) in turkeys of the experimental group and by 443.15% (5.4-fold) in the control group, whereas the area of the mucous membrane without lymphoid tissue decreased (by 10.56%, 1.1-fold in the experimental group and by 8.91%, 1.1-fold in the control group) (Table 1). In turkeys of the experimental group, the most intensive increase in the area of lymphoid tissue was observed from day 1 to day 10 (by 121.83%, 2.2-fold), whereas in the control group it occurred from day 20 to day 30 (by 92.73%, 1.9-fold). In both studied groups, this indicator increased with lower intensity from day 40 to day 50: in the experimental group by 14.28% (1.1-fold, $P < 0.05$) and in the control group by 25.15% (1.2-fold).

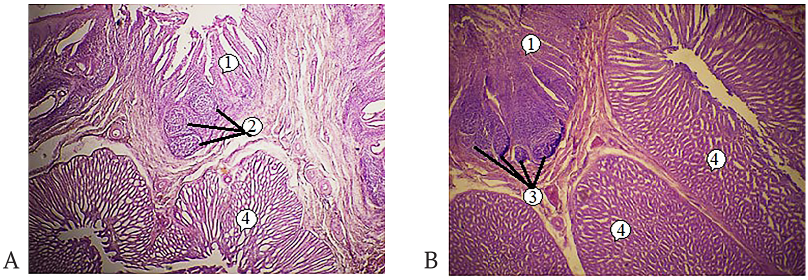

Figure 10. Clusters and chains of lymphoid nodules in the lamina propria of the mucous membrane of the glandular part of the stomach of turkeys from the experimental group at 40 days (A) and 50 days of age (B)

Note: *1 – superficial tubular glands; 2 – clusters of lymphoid nodules; 3 – chains of lymphoid nodules; 4 – lobules of deep glands. Stained with haematoxylin and eosin, ×80 (A, B)*

Source: *authors' own work*

With increasing age of turkeys in both studied groups, the development and increase in the content of individual structural and functional elements of lymphoid tissue were observed (Table 2). In day-old turkeys, lymphoid tissue was represented only by the first level of organisation – the diffuse form of lymphocytes – which accounted for 100%. With increasing age, a gradual decrease in the proportion of diffuse lymphoid tissue was observed due to the development of other structural and functional elements. Notably, in turkeys of the experimental group, the decrease in this indicator occurred more intensively than in the control group. Over the entire study period, the area of diffuse lymphoid tissue decreased by 49.54% (2-fold) in the experimental group and by 42.11% (1.7-fold) in the control group. A reduction in the rate of change of this indicator was recorded in the experimental group from day 10 to day 20 (by 21.91%, 1.3-fold), and in the control group from day 20 to day 30 (by 15.39%, 1.2-fold). The smallest decrease in diffuse lymphoid tissue was observed in turkeys aged from day 40 to day 50 (by 5.52%, 1.0-fold, $P < 0.05$) in the experimental group and from day 1 to day 10 (by 5.95%, 1.0-fold) in the control group.

Table 2. Content of structural levels of lymphoid tissue in the immune formations of the glandular part of the turkey stomach, %, M ± m, n = 6

Group of birds	Diffuse form	Pre-nodules	Lymphoid nodules	
			primary	secondary
		Day 1		
Hatching	100	-	-	-
		Day 10		
Experimental	81.48 ± 3.09	6.03 ± 1.41	12.49 ± 1.67	-
Control	94.05 ± 2.22	5.95 ± 2.22	-	-
		Day 20		
Experimental	63.63 ± 1.52	7.23 ± 0.79***	12.62 ± 0.58***	16.52 ± 0.86
Control	87.11 ± 1.91	6.68 ± 1.72**	6.21 ± 1.05	-
		Day 30		
Experimental	59.12 ± 0.68	8.38 ± 1.08	13.27 ± 1.14	18.73 ± 1.51
Control	73.70 ± 1.28	6.70 ± 1.30***	7.21 ± 1.20**	12.39 ± 0.36

Table 2. Continued

Group of birds	Diffuse form	Pre-nodules	Lymphoid nodules	
			primary	secondary
Day 40				
Experimental	53.41 ± 2.63**	9.29 ± 0.47	13.62 ± 1.04	23.68 ± 1.95
Control	67.17 ± 2.25	7.38 ± 1.41**	9.96 ± 0.48*	15.49 ± 1.01**
Day 50				
Experimental	50.46 ± 3.21*	9.53 ± 0.41	13.89 ± 0.71***	26.12 ± 2.28
Control	57.89 ± 1.16	8.62 ± 1.38**	13.08 ± 1.36	20.41 ± 0.67

***Note**: * – P < 0.05; ** – P < 0.01; *** – P < 0.001 compared with the corresponding value of the previous parameter*
***Source**: authors' own work*

As noted above, in turkeys of both groups, pre-nodules were recorded from day 10. Primary lymphoid nodules in the experimental group were detected from day 10, and secondary ones from day 20, whereas in the control group primary lymphoid nodules were observed from day 20 and secondary ones from day 30. It should be noted that the content of pre-nodules and lymphoid nodules in the experimental group slightly exceeded that in the control group. From 10 to 50 days of age, the area of pre-nodules increased 1.6-fold (by 21.11%) in the experimental group and 1.4-fold (by 44.87%) in the control group. Particularly pronounced growth was observed in the experimental group from day 10 to day 20 (by 19.90%, 1.2-fold, P < 0.001), whereas in the control group it occurred from day 40 to day 50 (by 16.80%, 1.2-fold, P < 0.01). With lower intensity, the content of pre-nodules increased from day 40 to day 50 (by 2.58%) in the experimental group and from day 20 to day 30 (by 0.20%, P < 0.001) in the control group.

With increasing age of the birds, an increase in the area of both primary and secondary lymphoid nodules was observed, reaching maximum values at day 50. Nodules with germinal centres were somewhat more numerous than those without them. An increase in the content of primary lymphoid nodules was recorded with slightly greater intensity in the experimental group from day 20 to day 30 (by 5.2%, approximately 1.0-fold) and in the control group from day 30 to day 40 (by 38.14%, 1.4-fold, P < 0.05). With lower intensity, this indicator increased in the experimental group from day 40 to day 50 (by 2.0%, P < 0.001) and in the control group from day 20 to day 30 (by 16.10%, P < 0.01). The greatest increase in the area of secondary lymphoid nodules was observed in the experimental group from day 30 to day 40 (by 26.43%, 1.3-fold) and in the control group from day 40 to day 50 (by 31.76%, 1.3-fold). With somewhat lower intensity, this indicator increased in the experimental group from day 40 to day 50 (by 10.30%) and in the control group from day 30 to day 40 (by 25.02%, P < 0.01).

The shape of lymphoid nodules in the glandular part of the stomach was recorded as round or oval. Some of them were partially bounded by a connective tissue capsule. From such nodules, lymphocytes migrated into the surface glandular epithelium and the loose fibrous connective tissue. In turkeys of both studied groups, nodules with germinal centres were somewhat larger than primary nodules without germinal centres. Their size increased with the age of the birds (Table 3).

Table 3. Dimensions of lymphoid nodules in the glandular part of the turkey stomach, M ± m, μm (n = 6)

Group of birds	Lymphoid nodules					
	primary			secondary		
	round	oval		round	oval	
		length	width		length	width
Day 10						
Experimental	107.83 ± 0.65	205.0 ± 1.87	135.50 ± 1.03	-	-	-
Control	-	-	-	-	-	-
Day 20						
Experimental	149.16 ± 1.99	237.17 ± 3.79	155.0 ± 1.68	159.83 ± 2.34	248.17 ± 4.73	167.33 ± 3.36
Control	129.67 ± 1.49	217.50 ± 2.80	142.67 ± 1.68	-	-	-
Day 30						
Experimental	156.17 ± 1.74**	253.33 ± 4.73*	162.17 ± 2.52	171.67 ± 1.56	267.17 ± 1.37	170.83 ± 1.40
Control	150.67 ± 1.06*	236.67 ± 1.49	146.83 ± 1.62***	158.17 ± 2.52	261.17 ± 0.87	155.17 ± 1.40
Day 40						
Experimental	178.17 ± 5.51	267.83 ± 4.20	164.17 ± 2.71	205.67 ± 2.62	275.83 ± 2.12	172.5 ± 0.65
Control	166.50 ± 1.68	257.17 ± 2.27	155.17 ± 1.74**	195.17 ± 3.79	270.50 ± 4.29	165.17 ± 2.37*
Day 50						
Experimental	204.5 ± 1.03	273.17 ± 3.05	181.67 ± 2.98	221.67 ± 4.42	291.83 ± 2.89	184.67 ± 2.12
Control	186.00 ± 1.87	270.50 ± 1.40	173.33 ± 3.24**	206.33 ± 4.61*	281.67 ± 2.86	170.66 ± 3.74**

Note: ** – P < 0.05; ** – P < 0.01; *** – P < 0.001 compared with the corresponding value of the previous parameter*
Source: *authors' own work*

As noted above, on day 10 of life in turkeys of the experimental group, primary lymphoid nodules were detected in the glandular part of the stomach, whereas in the control group these immune formations were observed from day 20. Over the period from day 20 to day 50, in the experimental group the size of these nodules increased by 37.10% (1.4-fold for the diameter of round nodules), by 15.17% (1.2-fold for the length of oval nodules), and by 17.21% (1.2-fold for the width of oval nodules). At the same time, in the control group, these indicators increased by 43.44% (1.4-fold), 24.36% (1.2-fold), and 21.49% (1.2-fold), respectively. The most intensive increase in the diameter of round primary lymphoid nodules was observed in the experimental group from day 40 to day 50 (by 14.78%), and in the control group from day 20 to day 30 (by 16.19%, P < 0.05). The length of oval primary lymphoid nodules in turkeys of both groups increased most intensively from day 20 to day 30 (by 6.81%, P < 0.05 in the experimental group and by 8.81% in the control group), while the width of oval primary lymphoid nodules increased most from day 40 to day 50 (by 10.66% in the experimental group and by 11.70%, P < 0.01 in the control group).

In turkeys of the experimental group, secondary lymphoid nodules in the glandular part of the stomach were recorded from day 20, whereas in the control group they were observed from day 30. With increasing age, their size increased, reaching maximum values at 50 days of age. Thus, over the period from day 30 to day 50, the diameter of round nodules and the length and width of oval secondary lymphoid nodules in the experimental group increased by 29.13% (1.3-fold), 9.23% (1.1-fold), and 8.10% (1.1-fold), respectively, while in the control group these values increased by 30.45% (1.3-fold), 7.85% (1.1-fold), and 9.90% (1.1-fold), respectively. The most intensive increase in the diameter of round secondary lymphoid nodules in both groups was observed from day 30 to day 40 (by 19.81% and 23.39%, respectively). Meanwhile, the length and width of oval secondary lymphoid nodules in the experimental group increased most from day 40 to day 50 (by 5.80% and 7.05%, respectively), whereas in the control group, the length increased most from day 40 to day 50 (by 4.13%) and the width from day 30 to day 40 (by 6.44%, $P < 0.05$).

In turkeys of both the experimental and control groups, on days 1, 10, and 20, lymphoid tissue of immune formations in the wall of the muscular part of the stomach was not detected. It was first observed as small, isolated accumulations of diffusely arranged lymphocytes at day 30. The diffuse lymphoid tissue had no clear boundaries and was located in the lamina propria of the mucous membrane between the columns of simple tubular, unbranched glands. In the areas where this tissue was localised, migration of lymphocytes between epithelial cells of the surface epithelium and glandular epithelium was observed. At 40 and 50 days of age in both groups, the content of diffuse lymphoid tissue increased but did not exceed 1.5%. In turkeys of these age groups, individual accumulations of diffuse lymphoid tissue were also recorded in the muscular and serous membranes in the form of small diffuse fields composed of small lymphocytes (Fig. 11). Pre-nodules and lymphoid nodules were not detected. However, in two of the six turkeys in the experimental group, isolated clusters (ranging from 2 to 3) of secondary lymphoid nodules were observed in the muscular part of the stomach on day 50. These were located in the deep regions of the circular layer of the muscular coat and were oval, triangular and rounded in shape (Fig. 12). The nodules were situated between bundles of smooth muscle cells arranged in a circular pattern. They were bounded by thin layers of loose fibrous connective tissue containing small blood vessels and nerve plexuses.

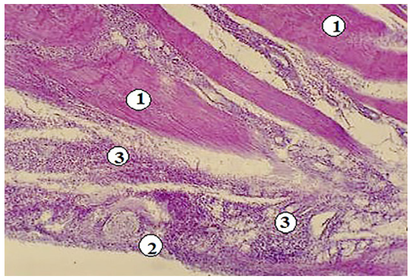

Figure 11. Diffuse lymphoid tissue in the muscular part of the stomach of a domestic turkey from the experimental group at 40 days of age

Note: *1 – muscular coat; 2 – serous membrane; 3 – diffuse lymphoid tissue. Stained with haematoxylin and eosin, ×100*

Source: *authors' own work*

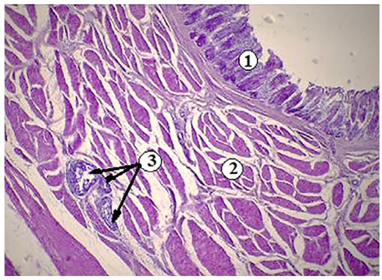

Figure 12. Secondary lymphoid nodules in the muscular part of the stomach of a 50-day-old turkey from the experimental group

Note: *1 – mucous membrane; 2 – muscular coat; 3 – clusters of secondary lymphoid nodules. Stained with haematoxylin and eosin, ×100*

Source: *authors' own work*

Thus, this scientific study presents the features of the histological structure of the stomach and its immune formations in vaccinated and unvaccinated turkeys, establishes the stages of lymphoid tissue development, and identifies the age groups of turkeys in which immune formations reach morphofunctional maturity, which is an important step in the development of immunomorphology in assessing the use of vaccination prophylaxis. The studies conducted have established the characteristics of the histostructure of the turkey stomach wall and the histotopography of its immune structures at various stages of postnatal development. It was determined that in the glandular part of the stomach, lymphoid tissue undergoes successive stages of structural and functional organisation – from a diffuse form to secondary lymphoid nodules, with a gradual increase in its area, a more complex structure, and an increase in the size of the nodular forms. In the muscular part of the stomach, lymphoid tissue was detected significantly later and was characterised by less pronounced structural changes. The morphological data presented reflect the characteristics of the development of immune structures in the stomachs of turkeys during the post-vaccination period.

Conclusions

Histological studies have shown that the stomach of turkeys is divided into glandular and muscular parts, which differ in the structure of their walls and have different functional significance. In the glandular part of the stomach, the mucous membrane is distinguished by considerable thickness and the presence of superficial tubular complexes of glands, whereas in the muscular part the muscular coat predominates, consisting of two layers of muscle bundles of various shapes with an oblique arrangement of myocytes. Vaccination of turkeys at one day of age against infectious diseases stimulates the development of immune formations in the glandular part of the stomach and accelerates their morphofunctional maturity. Thus, diffuse lymphoid tissue was recorded from day 1 in turkeys of the experimental group, while pre-nodules and primary lymphoid nodules were detected on day 10, and secondary lymphoid nodules on day 20. In contrast, in turkeys of the control group, pre-nodules were observed on day 10, primary lymphoid nodules on day 20, and secondary nodules on day 30 of life. The presence of secondary lymphoid nodules indicates morphofunctional maturity of lymphoid tissue and, accordingly, of immune formations. In turkeys of both studied groups, lymphoid tissue in the glandular part of the stomach was mainly located at the base of mucosal folds beneath the epithelial layer, between and beneath the superficial tubular glands, and around the excretory ducts of the glands. In turkeys of the experimental group at 30 days of age, individual lymphoid nodules were detected in the lobules of the deep glands of the submucosa, and by day 50 they were also observed in the muscular coat. With increasing age (from day 1 to day 50), an uneven increase in the area of lymphoid tissue in the mucous membrane was observed in both groups, rising by 525.38% (6.2-fold) in the experimental group and by 443.15% (5.4-fold) in the control group. At the same time, the area of the mucous membrane without lymphoid tissue decreased by 10.56% (1.1-fold) in the experimental group and by 8.91% (1.1-fold) in the control group. With increasing age, the size of lymphoid nodules also increased, with values in vaccinated birds slightly exceeding those in unvaccinated ones.

The obtained results indicate active formation of elements of GALT, which ensure the implementation of local immune reactions in the stomach wall of birds. These findings make it possible to assess the immunological status of turkeys at different age periods and to evaluate the effectiveness of veterinary preventive measures in poultry production systems. Prospects for further research may be aimed at studying the structural and functional organisation of immune formations in the stomach of older turkeys under vaccination and conducting comparative assessments with other species of domestic poultry.

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Conflict of Interest

None.

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Морфологічні особливості імунних утворень шлунку індиків у поствакцинальний період

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Анотація. Через органи травлення в організм птахів надходить до 70 % антигенного матеріалу, який є етіологічним фактором і призводить до низки захворювань. У зв'язку з цим, у їх стінці розвиваються імунні утворення, клітини яких забезпечують захист організму від агентів, що несуть ознаки генетично чужорідної інформації. Метою цієї роботи було простежити розвиток імунних утворень шлунка індиків за вакцинації і встановити строки їх морфофункціональної зрілості. Для дослідження були відібрані 66 особин індиків породи Біг-6, яких розділили на дослідну (вакциновану) і контрольну (невакциновану) групи. Матеріал для проведення гістологічних досліджень відбирали на першу, 10, 20, 30, 40 і 50 доби життя. Показано, що у індиків дослідної і контрольної груп, структурна організація лімфоїдної тканини, яка формує основу імунних утворень, виникає поетапно – від дифузних скупчень лімфоцитів до утворення передвузликів та лімфоїдних вузликів. Так, у індиків добового віку в залозистій частині шлунка спостерігалися локальні скупчення дифузно розташованих лімфоцитів, які мали тісний контакт з епітеліоцитами поверхневого епітелію та епітелію трубчастих залоз. На 10 добу у індиків дослідної і контрольної групи реєструвалися передвузлики з більш щільним розташуванням лімфоцитів, а в індиків дослідної групи – ще й первинні лімфоїдні вузлики, що були обмежені оболонкою. Останні, у індиків контрольної групи виявлялися починаючи з 20 доби. Вторинні лімфоїдні вузлики зі світлими центрами у залозистій частині шлунка спостерігалися у індиків дослідної групи з 20 доби, а в контрольної – з 30 доби, що свідчило про стан морфофункціональної зрілості імунних утворень. У м'язовій частині шлунка імунні утворення були розвинуті слабо. У індиків обох досліджуваних груп поодинокі скупчення лімфоцитів реєструвалися з 30 доби, а в окремих особин дослідної групи на 50 добу виявлялися угрупування вторинних лімфоїдних вузликів у глибоких ділянках м'язової оболонки. Отримані результати дають можливість з'ясувати механізми специфічної імунної відповіді, пов'язані із Т- і В-клітинами, що сприятиме розробці нових ефективних стратегій для боротьби з хворобами птиці

Ключові слова: гістологічні дослідження; гістотопографія; дифузна лімфоїдна тканина; передвузлики; лімфоїдні вузлики; морфофункціональна зрілість; свійська птиця