



The effect of vaccination on the development of immune formations in the esophagus of turkeys

Natalya Mazur

Postgraduate Student

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

<https://orcid.org/0009-0004-5747-4335>

Nadiia Dyshliuk*

Doctor of Veterinary Sciences, Professor

National University of Life and Environmental Sciences of Ukraine

03041, 15 Heroiv Oborony Str., Kyiv, Ukraine

<https://orcid.org/0000-0003-4753-9356>

Abstract. Vaccination of poultry provides protection against a range of bacterial and viral pathogens and depends on the development of immune structures within the digestive organs, where immune responses arise under the influence of antigens. These responses are manifested by the production of antibodies, the appearance of effector cells, and the formation of immunological memory specific to a given foreign agent. The aim of the present study was to examine the development of immune structures in the oesophagus and its dilated part – the crop – and to determine the timeline of their complete morphofunctional maturation in vaccinated and unvaccinated birds. For histological examination, material was collected from 66 Big-6 turkeys aged from 1 to 50 days, divided into an experimental (vaccinated) group and a control (unvaccinated) group. Classical histological staining methods were applied, including haematoxylin and eosin, Van Gieson, Mallory, and Weigert techniques, as well as silver impregnation with silver nitrate according to Kelemen. In vaccinated turkeys, immune formations in the oesophagus and crop were first observed at 20 days of age and were represented by local aggregations of diffuse lymphoid tissue, with the crop also containing pre-nodular structures. These formations were located beneath the epithelial layer in the lamina propria of the mucosa, within the submucosa, near the oesophageal glands, and directly within the glandular tissue. Migration of lymphoid cells into the lower layers of the epithelium

Suggested Citation:

Mazur, N., & Dyshliuk, N. (2025). The effect of vaccination on the development of immune formations in the esophagus of turkeys. *Ukrainian Journal of Veterinary Sciences*, 16(2), 46-62. doi: 10.31548/veterinary2.2025.46.

*Corresponding author



was observed from the surface of these aggregations. By 30 days of age, the lymphoid tissue in this group was morphofunctionally mature, with the presence of lymphoid nodules both with and without germinal centres. In unvaccinated turkeys, immune structures in the oesophagus and crop were first detected at 30 days of age and were represented by diffusely distributed lymphocytes. Morphofunctional maturity of these structures, indicated by the presence of all levels of lymphoid tissue organisation, was observed at 40 days of age. The obtained results are of practical importance and should be considered during the planning and implementation of prophylactic and therapeutic measures in turkey rearing

Keywords: digestion apparatus; lymphoid tissue; lymphoid nodules; lymphocytes; poultry

Introduction

One of the priority tasks of epizootic wellbeing of industrial poultry farms is immunoprophylaxis in order to prevent the occurrence of infectious diseases and increase the viability of poultry. Prophylactic vaccines, especially against dangerous viral infections, are carried out in the first days after the chick hatches, or during incubation. During this process, a weakened pathogen, which is unable to induce a pathological process, “populates” the cells where it parasitises. Field strains of the pathogen can no longer enter these cells. After a while, the bird needs to be revaccinated.

According to T.S. Budnik & S.V Gural-ska (2022), the effectiveness of revaccination of poultry against viral and bacterial infections depends on the development of peripheral immune organs, which are based on lymphoid tissue. The latter contains numerous lymphocytes located in a mesh-like network of reticular tissue. In the immune organs, through the action of antigens (foreign substances), as noted by C. Ceccopieri & J.P. Madej (2024), lymphocytes differentiate into effector cells responsible for the development of specific (cellular and humoral) immunity. The process of morphofunctional differentiation and specialisation of immune organs occurs in a specific order: from the formation of a diffuse form as a result of the expulsion of lymphocytes from

the lumen of blood vessels, to the emergence of prenodules without clearly defined boundaries and membrane, and the appearance of primary (without germinal centers) and secondary (with germinal centers) lymphoid nodules. The presence of secondary lymphoid nodules in the lymphoid tissue, as stated by N.J. Monisha *et al.* (2024a), signifies both its own morphofunctional maturity, as well as that of the structures that it forms, i.e. the ability to give a complete immune response in the form of a cellular reaction complex due to action of foreign substances with antigenic properties.

After hatching of the bird, its digestive organs quickly adapt to intake of feed components and water from the external environment. As noted by M.A.A. Mahdy & E.S.I. Mohammed (2024), a significant amount of antigens enters the organism in the form of antigen complexes – viruses, bacteria, foreign cells – which stimulate the development of the lymphoid tissue associated with the mucous membrane. Significant development of this tissue is correlated to the absence of lymph nodes and the pharyngeal lymphoid ring in birds, which are present in mammals. Moreover, birds, as noted by N. Aydin *et al.* (2022) and N. Dyshliuk *et al.* (2024), lack certain organs of the oral cavity (cheeks, teeth, gums, etc.), hence food is not retained in the oral cavity. Among the digestive

organs of poultry, as found by R. Al-Musawi & S. Ali Al-Khakani (2024), of special note is the esophagus, a tube-shaped organ that connects the oropharynx with the proventriculus. In certain bird species, according to N.J. Monisha *et al.* (2024b), singular clusters of peripheral lymphoid tissue, organised as diffuse form of lymphocytes and lymphoid nodules, were found in the walls of esophagus.

Despite the importance of immune formations of the digestive system in the development of specific immunity, their histotopography, microscopical and functional features are relatively well studied in chickens, ducks, pigeons and wild birds with different foraging activity, as noted by H.M. Ali *et al.* (2023), R. Sinha *et al.* (2023) and N.J. Monisha *et al.* (2024a). Literary sources on the effects of vaccination and the onset of morphofunctional maturity of immune formations of the esophagus of turkey are limited, while those that describe the development of the formations in terms of age are absent.

For this reason, the purpose of this work was to study the postnatal morphogenesis of immune formations of the esophagus and its extension in unvaccinated and vaccinated turkeys. The study is relevant and allows to evaluate the effectiveness of vaccine prophylaxis on the tissue and organ levels.

Literature Review

According to T. Budnik (2022), the effectiveness of preventive and therapeutic methods depends entirely on the condition of the immune system of birds. In turn, the functioning of immune system is possible only under the condition of a tight link of all specific immune responses and factors of innate immune reactivity. As noted by N.I. El-naseery *et al.* (2021) and E.C. Lavelle & R.W. Ward (2022), organs of the bird immune system are divided into central and peripheral. The emergence of blood cells, as well as the development and maturation

of lymphocytes without significant influence of antigens, occur in the central organs. T-lymphocytes are formed in the thymus, while B-lymphocytes – in the cloacal sac, or the bursa of Fabricius. Along the blood flow, T- and B-lymphocytes are carried into the peripheral organs of the immune system (spleen, lymph nodes – in waterfowl, Harderian gland, immune formations of digestive and respiratory organs, and of skin), where their mature forms interact with antigens, which leads to the activation of the immune system and subsequent development of cellular and humoral immunity.

One of the initial digestive organs, where feed enters and immune formations are developed, as stated by H. Sağsöz & N. Lıman (2009), V. Khomich *et al.* (2020) and A. Nahed *et al.* (2021) is the esophagus, characterised by its appearance of a long elastic tube. The authors found that the microscopic structure of the wall of this organ in birds of various trophic specialisations depends significantly on the way the birds obtain food. Its mucous membrane is lined with stratified squamous epithelium, which has varying degrees of keratinisation and contains glands, the size and shape of which depends on the ecology of nutrition. In the majority of bird species, next to the glands, diffuse lymphocytes and lymphoid nodules can be found, the amount of which considerably increases on the border of the esophagus and the glandular part of the stomach. M.A.A. Mahdy & E.S.I. Mohammed (2024) found that the diffuse lymphoid tissue includes small, medium and large lymphocytes. There are also macrophages, pseudoeosinophils, eosinophils, and mast cells.

C.M. Kotz (2002) showed that the mucous membrane of the esophagus of herons forms longitudinal wave-like folds with various configurations of bends and contains densely packed esophageal glands in the lamina propria of the mucous membrane. Lymphocytes in the

middle and caudal sections of the esophagus of the grey heron are localised mainly near the basal membrane of secretory parts of glands. The author makes assumptions regarding the possibility of expulsion of these cells onto the surface of epithelial layer, which, along with the secretion of glands, protect the mucous membrane. The lymphocytes of the middle section of the esophagus form clusters in the form of diffuse lymphoid tissue, and in the area of its transition into the stomach – 9-10 oval lymphoid nodules, which is possibly related to food retention during periods of increased foraging.

H.M. Ali *et al.* (2023) noted that the shape of the folds of mucous membrane of esophagus of pigeons differs in its individual parts, and also depends on the age group of the birds. The cervical part of the organ in young pigeons had primary folds of the mucous membrane with a small number of secondary folds, while in adults they were strongly folded and divided into primary, secondary and tertiary. In the plane of the folds, singular accumulations of diffuse lymphoid tissue were recorded. H. Hamoda & A. Farag (2018) found that the lymphoid tissue of the esophagus of pigeons is located in the lamina propria of the mucosa and the submucosa in the form of diffuse form and lymphoid nodules. Individual nodules were also found in muscular and serous membranes. In opinion of the authors, lymphoid tissue of the esophagus may serve

as an indicator for understanding the immune response after oral administration of vaccines.

Materials and Methods

Histological studies were carried out during years 2024-2025 in the educational, scientific, and production laboratory “Center for Biomorphological Technologies” of the Department of Vertebrate Biomorphology named V.G. Kasyanenko of the National University of Life and Environmental Sciences of Ukraine (Kyiv). All poultry manipulations in the experiment were carried out in accordance with the requirements of international principles of European convention for the protection of vertebrate animals used for research and other scientific purposes (1986) i Law of Ukraine No. 3447-IV (2006). Material for the research was collected, taking into account the age, from 66 heads of clinically healthy turkeys (females) of the Big-6 breed aged one, 10, 20, 30, 40 and 50 days, which were divided into two groups. Turkeys from the first (experimental) group were vaccinated according to the scheme of vaccine prophylaxis and therapeutic treatments of the poultry farm TOVSP “Volodar” of the Bila Tserkva district of Kyiv region (Table 1). Birds from the second (control) group were not vaccinated. Birds from both groups were kept in conditions of a poultry farm and were fed fodder corresponding to the diet of the farm.

Table 1. Scheme of vaccine prophylaxis and therapeutic treatments of the poultry farm TOVSP “Volodar”

No.	Disease	Name of the vaccine (drug)	Bird age, in days
1	Virulent Newcastle and Marek's disease	Vectormune ND – vector vaccine, manufacturer: Ceva-Phylaxia Veterinary Biological Company 1107, Budapest, Hungary (in incubator)	0
2	Coccidiosis	Immunocox-T - live attenuated vaccine, manufacturer: Ceva Animal Health Inc., Canada	1
3	Turkey viral rhinotracheitis	Hipraviar-SHS - live attenuated freeze-dried vaccine, manufacturer: Laboratorios Hipra, Spain	7
4	Virulent Newcastle disease	Cevac NEW L – live attenuated freeze-dried vaccine, manufacturer: Ceva-Phylaxia Veterinary Biological Company 1107, Budapest, Hungary	14

Table 1. Continued

No.	Disease	Name of the vaccine (drug)	Bird age, in days
5	Turkey viral rhinotracheitis	Hipraviar-SHS - live attenuated freeze-dried vaccine, manufacturer: Laboratorios Hipra, Spain	21
6	Turkey hemorrhagic enteritis	Г.Е. BAK – vaccine against turkey hemorrhagic enteritis, manufacturer: APKO Laboratorios Ltd, USA	28
7	Turkey viral rhinotracheitis індиків	Hipraviar-SHS - live attenuated freeze-dried vaccine, manufacturer: Laboratorios Hipra, Spain	42

Source: developed by the authors

The work uses histological and morphometrical methods of studying the esophagus of turkeys. For this purpose, dissection of carcasses of control and experimental bird groups was performed with respect to age, and the esophagus with its extension – the crop – was dissected. For histological studies, pieces about 1 cm in size were picked from the esophagus and the crop, placed into histological cassettes and immersed in a fixing fluid – 10% neutral buffered formalin for preservation of histological structures. The fixation period lasted for 48 hours. Next, the material was thoroughly rinsed under running water for 24 hours to remove the fixative, and dehydrated gradually in alcohol of increasing concentration – from 60° to 100° (1-3 h). For better absorption of paraffin by the material, it was first ran rinsed in a solution of alcohol with chloroform (1:1), then in two parts of pure chloroform, and then in a solution of chloroform with paraffin (in a thermostat at 37°C). The material in two parts of chloroform was kept in a thermostat at 55-56°C (2-3 h). To pour the material and obtain blocks, a pouring station with metal molds was used.

Histological sections, 5-10 μm thick, were prepared on the sledge microtome MPS-2 (MedTech-Price, Ukraine), stained with Carazzi hematoxylin solution and eosin solution according to Van Gieson, Mallory and Weigert, and impregnated according to Kelemen. To calculate the area of lymphoid tissue in individual parts of the esophagus, the point counting

method was employed using a binocular microscope with a measuring grid. The size of the lymphoid nodules was determined using the Olympus CX 43 microscope (Labdepo, Japan) and an ocular micrometer. Some histological specimens were photographed with a digital camera mounted in the Primo Star microscope (Carl Zeiss, Germany) and connected to a personal computer. The obtained results were recorded in protocols, and the statistical analysis of numerical values was performed using the software StatSoft Statistica 13.1 (2016). The following were calculated: the arithmetic mean and its error ($M \pm m$), statistical significance (P) according to Student and significance criteria – $P < 0.05$; $P < 0.01$; $P < 0.001$.

Results and Discussion

Based on the obtained results, it was found that the esophagus of turkeys is a tube-shaped organ, which is divided into two sections along its length: cervical and thoraco-abdominal. Before the thoraco-abdominal part of the organ, its wall expands and forms the crop. The latter is a sac where fodder is stored before entering the stomach. A.A. Alsanosy *et al.* (2021) and M.A.A. Mahdy & E.S.I. Mohammed (2024) noted that the crop is present only in granivorous birds (chickens, turkeys, pigeons), and is absent in waterfowl (geese, ducks). According to H.M. Ali *et al.* (2023), the esophagus of pigeons is divided into three sections: anterior (cervical), middle (crop), and posterior (thoracic).

The microscopic structure of the esophagus walls of turkeys from the experimental and the control groups is similar. The mucous membrane with a submucosal base forms 5-8 longitudinal folds, which are separated by several, less noticeable folds. Folding is best expressed in the cranial part of the organ (Fig. 1A). The shape of the folds is predominantly finger-shaped, less frequently – leaf-shaped, while in small folds – clip-shaped.

The inner layer of the mucous membrane of turkey esophagus is lined by a thick, stratified squamous epithelium with weak keratinisation, the process of which is best observed in the upper part of the organ (Fig. 1B). It is coated with a layer of mucus that, according to X. Zhang (2018) and T. Kataa & S.M. Ahmed (2022) protects the esophagus of birds from chemical and mechanical damage, as well as from pathogenic microorganisms and acts as a lubrication medium. The principal cell differentiation pathway of the superficial epithelium is represented by epithelial cells at

successive stages of differentiation. In the epithelial cells of the basal layer, which are arranged in one row and have a cylindrical shape, the nuclei are oval, with a relatively loose chromatin structure and a well-defined nucleolus. Above them, there are polygonal-shaped cells of the spinous layer, followed by flat epithelial cells with partially destroyed nuclei. Under the epithelium, there is a layer of loose fibrous connective tissue, which forms the lamina propria of the mucous membrane. In the form of papillae, it digs into the lower layers of the epithelium. In this layer, elastic and collagen fibers, numerous small blood vessels and the glandular apparatus are found. The latter, according to L.P. Kharchenko (2014), functions by moistening the feed and providing mechanical protection. The author determined that the extent of development of the glandular apparatus depends on the type of nutrition of birds. Feeding on insects with a hard chitinous exoskeleton or on dry and hard plant-based fodder contributes to the formation of a well-developed glandular apparatus.

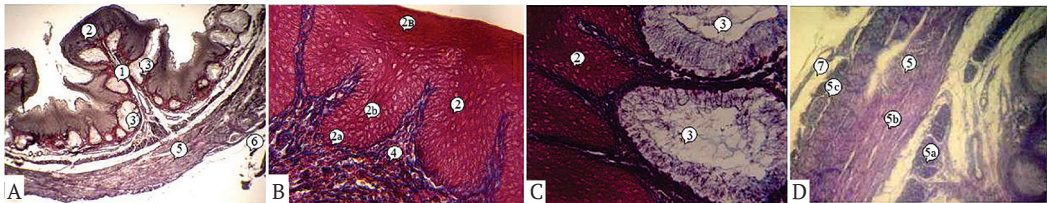


Figure 1. The cervical part of the esophagus of a control group turkey

Note: A – one-day old; B and C – 40-days old; D – Thoraco-abdominal section of the esophagus of experimental group turkey aged 50 days. Histological preparations: 1 – fold of the mucous membrane; 2 – epithelium: 2a – basal layer, 2b – stratum spinosum, 2c – apical layer; 3 – glands; 4 – papillae of the lamina propria of the mucous membrane; 5 – muscular membrane: 5a – inner longitudinal layer, 5b – middle circular layer, 5c – outer longitudinal layer; 6 – adventitia and 7 – serous membrane. Staining according to Van Gieson's method $\times 40$ (A), according to Mallory's method $\times 100$ (B, C), Carazzi hematoxylin and eosin $\times 80$ (D)

Source: developed by the authors

According to research, the glandular apparatus of turkeys is represented by packages of alveolar-tubular esophageal glands with mucous secretions. The glands had an unequal shape, a substantial size and were separated from

neighbouring glands by layers of loose fibrous connective tissue (Fig. 1C). Each gland was composed of terminal sections, formed by cylindrical epithelium and opening into the central cavity. External packages of glands are limited

by delicate bundles of collagen fibers, which were involved in the formation of their sheath. Between the glands, a well-developed capillary network of vessels was observed. The excretory ducts of the glands were directed to the surface of the mucous membrane. E. Rajabi & A. Nabipour (2009) and N.I. El-naseery *et al.* (2021) determined that glands are not present in all birds. In pigeons and parrots, they are absent in the cervical section of the esophagus and the crop.

The muscularis mucosae is located under the packages of esophageal glands. It is weakly defined, and is intermittent in some areas. The muscularis mucosae is formed by separate bundles of smooth sickle-shaped muscle cells of a longitudinal direction. Submucosal base, in the form of thin connective tissue plate, separates muscularis mucosae from the muscular layer. Individual elastic fibers, blood vessels, nerve plexuses and nerve nodes can be found in it. In the area of folds, the submucosa is thickened. The muscle layer along the entire esophagus is well-developed and is composed of three layers of smooth muscle cells: outer longitudinal, middle circular, and inner longitudinal (Fig. 1D). Among the layers, the middle one is the most developed, and is almost twice as thick as the longitudinal layers. The inner longitudinal layer goes deep into the large folds of the mucous membrane. Bundles of muscle cells and their layers are separated by thin layers of loose fibrous connective tissue, in which vessels and elements of the intermuscular nerve plexus are recorded (Fig. 2).

F. Taki-El-Deen (2017) determined that in some bird species (domestic ducks and waders), the muscle tissue of the esophagus has a bilayer structure and is formed by an outer circular and an inner longitudinal layers. Externally, the esophagus is surrounded by the adventitious membrane in the cervical part and by the serous membrane in the thoraco-abdominal part. The adventitious and serous membranes

are formed by loose fibrous connective tissue, which in the thoraco-abdominal part of the esophagus is covered by one layer of mesothelial cells (simple squamous epithelium). In those membranes, clusters of fat cells, small blood vessels, individual nerve nodes and nerve plexuses are found.



Figure 2. Elements of the intermuscular nerve plexus and vessels in the muscular layer of the thoraco-abdominal section of the esophagus of a 20-day old turkey from the control group
Note: histological preparation: 1 – muscular layer; 1a – intermuscular nerve plexus, 1b – blood vessel; 2 – serous membrane. Staining according to Mallory's method $\times 100$
Source: developed by the authors

In turkeys from the experimental group aged one and 10 days, immune formations in the wall of the esophagus were not observed. They were first detected at the age of 20 days. Immune formations were presented only as diffuse form of lymphocytes without visible thinning and densification. They were present locally under the epithelial layer and in deep areas of the lamina propria of the mucous membrane as single- and multilayered lymphoid lanes (chains), singular clusters of irregular shape, as well as near small blood vessels, terminal sections of esophageal glands and their excretory ducts (Fig. 3A).

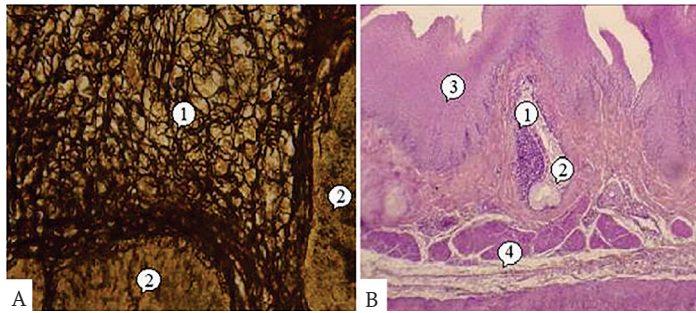


Figure 3. Diffuse lymphoid tissue of the cervical part of the esophagus of a 20-days old turkey from the experimental group

Note: A – between glands, B – in the secretory section of the gland. Histological preparations: 1 – diffuse lymphoid tissue; 2 – esophageal glands; 3 – epithelial layer; 4 – muscle layer. Silver nitrate impregnation according to Kelemen $\times 400$ (A), Staining with Carazzi hematoxylin and eosin $\times 80$ (B)

Source: developed by the authors

In the areas where immune formations are located, the latter came into contact with the epithelial layer, which is part of the lymphocyte microenvironment, or migrated into the lower layers of the epithelium. R. Sinha *et al.* (2023) found that among the lymphoid cells that have migrated into the epithelium, B-lymphocytes predominate, while the number of T-lymphocytes is much smaller, and plasma cells are found on the periphery of such clusters. Individual clusters of diffuse lymphoid tissue were also recorded in the wall of the secretory sections of glands between gland cells (Fig. 3B). As noted by N. Dyshliuk *et al.* (2024), local lymphoid tissue of the esophagus of birds along with its components is necessary for local immune responses. Diffuse lymphoid tissue was presented as a reticular tissue with a dense network of sinuous fibers, which were interconnected and formed uniform small and large network. The cytoplasm of reticulocytes formed short appendages. Their nuclei were irregularly shaped and contained a significant amount of heterochromatin. Between the elements of reticular tissue, lymphoid cells could be found. Some of them were connected to lymphoid chains. At the age of 30 days of turkeys from the experimental group,

the development of diffuse lymphoid tissue continued, which appeared in some areas of the submucosal base on the border with the muscular layer. Inside and outside the tissue, singular lymphoid prenodules and nodules (primary and secondary) were recorded, the base of which, as is of diffuse lymphoid tissue, is formed by reticular tissue (Fig. 4A)

The prenodules were small in size and appeared as denser clusters of cells, lacking a membrane surrounding them. On the base of the prenodules, primary lymphoid nodules were formed, which suggested that lymphoid tissue was starting to transform into a state of functional maturity. In such nodules, lymphoid cells, represented mainly by small and medium shapes, were located tightly next to each other. Around them, a membrane composed of delicate collagen, elastic and reticular fibers was visible. In the central part of the primary nodules, the reticular fibers were well-developed and interconnected, and formed a large-cell mesh. As a result of the action of antigens, primary lymphoid nodules turned into secondary lymphoid nodules, the appearance of which, as noted by H. Hamoda & A. Farag (2018), is connected to high levels of immune system activity, hence they are considered to be the main

morphofunctional (anatomical) units of the immune system at the peak of their activity. They are one of the most important morphological components of the lymphoid tissue. M. Markowska *et al.* (2017) divided lymphoid nodules of the peripheral organs of the immune

system into two types: perivascular, which are associated with the wall of hemo- and lympho-microcirculatory channel, and lymphoepithelial, which are specialised nodules of mucous membranes that are directly involved in a specific response to a particular antigen.

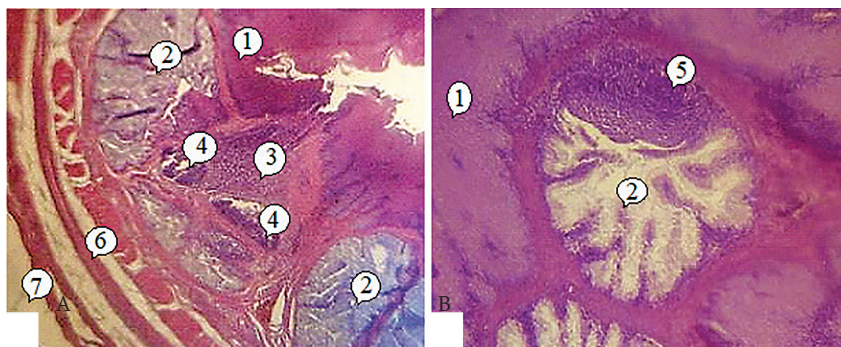


Figure 4. Immune formations in the esophagus of turkeys from the experimental group

Note: A – immune formations of the thoraco-abdominal section of the esophagus of a 30-days old turkey from the experimental group; B – primary lymphoid nodule in the wall of gland of the cervical section of the esophagus of a 40-days old turkey from the experimental group. Histological preparations: 1 – epithelial layer; 2 – cesophageal glands; 3 – diffuse lymphoid tissue; 4 – secondary lymphoid nodules; 5 – primary lymphoid nodule; 6 – muscular layer; 7 – serous membrane. Staining with Carazzi hematoxylin and eosin $\times 80$ (A), $\times 100$ (B)

Source: developed by the authors

According to the results of the study, a dark peripheral zone is noticeable in secondary lymphoid nodules – a mantle with a dense arrangement of primarily small lymphocytes, and a lighter (on histological sections) – germinal center. The latter, as noted by C. Ceccopieri & J.P. Madej (2024), increased in size under antigenic effect on the organism and contained a significant amount of mitotically dividing cells of the lymphoid lineage. In the germinal center, reticular fibers were either absent, or present as short fragments. However, in the membrane of secondary lymphoid nodules, all three types of fibers (reticular, collagen, and elastic) were observed. According to H. Hamoda & A. Farag (2018), impaired function of the germinal centers can lead to immunodeficiency and an increased sensitivity to infectious diseases. The authors have determined that

disappearance, or reverse development, of lymphoid nodules is observed with age-related involution of lymphoid formations and a simultaneous decrease in the amount of diffuse lymphoid tissue.

At ages 40 and 50 days in vaccinated turkeys, infiltration of the apical and glandular epithelia and the lamina propria of the mucous membrane is even more prominent. Lymphoid nodules were detected also in the secretory sections of the esophageal glands (Fig. 4B). In turkeys from the control group, minor clusters of immune formations were first detected starting at 30 days of age, and were represented by local clusters of diffuse lymphoid tissue. Single lymphocytes were found in the epithelium, which were located mainly in the basal layer between epithelial cells. At the age of 40 and 50 days, single lymphoid nodules with and

without germinal centers were observed. As found by L.P. Kharchenko (2014) and O. Lykova & L.P. Kharchenko (2021), lymphoid nodules of the esophagus are not present in all birds. They were not found by the authors in the European bee-eater and the chaffinch, and found in the rook, pheasant and quail. The observed nodules in birds of certain species had a close

morphofunctional connection with the glands. According to the authors, presence or absence of the nodules in the esophageal wall correlates to their feeding specialisation. Immune formations in the mucosa of esophagus of turkeys from experimental and control groups occupy an unequal area and their content is greater in vaccinated birds (Table 2).

Table 2. Area of immune formations in the mucous membrane of esophagus of turkeys, %, $M \pm m$, $n = 6$

Age group, in days	Bird group	
	experimental	control
20	1.13 ± 0.06	-
30	2.10 ± 0.17***	1.28 ± 0.03
40	3.83 ± 0.29***	2.16 ± 0.17***
50	4.16 ± 0.14	2.54 ± 0.20

Note: $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to the indicator in the previous group

Source: developed by the authors

As noted above, in turkeys of the first group immune formations of the esophagus started appearing at 20 days of age, while in the second – at 30 days of age. In general, in poultry from the experimental group (from 20 to 50 days of age), their area increased by 268.14% (3.7 times), while in control (from 30 to 50 days of age) – by 98.44% (almost 2 times). The increase in the area of immune formations of the esophagus occurred unevenly. In turkeys from the experimental group, this value increased most intensively from 20 to 30 days of age (by 85.84%,

$P < 0.001$), while in control – from 30 to 40 days of age (by 68.75%, $P < 0.001$). It was increasing less intensively in turkeys of 40 to 50 days of age (by 8.62% in experimental and by 17.59% in control groups). The lymphoid nodules of the esophagus of turkeys from both groups were primarily oval, round, and, some of them, triangular and pyriform with rounded tips. In the experimental group, they appeared at 30 days, while in the control group – at 40 days. Moreover, the sizes of secondary lymphoid nodules were relatively larger than that of primary (Table 3).

Table 3. Sizes of lymphoid nodules of the esophagus of turkeys, μm , $M \pm m$, $n = 6$

Bird groups	Lymphoid nodules					
	Primary			Secondary		
	round	oval length	oval width	round	oval length	oval width
	30 days					
Experimental	138.0 ± 8.03	250.0 ± 33.62	156.83 ± 18.21	154.5 ± 14.01	275.5 ± 11.11	162.83 ± 10.02
Control	-	-	-	-	-	-
	40 days					
Experimental	170.17 ± 13.73*	278.0 ± 17.93	167.67 ± 14.07*	183.83 ± 7.04*	289.0 ± 13.07	177.17 ± 14.63
Control	141.33 ± 11.27	264.83 ± 15.22	157.0 ± 4.86	155.5 ± 13.82	278.17 ± 13.88	163.17 ± 5.13

Table 3. Continued

Bird groups	Lymphoid nodules					
	Primary			Secondary		
	round	oval		round	oval	
	length	width		length	width	
Experimental	185.17 ± 10.18	286.5 ± 13.64	171.83 ± 7.22	186.5 ± 4.39	295.83 ± 8.16*	180.17 ± 4.76
Control	155.67 ± 6.97*	274.83 ± 24.22	158.0 ± 18.87	157.83 ± 7.04	280.17 ± 15.04	171.17 ± 15.59

Note: *P < 0.05; **P < 0.01; ***P < 0.001 compared to the indicator in the previous group

Source: developed by the authors

In summary, in turkeys from the experimental group (from 30 to 50 days) the diameter of round primary nodules increased by 34.18%, the length of oval nodules – by 14.60%, their width – by 9.56%, while the same parameters in secondary nodules increased by 20.71%, 7.38 and 10.65% respectively. The diameter, length and width increased most intensively from 30 to 40 days, in primary lymphoid nodules – by 23.31 (P < 0.05), 11.2 and 6.91% (P < 0.05) respectively, and in secondary lymphoid nodules – by 18.98%, 4.9 and 8.81% respectively. In turkeys from the control group (from 40 to 50

days), the diameter of primary lymphoid nodules increased by 10.15%, length – by 3.77%, and width – by 0.64%, while in secondary lymphoid nodules the same parameters increased by 1.50%, 0.72 and 4.9% respectively. With the preservation of feed, the mucous membrane of the crop of turkeys from the experimental and control groups had a smooth surface with periodic low folds, while in the absence of feed, the folds were high and of considerable sizes. In the crop, the glandular part (from the esophagus) was clearly visible, as well as its opposite – non-glandular part (Fig. 5A).

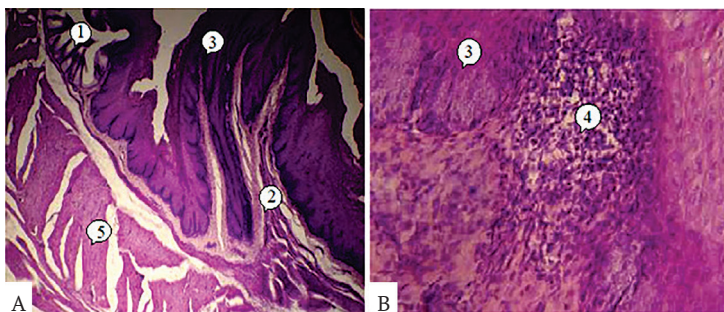


Figure 5. Structural and immune changes in the crop of turkeys from control and experimental groups

Note: A – glandular and non-glandular parts of the crop of a 20-days old turkey from the control group; B – migration of lymphoid cells into the epithelium of the crop of a 20-days old turkey from the experimental group. Histological preparations: 1 – glandular part of the crop; 2 – non-glandular part of the crop; 3 – epithelial layer; 4 – diffuse lymphoid tissue; 5 – muscular layer. Staining with Carazzi hematoxylin and eosin ×100 (A), ×400 (B)

Source: developed by the authors

The general microstructure of the crop of turkeys is similar to the cervical section of the esophagus, that is, its wall is formed by three

membranes: mucous, muscular and adventitious (Fig. 5A). In the mucous membrane, a thicker stratified squamous epithelium with many rows

of epithelial cells was clearly visible, which was in contact with the cavity of the crop. Under the epithelium, a layer of loose fibrous connective tissue with blood vessels and nerves could be found, between the elements of which – in the glandular part of the crop – packages of glands were located, while in the non-glandular part – they were absent. Bundles of smooth muscle cells formed a weakly defined, intermittent muscularis mucosae, under which loose fibrous connective tissue is located, forming a sub-mucosa. In the muscular membrane along the perimeter of the crop, in some of its sections, the outer longitudinal layer was not observed.

In turkeys from the experimental group aged one and 10 days, immune formations in the mucous membrane of the crop were also not present. They were first recorded in its non-glandular and glandular parts in turkeys aged 20 days. Immune formations were present as diffuse form and individual diffuse clusters of lymphocytes that formed prenules in some places. Such clusters were registered predominantly under the epithelium and near esophageal glands. Prenules were located both in the diffuse lymphoid tissue and outside it. From the surface of immune formations,

migration of lymphocytes into basal and spinous layers of the epithelium (Fig. 5B). Near or directly inside the clusters, blood vessels could be found. At the ages 30, 40 and 50 days of turkeys from the experimental group, an increase in amount of intraepithelial lymphocytes was observed, primary and secondary lymphoid nodules were detected. Diffuse form and prenules were recorded in the wall of the glandular apparatus as well.

In turkeys from the control group, immune formations were first observed at the age of 30 days. Local clusters of diffuse lymphoid tissue were located primarily under the epithelium. Prenules and lymphoid nodules were absent. In birds aged 40 and 50 days, an increase in amount of clusters of lymphoid tissue was observed, which was present in all forms, including secondary lymphoid nodules. The arrangement of reticular fibers in diffuse lymphoid tissue and lymphoid nodules of the crop of turkeys of both groups is similar to that of the esophagus. The area of immune formations in the mucous membrane of the crop of turkeys of both groups changes with increase in their age, moreover, it is somewhat larger in vaccinated birds (Table 4).

Table 4. Area of immune formations in the mucous membrane of the crop of turkeys, %, $M \pm m$, $n = 6$

Age group, in days	Bird group	
	experimental	control
20	1.39 ± 0.15	-
30	2.02 ± 0.05**	1.54 ± 0.12
40	4.09 ± 0.43***	3.06 ± 0.23***
50	5.18 ± 0.17*	4.02 ± 0.17**

Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared to the indicator in the previous group

Source: developed by the authors

In summary, in turkeys from the experimental group (from 20 to 50 days), this value increased by 272.66% (by 3.7 times). Its most intensive increase was observed between 30

and 40 days of age (by 102.47%, $P < 0.001$), least intensive – in birds aged 40 to 50 days (by 26.65%) In turkeys from the control group (from 30 to 50 days), the area of immune

the esophagus and the protrusion of its wall – the crop – in turkeys of the Big-6 breed with respect to age, which is an important criterion during the preventive immunisation of birds, was studied. It has been shown that in vaccinated and unvaccinated turkeys, the development of lymphoid tissue, which forms the basis of immune formations, occurred in the same sequence (formation of diffuse form, prenodules, primary and secondary nodules), however this process proceeded more intensively in vaccinated poultry. Immune formations were recorded in the plane of the mucous membrane and had a close morphofunctional connection with the epithelial layer, forming a “lymphoepithelium”, the esophageal glands, and in deep areas of the submucosa at the border with the muscular layer. Reticular fibers, intertwined, formed a fine- and large-cell network in the diffuse lymphoid tissue, surrounded the lymphoid nodules, forming their membrane, and were found in the center of primary lymphoid nodules, or in secondary lymphoid nodules as separate fragments. Collagen and elastic fibers in the form of thin bundles were recorded only in the membrane of lymphoid nodules, but not in their central part. In vaccinated turkeys, diffuse lymphoid tissue in the walls of the esophagus and the crop was first recorded at the

age of 20 days, and lymphoid nodules appeared at the age of 30 days, while in unvaccinated birds diffusely arranged lymphocytes appeared on the 30th day, and lymphoid nodules on the 40th. The area of immune formations of turkeys from both groups increased with age, and was somewhat larger in vaccinated birds. Lymphoid nodules had a predominantly round and oval shape. Their sizes changed with increasing age of the birds and reached maximum values in birds aged 50 days, moreover, the diameter of the round and the length and width of the oval secondary lymphoid nodules exceeded those of primary nodules. Prospects for further studies could be aimed at studies of the features of the structure and functions of immune formations of the esophagus of turkeys in later stages of the postnatal period of ontogenesis, and at conducting a comparative assessment with other species of domestic poultry.

Acknowledgements

None.

Funding

The study was not funded.

Conflict of Interest

None.

References

- [1] Ali, H.M., Ali, K.A., & Taha, A.M. (2023). [Comparative anatomical, histological, and electron microscopical studies on the cervical region of the esophagus in some birds with different diet habits](#). *Journal Advanced Veterinary Research*, 13(2), 181-187.
- [2] Al-Musawi, R.a., & Ali Al-Khakani, S.s. (2024). Comparative morphological and histochemical study of esophagus, in eurasian collared dove (*Streptoplia decaocto*) and buzzard (*Beuteo beuteo vulpinus*). *Journal of Anatomy and Physiology*, 5(3), 58-65. [doi: 10.36346/sarjap.2024.v05i03.004](#).
- [3] Alsanosy, A.A., Noreldin, A.E., Elewa, Y.H.A., Mahmoud, S.F., Elnasharty, M.A., & Aboelnour, A. (2021). Comparative features of the upper alimentary tract in the domestic fowl (*Gallus gallus domesticus*) and kestrel (*Falco tinnunculus*): A morphological, histochemical, and scanning electron microscopic study. *Microscopy and Microanalysis*, 27(1), 201-214. [doi: 10.1017/S1431927620024812](#).

- [4] Aydin, N., Akbalik, M.E., Topaloglu, U., & Bayram, B. (2022). Histological and histochemical profile of mucins in glands in partridge (*Alectoris chukar*). *Journal of Faculty of Veterinary Medicine Erciyes University*, 19(3), 175-181. doi: [10.32707/ercivet.1204251](https://doi.org/10.32707/ercivet.1204251).
- [5] Budnik, T., Huralska, S., Pinsky, O., Hryshchuk, H., & Honcharenko, V. (2022). Histoarchitectonics of the Harderian gland of chickens in the postvaccination period. *Scientific Horizons*, 25(12), 32-40. doi: [10.48077/scihor.25\(12\).2022.32-40](https://doi.org/10.48077/scihor.25(12).2022.32-40).
- [6] Budnik, T.S., & Guralaska, S.V. (2022). Cyto and histoarchitectonics of the chicken spleen in the postvaccination period. *Ukrainian Journal of Veterinary and Agricultural Sciences*, 5(3), 13-17. doi: [10.32718/ujvas5-3.03](https://doi.org/10.32718/ujvas5-3.03).
- [7] Ceccopieri, C., & Madej, J.P. (2024). Chicken secondary lymphoid tissues – structure and relevance in immunological research. *Animals*, 14(16), article number 2439. doi: [10.3390/ani14162439](https://doi.org/10.3390/ani14162439).
- [8] Dyshliuk, N., Huralska, S., Kot, T., Mazurkevych, T., Stehnei, Zh., & Usenko, S. (2024). Morphology of the gastrointestinal tract and its lymphoid tissue in the Common Blackbird (*Turdus merula*). *Acta Fytotechnica et Zootechnica*, 27(1), 35-45. doi: [10.15414/afz.2024.27.01.35-45](https://doi.org/10.15414/afz.2024.27.01.35-45).
- [9] El-naseery, N.I., Mohammed, A.A.A., Abuel-Atta, A.A., & Ghonimi, W.A.M. (2021). Species-specific differences of the avian oesophagus: Histological and ultrastructural study. *Anatomia, Histologia, Embryologia*, 50(5), 788-800. doi: [10.1111/ahc.12721](https://doi.org/10.1111/ahc.12721).
- [10] European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes. (1986, March). Retrieved from https://zakon.rada.gov.ua/go/994_137.
- [11] Hamoda, H., & Farag, A. (2018). Histological characterizations of the gut associated lymphatic tissue in pigeon. *Alexandria Journal of Veterinary Sciences*, 59(2), 157-164. doi: [10.5455/ajvs.16178](https://doi.org/10.5455/ajvs.16178).
- [12] Kataa, T., & Ahmed, S.M. (2022). [Anatomical and histological study of esophagus in small geese \(*Anseer anseer*\)](#). *Journal of Medicinal Plants Studies*, 10(4), 226-229.
- [13] Kharchenko, L.P. (2014). [Macroscopic structure of the stomach of birds of different trophic specializations](#). *Biology and Valeology*, 16, 62-70.
- [14] Khomich, V.T., Usenko, S.I., & Dyshliuk, N.V. (2020). Morphofunctional features of the esophageal tonsil in some wild and domestic bird species. *Regulatory Mechanisms in Biosystems*, 11(2), 207-213. doi: [10.15421/022030](https://doi.org/10.15421/022030).
- [15] Kotz, C.M. (2002). [Lymphoid formations of the digestive tract of birds of the Chaplevi family](#). *Biology and Valeology*, 5, 14-26.
- [16] Lavelle, E.C., & Ward, R.W. (2022). Mucosal vaccines-fortifying the frontiers. *Nature Reviews Immunology*, 22, 236-250. doi: [10.1038/s41577-021-00583-2](https://doi.org/10.1038/s41577-021-00583-2).
- [17] Law of Ukraine No. 3447-IV “On the Protection of Animals from Cruelty”. (2006, February). Retrieved from <https://zakon.rada.gov.ua/laws/show/3447-15#Text>.
- [18] Lykova, O., & Kharchenko, L.P. (2021). Nutrition ecology and morpho-functional organization of the digestive system of the black tern *Chlidonias niger* (Linnaeus, 1758). *Biodiversity, Ecology and Experimental Biology*, 22(2), 82-90. doi: [10.34142/2708-5848.2020.22.2.09](https://doi.org/10.34142/2708-5848.2020.22.2.09).
- [19] Mahdy, M.A.A., & Mohammed, E.S.I. (2024). Anatomical, histological, and scanning electron microscopic features of the esophagus and crop in young and adult domestic pigeons (*Columba livia Domestica*). *BMC Veterinary Research*, 20, article number 428. doi: [10.1186/s12917-024-04147-z](https://doi.org/10.1186/s12917-024-04147-z).

- [20] Markowska, M., Majewski, P.M., & Skwarło-Sońta, K. (2017). Avian biological clock – immune system relationship. *Developmental Comparative Immunology*, 66, 130-138. doi: [10.1016/j.dci.2016.05.017](https://doi.org/10.1016/j.dci.2016.05.017).
- [21] Monisha, N.J., John, A.S., Sojol, S.H., Islam, R., Sultana, N., & Islam, M.R. (2024a). Histomorphometry of the gastrointestinal tract of the broiler and cock chicken in Bangladesh. *Bangladesh Journal of Veterinary Medicine*, 22(2), 33-42. doi: [10.33109/bjvmjd2024am1](https://doi.org/10.33109/bjvmjd2024am1).
- [22] Monisha, N.J., Sojol, S.H., John, A.S., Islam, R., Haque, Z., Ali Khan, A.H.N., & Islam, M.R. (2024b). Histomorphometry of gastrointestinal tract mucosae of sonali and indigenous chickens in Bangladesh. *Journal of Science and Technology Research*, 6(1), 33-44. doi: [10.3329/jscitr.v6i1.77373](https://doi.org/10.3329/jscitr.v6i1.77373).
- [23] Nahed, A., Lamiaa, E.M., & Ghada, M. (2021). Comparative histological study of esophagus and liver in some aquatic birds. *International Journal of Advanced Research*, 9, 50-55. doi: [10.21474/IJAR01/12416](https://doi.org/10.21474/IJAR01/12416).
- [24] Rajabi, E., & Nabipour, A. (2009). Histological study on the oesophagus and crop in various species of wild bird. *Avian Biology Research*, 2(3), 161-164. doi: [10.3184/175815509X12474789336122](https://doi.org/10.3184/175815509X12474789336122).
- [25] Sağsöz, H., & Lıman N. (2009). Structure of the oesophagus and morphometric, histochemical-immunohistochemical profiles of the oesophageal gland during the post-hatching period of Japanese quails (*Coturnix coturnix japonica*). *Anatomia, Histologia, Embryologia*, 38(5), 330-340. doi: [10.1111/j.1439-0264.2009.00947.x](https://doi.org/10.1111/j.1439-0264.2009.00947.x).
- [26] Sinha, R., Kumar, P., Kumar, A., & Dar, Y. (2023). Morphological and morphometrical study on oesophagus of Japanese quail (*Coturnix japonica*). *Journal of Animal Research*, 13(03), 387-391. doi: [10.30954/2277-940X.03.2023.12](https://doi.org/10.30954/2277-940X.03.2023.12).
- [27] Taki-El-Deen, F. (2017). [Comparative microscopic study on the tongue, oesophagus and stomach of two different birds in Egypt](#). *The Egyptian Journal of Hospital Medicine*, 67(1), 359-365.
- [28] Zhang, X., Patil, D., Odze, R.D., Zhao, L., Lisovsky, M., Guindi, M., Riddell, R., Bellizzi, A., Yantiss, R.K., Nalbantoglu, I., & Appelman, H.D. (2018). The microscopic anatomy of the esophagus including the individual layers, specialized tissues, and unique components and their responses to injury. *Annals of the New York Academy of Sciences*, 1434(1), 304-318. doi: [10.1111/nyas.13705](https://doi.org/10.1111/nyas.13705).

Вплив вакцинації на розвиток імунних утворень у стравоході індиків

Наталя Мазур

Аспірант

Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0009-0004-5747-4335>

Надія Дишлюк

Доктор ветеринарних наук, професор

Національний університет біоресурсів і природокористування України
03041, вул. Героїв Оборони, 15, м. Київ, Україна
<https://orcid.org/0000-0003-4753-9356>

Анотація. Вакцинація птахів забезпечує захист від низки бактеріальних та вірусних збудників і залежить від розвитку імунних утворень органів травлення, в яких під впливом антигенів виникає імунна відповідь, що проявляється у продукуванні антитіл, появи клітин-ефекторів і формуванні імунологічної пам'яті на конкретний чужерідний об'єкт. Метою роботи було з'ясувати розвиток імунних утворень стравоходу та його розширення – вола і встановити терміни їх повної морфофункціональної зрілості у вакцинованої та невакцинованої птиці. Для проведення гістологічних досліджень матеріал відібрали від 66 голів індиків породи Біг-6 віком від однієї до 50 діб, яких поділили на дослідну (вакциновану) та контрольну (невакциновану) групи. При виконанні роботи використовували класичні методи фарбування гістопрепаратів гематоксиліном та еозином, за Ван Гізон, Малорі, Вейгертом та імпрегнували аргентуму нітратом за Келеменом. У вакцинованих індиків імунні утворення стравоходу та вола вперше реєстрували з 20-добового віку, які представлені локальними скупченнями дифузної лімфоїдної тканини, а у волі ще й перед вузликами. Вони знаходилися під епітеліальним шаром у власній пластинці слизової оболонки, підслизовій основі, поблизу стравохідних залоз та безпосередньо в них. Із поверхні скупчень простежувалася міграція лімфоїдних клітин у нижні шари епітелію. У 30-добовому віці індиків цієї групи лімфоїдна тканина стає морфофункціонально зрілою. У ній реєструвалися лімфоїдні вузлики без світлих та із світлими центрами. У невакцинованих індиків вперше імунні утворення стравоходу і вола виявлялися з 30-добового віку і вони були представлені дифузно розміщеними лімфоцитами, а в 40-добовому віці встановлена їх морфофункціональна зрілість, тобто виявлені всі рівні структурної організації лімфоїдної тканини. Отримані результати важливо враховувати при проведенні профілактично-лікувальних заходів під час вирощування індиків

Ключові слова: апарат травлення; лімфоїдна тканина; лімфоїдні вузлики; лімфоцити; птиця