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Patterns of formation of the lymphoid tissue of the oesophageal tonsil in ducks at the early stages of the postnatal period of ontogenesis

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Abstract. The relevance of studying the oesophageal tonsil of ducks at the early stages of the postnatal period of ontogenesis is linked to the need to analyse how the levels of structural organisation of its lymphoid tissue are established. In this context, the aim of the research was to determine the localisation and form of the lymphoid tissue of the oesophageal tonsil in Blagovarsky cross broiler ducks from 1 to 240 days of age. Morphometric investigations were carried out and

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the topography of the oesophageal tonsil was determined in ducks of the studied age groups. Biological material was fixed in a 10% solution of neutral formalin. For histological examination, paraffin blocks were prepared on a sledge microtome and histological sections were obtained, which were stained to reveal the structural features and area of the tissue components of the oesophageal tonsil. It was established that in ducks, the oesophageal tonsil was macroscopically visualised at the transition of the mucous membrane of the caudal part of the oesophagus into the glandular part of the stomach. Changes in its length and width were asynchronous. Up to 5 days of age, the lymphoid tissue of the oesophageal tonsil was represented only by the diffuse form. In ducks aged 10 days and older, primary and secondary nodules appeared. By 15 days of age, the oesophageal tonsil had reached morphofunctional maturity and was characterised by fully developed lymphoid tissue. In day-old ducklings, an infiltration of the glandular portions of the oesophageal glands and the excretory ducts by lymphocytes was observed. With increasing age, intensive colonisation of the epithelium of the oesophageal glands and the excretory ducts by lymphoid cells was recorded. As a result, the lumina of the glands narrowed and transformed into crypt-like openings that opened into the lumen of the oesophagus. The data obtained on the development of the lymphoid tissue of the oesophageal tonsil in broiler ducks during the initial age phases can be used to optimise rearing processes, improve productive performance and refine vaccination strategies to prevent dangerous infectious diseases

Keywords: immune formations; lymphoid nodules; morphometric investigations; poultry; mucous membrane of the oesophagus

Introduction

In phylogeny, the organs of haematopoiesis and lymphopoiesis of birds occupy a special place. In birds, lymphoid tissue is for the first time separated from myeloid tissue, although both branches remain closely connected, forming a single haematopoietic system (Pendl & Schmidt, 2024). The formation of lymphoid tissue in ontogenesis takes place in direct contact with blood vessels, which mirrors its phylogenetic development and its dependence on the vascular bed (Hantusch, 2024). A characteristic feature of birds is the presence of another central organ of haematopoiesis and lymphopoiesis – the cloacal bursa (bursa of Fabricius), which is not found in other cloacal animals. In the course of phylogeny, birds also developed the Harderian gland, typical of vertebrates except for primates and humans, which has been described in detail in the works of I.R. Tizard (2024) and A. Jamil *et al*. (2024). An oesophageal tonsil has likewise been identified, which is unique for this class and is not found in other vertebrate species (Capotă *et al*., 2025).

Another important sign of the transition of the immune system to a higher level of structural and functional organisation is the formation in waterfowl of structures similar to the lymph nodes of mammals. These were studied by C. Ceccopieri & J.P. Madej (2024) and H. Pendl & R.E. Schmidt (2024). As shown by these authors, in birds, due to the absence of classical lymph nodes, the diffuse lymphoid tissue is extremely well-developed. It forms in places of potential antigen penetration and sites of antigenic stimulation. An example of such tissue is the lymphoid tissue associated with mucous membranes (MALT – mucous associated lymphoid tissue), which includes

bronchus-associated lymphoid tissue (BALT – bronchus associated lymphoid tissue), nasal-associated lymphoid tissue (NALT – nasal associated lymphoid tissue) and gut-associated lymphoid tissue (GALT – gut associated lymphoid tissue). Additional, though less described, components of MALT include conjunctiva-associated lymphoid tissue (CALT – conjunctiva associated lymphoid tissue), larynx-associated lymphoid tissue (LALT – larynx associated lymphoid tissue) and lymphoid tissue associated with the excretory ducts of the salivary glands (DALT – salivary duct associated lymphoid tissue). According to the conclusions of C. Ceccopieri & J.P. Madej (2024), owing to the activity of immunocompetent cells – dendritic cells, T- and B-lymphocytes – MALT contributes to the formation of long-term local immunity.

The bodies of waterfowl are exposed to increased antigenic load, which is determined by the habitat. Therefore, the immune system of these birds includes both lymph nodes and MALT. The oesophageal tonsil in birds belongs to the peripheral organs of haematopoiesis and lymphopoiesis and is part of the group of lymphoid formations associated with mucous membranes (MALT – mucous associated lymphoid tissue). According to H.M. Ali *et al*. (2023), this organ plays a key role in shaping the local immune response to pathogens entering the body through the digestive tract.

For this reason, investigation of the morphofunctional features of the oesophageal tonsil in ducks is an important task for a deeper understanding of the immune mechanisms in these birds. Analysis of age-related changes in its structure makes it possible to identify stages in the development of the immune system and to optimise housing conditions, feeding, and vaccination to ensure high health status and productivity. The aim of the study was to determine the patterns of formation of the lymphoid tissue of the oesophageal tonsil in ducks from hatching to the onset of sexual maturity. To achieve this aim, the following tasks were set: to determine the macroscopic indices and features of the microstructure of the lymphoid tissue, its interrelations with the oesophageal glands and the surface epithelium of the mucous membrane; to determine the area of the lymphoid tissue, the levels of its organisation and its interrelations with the components of the mucosa.

Literature Review

Current scientific research points to the leading role of the organs of haematopoiesis and lymphopoiesis in maintaining immune homeostasis in animals. Peripheral lymphoid formations, which are the first to come into contact with environmental antigens, are particularly important in this respect. The organs of haematopoiesis and lymphopoiesis in animals are divided into central and peripheral. In the central organs, blood cells develop; in the peripheral organs, under the influence of antigens, lymphocytes differentiate into effector cells that ensure immunity and the elimination of foreign agents from the body (Akhand & Ahsan, 2023).

The central organs are situated in regions of the body protected from the direct action of antigens, whereas the peripheral organs are located at the sites where antigens enter the organism. As noted by J.I. Park *et al*. (2023) and L.V. Kadirova (2024), most antigens enter with feed and water through the digestive tract, in the wall of which about 70% of the lymphoid tissue of the body and 80% of its immunocompetent cells are concentrated. It is lymphoid tissue that forms the functional basis of the peripheral organs of haematopoiesis and lymphopoiesis.

One of the key components of the immune system of birds is the oesophageal tonsil, which belongs to the lymphoid formations associated with mucous membranes (MALT – mucous

associated lymphoid tissue) and functions as a peripheral organ of haematopoiesis and lymphopoiesis. According to F. Davison (2022), because representatives of this class lack the pharyngeal lymphoid ring of Pirogov-Waldeyer, the oesophageal tonsil is the first organ to respond to antigens entering with feed and water. It is located in the mucous membrane of the caudal part of the oesophagus, at the boundary with the glandular part of the stomach, and is represented by diffuse and nodular forms of lymphoid tissue (Zeinali *et al*., 2024). M. Rochman *et al*. (2025) confirmed that this formation actively responds to antigenic stimuli entering via the oesophagus and plays an important role in the local immune response to pathogens that penetrate the organism through the digestive tract.

The morphofunctional features of the oesophageal tonsil in birds, particularly in ducks, as indicated by J. Mehrzad *et al.* (2024), are of considerable scientific interest, since the study allows a deeper understanding of the mechanisms of immune response to external stimuli. Analysing age-related changes in the structure and function of this organ helps to identify critical periods in the development of the immune system, to optimise the conditions of housing and feeding, and to improve vaccination strategies aimed at increasing productivity and maintaining health. The oesophageal tonsil in ducks, as shown by N.I. El-Naseery *et al*. (2021), begins to form as early as the fifth day after hatching, which underlines its important role in the early development of the immune response. During the development of the organ, significant changes occur in the total amount of lymphoid tissue and in the levels of its structural organisation. The total amount of lymphoid tissue reaches a maximum at 30 days of age and then gradually decreases. According to J. Mehrzad *et al.* (2024), this may be related to adaptation to constant antigenic stimuli and the establishment of a stable immune response. With age, a redistribution of lymphoid tissue takes place, with a decrease in its diffuse form and an increase in the proportion of connective tissue, which points to changes in the lymphopoietic processes of the organ.

A crucial indicator of the function of the oesophageal tonsil is the presence of a substantial number of immunocompetent cells. C. Ceccopieri & J.P. Madej (2024) noted that the presence of T- and B-lymphocytes, plasma cells, macrophages, and monocytes ensures the development of cellular and humoral immunity. In older birds, the number decreases, which, in the opinion of J. Mehrzad *et al*. (2024), may be associated either with the achievement of a stable immune response or with general changes in the functioning of the immune system.

External factors, vaccination in particular, also influence the morphogenesis of the oesophageal tonsil. S. Guralska *et al*. (2023) reported that in vaccinated birds, its morphofunctional maturity occurs earlier, which confirms the role of antigenic stimulation in the development of the immune system. This also highlights the importance of the organ as an indicator of the effectiveness of vaccination strategies (Lestari *et al*., 2023). In turn, N. Yıldırım (2024) showed that age-related changes in the structure and function of the tonsil have practical significance for adjusting vaccination methods, treating infections and conducting immunological monitoring, since the regenerative capacity of the organ declines with age. Thus, studying the morphofunctional changes in the oesophageal tonsil in ducks is necessary for a better understanding of the mechanisms of immunity formation in this species. Knowledge of age-related changes in the oesophageal tonsil will help to optimise methods of housing, feeding and vaccination, as well as to improve disease prevention strategies in poultry farming.

Materials and Methods

The research was carried out from September 2023 to March 2025 in the Teaching-Scientific-Production Laboratory "Centre of Biomorphological Technologies" of the Department of Vertebrate Biomorphology named after Academician V.H. Kasyanenko. The present work continues investigations performed in 2012-2013, the results of which were partly published in scientific articles by V.T. Khomych & S.I. Usenko (2012; 2013). In the publication by V.T. Khomych & S.I. Usenko (2012) information was presented on the localisation of the lymphoid tissue of the oesophageal tonsil in the lamina propria and submucosa of the oesophageal mucosa in ducks aged 1-20 days. The age of the ducks at which different levels of structural organisation of lymphoid tissue (diffuse form, prenodules, primary and secondary lymphoid nodules) appear and its area in the mucous membrane were determined. In the publication by V.T. Khomych & S.I. Usenko (2013) the investigation of the lymphoid tissue of the oesophageal tonsil in ducks aged 25-120 days was continued and morphometric indices of the oesophageal tonsil, the area of its lymphoid tissue and the levels of its development were provided. For a full understanding of the patterns of development of the oesophageal tonsil in ducks from hatching to the onset of sexual maturity (180 days) and its subsequent development, the present study used the findings of the investigations from 2012 to 2013 and extended the examination of this immune formation in ducks aged 150-240 days. In addition, the studies from 2012 to 2013 were supplemented. Specifically, in ducks aged 1-25 days, macroscopic indices of the oesophageal tonsil were determined; in ducks aged 1-120 days, changes in the linear measurements of the mucosal folds of the oesophagus in the region of the oesophageal tonsil and in adjacent areas, and the relationships between the lymphoid tissue and the oesophageal glands, were examined.

Material for the study was taken from 68 Blagovarsky cross broiler ducks aged 1 day, 5, 10, 15, 20 and 25 days (six birds in each age group) and 30, 60, 90, 120, 150, 180, 210 and 240 days (four birds in each age group). Preventive vaccinations were not carried out in these birds. After ether anaesthesia, euthanasia was performed by acute exsanguination in accordance with the general ethical principles of O.H. Reznikov (2003). The authors state that the provisions of the Law of Ukraine No. 3447-IV (2006) were compiled with and the ARRIVE recommendations (n.d.) were taken into account. As this research was post-mortem, the standards of OIE/WOAH (n.d.) were followed.

After opening the carcasses of the ducks, the caudal part of the oesophagus and the cranial part of the glandular stomach were dissected in the thoraco-abdominal cavity and cut longitudinally with fine-pointed scissors. In the caudal part of the oesophagus, the topography, relief, and colour were recorded. The length and greatest width of the oesophageal tonsil were measured with callipers and a ruler (DSTU EN ISO 13385-1:2018, 2018). After sampling material for histological examination, it was labelled and fixed in a 10% aqueous solution of neutral formalin. The next stage was the preparation of histological sections. For this purpose, the fixed material was washed in tap water, dehydrated in ethanol solutions of increasing concentration and then embedded in paraffin. Histological sections 5-10 μm thick were cut on an MPS-2 sledge microtome (Tochmedpribor, Ukraine). The obtained histological slides were stained with Carazzi haematoxylin and eosin, according to Mallory, Van Gieson and Weigert (Horalskyi *et al.*, 2015). The structural components of the lymphoid tissue (reticular fibres) were demonstrated by

impregnating the sections with a 1% aqueous solution of silver nitrate. After staining and impregnation, the histological preparations were examined using Olympus (Japan) and Levenhuk (China) light microscopes.

The results obtained were recorded in protocols, and the numerical indices were processed statistically on a personal computer using Excel and Statist software. Statistical processing of the data included calculation of the mean value, the standard error of the mean and the P-value for testing the statistical significance of the results, in accordance with the requirements of morphological studies. A P-value of less than 0.05 was considered statistically significant. These methods made it possible to identify patterns in changes in lymphoid tissue in the different age groups of ducks and to assess the statistical significance of the findings obtained.

Results and Discussion

In conformity with the results of the investigations, the anatomically defined location of the oesophageal tonsil in ducks is the mucous membrane of the caudal part of the oesophagus in the region where it passes into the glandular part of the stomach. The mucous membrane of the oesophagus forms folds, owing to which the lumen of the oesophagus widens as the bolus of feed passes through. The position of the lymphoid tissue of the oesophageal tonsil is closely related to the longitudinal folds of the oesophagus. In the age groups of ducks studied, the mucous membrane forms 7-12 folds, in which the tonsil is localised (Fig. 1).

A B

Figure 1. Oesophageal tonsil of ducks of different ages
***Note**: A – at 5 days of age; B – at 120 days of age. Macroscopic specimen*
***Source**: V.T. Khomych & S.I. Usenko (2012; 2013)*

Macroscopically, the oesophageal tonsil was identified in ducks at 5 days of age and appeared as a pale band. The width of the band corresponded to the perimeter of the oesophagus and measured 1.97 ± 0.02 cm, while its length was 0.9 ± 0.06 cm. By 10 days of age, the oesophageal tonsil had the form of a yellowish ring-shaped, nodular band. This appearance remained the same in ducks of all older age groups, and only its linear dimensions (length and width) changed. V.R. Indu & K.M. Lucy (2021) reported that in hens, the oesophageal tonsil appeared as isolated pale patches located at the base of the folds.

Changes in the linear dimensions (length and width) of the oesophageal tonsil of ducks with age were asynchronous and periodic. The most intensive increase in the length of the oesophageal tonsil was observed in ducks aged 5-10, 10-15 and 60-90 days, by 37.8% ($P < 0.01$), 33.1% ($P < 0.001$) and 31.1% ($P < 0.001$) respectively, while the width increased most noticeably between 20 and 25 days, by 48.54%

(P < 0.001). The greatest values of length and width of the oesophageal tonsil were recorded in 120-day-old ducks, at 3.78 ± 0.25 cm and 0.84 ± 0.11 cm respectively (Fig. 2).

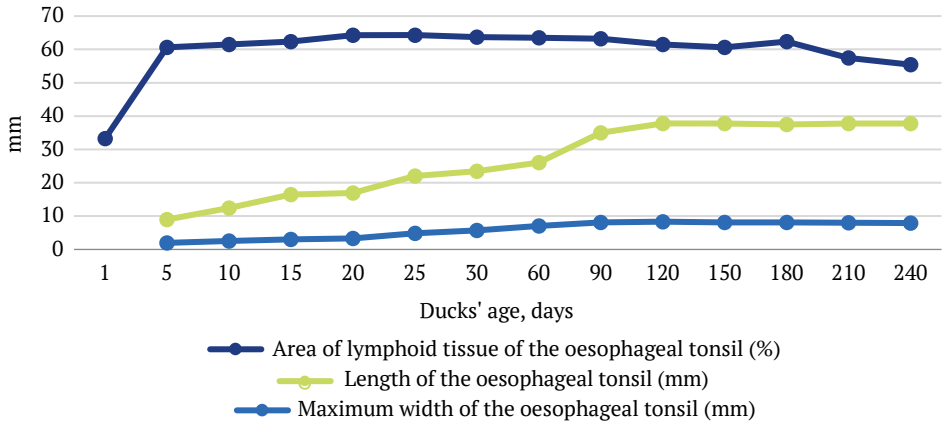


Figure 2. Morphometric indices of the oesophageal tonsil at the early stages of the postnatal period of ontogenesis and the area of its lymphoid tissue
***Note**: different colours indicate the area, length, and width of the oesophageal tonsil*
***Source**: developed by the authors*

Subsequently, a tendency towards a decrease in the width of the oesophageal tonsil was observed. The most marked reduction in this index, by 2.4% (P < 0.001), was found in ducks aged 120-150 days, when the width of the tonsil decreased to 0.81 ± 0.14 cm. In the age groups of 180 and 210 days, the width of the oesophageal tonsil remained almost unchanged (0.81 ± 0.8 cm and 8 ± 0.1 cm respectively). By 240 days of age it decreased by a further 1.25% (P < 0.001) and was 0.79 ± 0.02 cm. In ducks aged 150-240 days, the length of the oesophageal tonsil remained almost unchanged and ranged from 3.79 ± 0.21 cm to 3.78 ± 0.38 cm. L.O. Buhai (2008), who examined this immune formation in Muscovy ducks from the first day of life to sexual maturity, also reported periodic and asynchronous growth and development of the oesophageal tonsil.

Analysis of the relationship between the development of the area of lymphoid tissue of the oesophageal tonsil and its macroscopic indices showed that the area of lymphoid tissue of the oesophageal tonsil in the mucous membrane of the oesophagus reached its maximum values in ducks at 20 days of age and remained almost unchanged in 25-day-old ducks. In contrast, the linear dimensions reached the maximum values only in 120-day-old ducks. In older age groups (150-240 days), as noted above, the length and width of the oesophageal tonsil remained almost unchanged. The area of lymphoid tissue (Fig. 2), however, tended to decrease, except in the group of ducks aged 180 days, in which a slight increase in this index was recorded (by 2.9%, P < 0.001). Thus, quantitative changes in the area of lymphoid tissue of the oesophageal tonsil did not influence changes in its macroscopic indices.

In the region where the oesophageal tonsil is located, thickening of the wall of the oesophageal mucosa relative to adjacent areas was also noted. According to the results obtained, this is related to an increase in the height and maximum width of the folds of the mucous membrane in the region of the oesophageal tonsil

compared with those in neighbouring parts of the oesophagus. In day-old ducks, the linear measurements of the mucosal folds in the area of the oesophageal tonsil already substantially exceeded the corresponding indices for folds in adjacent portions of the oesophagus (Fig. 3).

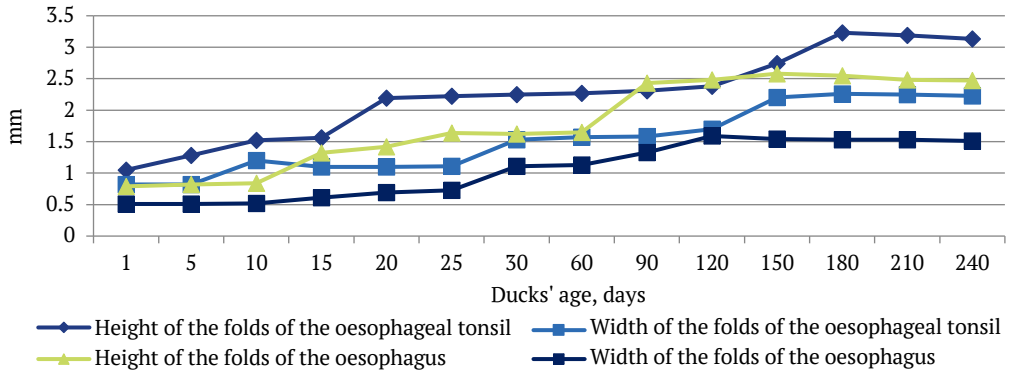


Figure 3. Linear measurements of the folds of the mucous membrane of the oesophagus and the oesophageal tonsil of ducks
***Note**: different colours indicate the height of the folds of the oesophageal tonsil and of the oesophagus*
***Source**: developed by the authors*

The height of the folds in the region of the oesophageal tonsil was 28% greater and the width 54.1% greater than in the adjacent part of the oesophagus. Changes in these indices with age were uneven and asynchronous. During the first 5 days of life, the height, and maximum width of the folds increased by 28% (that is, doubled) ($P < 0.05$) and 5.2% ($P < 0.05$) respectively. In the same period, the area of lymphoid tissue of the oesophageal tonsil increased most markedly – by 82% ($P < 0.001$). The maximum increase in the height of the folds of the oesophageal tonsil compared with adjacent parts of the oesophagus was recorded in ducks aged 15-20 days (by 99%, $P < 0.001$) and remained unchanged up to 25 days of age. In other age groups, this index fluctuated between 18.8% ($P < 0.001$) and 56% ($P < 0.001$). The width of the folds in the region of the oesophageal tonsil compared with the folds in adjacent areas of the oesophagus increased most intensively at 10-15, 15-20 and 20-25 days of age, by 116% ($P < 0.001$), 106% ($P < 0.001$) and 125% ($P < 0.001$) respectively. In the other age groups, this index also fluctuated, between 46% ($P < 0.001$) and 82.7% ($P < 0.001$).

Uneven and asynchronous changes in the indices of height and width of the mucosal folds of the oesophagus in the region where the oesophageal tonsil is located were also observed when analysed by age. In day-old ducks, the height of the folds in the area of the oesophageal tonsil was 1.05 ± 0.04 mm and the width 0.79 ± 0.01 mm. By 5 days of age, the height of the folds had increased by 22% ($P < 0.001$), while the width remained unchanged. The area of lymphoid tissue in the mucosa increased by 82% ($P < 0.001$). With age, these indices changed unevenly. The most intensive increase in the height of the folds, by 40.4%, occurred in birds between 15 and 20 days of age. At this time, the content of lymphoid tissue of the oesophageal tonsil increased by 3.1% ($P < 0.001$). The width of the folds in the region of the oesophageal tonsil increased maximally in ducks aged 10-15 days and 60-90 days, by 57.1% ($P < 0.01$)

and 53.3% (P < 0.001) respectively. The area of lymphoid tissue of the oesophageal tonsil in ducks aged 10-15 days decreased by 2% (P < 0.001), while in those aged 60-90 days it increased by 0.8% (P < 0.001). The greatest values of the height of the mucosal folds in the region of the oesophageal tonsil were reached in ducks at 180 days of age and amounted to 3.23 ± 0.06 mm, while the greatest width was found in 150-day-old ducks and was 2.58 ± 0.05 mm. In ducks aged 150-180 days, the area of lymphoid tissue increased by 2.9% (one of the highest indices) (P < 0.001). In older birds, these indices decreased slightly (height by 1.2-1.9%, width by 0.4-2.7%), and in 240-day-old ducks, the indices were 3.13 ± 0.05 mm and 2.47 ± 0.05 mm respectively. At the same time, a decrease in the area of lymphoid tissue was observed in ducks aged 210 and 240 days, by 7.9% (P < 0.001) and 3.6% (P < 0.001) respectively.

Thus, the uneven increase in the linear measurements of the folds of the mucous membrane of the oesophagus in the region of the oesophageal tonsil, both with age and in comparison with adjacent parts of the oesophagus, is related not only to the growth of the body of the ducks but also to the development of lymphoid tissue in the mucosa. Asynchronous development of the lymphoid tissue of the oesophageal tonsil was observed, influencing increases in the height and width of oesophageal folds oriented in different planes. Such changes in the linear indices of the folds in the region of the oesophageal tonsil compared with adjacent parts of the oesophagus indicate that the development of its lymphoid tissue proceeded unevenly and was oriented in different planes. Thickening of the oesophageal wall in the region of the oesophageal tonsil has also been reported in hens by I. Oláh *et al.* (2003) and C. Casteleyn *et al.* (2010), and in ducks by H.H. Donmez *et al*. (2012).

Microscopic examination confirmed that the general plan of the structure of the oesophageal wall in the region of the oesophageal tonsil is characteristic of ducks of all age groups studied. The wall of the oesophagus consists of mucosal, muscular and serous coats. The mucous membrane forms longitudinal folds involving all its components: epithelium, lamina propria, muscularis mucosae and submucosa. The epithelium covering the mucosa of the oesophagus is stratified squamous; immediately adjacent to the glandular part of the stomach it is replaced by the simple columnar glandular epithelium characteristic of this part of the stomach. In ducks of all age groups, the muscularis mucosae is poorly developed and represented by individual smooth muscle cells. In the lamina propria and submucosa, which consist of loose fibrous connective tissue, the oesophageal glands are located. These glands produce a mucous secretion that is discharged onto the surface of the mucosa through the excretory ducts. Near the glandular part of the stomach, lobules of the deep glands of this part of the stomach were also found in the submucosa of the oesophagus (Fig. 4).

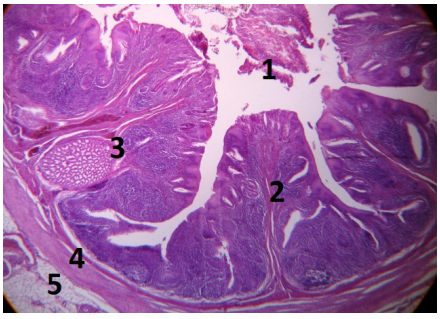
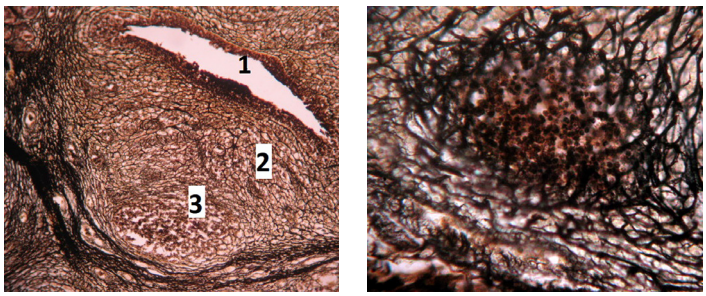


Figure 4. Oesophageal tonsil of a duck aged 15 days
***Note**: 1 – lumen of the oesophagus; 2 – fold of the mucous membrane; 3 – lobule of the deep glands of the glandular part of the stomach; 4 – muscular coat of the oesophagus; 5 – serous coat of the oesophagus. Haematoxylin and eosin stain, ×40*
***Source**: authors' own data*

The excretory ducts of the glands open on the surface of the mucosa of the glandular part of the stomach. The muscular coat of the oesophagus is formed by smooth muscle tissue arranged in three layers. The inner and middle layers are well-developed. In the inner layer the smooth muscle cells run longitudinally, and in the middle layer, these cells run circularly. The outer layer of the muscular coat is poorly developed and represented by small bundles of longitudinally oriented smooth muscle cells. In older ducks, the inner layer of the muscular coat locally projects into the folds of the mucosa. The serous coat of the oesophagus in the region of the oesophageal tonsil is formed by loose fibrous connective tissue covered externally by mesothelium.

The lymphoid tissue that provides the functional features of the oesophageal tonsil is represented by diffuse and nodular forms. It is based on reticular tissue, the arrangement of whose fibres depends on the level of development of the lymphoid tissue. In diffuse lymphoid tissue, the reticular fibres lack a definite orientation, are closely packed and form a network with small meshes containing lymphoid cells (Fig. 5).



A B

Figure 5. Architectonics of reticular fibres in the oesophageal tonsil of a duck
***Note**: A: 1 – excretory duct of an oesophageal gland; 2 – diffuse lymphoid tissue; 3 – lymphoid nodule; ×100. B – formation of a lymphoid nodule, ×400. Impregnation with silver nitrate*
***Source**: authors' own data*

The architectonics of the reticular fibres in the lymphoid nodules has specific features. In lymphoid nodules without germinal centres, the reticular fibres are arranged more loosely and form a meshwork with large spaces. In lymphoid nodules with pale germinal centres, the reticular fibres are usually absent. The nodules are bounded by a capsule composed of densely arranged reticular fibres together with isolated collagen and elastic fibres.

In ducks of all age groups, the lymphoid tissue of the oesophageal tonsil is located in the lamina propria and submucosa of the oesophageal mucosa (Fig. 4). Lymphoid cells surround blood vessels, the secretory portions of the oesophageal glands and the ducts. These cells locally infiltrate the epithelium of the glands and the mucosa. Near the glandular part of the stomach, lymphoid tissue is localised around the lobules of its deep glands, some of which are infiltrated by lymphoid cells. In older ducks, small accumulations of lymphoid cells were also found in the lumina of some glands. In the regions where the oesophageal tonsil is located, the structure of the components of the mucosa changes. In the lamina propria and submucosa, in addition to collagen and elastic fibres, local accumulations of reticular meshwork typical of diffuse lymphoid tissue and lymphoid nodules were observed (Fig. 5).

The development of the lymphoid tissue of the oesophageal tonsil takes place gradually. In day-old and 5-day-old ducks, the lymphoid tissue is represented only by the diffuse form (Fig. 6). In ducks aged 10 days, lymphoid nodules without germinal centres appeared in the oesophageal tonsil. Lymphoid nodules with pale (germinal) centres were found in ducks aged 15 days, which is a sign of an immune response to antigenic stimulation (Fig. 7). This is consistent with the conclusions of V. Logvinova & A. Oliyar (2021) and N. Mazur & N. Dyshliuk (2025), who determined that the formation of lymphoid tissue associated with the mucosa of the digestive organs takes place in four stages: diffuse lymphoid tissue, prenodules, primary and secondary lymphoid nodules.

Figure 6. Lymphoid tissue in the mucous membrane of the oesophagus in the region of the oesophageal tonsil of a day-old duck
***Note**: 1 – fold of the mucous membrane of the oesophagus; 2 – oesophageal glands; 3 – lymphoid tissue in the lamina propria and submucosa of the mucous membrane; 4 – lumen of an oesophageal gland; 5 – lobule of the deep glands of the glandular part of the oesophagus; 6 – muscular coat of the oesophagus. Impregnation with silver nitrate, ×100*
***Source**: authors' own data*

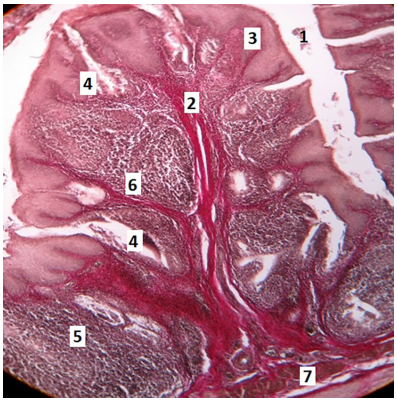


Figure 7. Formation of lymphoid nodules with germinal centres in the oesophageal tonsil of a 15-day-old duck
***Note**: 1 – lumen of the oesophagus; 2 – fold of the mucous membrane of the oesophagus; 3 – epithelium; 4 – oesophageal glands and the ducts; 5 – lymphoid tissue in the lamina propria and submucosa of the mucous membrane; 6 – lymphoid nodule with a germinal centre; 7 – muscular coat of the oesophagus. Van Gieson staining, ×100*
***Source**: authors' own data*

Diffuse and nodular forms of lymphoid tissue of the oesophageal tonsil in ducks occupy different areas. The area of diffuse lymphoid tissue is the largest, whereas that of the nodular forms is considerably smaller. The ratio of these indices changes with increasing age of the ducks (Fig. 8). In day-old and 5-day-old ducks, the lymphoid tissue is 100% represented by the diffuse form. As the ducks grow older, its content decreases unevenly. By 180 days of age, the area of the diffuse form of lymphoid tissue was 63.77 ± 0.51%. In older age groups, its area increased: in 210-day-old ducks by 7.8%, and in 240-day-old ducks by a further 1%, reaching 71.14 ± 0.23% of the area of the mucous membrane. These changes in the content of diffuse lymphoid tissue of the oesophageal tonsil are partly related to the development within it of lymphoid nodules.

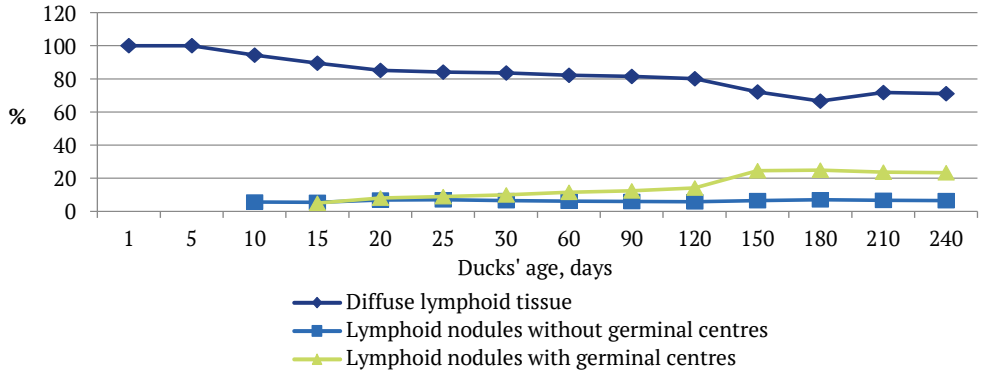


Figure 8. Content of structural components of lymphoid tissue of the oesophageal tonsil in ducks at different ages
***Note**: different colours indicate the content of lymphoid nodules at different ages of ducks*
***Source**: developed by the authors*

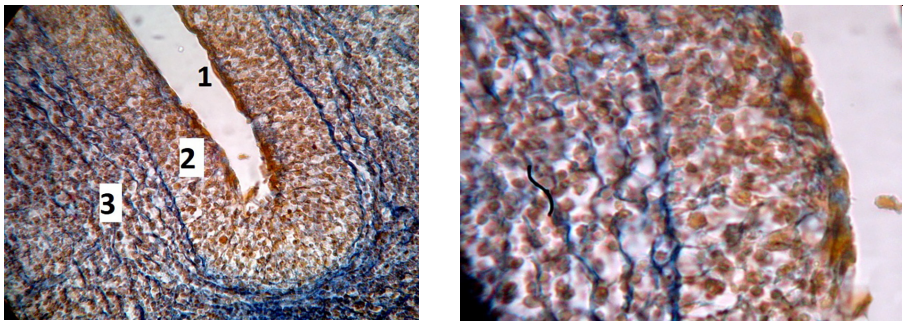
In ducks at 10 days of age, lymphoid nodules without germinal centres occupied 5.7 ± 0.24% of the area of the lymphoid tissue of the oesophageal tonsil. The amount increased markedly up to 20 days of age. With increasing age of the ducks, only slight changes in this index were recorded. In older age groups (150-240 days), the area of lymphoid nodules without germinal centres reached its highest values at 180 days of age, at 6.96 ± 0.32%. Afterwards it decreased evenly by 2.6% ($P < 0.01$) and in 240-day-old ducks was 6.52 ± 0.41%.

Lymphoid nodules with germinal centres in the oesophageal tonsil of ducks aged 15 days occupied 5.00 ± 0.64% of the area of lymphoid tissue. The area of these nodules in the lymphoid tissue of the oesophageal tonsil increased unevenly up to 180 days of age and amounted to 24.90 ± 0.38%. The most active formation of lymphoid nodules with germinal centres was observed in ducks aged 20 and 150 days: the area increased by 62% and 73.7% respectively. The maximum value (24.90 ± 0.38% of the area of lymphoid tissue of the oesophageal tonsil) was reached in ducks aged 180 days. In the older age groups (210 and 240 days), the area of these nodules decreased slightly, by 4.5% and 1.2% respectively, and in 240-day-old ducks it was 23.39 ± 0.19%. From 20 days of age, the content of lymphoid nodules with germinal centres clearly exceeded the content of nodules without germinal centres. A similar pattern of development of the lymphoid tissue of the oesophageal tonsil in hens at different ages was reported

by N.V. Dyshliuk (2009). In day-old hens, the author identified diffuse lymphoid tissue; at 10 days of age, primary lymphoid nodules appeared, and by 20 days secondary lymphoid nodules were present, while the content of diffuse lymphoid tissue increased with age.

The lymphoid nodules of the oesophageal tonsil in ducks of all age groups studied are mainly round or oval. Nodules with germinal centres are larger than those without these centres. In round nodules, the diameter of the former reached its maximum in ducks aged 150 days, and of the latter in ducks aged 180 days, at 198.82 ± 1.67 μm and 239.7 ± 1.84 μm respectively. Oval nodules reached the greatest size in ducks at 180 days of age. The length and maximum width were 304.56 ± 2.59 μm and 210.09 ± 2.43 μm (without germinal centres) and 349.68 ± 4.20 μm and 252.39 ± 3.16 μm (with germinal centres) respectively. T.A. Mazurkevych & V. Khomych (2017) also observed lymphoid nodules of such shapes in the lymphoid formations of the intestine of ducks.

Oesophageal glands and the excretory ducts deserve particular attention in the formation of the oesophageal tonsil in ducks. With increasing age, considerable changes were observed in the shape, content, and localisation in the mucous membrane of the oesophageal tonsil. In day-old ducks, the oesophageal glands were small and elongated-oval. These glands were located in the lamina propria of the mucous membrane at the base of the folds, on the lateral surfaces and at the tips. By 5 days of age the size had increased, and the glands had become round-oval, sometimes with star-shaped depressions. From the first day of life, lymphocytic infiltration of the secretory portions of the oesophageal glands and the excretory ducts was noted. This process became more intense with age. As a result, the epithelium thickened (Fig. 9) and the lumina of the glands narrowed, although the lumina did not disappear completely. The lumina were transformed into crypt-like openings surrounded by lymphoepithelium, opening onto the surface of the oesophagus.



A B

Figure 9. Formation of lymphoepithelium in the excretory ducts of the oesophageal glands in the oesophageal tonsil of ducks
***Note**: A: 1 – lumen of the excretory duct of an oesophageal gland; 2 – lymphoepithelium; 3 – lymphoid tissue, ×100. B – epithelium of an oesophageal gland infiltrated by lymphoid cells, ×400. Mallory staining*
***Source**: authors' own data*

The altered glands were found at the base of the folds of the mucous membrane and on the lateral surfaces. From 60 days of age, oesophageal glands in the region of the oesophageal tonsil were noted only beneath the epithelium, and from 180 days of age also at the

tips of some folds. In day-old ducks, oesophageal glands occupied 19.1 ± 0.67% of the area of the mucous membrane. With increasing age, up to 120 days, the area of the secretory portions of the oesophageal glands and the excretory ducts steadily decreased. The most intensive decrease occurred at 15, 60 and 90 days of age, by 31.6% ($P < 0.001$), 34.2% ($P < 0.01$) and 52.7% ($P < 0.001$) respectively. This process was due to an increase in the area of lymphoid tissue of the oesophageal tonsil. In ducks aged 120 and 150 days, the oesophageal glands occupied the smallest area, only 3.07 ± 0.06% of the mucous membrane (Fig. 10).

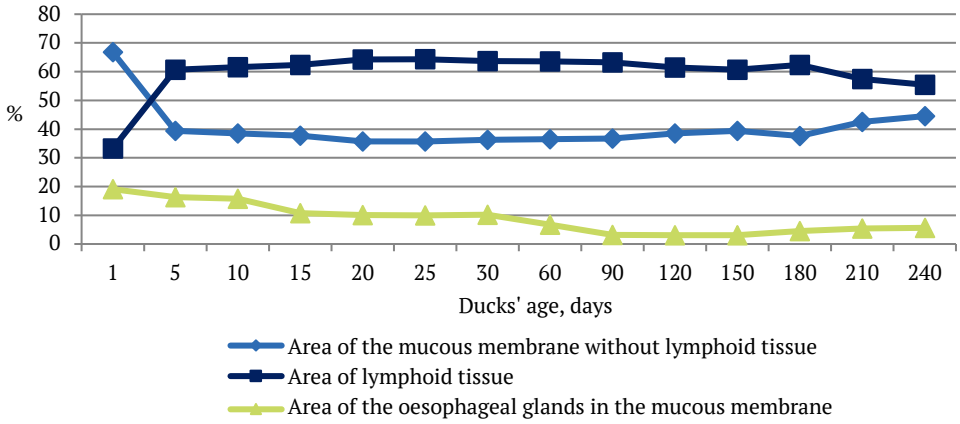


Figure 10. Area of lymphoid tissue and oesophageal glands of the mucous membrane of the oesophagus in ducks aged from 1 to 240 days
***Note**: different colours indicate the areas of different tissues*
***Source**: developed by the authors*

From 150 days of age, an increase in the area of the oesophageal glands was recorded. In ducks aged 180, 210 and 240 days, this index increased by 46.3% ($P < 0.001$), 20.9% ($P < 0.001$) and 4.4% ($P < 0.001$) respectively. In 240-day-old ducks, the area of the oesophageal glands accounted for 5.67 ± 0.07% of the mucous membrane. Conversely, in ducks of this age, the area of lymphoid tissue of the oesophageal tonsil decreased.

The results obtained in this work are consistent with the findings of other authors. The anatomically defined location of the oesophageal tonsil is the mucous membrane of the caudal part of the oesophagus at the transition to the glandular part of the stomach, as noted by V.R. Indu & K.M. Lucy (2021) and N.V. Dyshliuk (2009). The position of the lymphoid tissue of the oesophageal tonsil is closely related to the longitudinal folds of the oesophagus, as also reported by N. Nagy *et al.* (2005) and L.O. Buhai (2008). Thickening of the oesophageal wall in the region of the oesophageal tonsil was described by C. Casteleyn *et al.* (2010). In the present study, it was established that this occurs owing to an increase in the width and height of the mucosal folds during the development of lymphoid tissue. Increases in the linear parameters of the tonsil occur with increasing age of the ducks and are characterised by periodicity and asynchronicity. L.O. Buhai (2008) observed the same phenomenon in Muscovy ducks.

Functional maturity of the lymphoid tissue of the oesophageal tonsil (the presence of lymphoid nodules with germinal centres) was

recorded at 15 days of age. This fully corresponds to the data of A. Friedman *et al*. (2012), who reported that, in hens after hatching, protection against foreign antigens is provided by maternal IgA, and that a specific adaptive immune response matures during the first two weeks after hatching. T.A. Mazurkevych & V. Khomych (2017) established that in Blagovarsky cross broiler ducks full morphofunctional maturity of Peyer's patches of the duodenum, jejunum and caecum occurs at 15 days of age. With increasing age of the ducks, an increase in the content of lymphoid tissue of the oesophageal tonsil and its components was recorded. The formation of lymphoepithelial structures in the mucous membrane of the oesophagus in the region of the oesophageal tonsil is associated with the development of its lymphoid tissue. Colonisation of the oesophageal glands and the ducts by lymphoid cells led to the appearance of crypt-like structures. The presence of crypts in the oesophageal tonsil of hens was observed by V.R. Indu *et al*. (2020).

Thus, the results of the study confirm that in ducks the oesophageal tonsil is located in the mucous membrane of the caudal part of the oesophagus, where it is associated with longitudinal folds that contribute to widening of the oesophageal lumen during the passage of the bolus of feed. With increasing age, asynchronous and periodic changes are observed in the linear parameters of the tonsil, in particular its length, width, and the area of its lymphoid tissue, indicating a complex developmental process in this organ. In addition, changes in the morphology of the oesophageal tonsil, including the development of crypt-like structures, reflect the gradual maturation of this organ, which is important for effective immune protection in the early postnatal period in ducks.

Conclusions

The development and morphological changes of the oesophageal tonsil in ducks during the postnatal period of ontogenesis are important for understanding the functioning of the avian immune system. This organ in ducks aged 1 to 240 days in the postnatal period of ontogenesis is located in the caudal part of the oesophagus at the border with the glandular part of the stomach. Its site of localisation is the mucous membrane of the oesophagus, namely the lamina propria and the submucosa. Macroscopically, the tonsil was identified in 5-day-old ducks as a pale band. From 10 days of age, it took the form of a yellowish ring-shaped nodular band. The linear dimensions of the oesophageal tonsil increased with age, which is related not only to the physical growth of the ducks but also to the development of the lymphoid tissue of the oesophageal tonsil. In the region of the tonsil, thickening of the longitudinal folds of the oesophageal mucosa was observed in comparison with those in adjacent areas. Lymphoid cells were already found in the mucous membrane of the oesophagus in the region of the tonsil in day-old ducks, forming diffuse lymphoid tissue. Nodular levels of development of the lymphoid tissue appeared gradually. Lymphoid nodules without germinal centres were present in the lymphoid tissue of the tonsil in ducks at 10 days of age, and nodules with germinal centres appeared at 15 days of age, indicating maturity of the lymphoid tissue of the tonsil. The content of the components of lymphoid tissue changed with age. Diffuse lymphoid tissue reached its maximum in 30-day-old ducks and decreased by 240 days of age. The area of lymphoid nodules increased with age, especially that of nodules with germinal centres, but decreased after the onset of sexual maturity (180 days). This may indicate the beginning of involution of the oesophageal tonsil, which is typical of organs of haematopoiesis and lymphopoiesis. In the region of the oesophageal tonsil, structural changes occurred in the oesophageal glands and the excretory ducts. The epithelium was

infiltrated by lymphoid cells, transforming into lymphoepithelium. The secretory portions of the oesophageal glands and the ducts formed crypt-like structures opening onto the surface of the oesophagus. The patterns established – the development of the oesophageal tonsil and the components of its lymphoid tissue, the linear dimensions of the longitudinal folds of the tonsil and adjacent parts of the oesophagus, and the area of the oesophageal glands at different ages (1-240 days) in the early stages of the postnatal period of ontogenesis in Blagovarsky cross ducks – were characterised by asynchronicity and periodicity, which should be taken into account when planning vaccination strategies in poultry farming. To determine the age and characteristics of involution of the oesophageal tonsil, further studies in older ducks are needed. Such work is important for optimising preventive measures aimed at avoiding dangerous infectious diseases.

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Conflict of Interest

None.

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Закономірності формування лімфоїдної тканини стравохідного мигдалика в качок на ранніх етапах постнатального періоду онтогенезу

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Анотація. Актуальність дослідження стравохідного мигдалика качок на ранніх етапах постнатального періоду онтогенезу зумовлена необхідністю проведення аналізу становлення рівнів структурної організації його лімфоїдної тканини. У зв'язку з цим, метою дослідження було виявлення локалізації та форми лімфоїдної тканини стравохідного мигдалика бройлерних качок Благоварського кросу віком від 1 до 240 діб. Проводили морфометричні дослідження та визначали топографію стравохідного мигдалика у качок досліджуваних вікових груп. Фіксацію біологічного матеріалу проводили у 10 % розчині нейтрального формаліну. При проведені гістологічних досліджень виготовляли парафінові блоки на санному мікротомі та отримували гістозрізи, які зафарбовували для виявлення особливостей структури і площі тканинних компонентів стравохідного мигдалика. Встановлено, що стравохідний мигдалик у качок макроскопічно візуалізувався в ділянці переходу слизової оболонки каудальної частини стравоходу в залозисту частину шлунку. Зміни його довжини і ширини характеризувались асинхронністю. Лімфоїдна тканина стравохідного мигдалика до 5-добового віку качок представлена лише дифузною формою. У птахів віком 10 і більше діб з'являлися первинні і вторинні вузлики. На 15 добу стравохідний мигдалик набував морфофункціональної зрілості та характеризувався повністю сформованою лімфоїдною тканиною. У добових качок встановили інфільтрацію лімфоцитами залозистих відділів стравохідних залоз та вивідних проток. З віком

спостерігалося інтенсивне заселення лімфоїдними клітинами епітелію стравохідних залоз та вивідних проток. У результаті просвіти залоз звужувалися та перетворювалися на криптоподібні отвори, що відкриваються в просвіт стравоходу. Отримані дані щодо розвитку лімфоїдної тканини стравохідного мигдалика у бройлерних качок у початкові вікові фази можуть бути використані для оптимізації процесів вирощування, підвищення продуктивних показників і вдосконалення стратегій вакцинації з метою запобігання небезпечним інфекційним хворобам

Ключові слова: імунні утворення; лімфоїдні вузлики; морфометричні дослідження; свійська птиця; слизова оболонка стравоходу

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Echocardiographic characteristics of the morphofunctional parameters of the heart in clinically healthy rabbits

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Abstract. Rabbits are prone to developing cardiovascular system pathologies and require modern methods of diagnosis and treatment. One of the most informative and safe tools for assessing the condition of the heart is echocardiography. In this regard, the aim of the study was to determine the reference values for echocardiographic measurements in one-dimensional and two-dimensional modes in clinically healthy rabbits without the use of sedation. To diagnose the condition of the cardiovascular system in rabbits, echocardiography was used – a method that allowed for a comprehensive assessment of the morphology, size and contractile function of the heart structures using parasternal access. The study was focused on obtaining quantitative and qualitative parameters of the morphofunctional state of the main structures of the heart using echocardiography, in particular the left and right ventricles, interventricular septum, left atrium and valve apparatus. During the study, key parameters of left ventricular contractility in clinically healthy rabbits were determined. Age dynamics of the studied parameters were revealed: at 4.5 months of age, the end-diastolic size was up to 14.71 ± 1.51 mm, and the end-systolic size was up to 8.88 ± 1.64 mm. At the same time, the fractional shortening (39 ± 11%) and ejection fraction (79 ± 22%) remained relatively stable, indicating physiological adaptation of the heart to increasing body weight. In particular, a correlation was established between an increase in the total body weight of animals and an increase in the structural dimensions of the heart. Thus, it has been proven that echocardiography is a highly informative, safe and valuable tool for assessing the condition of the heart in rabbits. As a result, the scientific information presented in the article is of practical value for veterinary cardiologists and general practitioners, providing a basis for the timely diagnosis and effective treatment of heart disease in rabbits

Keywords: transthoracic echocardiography; ultrasound diagnostics; cardiology; laboratory animals; contractility indices; haemodynamics; reference values

Introduction

The development of cardiological science and clinical practice determines the need to develop and standardise the values of morphofunctional parameters of the heart for different animal species, in particular rabbits, which are widely used in experimental studies. Transthoracic echocardiography is one of the most common and accessible methods for non-invasive assessment of cardiac activity in both humans and animals. A number of researchers, including A.E.G.W. Marques *et al*. (2020), have reported that rabbits can be used as a model in cardiovascular research. The cardiac system of rabbits is similar in structure and electrophysiological characteristics to that of other mammals, making them a valuable model for studying the mechanisms of cardiac pathology and developing new therapeutic approaches. In addition, rabbits are widely used as model subjects in scientific research and experimental work, as well as being kept as pets. This necessitates the determination of the physiological parameters of their cardiac indicators for the timely diagnosis of pathologies.

As noted by V. Jekl *et al.* (2022), echocardiography is a non-invasive method for assessing cardiovascular function, which is used in both experimental research and clinical practice. With the increase in the lifespan of rabbits kept as companion animals, the incidence of heart disease is increasing, positioning echocardiography as an important diagnostic tool alongside clinical assessment, chest X-ray and electrocardiography. According to

N. Ngosurachet *et al.* (2022), various imaging modes are used during echocardiography to analyse the morphometric parameters of the myocardium and assess the contractile function of the left ventricle. Innovative technologies, in particular two-dimensional spectral tracking (2D speckle tracking echocardiography), according to A.Y. Kabakov *et al*. (2021), open up new opportunities for detailed analysis of myocardial characteristics.

C.J. Orcutt & R.L. Malakoff (2020) found that the incidence of cardiovascular disease in rabbits usually increases with age, especially in animals older than four years, but the pathology can also occur in younger individuals. M.G. Dilek & H. Erden (2019) showed that the age and body weight of rabbits significantly affect the morphology of the heart, in particular the size of the chambers and the thickness of the myocardial walls. They also found that young rabbits may have an increased ejection fraction with a stable fractional shortening, and that the use of sedation in accordance with protocols leads to a decrease in systolic parameters compared to the active state of the animal. As noted by E.V. Praag (2015), additional factors contributing to the development of cardiovascular disease include nutritional deficiencies, excess weight, and stress factors.

According to S. Ozawa *et al.* (2021), cardiac pathologies are common in rabbits, including degenerative valve changes, cardiomyopathy, and myocarditis. The incidence of cardiovascular disease in the population is estimated at approximately 2.6% with associated symptoms (murmurs, arrhythmias, shortness of breath). Diagnosis is based on a comprehensive examination, but chest X-rays do not allow for assessment of heart function, and their interpretation is complicated by the small size of the chest. Electrocardiography allows for the determination of electrical activity but does not reflect structural characteristics. Thus, echocardiography is considered the method of choice.

M. Kaya & M.A. Cetinkaya (2025) analysed the results of a comprehensive study of 58 healthy adult New Zealand white rabbits, which aimed to determine reference intervals for radiographic cardiac indices. The authors found that vertebral heart size (VHS) measurements were 7.94 ± 0.31 vertebrae (range 7.2-8.6 vertebrae) in the right lateral projection and 8.67 ± 0.33 vertebrae (7.8-9. 2 vertebrae). At the same time, the size of the left atrium and its relationship with other heart structures showed marked gender independence, which opens up opportunities for the development of universal reference values. A study by M.G. Sousa *et al*. (2020) confirmed that most echocardiographic parameters of rabbits are similar to those of other rodents, which facilitates their use as experimental models. Despite the available data, N. Ngosurachet *et al.* (2022) noted significant variability in the obtained indicators, which complicates their clinical use. At the systemic level, the British Society of Echocardiography proposed a comprehensive approach that emphasised the importance of clinical context and indexing to body size parameters (Harkness *et al.*, 2020).

An analysis of current research shows that there is a global lack of systematic and standardised reference values for morphological and functional parameters of the heart in healthy rabbits. This gap complicates the correct interpretation of results and the differentiation of physiological changes from pathological ones. Given the relevance of the topic, the aim of this study was to establish such parameters for B-mode (two-dimensional imaging of heart structures) and M-mode echocardiography (assessment of wall and valve motion over time) that can serve as a basis for improving the diagnosis, treatment, and prevention of cardiovascular pathologies.

Materials and Methods

The research was conducted in June 2025 at the Department of Veterinary Surgery named

after Academician I.O. Povazhenko of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine. The object of the study was 13 clinically healthy female rabbits of the Hiplus breed from a single line. The age of the animals at the time of measurement was between 3 and 4.5 months; only non-pregnant and non-lactating individuals were selected for the group. Given the specifics of establishing reference values, this study was pilot in nature and aimed to establish indicative normative indicators that require further verification on expanded population samples. To ensure the statistical power and scientific validity of the results obtained with a given number of animals, the experiment was designed as a longitudinal study (repeated measures design). This approach involved conducting a series of sequential echocardiographic examinations of each individual, which made it possible to obtain a representative data set for analysis. Particular attention was paid to the homogeneity of the sample: the animals were identical in breed, sex, and lineage and were kept in uniform conditions. This minimised interindividual variability caused by genetic or environmental factors and significantly increased the internal validity of the study.

All rabbits were kept at the Department of Veterinary Surgery named after Academician I.O. Povazhenko of the National University of Life and Environmental Sciences of Ukraine and were housed in individual mesh cages measuring 30x50x45 cm in a separate room with an area of 20 m². The cages were equipped with bunker feeders, a nipple watering system and plastic ramps (24x40 cm) to prevent limb injuries. The space ratio per animal, temperature and ventilation parameters of the holding room complied with the SOU 85.2-37-736:2011 (2011) standard. The experimental study was conducted in accordance with the ARRIVE (n.d.) recommendations, Law of Ukraine No. 3447-IV (2006) and Directive 2010/63/EU (2010).

Echocardiographic examination was performed on the same individuals three times at different ages (3 months, 3.5 months and 4 months), which allowed to track the dynamics of the parameters under study. At the time of the echocardiographic examination, a veterinarian performed a clinical examination of the animals using physical examination methods, which included an assessment of the rabbits' physique, the condition of their coat and skin, and selective measurement of body temperature, pulse rate and respiratory movements. At the beginning of the study, the rabbits were weighed and transported in animal carriers to the ultrasound diagnostics room. Assistants secured the animals and shaved the fur on the chest wall (corresponding to the topographical boundaries of the heart) using an animal clipper, after which they applied ultrasound gel to improve contact with the skin.

Echocardiographic examination of rabbits was performed using a modern veterinary ultrasound scanner SIUI Apogee 2100V (China). To obtain high-quality images, a C6LN microconvex sensor with parameters R17.5, a frequency range of 4.5–7.5 MHz and a central frequency of 6 MHz was used, which allowed for a detailed assessment of cardiac structures in rabbits. During the examination, the rabbits were placed in a right lateral position, which provided the most optimal access to the chest and facilitated the high-quality acquisition of ultrasound data. The study itself was conducted in long and short right parasternal projections, which corresponded to the generally accepted standards of veterinary echocardiography for small animals. It is important to note that echocardiography in rabbits was performed without sedation, which minimised additional stress and the effect of drugs on cardiac function.

In two-dimensional echocardiography, the diameter of the left atrium (LA) and aorta (Ao) was measured at the level of the aorta and aortic valves in the right parasternal short-axis view. The ratio of the left atrium diameter to the aortic diameter (LA/Ao) was automatically calculated by the ultrasound system. M-mode echocardiography was performed to measure the size of the chamber and the thickness of its wall in the right parasternal four-chamber projection along the short axis. The thickness of the interventricular septum at end-diastole (IVSd) and end-systole (IVSs) was measured, the internal diameter of the left ventricle at the end of diastole (LVIDd) and systole (LVIDs), (LVIDs)), as well as the thickness of the posterior wall of the left ventricle at the end of diastole (LVPWd) and systole (LVPWs). Fractional shortening (FS) and ejection fraction (EF) were determined and calculated using ultrasound system software according to the Teichholz method.

Statistical data processing was performed using descriptive statistics to determine the mean values and standard deviation (M ± SD) in different age groups. Additionally, Pearson's correlation analysis was performed to assess the relationship between echocardiographic parameters and rabbit body weight. This analysis was aimed at identifying the relationship between the structural and functional parameters of the heart (measured in B and M modes) and the individual body weight of each animal within one age point (i.e., separately for 3 months, 3.5 months, and 4.5 months). No comparison of correlation relationships between different age groups was performed, nor was it the purpose of this study. The analysis allowed to assess the strength of the linear relationship between the parameters and body weight at a specific moment of ontogenesis. The significance of the correlation coefficient was assessed using the t-test with df = n – 2. A two-tailed level of statistical significance was accepted at $P < 0.05$. To compare parameters between age points, Student's paired t-test was used.

Results and Discussion

In order to assess the age-related dynamics of the morphofunctional parameters of the heart in clinically healthy rabbits, an analysis of echocardiographic parameters was performed in three consecutive age periods. Given the longitudinal nature of the study design (repeated measures design), this allowed to track individual and group variability in cardiac structures as the animals grew. Descriptive statistical characteristics of the studied parameters (M ± SD) are presented in Table 1.

Table 1. Echocardiographic parameters in rabbits of different ages, n = 13, M ± SD

Parameter	Age of animals		
	3 months	3.5 months	4.5 months
Body weight (BW), g	3,400.00 ± 1,366.35	4,295.38 ± 422.02	4,438.46 ± 529.93
Heart rate (HR), bpm	254.38 ± 29.26	229.85 ± 27.09	248.77 ± 49.46
B-mode			
Aortic diameter (Ao), mm	8.12 ± 0.75	8.24 ± 0.53	8.57 ± 0.96
Left atrium (LA), mm	9.85 ± 0.73	10.15 ± 0.88	11.02 ± 1.27
Left atrium to aorta ratio (LA/Ao)	1.22 ± 0.12	1.24 ± 0.14	1.27 ± 0.24
Pulmonary artery (PA), mm	7.38 ± 0.62	7.45 ± 0.43	7.62 ± 0.92
Pulmonary vein (PV), mm	2.45 ± 0.50	2.80 ± 0.56	2.69 ± 0.72
Right pulmonary artery (RPA), mm	2.88 ± 0.58	3.12 ± 0.67	3.19 ± 0.89
Pulmonary vein to right pulmonary artery ratio (PV/RPA)	0.86 ± 0.10	0.91 ± 0.11	0.86 ± 0.12

Table 1. Continued

Parameter	Age of animals 3 months	3.5 months	4.5 months
B-mode			
Mitral valve E-point to septal separation (EPSS), mm	2.48 ± 0.52	2.75 ± 0.79	3.24 ± 1.66
M-mode			
Left ventricular posterior wall thickness at end-diastole (LVPWd), mm	4.10 ± 0.64	4.13 ± 0.71	4.25 ± 0.99
Interventricular septum at end-diastole (IVSd), mm	3.86 ± 0.62	3.92 ± 0.29	4.05 ± 0.66
Left ventricular internal diameter at end-diastole (LVIDd), mm	14.14 ± 1.31	14.21 ± 1.46	14.71 ± 1.51
Left ventricular posterior wall thickness at end-systole (LVPWs), mm	5.73 ± 0.94	5.42 ± 0.95	5.85 ± 1.63
Interventricular septum at end-systole (IVSs), mm	5.15 ± 0.73	5.65 ± 0.61	6.14 ± 1.52
Left ventricular internal diameter at end-systole (LVIDs), mm	9.06 ± 0.54	8.97 ± 1.47	8.88 ± 1.64
Fractional shortening (FS), %	35.0 ± 8.0	37.0 ± 7.0	39.0 ± 11.0
Ejection fraction (EF), %	71.0 ± 16.0	74.0 ± 14.0	79.0± 22.0

***Note**: there were no statistically significant results for the studied parameters compared to their values in 3-month-old rabbits*
***Source**: authors' own work*

The results show that there was only a tendency for the body weight of rabbits to increase with age. Thus, the body weight of 3.5-month-old rabbits differed by 26.33% ($P < 0.05$) compared to 3-month-old rabbits. The observed tendency towards an increase in body weight in rabbits from 3 to 4.5 months of age indicated a regular gain in body mass during growth. Age-related changes in cardiac dimensions in rabbits are consistent with the findings of M.G. Dilek & H. Erden (2019) who reported a gradual increase in cardiac chamber dimensions during rabbit growth. The results confirm that the most intensive growth of the studied parameters was observed during puberty, which correlated with an overall increase in the body weight of the animals. In addition, the thickening of the left ventricular myocardium indicates the adaptation of the cardiovascular system to the growing haemodynamic needs of the body.

The diameter of the aorta tended to increase with the age of the rabbits, reflecting natural morphological changes in cardiovascular structures during postnatal development. From 3 to 4.5 months of age, the size of the left atrium increased by 11.88% ($P < 0.01$), which may indicate adaptive changes in atrial volume to ensure cardiac output. The ratio of the left atrium to the aorta remained relatively stable, indicating that the proportions between the atrium and the aorta were maintained as the rabbits grew. The results obtained are consistent with similar studies by M.G. Dilek & H. Erden (2019) on New Zealand white rabbits, where the authors observed an increase in the systolic and diastolic diameters of the left ventricle, as well as the size of the aortic root, which correlated with an increase in body weight.

The diameter of the pulmonary artery tended to increase over the entire period, while

the sizes of the PV and RPA increased unevenly, reflecting individual growth rates and adaptive changes in the heart muscle. The PV/RPA ratio remained stable (0.86-0.91), indicating that optimal heart chamber proportions were maintained. The EPSS distance with the age of rabbits showed a tendency to increase by 30.65% compared to that in 3-month-old animals, which may indicate an adaptive change in left ventricular systolic function.

LVPWd and IVSd values tended to increase, reflecting morphological maturation of the heart muscle. The left ventricular diameter in diastole gradually increased throughout the study period, while the left ventricular diameter in systole remained relatively stable, indicating preserved normal contractility. The thickness of LVPWs and IVSs increased moderately without reaching statistical significance, reflecting the adaptation of the heart muscle to increased load against the background of a tendency to change body weight. The tendency to increase these parameters may reflect the optimisation of the pumping efficiency of the heart, which is important for differentiating physiological growth from pathological hypertrophy.

To assess the effect of body weight on cardiovascular parameters in 3-month-old rabbits, a Pearson correlation analysis was performed. The correlation coefficient (r) between the animal's body weight and the main echocardiographic parameters in B- and M-modes was determined. The use of this statistical approach made it possible to establish the degree of dependence between the overall somatic growth of the organism and the proportional increase in the linear dimensions of the chambers and the thickness of the heart walls (Table 2).

Table 2. Correlation analysis between body weight and echocardiographic parameters in 3-month-old rabbits, n = 13

Parameter	M ± SD	r
Body weight, g	3,400.00 ± 1,366.35	
B-mode		
Aortic diameter (Ao), mm	8.12 ± 0.75	0.50***
Left atrium (LA), mm	9.85 ± 0.73	0.21*
Left atrium to aorta ratio (LA/Ao)	1.22 ± 0.12	-0.30**
Pulmonary vein (PV), mm	7.38 ± 0.62	0.17
Pulmonary vein (PV), mm	2.45 ± 0.50	0.27
Right pulmonary artery (RPA), mm	2.88 ± 0.58	0.33**
Pulmonary vein to right pulmonary artery ratio (PV/RPA)	0.86 ± 0.10	-0.10
Mitral valve E-point to septal separation (EPSS), mm	2.48 ± 0.52	0.02
M-mode		
Left ventricular posterior wall thickness at end-diastole (LVPWd), mm	4.10 ± 0.64	-0.31**
Interventricular septum at end-diastole (IVSd), mm	3.86 ± 0.62	-0.45***
Left ventricular internal diameter at end-diastole (LVIDd), mm	14.14 ± 1.31	0.14
Left ventricular posterior wall thickness at end-systole (LVPWs), mm	5.73 ± 0.94	-0.20*
Interventricular septum at end-systole (IVSs), mm	5.15 ± 0.73	0.02
Left ventricular internal diameter at end-systole (LVIDs), mm	9.06 ± 0.54	-0.13
Fractional shortening (FS), %	35 ± 8	0.15
Ejection fraction (EF), %	71 ± 16	0.15

***Note**: * – P < 0.05; ** – P < 0.01; *** – P < 0.001, statistically significant indicators, correlated with body weight in 3-month-old rabbits*
***Source**: authors' own work*

In B-mode, there was a moderate positive correlation between rabbit body weight and aortic diameter ($P < 0.001$), indicating an increase in Ao in rabbits with greater body weight. A weak positive correlation was found between the left atrial index and the body weight of rabbits ($P < 0.05$), while the LA/Ao ratio was moderately negatively correlated ($P < 0.01$), which may reflect proportional changes in the structure of the atrium relative to the aorta. A weak positive correlation was found between the diameter of the PA and PV indices of rabbits and body weight, which was not statistically significant. The right pulmonary artery index in rabbits had a weak but significant positive correlation ($P < 0.01$), indicating an increase in this parameter in rabbits with higher body weight. The PV/RPA and EPSS ratios did not show a significant relationship with the body weight of rabbits. In M-mode, a moderate negative correlation with body weight ($P < 0.01$) was determined for IVSd and LVPWd thickness, indicating adaptive morphological changes in the heart in rabbits of different weights. Weak or insignificant correlations were observed for other M-mode parameters.

Correlation analysis of rabbit body weight with echocardiographic parameters at 3.5 months of age is presented in Table 3. Thus, in rabbits aged 3.5 months, during echocardiographic examination in B-mode, a moderate positive correlation was observed between body weight and aortic diameter ($P < 0.001$), indicating an increase in Ao in rabbits with greater body weight. A positive correlation was found between left atrial indices and body weight in rabbits ($P < 0.05$). No significant relationship was found between LA/Ao indices and pulmonary artery diameter and body weight in rabbits. A weak positive correlation was found between the pulmonary vein index and the body weight of rabbits ($P < 0.05$), while the RPA and EPSS indices did not show a statistically significant dependence. A moderate positive correlation was found between the PV/RPA ratio and the body weight of rabbits ($P < 0.01$).

Table 3. Correlation analysis between body weight and echocardiographic parameters in 3.5-month-old rabbits, n = 13

Parameter	Mean ± SD	r
Body weight, g	4,295.38 ± 422.02	
B-mode		
Aortic diameter (Ao), mm	8.24 ± 0.53	0.42***
Left atrium (LA), mm	10.15 ± 0.88	0.23*
Left atrium to aorta ratio (LA/Ao)	1.24 ± 0.14	-0.08
Pulmonary vein (PV), mm	7.45 ± 0.43	0.01
Pulmonary vein (PV), mm	2.80 ± 0.56	0.19*
Right pulmonary artery (RPA), mm	3.12 ± 0.67	0.05
Pulmonary vein to right pulmonary artery ratio (PV/RPA)	0.91 ± 0.11	0.25**
Mitral valve E-point to septal separation (EPSS), mm	2.75 ± 0.79	0.05
M-mode		
Left ventricular posterior wall thickness at end-diastole (LVPWd), mm	4.13 ± 0.71	0.05
Interventricular septum at end-diastole (IVSd), mm	3.92 ± 0.29	0.11
Left ventricular internal diameter at end-diastole (LVIDd), mm	14.21 ± 1.46	0.02
Left ventricular posterior wall thickness at end-systole (LVPWs), mm	5.42 ± 0.95	-0.02
Interventricular septum at end-systole (IVSs), mm	5.65 ± 0.61	-0.27**

Table 3. Continued

Parameter	Mean ± SD	r
M-mode		
Left ventricular internal diameter at end-systole (LVIDs), mm	8.97 ± 1.47	0.08
Fractional shortening (FS), %	37 ± 7	-0.10
Ejection fraction (EF), %	74 ± 14	-0.10

***Note**: * – P < 0.05; ** – P < 0.01; *** – P < 0.001, statistically significant indicators, correlated with body weight in rabbits at 3.5 months of age*
***Source**: authors' own work*

In M-mode, the most pronounced negative correlation was between the left ventricular interventricular septal thickness in systole and the body weight of rabbits (P < 0.01). No statistically significant correlations were found for other M-mode parameters, such as LVPWd, IVSd, LVIDd, LVPWs, and LVIDs. The shortening and ejection fractions remained independent of the body weight of rabbits. The results obtained are consistent with the data of studies by M.S. Young *et al*. (1990) and M. Kaya & M.A. Cetinkaya (2025), who noted the stability of functional parameters in healthy rabbits, which, as a rule, did not depend on body weight.

The correlation analysis of rabbit body weight with echocardiographic parameters at 4.5 months of age is presented in Table 4. As a result of assessing the effect of body weight on the cardiovascular parameters of rabbits and body weight in B-mode, it was found that the LA/Ao ratio was moderately negatively correlated with the body weight of rabbits (P < 0.001). The results obtained indicate a change in the proportions of the atrium relative to the aorta in rabbits with a higher body weight. A weak negative correlation was found between PV parameters and the PV/RPA ratio with the body weight of rabbits (P < 0.01), while no statistically significant relationship was found for Ao, LA, PA and RPA indicators. EPSS tended to have a negative relationship, but did not reach the level of significance.

Table 4. Correlation analysis between body weight and echocardiographic parameters in 4.5-month-old rabbits, n = 13

Parameter	Mean ± SD	r
Body weight, g	4,438.46 ± 529.93	
B-mode		
Aortic diameter (Ao), mm	8.57 ± 0.96	-0.08
Left atrium (LA), mm	11.02 ± 1.27	-0.11
Left atrium to aorta ratio (LA/Ao)	1.27 ± 0.24	-0.51***
Pulmonary vein (PV), mm	7.62 ± 0.92	-0.18
Pulmonary vein (PV), mm	2.69 ± 0.72	-0.27**
Right pulmonary artery (RPA), mm	3.19 ± 0.89	-0.11
Pulmonary vein to right pulmonary artery ratio (PV/RPA)	0.86 ± 0.12	-0.27**
Mitral valve E-point to septal separation (EPSS), mm	3.24 ± 1.66	-0.29
M-mode		
Left ventricular posterior wall thickness at end-diastole (LVPWd), mm	4.25 ± 0.99	0.02
Interventricular septum at end-diastole (IVSd), mm	4.05 ± 0.66	-0.18
Left ventricular internal diameter at end-diastole (LVIDd), mm	14.71 ± 1.51	-0.45***
Left ventricular posterior wall thickness at end-systole (LVPWs), mm	5.85 ± 1.63	-0.48***
Interventricular septum at end-systole (IVSs), mm	6.14 ± 1.52	-0.36***

Table 4. Continued

Parameter	Mean ± SD	r
M-mode		
Left ventricular internal diameter at end-systole (LVIDs), mm	8.88 ± 1.64	0.14
Fractional shortening (FS), %	39 ± 11	-0.42***
Ejection fraction (EF), %	79 ± 22	-0.42***

***Note**: * – P < 0.05; ** – P < 0.01; *** – P < 0.001, statistically significant indicators, correlated with body weight in rabbits at 4.5 months of age*
***Source**: authors' own work*

In M-mode, moderate and pronounced negative correlations were observed for most structural and functional parameters: LVIDd (P < 0.001), LVPWs (P < 0.001), IVSs (P < 0.001), FS (P < 0.001) and EF (P < 0.001). No statistically significant correlations with rabbit body weight were found for other M-mode parameters, such as LVPWd, IVSd and LVPWs. The data obtained may indicate that myocardial wall thickness in diastole and end-systolic ventricular diameter are more stable parameters that are less dependent on body weight fluctuations during this age period compared to active contractility indicators. To assess the effect of body weight on cardiovascular parameters in 4.5-month-old rabbits in B-mode, it was found that the LA/Ao ratio was moderately negatively correlated with body weight (P < 0.001), indicating a change in the proportions of the atrium relative to the aorta in rabbits with greater body weight. A weak negative correlation was found between pulmonary vein indices and the PV/RPA ratio with the body weight of rabbits (P < 0.01), while no statistically significant relationship was found for Ao, LA, PA, and RPA. The EPSS indicator showed a tendency towards a negative correlation with the body weight of rabbits.

Comparing the results obtained at different ages of rabbits, it can be noted that in the early stage of the postnatal period of ontogenesis, an increase in the body weight of rabbits was associated with a proportional increase in Ao, which corresponded to the normal development of the cardiovascular system. By 4.5 months of age, the proportional relationship weakened, probably due to individual growth rates or adaptive changes in cardiac output. Based on the LA to Ao ratio, a change in the proportions of the atrium relative to the aorta can be observed. At the beginning of the study, the growth of the atrium in rabbits was proportional to the aorta. However, by 4.5 months of age, rabbits with a higher body weight had a relatively lower intensity of growth of LA parameters compared to Ao, which explains the strong negative correlation. This may be an adaptive mechanism to maintain optimal cardiac output. In 3-month-old rabbits, changes in body weight stimulated the growth of the heart ventricles. However, in older animals, there was a regulatory decrease in the thickness and volume of the heart chambers in relative proportions, possibly due to the adaptation of the heart to physiological load. The adaptive thickening of the interventricular septum of the heart, which stabilised over time, reflected the physiological maturation of the heart.

The publication by A.P. Fontes-Sousa *et al*. (2006) describes M-modal and Doppler reference values for healthy male New Zealand white rabbits. In this study, the average heart rate during echocardiography was 155.0 ± 29 beats per minute. At the same time, the authors also studied morphometric indicators: the thickness of the interventricular septum, which was 2.03 ± 0.37 mm in diastole and 3.05 ± 0.45 mm in systole; the internal dimension of the left ventricle – 14.37 ± 1.49 mm in diastole and

10.25 ± 1.22 mm in systole, and the thickness of the free wall – 2.16 ± 0.25 mm in diastole and 3.48 ± 0.55 mm in systole. The results of the functional parameters studied showed a fractional shortening of 30.13 ± 2.98% and an ejection fraction of 61.29 ± 4.66%, indicating good systolic heart function in these animals. Changes in functional parameters (FS, EF) indicate adaptive regulation of systolic function as a result of an increase in the body weight of rabbits and a decrease in the correlation between heart function and body weight in the early stages of development of these animals. The results obtained coincide with the results of studies conducted by Z. Popovi *et al*. (2005), D. Casamian-Sorrosal *et al.* (2014b) and N. Ngo-surachet *et al*. (2022), who proved that functional parameters are scaled allometrically according to the size of the heart ventricles and the duration of their filling, which leads to their relative stability during the growth and development of the organism. As noted by M.G. Dilek & H. Erden (2019), establishing reference values for echocardiographic parameters for healthy rabbits, taking into account their age and body weight, is extremely important for the correct interpretation of data. The results obtained in the current study can serve as a basis for comparing similar parameters in experimental models of certain pathological conditions. For example, understanding the physiological development of the heart in rabbits is the basis for studying various pathologies, since rabbits are used to model conditions that lead to cardiac remodelling, such as hypertrophy. In particular, in rabbits with obesity and hypertension, echocardiography was used to diagnose the development of concentric and eccentric hypertrophy, as well as diastolic filling disorders, which, according to S. Minegishi *et al*. (2011), must be differentiated from physiological growth.

The shortening fraction and ejection fraction are key indicators for assessing left ventricular systolic function. FS is a one-dimensional parameter, while EF is a volumetric parameter (Levy & Deutschman, 2004). The sensitivity of FS to symmetrical LV contraction makes EF a more accurate parameter in cases of asymmetrical remodelling, but both indicators correlate closely in animals with normal LV geometry (Szmitko *et al.*, 2003). Since the present study demonstrated preservation of normal geometry and contractility of the left ventricle (LVIDs remained stable), both FS and EF are informative. As reported by X. Chen *et al.* (2022), echocardiographic measurements in rabbits also demonstrated high reproducibility, making them a reliable tool for longitudinal studies. Understanding the development of the cardiovascular system in the postnatal period of ontogenesis is critical for assessing normal heart morphology and function, as well as for establishing baseline indicators during experimental studies. Rabbits are an important model for cardiovascular research because they are relatively accessible, yet, as noted by D. Casamian-Sorrosal *et al*. (2014a) and M.G. Dilek & H. Erden (2019), large enough to perform detailed anatomical and physiological experiments. Echocardiography allows for non-invasive assessment of the structural and functional parameters of the heart, and their correlation with body weight, according to T. Hornyik *et al.* (2021), can serve as a marker of physiological growth and adaptation of the cardiovascular system.

The data obtained in the current study emphasised the importance of understanding the dynamics of changes in cardiac structures in response to the growth and development of the organism under controlled conditions. The study revealed regular age-related changes in the structure and function of the heart in rabbits, which closely correlated with body weight gain. The structural parameters of the heart predominantly correlated positively

with body weight in the early postnatal period of ontogenesis, while functional indicators (shortening and ejection fractions) remained stable or changed less significantly, indicating physiological maturation of the heart and its adaptation to increased load during the period of active growth of the animal.

Conclusions

As a result of the conducted study, reference morphofunctional cardiac parameters of clinically healthy rabbits were established and systematised across three consecutive age periods using B-mode and M-mode echocardiography. Age-related changes in the structure and function of the rabbit heart were identified, which correlated with the increase in body weight. In particular, the growth dynamics of the animals were accompanied by a regular increase in Left ventricular internal diameter at end-diastole (LVIDd), mm and Left ventricular internal diameter at end-systole (LVIDs), mm, as well as the thickness of the ventricular walls (Interventricular septum (IVS) and Left ventricular posterior wall (LVPW)) during different phases of the cardiac cycle. Structural cardiac parameters increased proportionally with the growth of the animals, whereas functional cardiac indices indicated maintenance or improvement of contractile efficiency. These results provide a basis for further comparison of normal and pathological cardiac morphology during the postnatal period of rabbit ontogenesis.

Analysis of the obtained results demonstrated that in 3-month-old rabbits the most pronounced changes in the heart depending on body weight dynamics were observed for Aortic diameter, Pulmonary vein, Interventricular septum at end-diastole and Left ventricular posterior wall thickness at end-diastole, which emphasised the influence of body weight on structural cardiac parameters, whereas functional parameters (Fractional shortening, ejection fraction and mitral valve E-point to septal separation) remained practically unchanged. In rabbits aged 3.5 months the most significant dependence of cardiac parameters on body weight was observed for aortic diameter, left atrium, pulmonary vein, pulmonary vein to right pulmonary artery ratio and interventricular septum at end-systole, whereas most other structural and functional cardiac parameters remained practically independent of body weight. This indicates that structural cardiac parameters continued to correlate with body weight, while functional cardiac indices remained stable and were not influenced by minor changes in body weight at this age period. In rabbits aged 4.5 months a clear tendency towards negative correlation between body weight and structural as well as functional cardiac parameters was observed, particularly with regard to ventricular wall thickness, ventricular dimensions and fractional shortening and ejection fraction. This indicates that during this age period body weight became an important factor influencing both cardiac morphology and cardiac function in rabbits. Changes in correlation parameters with age may be explained by physiological maturation of the heart, body growth of rabbits and adaptation of the myocardium to increasing load, which is typical for growth and development during the postnatal period of ontogenesis. Prospects for further research include the study of echocardiographic parameters in rabbits and comparative analysis of reference values in other breeds and age groups. This will provide a basis for the development of unified cardiological standards necessary for timely diagnosis and monitoring of treatment effectiveness of cardiovascular diseases in veterinary practice.

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Conflict of Interest

None.

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Ехокардіографічна характеристика морфофункціональних параметрів серця у клінічно здорових кролів

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Анотація. Кролі схильні до розвитку патологій серцево-судинної системи та потребують сучасних методів діагностики й лікування. Одним із найбільш інформативних і водночас безпечних інструментів оцінки стану серця є ехокардіографія. У зв'язку з цим, мета дослідження полягала у визначенні референтних значень для ехокардіографічних вимірювань в одномірному та двомірному режимах у клінічно здорових кролів без застосування седації. Для діагностування стану серцево-судинної системи кролів використали ехокардіографію – метод, який дозволив комплексно оцінити морфологію, розміри та скорочувальну функціональність структур серця, використовуючи парастернальний доступ. Дослідження було спрямоване на отримання кількісних та якісних показників морфофункціонального стану основних структур серця за допомогою ехокардіографії, зокрема лівого і правого шлуночків, міжшлуночкової перегородки, лівого передсердя та клапанного апарату. У ході дослідження визначено ключові параметри скоротливості лівого шлуночка у клінічно здорових кролів. Виявлено вікову динаміку досліджуваних показників: у 4,5-місячному віці кінцево-діастолічного розміру до 14,71 ± 1,51 мм, а кінцево-систолічного розміру до 8,88 ± 1,64 мм. При цьому фракція укорочення

(39 ± 11 %) та фракція викиду (79 ± 22 %) залишалися відносно стабільними, що свідчить про фізіологічну адаптацію серця до зростаючої маси тіла. Зокрема, встановлено кореляційний зв'язок між збільшенням загальної маси тіла тварин та зростанням структурних розмірів серця. Таким чином, доведено, що ехокардіографія є високоінформативним, безпечним та цінним інструментом для оцінки стану серця у кролів. В результаті, наукова інформація, представлена у статті, має практичну цінність для ветеринарних кардіологів та лікарів загальної практики, забезпечуючи основу для своєчасної діагностики та ефективного лікування серцевих захворювань у кролів

Ключові слова: трансторакальна ехокардіографія; ультразвукова діагностика; кардіологія; лабораторні тварини; показники скоротливості; гемодинаміка; референтні значення

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Amino acids of the biliary system in rats with tetracycline-induced hepatosis and the use of milk phospholipids

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Abstract. The relevance of the study is due to the high frequency of drug-induced liver injury in mammals and insufficient study of the issue of amino acid metabolism disorders in acute forms of hepatopathy of the corresponding genesis. In this regard, the study was aimed at identifying specific changes in the profile of free amino acids in the bile and liver of rats with tetracycline-induced fatty hepatosis, as well as determining the corrective effect of the phospholipid fraction of milk. The leading approach in studying this problem was an experiment on laboratory animals with modelling of fatty hepatosis due to the cytotoxic effect of tetracycline, followed by the selection of bile and liver samples, followed by studying the spectrum of free amino acids using thin-layer chromatography with ninhydrin staining. It was found that the general pattern of changes in the hepatobiliary system of diseased rats indicated a violation of bile acid conjugation processes and mitochondrial dysfunction, as well as a pronounced blockage of the use of free amino acids in metabolic processes. At the same time, the use of phospholipid-containing therapy in rats with tetracycline-induced fatty hepatosis activated the use of the intrahepatic reserve of amino acids involved in bile acid

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conjugation and antioxidant protection, in particular sulphur-containing representatives and the total fraction of glycine/serine/glutamine. It was determined that the bile of these rats also had a reduced content of glycine and taurine-containing amino acid fractions in the bile of these rats, with a simultaneous increase in the level of the total alanine/tyrosine/threonine and arginine/ornithine/lysine fractions, confirming the activation of detoxification processes in the liver involving free amino acids. It was found that the use of the phospholipid fraction of milk reduced the manifestations of disturbances in the intermediate metabolism of amino acids and contributed to the restoration of the content of leucine- and valine-containing fractions in the bile and liver of sick rats. The established patterns are of practical value for laboratory diagnosis and preclinical evaluation of hepatoprotective agents, since the amino acid profile of the hepatobiliary system can be used as a sensitive indicator of the severity of liver injury and as a criterion for determining the effectiveness of therapy

Keywords: chromatography; amino acid fractions; liver; bile; corrective therapy; tetracycline hydrochloride; laboratory diagnostics

Introduction

Drug-induced liver injury (DILI) is considered one of the most challenging problems in modern hepatology, as it is a common cause of acute liver failure, masquerades as other lesions, and requires clear diagnostic and monitoring algorithms. In practical guidelines, R.J. Fontana *et al*. (2023) noted that the management of patients with DILI should be based on a standardised approach that combines clinical assessment, biochemical markers, and analysis of potentially hepatotoxic drugs (including herbal and dietary supplements). Summarising current understanding of the pathogenesis of this hepatopathology, J. Skat-Rørdam *et al.* (2025) emphasised that the key mechanisms of DILI remain mitochondrial dysfunction, oxidative stress and immune-mediated injury to hepatocytes, and therefore risk assessment should be based on the evaluation of these links. At the same time, S. Thakur *et al.* (2024) emphasised that biomarker panels (rather than individual indicators) capable of reflecting various components of pathogenesis, from oxidative stress to cholestasis and inflammation, are becoming increasingly important for hepatotoxicity. Against this background, amino acid homeostasis is gaining increasing attention as the "central axis" of liver metabolism and its disorders.

In the context of metabolic hepatopathies, C. Fotakis *et al.* (2023) showed, based on metabolic profiling, that non-alcoholic fatty liver disease (NAFLD) is associated with reproducible metabolic patterns, including amino acid patterns, that reflect changes in energy and nitrogen metabolism. This is consistent with what M. Holeček (2024) described in detail, pointing to the role of alanine and glutamine as key carbon and nitrogen carriers for gluconeogenesis and the functional interaction of the liver with other organs. This finding makes amino acid shifts not only "markers" but also functionally significant components in pathology. Of particular interest are integral amino acid indices as tools for assessing metabolic imbalance and predicting the severity of liver injury. In particular, H. Enomoto *et al*. (2023) emphasised that the ratio of branched-chain amino acids to tyrosine is not only an indicator of amino acid imbalance but also a reflection of systemic metabolic shifts in chronic hepatopathies. A study

by J. Bugajska *et al*. (2023) demonstrated that the amino acid composition of bile can reflect the characteristics of pathological processes in the biliary tract. This reinforces the relevance of analysing the biliary spectrum of amino acids in elucidating metabolic changes in the hepatobiliary system. At the same time, A. Eguchi *et al*. (2025) showed that the profile of amino acid-conjugated bile acids in blood serum reflects the severity of chronic liver disease and has prognostic significance for the viability of the organism. In other words, changes in conjugation are not only a biochemical feature, but also a clinically and experimentally significant characteristic of the lesion.

The key link between amino acid metabolism and the detoxification function of the liver is the conjugation of bile acids with glycine and taurine, which is provided by the enzyme bile acid-CoA:amino acid N-acyltransferase (BAAT). Importantly, S.A.J. Trammell *et al*. (2023) identified BAAT not only as a bile acid conjugation enzyme, but also as a hepatic N-acyltaurine synthase for polyunsaturated fatty acids, i.e., BAAT forms a broader spectrum of bioactive lipid derivatives potentially relevant to models of drug-induced steatosis and cholestatic components of injury. The regulatory mechanisms of this axis complement the data of T. Miyazaki *et al*. (2023), who used an experimental model to demonstrate enhanced taurine biosynthesis and conjugation of bile acids with taurine through farnesoid X receptor (FXR)-dependent mechanisms, emphasising that the balance of taurine/glycine conjugates is a controllable and therefore potentially decisive link in the pathogenesis of hepatopathy. Against the backdrop of the search for non-fragmentary approaches to the correction of drug-induced hepatopathies, there is growing interest in nutritional strategies, primarily in milk phospholipids. Experimentally, Z. Wu *et al*. (2022) showed that the addition of milk fat globule membranes (MFGM) alleviates colitis and secondary liver injury by improving the mucosal barrier and modulating the microbiota, which is important for the gut-liver axis. Additionally, Q. Zhang *et al*. (2021) showed that adding MFGM to the diet can reduce the manifestations of hepatic steatosis under experimental conditions of metabolic stress, which forms a rational basis for testing the hepatoprotective potential of milk phospholipids and in a model of tetracycline-induced hepatosis. That is why a comprehensive study of amino acids in the biliary system of rats under conditions of tetracycline-induced liver injury and against the background of milk phospholipid administration represents a relevant research direction that combines mechanistic analysis (mitochondrial dysfunction/oxidative stress/bile acid conjugation) with potential nutritional correction.

Thus, current data highlight two key unresolved issues. First, simultaneous changes in the profile of free amino acids in the liver and bile in a model of acute drug-induced steatosis, in particular tetracycline-induced steatosis, have not yet been sufficiently characterised. Second, there is limited information on whether the phospholipid fraction of milk is capable of modifying these amino acid shifts through its effect on bile acid conjugation, antioxidant pathways, and complexes associated with glutathione metabolism.

The aim of the study was to reveal specific changes in the profile of free amino acids in the bile and liver of rats with tetracycline-induced fatty hepatosis and to determine the corrective effectiveness of the phospholipid fraction of milk in restoring the amino acid balance at the level of the hepatobiliary system. The objectives were, first, to quantitatively characterise changes in the profile of free amino acids in the bile and liver of rats relative to the control group; secondly, to determine whether the phospholipid fraction of milk modifies the deviations of

glycine-taurine and sulphur-containing amino acid groups associated with bile acid conjugation and antioxidant protection; and, thirdly, to evaluate the informativeness of the amino acid spectrum as a potential indicator of the effectiveness of correction, comparing the "Self-rehabilitation" groups without therapy and in the case of oral administration of milk phospholipids "Tetracycline + biologically active supplement FLP-MD" to laboratory rats.

Literature Review

In both veterinary and human medicine, the toxic effect of drugs on the liver is one of the leading problems in hepatopathology. Several interrelated pathogenetic mechanisms are distinguished in the development of DILI, including mitochondrial dysfunction, oxidative stress, and bile acid homeostasis imbalance, which significantly complicates the treatment of sick animals. J. Skat-Rørdam *et al*. (2025) added the activation of cell death signalling pathways to the list of drug-induced liver injury disorders. The experts emphasised the need to use experimental models in combination with comprehensive approaches and genetic tools for in-depth study of the pathogenesis of DILI and the search for reliable biomarkers. This pathogenesis of drug-induced liver injury is consistent with the phenotype of tetracycline-induced microvesicular steatosis, where the suppression of mitochondrial β -oxidation of fatty acids, which leads to the accumulation of small fat droplets in hepatocytes. The key links in this phenotype are dysregulation of lipid metabolism, membrane disorders and oxidative stress, so it seems reasonable to consider milk polar lipids as potential corrective factors.

Previous studies have noted the high efficacy of milk phospholipids (polar lipids) in models of liver injury. Thus, Z. Wu *et al*. (2022) in a model of acute colitis (induced by DSS) with secondary liver injury showed that inflammatory aggression was accompanied by an increase in the activity of aspartate aminotransferase and alanine aminotransferase in blood plasma and liver, while prophylactic administration of MFGM reduced these indicators and mitigated the manifestations of hepatic oxidative stress. This indicated the pronounced hepatoprotective potential of milk polar lipids. Further studies detailed the effect of milk polar lipids on fat metabolism in hepatic steatosis. In particular, Q. Zhang *et al*. (2021) demonstrated that the offspring of mice whose mothers received a high-fat diet developed pronounced hepatic steatosis: small fat vacuoles accumulated in hepatocytes, the content of triacylglycerols in blood serum and liver increased, and aspartate aminotransferase activity increased. In contrast, the addition of MFGM in the early postnatal period contributed to the restoration of triacylglycerol levels and aspartate aminotransferase activity and improved antioxidant defence parameters (decrease in malondialdehyde content, increase in superoxide dismutase activity). This was consistent with the suppression of *de novo* lipogenesis and restoration of mitochondrial function in the liver of these animals. A.L. Zhou & R.E. Ward (2024) also showed that a diet enriched with milk polar lipids caused a decrease in absolute and relative liver weight and a decrease in the expression of liver genes associated with lipid metabolism, including cholesterol. This confirms the beneficial effect of milk phospholipids on hepatic lipid metabolism under stress conditions. In a review of clinical studies, A.P. Kanon *et al*. (2024) showed that adding MFGM to the diet of adults was associated with a decrease in total cholesterol and low-density lipoproteins in the blood. This hypolipidemic effect was consistent with the hypothesis of the cardiometabolic benefits of milk phospholipids. Taken together, these results significantly complement experimental data on the effective correction of the lipid profile of blood plasma,

bile and liver in rats using the components of the dietary supplement "FLP-MD" in a model of tetracycline-induced hepatosis.

In modern research, increasing attention is being paid to the amino acid profile as a marker of liver injury. For example, J. Zhao *et al*. (2023) used Mendelian randomisation to prove a causal link between genetically determined levels of certain amino acids and the risk of metabolically associated fatty liver disease (MAFLD). In particular, it was shown that genetically determined increases in alanine levels and decreases in glutamine content cause a higher risk of developing MAFLD. These data emphasised that changes in amino acid composition are not only a consequence but also a possible trigger for the progression of steatosis. In turn, N.Y.T. Tan *et al*. (2024) proposed an integrated glutamate-serine-glycine index as a non-invasive indicator of MAFLD activity stage and response to therapy. Overall, the results obtained indicate that the amino acid profile is a promising platform for assessing the severity and prognosis of hepatopathy. At the same time, most studies focus on the analysis of blood plasma and practically do not consider synchronous changes in the amino acid spectrum in the liver and bile.

A separate area of research is the assessment of the phospholipid fraction of MFGM as a source of bioactive lipids. Literature data have shown that MFGM components such as phosphatidylcholine, sphingomyelin and phosphatidylethanolamine are capable of regulating lipid and cholesterol metabolism, improving the barrier function of the intestine and exhibiting anti-inflammatory properties. The reduction in cholesterol and low-density lipoprotein levels in adults when consuming MFGM, demonstrated in clinical studies, is consistent with the hypolipidemic and general health-promoting effects of milk polar lipids. These facts are comparable to the above experimental data on the correction of lipid metabolism in the liver under the influence of milk phospholipids. In addition, it is worth noting the role of individual amino acids in the course and treatment of animals with hepatopathology. A decrease in branched-chain amino acids (BCAA) during the progression of chronic liver disease is associated with a worsening prognosis, while their additional administration is recommended in patients with cirrhosis (especially in the presence of hepatic encephalopathy) to improve nutritional status and clinical outcomes. Moreover, M.C. Trillos-Almanza *et al*. (2024) showed that BCAAs and their metabolites contributed to a decrease in the intensity of activation of hepatic stellate cells (the main effectors of fibrogenesis) in *in vitro* experiments. Thus, the available literature data have shown that tetracycline-induced hepatosis is an experimentally validated model for studying DILI. Milk phospholipids and other components of milk fat globule membranes have a clinically proven ability to correct structural and metabolic disorders in the liver. At the same time, the amino acid profile is considered a sensitive marker of liver injury. However, simultaneous changes in the amino acid composition of the liver and bile in conditions of drug-induced hepatosis and its correction with milk phospholipids remain insufficiently characterised, which explains the need for this study.

Materials and Methods

An acute experiment on laboratory rats, followed by the collection of biological material samples and their preparation for amino acid composition analysis, was conducted in January 2024 at the vivarium of the Educational and Scientific Centre (ESC) "Institute of High Technologies" of the Taras Shevchenko National University (Kyiv), which is equipped with centralised water supply, sewage, ventilation and air conditioning. The research was conducted in

accordance with Directive 2010/63/EU (2010), Law of Ukraine No. 3447-IV (2006) and Order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 (2012). The amino acid composition of the prepared bile and liver samples was studied in the scientific laboratory of the same research centre during 2024. The research project was reviewed and approved by the Bioethics Committee of the National University of Life and Environmental Sciences of Ukraine, as confirmed by the decision of the Bioethics Committee of the National University of Life and Environmental Sciences (NULES) of Ukraine dated 10 October 2023, protocol No. 10.

Each group of animals was kept in separate cages on a standard vivarium diet. They had free access to food and drinking water. Before the start of the experiment, the rats were kept in quarantine with clinical examination for two weeks. Monitoring of changes in body weight and feed consumption by the test animals was carried out for 9 days.

Using the author's method by V. Gryshchenko *et al*. (2019), fatty hepatosis was modelled in laboratory rats. For this purpose, using a soft silicone probe, a 4% solution of tetracycline hydrochloride was administered intragastrically to the animals daily at a rate of 250 mg/kg of body weight for 7 days. As a result of intragastric administration of high doses of tetracycline hydrochloride, rats developed fatty hepatosis. Animals that received intragastric administration of tetracycline hydrochloride without further therapy were assigned to the "Self-rehabilitation" group (n = 6). Rats in the "Tetracycline + "FLP-MD" dietary supplement" group (n = 6) were administered an intragastric dose of a 1% solution of the FLP-MD dietary supplement (experimental series, NULES Ukraine), which contains milk phospholipids as the main active ingredient. The drug was administered intragastrically for 9 days – one hour before the administration of the antibiotic, as well as for two days after the completion of the course of antibiotics. The daily therapeutic dose of "FLP-MD" dietary supplement was 13.5 mg/kg of body weight (Patent of Ukraine No. 8651, 2009). Rats that were administered distilled water in a volume equivalent to the volume of the antibiotic and dietary supplement were included in the "Control" group (n = 6). The next group of animals, the "Preparation" group, consisted of clinically healthy animals that additionally received milk phospholipids as part of the dietary supplement "FLP-MD" (n = 6). During the experiment, the body weight of the rats was monitored daily using ORION OS-0K22 electronic scales (ORION ELECTRONICS LTD, Europe), adjusting the dosage of the drugs according to the established changes. On day 10, bile and liver samples were taken from rats in all groups for further analysis of their amino acid composition.

The spectrum of free amino acids was determined by the method of paper chromatography with ninhydrin staining, modified on the basis of the classical methods of R. Consden *et al.* (1944), S.M. Partridge (1948) and taking into account the approaches of S. Moore & W. H. Stein (1948) and R.J. Block *et al.* (1958), which allowed for a quantitative assessment of the amino acid composition of biological material. This method facilitated the separation of amino acids on chromatographic paper due to differences in the distribution coefficients between the mobile and stationary phases and is suitable for quantitative analysis in biomaterials, with the exception of proline and hydroxyproline, which give an atypical reaction with ninhydrin.

Bile sampling. Rats were fasted for 18 hours prior to surgery. Anaesthesia was achieved by intraperitoneal administration of thiopental sodium at a dose of 60 mg/kg. Approximately 5 minutes after administration of the anaesthetic, the animal was immobilised on its back. A median laparotomy was performed along the

midline of the abdomen, starting approximately 0.5 cm caudal to the xiphoid process; the length of the incision was 2.0-2.5 cm. The common bile duct and portal vein were identified at the hepatic hilum. At a distance of about 0.5 cm distal to the bifurcation of the duct, two ligatures were applied: one to hold the duct during manipulation, the other to fix the cannula. After delicate incision of the duct wall, a cannula was inserted and connected to a 200 µL micropipette. Bile collection began 30 minutes after cannulation and was performed every 30 minutes for 3 hours (a total of six samples: at 30, 60, 90, 120, 150, and 180 minutes), as described by D.O. Melnychuk *et al.* (2015).

Liver sampling. Biological material samples were collected under thiopental anaesthesia in rats. Liver samples were collected immediately after euthanasia of the rats. A portion of the organ weighing 300 mg was measured using torsion balances (model VT-500 torsion balance, Ukraine), after which 700 µL of physiological saline was added. The samples were homogenised using a (Lankai FSH-2A, China) over ice twice for 15-20 seconds at medium speeds, with a 30-second break between approaches. The homogenate was centrifuged in a high-speed microcentrifuge (Biosan Microspin 12, Latvia) after pre-cooling the rotor at 14.5 thousand rpm for 10-12 minutes.

Sample preparation. Since some amino acids have very similar *Rf* values and are not completely separated during one-dimensional chromatography, preliminary concentration and extraction of the sample was performed. Each sample (20 µL of liver sample homogenate) was applied to a small square of desulphurised filter paper (3.5×3.5 cm) – 20 µL three times per paper (each layer was dried before applying the next). The paper saturated with the sample was cut into small pieces and placed in a test tube with 2 mL of an extracting mixture of acetone:ethanol:water (7:2:1, by volume). This allowed the amino acids to be extracted (desorbed) from the paper into the solution and simultaneously separated from macromolecules and salts that could interfere with the analysis. After extraction, the solution was evaporated to dryness (in a stream of air at room temperature), and the residue was dissolved in 15 µL of distilled water. The resulting concentrate was applied to the starting line of the chromatographic paper in the form of three consecutive drops of 5 µL each (with a 15-minute interval between applications for drying). In this way, a compact concentrated spot was formed from each sample. The "cut paper elution" approach was adapted for amino acids as an author's modification (Hackman & Lazarus, 1956).

Chromatographic separation. Filtrak FN-1 chromatography paper (Germany) was used as the stationary phase. The mobile phase was a six-component mixture of solvents: isoamyl alcohol (Carlo Erba, France): n-butanol (Carlo Erba, France): glacial acetic acid (Merk, Germany): formic acid (BASF, Germany): distilled water: butylbenzyl ether (Thermo Fisher Scientific, USA) in a ratio of 21:8:10:1:10:1 (by volume) with the addition of 0.2% ninhydrin (Thermo Fisher Scientific, USA). Chromatography was performed in a sealed chamber using the ascending paper chromatography method, pre-saturated with mobile phase vapours (Consden *et al*., 1944; Williams & Kirby, 1948). The starting zones for sample application were located on a line 2-3 cm from the bottom edge of the paper. The development of the chromatogram lasted approximately 7 hours (the solvent passed through almost the entire length of the paper strip). If necessary, multiple solvent passes or extended development time (up to 12-20 hours) can be used to improve separation. However, in this case, a single pass over 7 hours was sufficient to separate most fractions.

Chromatogram development. After completion of chromatographic separation, the

paper strip was removed from the chamber and dried in a fume hood at room temperature (1 hour, until complete removal of solvent vapours). To visualise the amino acids, the chromatogram was placed in a drying oven (Labexpert 3015, Ukraine) at a temperature of 45°C for 120 minutes. During this time, ninhydrin (Thermo Fisher Scientific, USA), added to the mobile solvent, reacted with amino acids, forming coloured products directly on the paper. Thus, additional spraying with the reagent was not required. This gentle heating (temperature 45°C, 2 hours) ensured the gradual development of amino acid spots. For comparison, in standard methods, the paper chromatogram is immersed in a ninhydrin solution for several seconds after drying and then dried, followed by heating for 15 minutes at 60°C to reveal purple spots (Block *et al*., 1958). The approach used in this work, with the addition of ninhydrin directly to the eluent, simplified the procedure with a similar development effect.

Quantitative analysis. After development, the amino acid spots on the chromatogram were quantitatively evaluated by reflectance densitometry. The intensity of each spot's colour was measured on a DO-1M densitometer (Ukraine) in reflected light. The use of spectral reflectometry to evaluate ninhydrin staining on chromatograms is a well-known approach in the quantitative analysis of amino acids. For quantitative determination, calibration curves were constructed in advance: known amounts of standard amino acids were applied to chromatographic paper and, after the entire procedure (separation and development), the optical density of their spots was measured. Within the linear range of the ninhydrin reaction, the optical density of the colour is proportional to the amino acid content. The measured optical density of the spots of the test samples was compared with the calibration graph of the corresponding amino acid and used to determine the amount of this amino acid in the sample, taking into account the dilution made during sample preparation. If some amino acids did not separate (co-eluted) due to the proximity of *Rf*, their total content was noted together, or separation required additional measures (e.g., solvent change or two-dimensional chromatography).

Statistical processing of results. The experimental data obtained were processed using methods of variational statistics in the Microsoft Excel 2016 software environment. For each indicator, the arithmetic mean (*M*), standard deviation (*SD*) and standard error of the mean (*m*) were determined using the following formulas:

$$M = \frac{\sum x_i}{n}, \quad (1)$$

$$SD = \sqrt{\frac{\sum (x_i - M)^2}{n-1}}, \quad (2)$$

$$m = \frac{SD}{\sqrt{n}}, \quad (3)$$

where n – number of observations.

Calculations were performed using built-in Excel functions: = AVERAGE(range) – for the mean value; = STDEV.S(range) – for the standard deviation; =STDEV.S(range)/SQRT(COUNT(range)) – for the standard error of the mean. The significance of differences between means was tested using Student's t-test for independent samples with unequal variances (t-test, two-sample unequal variance) with a statistical significance level of $P < 0.05$.

Results and Discussion

To determine the features of the effect of tetracycline-induced hepatosis on the functioning of the hepatobiliary system of the organism and to clarify the corrective effectiveness of milk phospholipids, the amino acid composition of bile in rats was analysed. The obtained indicators of total amino acid fractions in the bile of experimental rats from different groups are

presented in Table 1. This made it possible to determine the direction of shifts in the amino acid profile of bile in the experimental rats under different experimental conditions.

Table 1. Concentration of free amino acids in the bile of rats with tetracycline-induced hepatosis and the use of milk phospholipids, mg% (M ± m, n = 6)

Total amino acid fraction	Clinically healthy rats, "Control" group	Rats with tetracycline-induced hepatosis, "Self-rehabilitation" group	Rats with tetracycline-induced hepatosis treated with the dietary supplement "FLP-MD", "Tetracycline + "FLP-MD" supplement" group	Clinically healthy rats treated with the dietary supplement "FLP-MD", "Preparation" group
Cysteine/ Cystine	0.48 ± 0.03	0.71 ± 0.02*	0.40 ± 0.02*	0.65 ± 0.06*
Arginine/ Ornithine/ Lysine	1.55 ± 0.07	2.77 ± 0.06*	1.95 ± 0.80*	2.20 ± 0.10*
Histidine/ Taurine/ Asparagine	0.97 ± 0.06	1.33 ± 0.08*	0.63 ± 0.04*	1.48 ± 0.09*
Glycine/ Serine/ Glutamine/ Aspartic acid	1.44 ± 0.09	3.16 ± 0.05*	1.03 ± 0.09*	2.30 ± 0.09*
Methionine/ Glutamic acid	1.00 ± 0.05	2.17 ± 0.08*	0.82 ± 0.05*	1.18 ± 0.05*
Alanine/ Tyrosine/ Threonine	1.50 ± 0.03	2.10 ± 0.07*	2.15 ± 0.09*	1.50 ± 0.07
Valine/ Norvaline/ Tryptophan	1.07 ± 0.05	1.67 ± 0.03*	0.85 ± 0.04*	0.98 ± 0.08
Leucine/ Norleucine/ Phenylalanine	0.88 ± 0.01	1.64 ± 0.10*	0.82 ± 0.18	0.97 ± 0.09

***Note**: * – P < 0.05 compared to the control group*
***Source**: authors' own work*

In the bile of rats in the "Self-rehabilitation" group, the cysteine/cystine fraction was 48% higher than the corresponding values in the "Control" group. This pattern reflected the activation of the methionine-homocysteine cycle and enhanced cysteine resynthesis during the recovery phase after tetracycline-induced oxidative stress. Cysteine is a limiting substrate for the synthesis of glutathione and taurine. These pathways are critical for the detoxification of reactive oxygen species and the conjugation of bile acids. Recent data from T. Miyazaki *et al*. (2023) confirmed that activation of farnesoid X receptors or NR1H4 stimulates both taurine synthesis and bile acid conjugation under the catalytic influence of BAAT, which requires sufficient supply of sulphur-containing amino acids. Thus, the increased content of cysteine/cystine in bile during the self-recovery stage can be interpreted as a consequence of the fact that the rate of their formation exceeds the intensity of their use in the

synthesis of glutathione and taurine. In the bile of sick rats in the "Tetracycline + FLP-MD dietary supplement" group, on the contrary, a 17% decrease in the total cysteine/cystine fraction was recorded compared to the control group. This may indicate that, despite correction with milk phospholipids, the cysteine reserve is still actively spent on restoring glutathione content and conjugating toxic forms of bile acids that accumulate during the modelling of tetracycline-induced hepatosis. Recent experimental work by J. Liu *et al.* (2025) has shown that an excess of hydrophobic bile acids, by activating FXR/SHP (small heterodimer partner), can inhibit the glutathione synthetase pathway, increasing the consumption of cysteine for antioxidant protection. Thus, lower values of the total cysteine/cystine fraction in bile are consistent with the idea of a continuing load on sulphur-containing amino acids even under therapeutic conditions. In clinically healthy animals in the "Preparation" group, which received only milk phospholipids, the concentration of the cysteine/cystine fraction in bile was 35% higher than in the control group. This may reflect mild stimulation of sulphur-containing metabolism and enhanced conjugation of bile acids against the background of improved membrane transport under the action of phospholipids.

In the bile of sick rats in the "Self-rehabilitation" group, the content of the total arginine/ornithine/lysine fraction was 79% higher than the corresponding values in the control group. This increase in values may be associated with the activation of the ornithine cycle and enhanced detoxification of nitrogen. Ornithine and arginine are key metabolites of this pathway, while lysine reflects the overall increase in protein catabolism. A similar dysregulation of the arginine-ornithine pathway in acute liver injury was described by M. Holeček *et al*. (1996), who recorded a decrease in arginine concentration against a background of increased ornithine content in blood plasma in four models of experimental liver injury in rats, which correlated with the severity of hepatocellular injury. Thus, the levels of these amino acids are considered to be sensitive biomarkers of acute hepatic injury. In the group of animals with hepatosis that received milk phospholipids (the "Tetracycline + "FLP-MD" dietary supplement" group), the total level of these amino acids was also significantly higher than the control by 26%, but lower than during self-rehabilitation. This can be interpreted as a partial normalisation of nitrogen metabolism against the background of the correction: milk phospholipids improved the structural state of hepatocytes, which contributed to the restoration of ornithine cycle enzyme activity. This reduced the need for "excessive" excretion of essential amino acids into bile. These conclusions are consistent with the data of S. Colosimo *et al*. (2023) on the reduction of hyperammonaemia and correction of nitrogen metabolism disorders using amino acid mixtures/modulating drugs in experimental models of metabolic hepatopathology.

In the bile of rats in the "Preparation" group, which received only milk phospholipids, a 42% increase in the concentration of the total arginine/ornithine/lysine fraction was also recorded relative to the control. This shift can be interpreted as moderate stimulation of protein and nitrogen metabolism and a certain adjustment of the ornithine cycle even in the absence of pronounced liver injury. The additional involvement of essential amino acids created better conditions for ammonia utilisation and maintenance of nitrogen homeostasis. This is consistent with data on the potential hepatoprotective role of L-arginine in experimental models of cholestatic liver injury. Y. Ozsoy *et al*. (2011) demonstrated that the use of L-arginine reduced the severity of biochemical and morphological signs of cholestatic injury, which is consistent with the idea of the anabolic

and hepatoprotective potential of additional amino acid administration.

In the bile of sick rats undergoing self-rehabilitation, the histidine/taurine/asparagine fraction was 37% higher than in the control group. Taurine is the end product of sulphur-containing amino acid metabolism and the main substrate for conjugation with bile acids, while histidine and asparagine reflect participation in buffering and anti-inflammatory reactions (via carnosine, histamine, amidation). An increase in their content in rat bile during the self-recovery phase may indicate the activation of bile acid conjugation reactions and the antioxidant and anti-inflammatory potential of hepatocytes. In line with this, a review by M. Stofan & G.L. Guo (2020) noted that FXR receptor activation is a key link in the regulation of bile acid synthesis, conjugation and detoxification and was considered an important mechanism for protecting the liver from metabolic and cholestatic stress. In the bile of sick rats in the "Tetracycline + "FLP-MD" dietary supplement" group, the total content of histidine/taurine/asparagine, on the contrary, was 35% lower than in the control group. This indicates that even with correction by milk phospholipids, the supply of taurine and related amino acids is still insufficiently restored and is likely to be intensively used for the conjugation of toxic forms of bile acids and the neutralisation of oxidative stress products. A. Eguchi *et al*. (2025) showed that in humans with chronic liver disease, a decrease in the glycine/taurine ratio in the serum conjugated bile acid fraction was closely associated with more pronounced deterioration of liver function, higher markers of fibrosis, and a less favourable prognosis for patient recovery. The bile of animals in the "Preparation" group, which received only milk phospholipids, showed the highest concentration of this cluster, which differed by 53% from the control. This can be interpreted as preventive "saturation" with taurine and related amino acids, which created favourable conditions for bile acid conjugation, maintenance of bile secretion and increased liver resistance to metabolic stress. Similarly, A. Gregor *et al.* (2021) showed in a calorie-restricted mouse model that this adaptation was accompanied by an increase in the level of free taurine and taurine-conjugated bile acids in the intestinal wall and an increase in the content of bile acids in the liver and blood plasma. The established patterns were considered an important component of the protective restructuring of bile acid and antioxidant homeostasis.

In the bile of sick rats in the "Self-rehabilitation" group, a sharp increase in the total content of glycine/serine/glutamine/aspartic acid was noted – more than 119% relative to the control. Glycine and glutamine are not only substrates for the synthesis of glutathione and urea, but also key donors in nitrogen detoxification reactions, while aspartic acid is involved in anion transport and the ornithine cycle. Such a pronounced increase in their levels in bile can be interpreted as a manifestation of enhanced protein catabolism and as a result of the activation of nitrogen metabolism during the stage of independent recovery after tetracycline injury. A similar trend towards an increase in the concentration of free amino acids in bile in conditions of hepatobiliary system damage was described by J. Bugajska *et al*. (2023), who found an increase in the content of most amino acids in bile in gallstone disease compared to the control group. On the other hand, M. Holeček (2024) emphasised that glutamine and aspartate are key participants in ammonium detoxification and ornithine cycle functioning in liver injury. Thus, the data obtained on the increase in the total content of the glycine/glutamine/aspartic acid fraction in bile was consistent with the idea of the enhanced participation of these amino acids in ensuring nitrogen homeostasis at the liver level. In sick

animals from the "Tetracycline + "FLP-MD" dietary supplement" group, against the background of milk phospholipid administration, the content of the total glycine/serine/glutamine/aspartic acid fraction in bile was 28% lower than the corresponding values in the control group. This may indicate that the corrective effect of milk phospholipids was manifested instead of the usual elimination of these amino acids in bile to intensive use in intracellular synthesis of proteins, glutathione and energy production necessary to ensure active regeneration of hepatocytes. A similar transition from catabolic to anabolic amino acid metabolism is described in the work of O. Rom *et al*. (2020), where glycine-containing therapy in experimental metabolic dysfunction-associated steatotic liver disease (MASLD)/NAFLD stimulated β-oxidation of fatty acids and glutathione synthesis in the liver, reducing steatosis, oxidative stress and fibrosis.

In the bile of animals in the "Preparation" group, which received only milk phospholipids, the content of the total fraction of glycine/serine/glutamine/aspartic acid was also significantly higher by 60% than in the control group. This probably reflected the physiological enhancement of bile formation under the influence of phospholipids, increased proliferation and restoration of the biliary tract epithelium, for which glycine and serine are key 'building' substrates. In support of this, S. Pan *et al*. (2021) emphasised that serine and glycine, through one-carbon metabolism, provide proliferating cells with nucleotides, proteins and membrane lipids, which is consistent with the interpretation of the results in this study regarding the established growth of this cluster as a marker of active renewal of the biliary tract epithelium.

In the bile of diseased rats in the "Self-rehabilitation" group, the content of the total methionine/glutamic acid fraction exceeded the control by 117%. Methionine is a methyl group donor and precursor of cysteine, and glutamate is a central node between nitrogen metabolism and energy cycles. Their "accumulation" in bile can be explained by impaired intrahepatic utilisation (methylation, glutathione synthesis) and enhanced transamination against the background of oxidative and lipid stress in tetracycline hepatosis. In sick animals from the "Tetracycline + "FLP-MD" dietary supplement" group, against the background of milk phospholipid use, the content of the total methionine/glutamic acid fraction was 18% lower than in the control group. It is likely that these amino acids were actively used in intracellular metabolism, in particular in the methionine-cysteine cycle, glutathione and protein synthesis, rather than being excreted in bile. In the bile of animals in the "Preparation" group, which received only milk phospholipids, a moderate but statistically significant 18% increase in the content of the total methionine/glutamic acid fraction was observed compared to the control. The observed changes can be explained by the activation of the methionine cycle and increased endogenous synthesis of phospholipids in the liver against the background of the intake of exogenous milk phospholipids. A summary of the data by G. Contarini & M. Povolo (2013) also showed that milk phospholipids can stimulate the restoration of the phospholipid composition of the liver and reduce its lipid overload, which is consistent with the proposed assumption about the activation of the methionine cycle against the background of phospholipid correction.

In the bile of sick rats in the "Self-rehabilitation" group, the content of the total fraction of alanine/tyrosine/threonine significantly increased by 40% relative to its values in the control group, and in rats from the "Tetracycline + "FLP-MD" dietary supplement" group against the background of milk phospholipid use, it increased by 43%. Alanine is a key substrate for gluconeogenesis and transamination, tyrosine

is an aromatic amino acid sensitive to the intensity of total proteolysis, while threonine is an essential component of glycoprotein and structural protein synthesis. Thus, a statistically significant increase in the total content of these fractions in both groups can be interpreted as a consequence of the enhanced mobilisation of these amino acids in hepatocytes in response to the development of experimental hepatopathology. In the bile of animals in the "Preparation" group, which received only milk phospholipids, the content of this total amino acid fraction remained unchanged.

At the same time, in the bile of sick rats in the "Self-rehabilitation" group, the content of the total fraction of valine/norvaline/tryptophan increased by 56% relative to the control. Valine belongs to branched-chain amino acids (BCAA), and tryptophan belongs to aromatic amino acids (AAA), so an increase in their total concentration in the secretion can be considered a manifestation of a shift in the BCAA/AAA balance during the recovery phase due to tetracycline-induced liver injury. K. Tajiri & Y. Shimizu (2018) emphasised that in patients with chronic diffuse liver injury and cirrhosis, there was a decrease in plasma concentrations of branched-chain amino acids against a background of an increase in aromatic amino acids, which led to a drop in the Fisher index (BCAA/AAA) index and the BCAA/tyrosine ratio. Such amino acid dysregulation is considered a typical sign of cirrhosis progression and a prognostic marker of its course. Against this background, the detected increase in the total content of valine/norvaline/tryptophan in the bile of rats during self-rehabilitation may indicate their compensatory elimination from the intracellular reserve due to the processes of bile formation and bile secretion. In the bile of rats in the "Tetracycline + "FLP-MD" dietary supplement" group, against the background of the use of milk phospholipids, the content of the total valine/norvaline/tryptophan fraction decreased by 21% compared to the control. This shift can be interpreted as meaning that, under the conditions of the correction applied, these amino acids are used more intensively by hepatocytes for protein synthesis and regeneration processes, compared to their losses in bile. M.C. Trillos-Almanza *et al*. (2024) noted that in patients with end-stage liver disease, BCAA levels were reduced, and their additional administration was considered as an approach to alleviating fibrosis and stimulating regeneration. In their work, the authors demonstrated that BCAAs and their metabolites reduce the activation of stellate cells in the livers of humans and rats. Against this background, the observed decrease in the total valine/norvaline/tryptophan fraction in bile during phospholipid therapy can be interpreted as a manifestation of more "economical" use of BCAAs in the liver to ensure recovery processes, while the absence of significant changes in these amino acids in rats in the "Preparation" group confirms that the nature of the redistribution of BCAA/AAA in bile is caused precisely by liver injury.

Only in sick rats in the "Self-rehabilitation" group did the content of the total leucine/phenylalanine fraction in bile differ significantly higher by 86% compared to the control, while in animals in the "Tetracycline + "FLP-MD" dietary supplement" and "Preparation" groups, these indicators remained unchanged. Leucine is a key branched-chain amino acid, and phenylalanine is a typical aromatic amino acid, so their joint increase in bile can be considered a manifestation of a pronounced amino acid imbalance at the stage of uncorrected recovery after tetracycline injury. Y. Zhang *et al*. (2024) noted that in chronic liver diseases, the BCAA/AAA ratio is a sensitive indicator of the degree of decompensation and prognosis, since a decrease in BCAA and a relative increase in AAA are closely related to the severity of liver injury.

Against this background, the fact that in the group with phospholipid correction, the total leucine/phenylalanine content did not differ from the control level, and in the animals of the "Preparation" group, only a tendency to increase was noted, further confirms the effectiveness of the corrective effect of milk phospholipids on the amino acid balance.

For a comprehensive characterisation of intermediate amino acid metabolism, an analysis of the intrahepatic composition of free amino acids was also performed, which directly reflected the metabolic state of hepatocytes in tetracycline-induced hepatosis against the background of correction with milk phospholipids. Table 2 presents the summarised results of individual total amino acid fractions in the liver of animals from different groups, which, in combination with the corresponding indicators in bile samples, allows to track changes in key links of nitrogen-excreting and bile-forming metabolism in the area of primary injury by toxic doses of tetracycline and, as a result, the development of fatty hepatosis.

Table 2. Content of free amino acids in liver samples of rats with tetracycline-induced fatty hepatosis and correction with milk phospholipids, mg% (M ± m, n = 6)

Total amino acid fraction	Clinically healthy rats, "Control" group	Rats with tetracycline-induced hepatosis, "Self-rehabilitation" group	Rats with tetracycline-induced hepatosis treated with the dietary supplement "FLP-MD", "Tetracycline + "FLP-MD" supplement" group	Clinically healthy rats treated with the dietary supplement "FLP-MD", "Preparation" group
Cysteine/Cystine	0.30 ± 0.03	0.41 ± 0.04*	0.17 ± 0.02*	0.44 ± 0.05*
Arginine/ Ornithine/Lysine	1.48 ± 0.14	2.74 ± 0.32*	0.86 ± 0.07*	2.10 ± 0.24
Histidine/ Taurine/ Asparagine	0.87 ± 0.07	2.02 ± 0.22*	0.57 ± 0.05*	1.57 ± 0.16*
Glycine/Serine/ Glutamine/ Aspartic acid	2.24 ± 0.20	3.73 ± 0.38*	1.33 ± 0.23*	4.06 ± 0.39*
Methionine/ Glutamic acid	2.36 ± 0.14	3.07 ± 0.24*	1.21 ± 0.19*	3.04 ± 0.23*
Alanine/ Tyrosine/ Threonine	1.20 ± 0.10	1.99 ± 0.09*	1.80 ± 0.17*	2.35 ± 0.20*
Valine/ Norvaline/ Tryptophan	0.82 ± 0.07	1.58 ± 0.14*	0.78 ± 0.04	1.79 ± 0.13*
Leucine/ Norleucine/ Phenylalanine	1.13 ± 0.08	1.82 ± 0.10*	2.10 ± 0.08*	2.70 ± 0.18*

***Note**: *P < 0.05 compared to the control group*
***Source**: authors' own work*

The amino acid profile in the liver of diseased rats with tetracycline-induced hepatosis (the "Self-rehabilitation" group) showed significant alterations. In particular, the content of the

total cysteine/cystine fraction in the liver of rats in this group increased by 37% compared with the control values, whereas in animals from the "Tetracycline + "FLP-MD" dietary supplement" group it decreased by 43%. In clinically healthy rats receiving milk phospholipids (the "Preparation" group), the cysteine/cystine content increased by 47% relative to the control. Thus, the development of tetracycline-induced fatty hepatosis was associated with depletion of the hepatic "thiol" profile, whereas administration of milk phospholipids to diseased animals shifted the balance toward accumulation of reduced sulphur-containing amino acids in hepatocytes. This pattern is probably due to the fact that toxic liver injury led to massive use of cysteine for glutathione synthesis and detoxification of active oxygen species, as well as its entry into the blood, which led to a decrease in the intrahepatic reserve. In contrast, the use of milk phospholipids in sick animals probably enhanced the synthesis and resynthesis of glutathione, partially restoring cysteine reserves in hepatocytes. In a review by M.T. Nguyen *et al.* (2025) on NAFLD therapy with glutathione, it was emphasised that the restoration of intracellular glutathione reserves is accompanied by a decrease in oxidative stress and liver enzyme activity, which indirectly confirms the key role of adequate cysteine supply in maintaining the redox homeostasis of hepatocytes. This is consistent with the results regarding an increase in the cysteine/cystine ratio when using phospholipid correction.

For the total fraction of arginine/ornithine/lysine in the liver of the experimental rats, two opposite trends were observed relative to the control. In the self-rehabilitation group of rats, the total content of the components of this heterogeneous fraction increased by 85% from the control level, while in the tetracycline-induced hepatosis and milk phospholipid administration group, it decreased by 42%. In clinically healthy animals, against the background of milk phospholipid administration, only a tendency to increase this indicator (by 42%) compared to the control was recorded. Thus, the tetracycline load on the liver in the experimental rats against the background of milk phospholipid administration was accompanied by depletion of arginine, ornithine and lysine depots, while in the case of spontaneous recovery, their compensatory increase was observed in the studied samples (the phenomenon of adaptive compensation). At the same time, intragastric administration of milk phospholipids to clinically healthy animals provoked only a tendency towards a further increase in the values of this fraction. At the molecular level, arginine and ornithine are key participants in the ornithine cycle, which detoxifies ammonia and protects the body, while lysine is the main essential amino acid sensitive to changes in total proteolysis and protein synthesis. The decrease in their total content in the liver during tetracycline exposure in rats is likely to be the result of changes in the ornithine cycle and arginase activity, accompanied by intense depletion of intracellular arginine.

In a review by S. Thakur *et al*. (2024) emphasised that metabolites of the ornithine cycle, including arginine and ornithine, can be considered promising biomarkers of hepatotoxicity, as their imbalance reflects the stress of ammonia detoxification and hepatocyte damage. In contrast, a sharp increase in the arginine/ornithine/lysine cluster in the liver during the self-rehabilitation phase can be interpreted as the activation of ureagenesis and anabolic processes aimed at restoring protein and nitrogen balance. This assumption was indirectly supported by experimental data from S. Al-Dalaen *et al*. (2016), which showed that the additional administration of L-arginine in CCl_4-induced hepatitis in rats enhanced the antioxidant protection of the liver and improved the morphological condition of hepatocytes,

indicating the hepatoprotective potential of arginine-dependent pathways. In a model of tetracycline-induced liver injury in rats, the increase in the total arginine/ornithine/lysine fraction in the case of self-rehabilitation and the tendency for its increase in clinically healthy animals against the background of milk phospholipid administration are also consistent with the idea that the restoration and maintenance of the optimal composition of amino acids in the ornithine cycle is an important link in the repair of toxic liver injury.

Quantitative characteristics of the total fraction of histidine/taurine/asparagine in rat liver samples (Table 2) showed opposite changes relative to the control. In animals in the "Self-rehabilitation" group, the total content of these amino acids increased by 132% from the control level. In animals in the "Tetracycline + "FLP-MD" dietary supplement" group, on the contrary, it decreased by 34%, while in animals in the "Preparation" group, which received milk phospholipids, it increased by 80% from the control. Thus, tetracycline-induced changes in the liver under conditions of milk phospholipid administration were accompanied by a marked depletion of histidine, taurine and asparagine, while in animals undergoing self-recovery and separately against the background of the administration of milk phospholipids to clinically healthy rats, this total content accumulated significantly.

This pattern is consistent with the functions of these amino acids. Histidine is a precursor of carnosine and histamine and acts as a buffer and antioxidant; taurine ensures bile acid conjugation, osmoregulation and has a pronounced antioxidant and anti-inflammatory effect; asparagine, with the participation of asparaginase, forms a "stress response" of hepatocytes, accumulating nitrogen and maintaining energy balance. A sharp decrease in the content of amino acids of this heterogeneous fraction in the liver of rats with tetracycline injury is associated with their intensive use in antioxidant and detoxification processes. On the other hand, a significant increase in the total content of histidine/taurine/asparagine during self-rehabilitation and in clinically healthy animals under the influence of milk phospholipids indicates the activation of recovery processes and the accumulation of substrates for antioxidant protection and membrane repair.

This interpretation is supported by experimental data from other researchers. Thus, W. Liu *et al*. (2008) showed that the addition of histidine and carnosine in chronic alcoholic liver injury in mice significantly reduced the activity of transaminases and markers of oxidative and inflammatory stress, which is consistent with the role of histidine as a component of the anti-inflammatory and antioxidant response. Against this background, a marked increase in the total fraction of histidine/taurine/asparagine in the liver under conditions of self-rehabilitation and against the background of the use of milk phospholipids was considered as a marker of the activation of hepatoprotective, antioxidant and reparative mechanisms, while its decrease in animals of the "Tetracycline + "FLP-MD" dietary supplement" group was considered an indicator of intensive involvement of these reserves.

The content of the total fraction of glycine/serine/glutamine/aspartic acid in rat liver samples underwent significant and mutually opposite shifts. In particular, in rats in the "Self-rehabilitation" group, the content of this total fraction increased by 67% from the control level, while in animals with tetracycline-induced liver injury in the "Tetracycline + "FLP-MD" dietary supplement" group, on the contrary, a decrease in its level by 41% from the control was noted. In clinically healthy animals in the "Preparation" group, against the background of the introduction of milk phospholipids, the content of this fraction in the liver reached its

highest values, increasing by 81% relative to the control group parameters. Thus, the use of tetracycline in toxic doses led to the intensive use of components of the heterogeneous fraction of glycine/serine/glutamine/aspartic acid, accompanied by a decrease in their content in liver samples, while in the case of self-recovery and when milk phospholipids were administered to clinically healthy animals, their content increased significantly. These amino acids are at the intersection of one-carbon metabolism, glutathione synthesis and ammonia detoxification. Glycine and serine are key donors of one-carbon groups and precursors of glutathione; glutamine and aspartic acid act as "reservoirs" for ammonium and replenish the Citric acid cycle. A sharp decrease in the levels of this fraction in the liver during tetracycline loading may be associated with the quantitative redistribution of these amino acids in the bodies of sick rats, which are also actively consumed in the neutralisation of toxic products of nitrogen metabolism and in the intensification of oxidative processes. Conversely, during self-rehabilitation and especially in clinically healthy rats that were administered milk phospholipids, an increase in the content of amino acids of this fraction in the liver parenchyma may reflect the activation of one-carbon metabolism and the preparation of the substrate base for glutathione synthesis and the restoration of energy and nitrogen homeostasis.

Thus, milk phospholipids probably contribute to the rebalancing of amino acids in the liver of animals from a catabolic, stressful state to an anabolic and cytoprotective one. This is consistent with the metabolic studies by R.P. da Silva *et al*. (2020), who emphasised that disturbances in the one-carbon metabolism of glycine/serine and glutamine content are a key link in the progression of fatty liver disease. Overall, these data are consistent with the results obtained regarding the intensive use of the hepatic fraction of glycine/serine/glutamine/ aspartic acid at the peak of tetracycline injury, which may be the result of switching between the acute stress phase and the repair phase and the enhancement of cytoprotective mechanisms, and its insufficient involvement in the recovery processes in animals during self-rehabilitation. At the same time, in clinically healthy rats that were administered milk phospholipids, their excessive mobilisation was established, which may have a positive effect on synthetic processes in hepatocytes.

In the case of the total fraction of methionine/glutamic acid in rat liver samples (Table 2), there were mixed but significant changes relative to the control. In the "Self-rehabilitation" group of rats, the total content of amino acids in this fraction increased by 30% relative to the control level, and in clinically healthy animals receiving milk phospholipids, it increased by 29%. In contrast, in sick rats in the "Tetracycline + "FLP-MD" dietary supplement" group, the quantitative parameters of this fraction decreased by 49% relative to the control. Thus, the administration of tetracycline hydrochloride in toxic doses to rats was accompanied by increased utilisation of the hepatic pool of methionine and glutamic acid, while in the self-recovery phase and against the background of the use of milk phospholipids in clinically healthy animals, moderate mobilisation of this group of amino acids was observed. This profile is consistent with the fact that methionine and glutamate are at the centre of one-carbon metabolism, S-adenosylmethionine and glutathione synthesis. A marked decrease in their total content in the liver of rats under tetracycline load can be interpreted as a sign of intensification of the methionine cycle and glutamate-dependent pathways in hepatocytes, which is important for maintaining the proper level of methylation and antioxidant protection in pathologically altered hepatocytes. Conversely,

an increase in the total fraction of methionine/glutamic acid during self-rehabilitation and against the background of the administration of milk phospholipids to clinically healthy animals is caused by their insufficient use in the above-mentioned processes.

At the same time, the quantitative parameters of the total alanine/tyrosine/threonine fraction in the liver of rats (Table 2) indicate similar trends in changes in animals from different experimental groups in relation to the control. In particular, in rats of the "Self-rehabilitation" group, the total content of amino acids of this fraction increased by 65% compared to the control, in animals of the "Tetracycline + "FLP-MD" dietary supplement" group – by 50%, and in clinically healthy rats that received milk phospholipids, it increased by 95%. Thus, the artificial administration of toxic doses of tetracycline hydrochloride to rats was accompanied by a marked increase in the content of the alanine/tyrosine/threonine fraction in hepatocytes, both under conditions of self-rehabilitation and in the case of prophylactic administration of milk phospholipids. Alanine is a key gluconeogenic substrate and marker of proteolysis, tyrosine belongs to aromatic amino acids sensitive to impaired liver metabolism, and threonine is involved in the synthesis of glycoproteins and can be converted to glycine/serine. A marked increase in this group of amino acids in tetracycline-induced injury can be interpreted as a consequence of increased breakdown of peripheral tissue proteins and reduced utilisation of these amino acids in liver dysfunction against a background of mitochondrial disorders. At the same time, a significant increase in the content of alanine/tyrosine/threonine in hepatocytes may reflect their mobilisation to ensure regenerative processes in hepatocytes, in particular activation of gluconeogenesis, protein synthesis and cell membrane repair, when these amino acids accumulate as important substrates for the energy and structural restoration of hepatocytes.

The established patterns are consistent with the findings of C. Fotakis *et al*. (2023), who demonstrated in a metabolomic study of blood serum from NAFLD patients that elevated concentrations of alanine and tyrosine are associated with greater disease severity, and that threonine levels in women are inversely related to the progression of steatosis. This emphasises that quantitative shifts in the total alanine/tyrosine/threonine fraction are a sensitive marker of liver metabolism, and their excessive accumulation in the liver against the background of milk phospholipid administration can be considered a reflection of activated but controlled adaptive-anabolic processes, in contrast to uncontrolled dysfunction in tetracycline injury.

Quantitative analysis of the total fraction of valine/norvaline/tryptophan in rat liver samples relative to the control showed a significant increase in the content of its components, especially pronounced in the "Self-rehabilitation" and "Preparation" groups. Thus, in rats of the "Self-rehabilitation" group, their level increased by 93% relative to the control. In animals of the "Preparation" group, which received milk phospholipids, the content of this amino acid fraction increased by 118% compared to the control. In rats from the "Tetracycline + "FLP-MD" dietary supplement" group, this indicator was finally restored. Such quantitative changes in the amino acid spectrum can be interpreted as the transition of the liver from an acute toxic phase to an anabolic-regenerative mode. Valine, as a representative of BCAA, is an important substrate for energy supply and protein synthesis, and tryptophan is important for the kynurenine and serotonin pathways, which modulate inflammation, microcirculation, and tissue regeneration. In tetracycline-induced liver cell injury in animals in the "Self-rehabilitation" group, the

use of these amino acids was more focused on the benefit of extrahepatic tissues and the immune response, so no significant changes were observed in the liver itself. In contrast, in sick animals in the "Tetracycline + "FLP-MD" dietary supplement" group, under conditions of phospholipid correction, there was an improvement in mitochondrial function and membrane integrity, which contributed to more active use of valine and tryptophan by hepatocytes, ensuring intensive protein synthesis, remodelling of the extracellular matrix and restoration of microvessels. A similar idea was supported by Y. Morine *et al.* (2022), who showed that in patients with hepatocellular carcinoma, the levels of essential amino acids, in particular valine and tryptophan, increased in both tumour liver tissue and blood serum before surgery, and decreased to normal values after liver resection. The authors interpreted this as a reflection of metabolic restructuring and a high demand for these amino acids in cells with enhanced growth and remodelling of liver tissue. In the context of the established changes in the model of tetracycline-induced liver injury, this allows to consider the increase in the total valine/norvaline/tryptophan fraction in sick rats in the case of self-rehabilitation as a marker of controlled regenerative-anabolic processes in the liver, and in clinically healthy animals against the background of milk phospholipid administration as a result of their active accumulation.

In the liver of rats, a consistent and statistically significant increase in the total leucine/norleucine/phenylalanine fraction relative to the control was observed in all experimental groups. In sick animals in the "Self-rehabilitation" group, the total content of these amino acids exceeded the control by 61%, in the "Tetracycline + "FLP-MD" dietary supplement" group by 86%, while in clinically healthy animals receiving milk phospholipids, it increased by 139% from control values. Thus, tetracycline-induced liver injury was accompanied by a pronounced accumulation of intracellular reserves of the amino acid groups leucine/norleucine/phenylalanine in hepatocytes, and with the additional administration of milk phospholipids to clinically healthy animals, these processes occurred more actively.

The established patterns should be interpreted taking into account that leucine, together with norleucine, are representatives of branched-chain amino acids with powerful anabolic and mTOR-activating potential, while phenylalanine belongs to aromatic amino acids that are sensitive to disturbances in liver metabolism. In acute tetracycline-induced hepatosis, the increase in the content of this mixed group of amino acids in the liver reflected a combination of enhanced proteolysis of peripheral tissues (the influx of leucine and phenylalanine from extrahepatic proteins) and limited oxidation of these amino acids by damaged mitochondria. In the self-rehabilitation group, and especially against the background of milk phospholipid administration, the further increase in the leucine/phenylalanine fraction content in the liver parenchyma should be interpreted as a switch to an anabolic-regenerative mode: phospholipids stabilise membranes and mitochondria, which facilitates the uptake of BCAA/AAA by hepatocytes and forms a reserve of substrates for intensive protein synthesis, cytoskeleton remodelling and synthesis of phospholipid components of membranes without signs of profound decompensation.

The classic clinical picture of amino acid imbalance in chronic liver disease was described by H. Enomoto *et al*. (2023), which contrasts to some extent with the results presented in this material. The authors showed that in patients with cirrhosis, blood serum is characterised by decreased levels of BCAAs (valine, leucine, isoleucine) and increased levels of aromatic amino acids, leading to a decrease in the

BCAA/AAA ratio and the BCAA/tyrosine ratio (BTR); low BTR correlates with the progression of fibrosis, sarcopenia, hypoalbuminaemia and a worse prognosis. Against this background, the unidirectional increase in BCAA and phenylalanine in the liver tissue of the experimental rats can be considered as a manifestation of adaptation at an early stage and intensification of regenerative processes in the modelling of acute tetracycline-induced injury and prophylactic phospholipid correction, rather than "classical" plasma amino acid imbalance characteristic of decompensated cirrhosis.

Thus, according to the results of experimental studies, certain patterns have been established regarding the quantitative redistribution in the hepatobiliary system of rats of most of the studied heterogeneous amino acid fractions depending on the functional state of the liver. This indicates significant changes in the intermediate metabolism of amino acids in the case of artificial reproduction of fatty hepatosis in rats, which is closely related to functional disorders of the liver, in particular the protein-synthesising ability of hepatocytes. The use of milk phospholipids in sick rats contributed to better utilisation of amino acids in synthetic and regenerative processes in hepatocytes, and even to the return of certain indicators to physiological limits: in bile – the leucine/norleucine/phenylalanine fraction, and in the liver – the valine/norvaline/tryptophan fraction. Overall, this indicates a positive corrective effect of the milk phospholipid-based dietary supplement, once again proving the close relationship between the structural state of hepatocytes and their metabolic activity.

Conclusions

The results of the experimental study demonstrated significant quantitative changes in heterogeneous amino acid fractions in the hepatobiliary system of rats depending on the functional state of the liver. In particular, in the bile of rats undergoing self-rehabilitation after acute toxic liver injury caused by tetracycline hydrochloride, a significant increase in the content of all studied amino acid fractions was observed. The most pronounced changes occurred in the fractions of arginine/ornithine/lysine (by 79%), glycine/serine/glutamine/aspartic acid (by 119%), methionine/glutamic acid (by 117%), and leucine/norleucine/phenylalanine (by 86%). At the same time, similar patterns were observed in the liver of animals in this group. Significant changes were recorded in the fractions of arginine/ornithine/lysine (by 85%), histidine/taurine/asparagine (by 132%), and valine/norvaline/tryptophan (by 93%). Among these, the arginine/ornithine/lysine fraction proved to be the most sensitive to the development of the acute form of hepatopathy, showing the most pronounced changes both in the bile and in the liver of the diseased animals. These patterns may indicate intensification of catabolic processes and blockage of the further utilisation of these amino acids in maintaining nitrogen homeostasis and an adequate level of antioxidant processes.

When milk phospholipids were administered to the diseased animals, opposite changes in the quantitative parameters of the amino acid profile of the hepatobiliary system were observed. In particular, in the bile of these animals the most pronounced decrease in content was recorded for the histidine/taurine/asparagine fraction (by 35%). At the same time, an increase in levels was detected only for two fractions: arginine/ornithine/lysine (by 26%) and alanine/tyrosine/threonine (by 43%). Restoration of quantitative characteristics was observed only for the leucine/norleucine/phenylalanine fraction. Similar patterns were detected in the liver samples of animals in this group. Under conditions of administration of milk phospholipids to diseased animals, the most sensitive changes

in the biliary system were observed in two amino acid fractions: alanine/tyrosine/threonine and leucine/norleucine/phenylalanine.

At the same time, in the bile of clinically healthy rats the most pronounced increase in concentration was observed for two amino acid fractions: histidine/taurine/asparagine and glycine/serine/glutamine/aspartic acid. Three fractions remained unchanged: alanine/tyrosine/threonine, valine/norvaline/tryptophan, and leucine/norleucine/phenylalanine. In liver samples from these animals, the greatest increases in content were detected in similar fractions: alanine/tyrosine/threonine (by 95%), valine/norvaline/tryptophan (by 118%), and leucine/norleucine/phenylalanine (by 139%). Elevated levels were also recorded for the histidine/taurine/asparagine fraction (by 80%) and the glycine/serine/glutamine/aspartic acid fraction (by 81%). Overall, this indicates specific features of the influence of milk phospholipids on certain links of nitrogen metabolism associated with the hepatobiliary system of mammals.

Thus, a pronounced corrective effect of milk phospholipids on intermediate amino acid metabolism was observed, as well as the possibility of using the most sensitive indicators as markers of the development of hepatopathology and of the effectiveness of therapy. In the future, it is planned to investigate substrates of the tricarboxylic acid cycle in order to clarify further metabolic pathways for the utilisation of free amino acids in the animal organism during the development of the drug-induced form of fatty hepatosis.

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Conflict of Interest

None.

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Амінокислоти біліарної системи в щурів за тетрацикліндукованого гепатозу і застосування фосфоліпідів молока

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Анотація. Актуальність дослідження зумовлена високою частотою медикаментозного ушкодження печінки в ссавців та недостатнім вивченням питання щодо порушень метаболізму амінокислот за гострої форми гепатопатії відповідного генезу. У зв'язку з цим, робота була спрямована на виявлення специфічних змін профілю вільних амінокислот у жовчі та печінці щурів за тетрацикліндукованого жирового гепатозу, а також на визначення коригувального ефекту дії фосфоліпідної фракції молока. Провідним підходом у дослідженні цієї проблеми був експеримент на лабораторних тваринах із моделюванням жирового гепатозу завдяки цитотоксичному ефекту дії тетрацикліну, з подальшим відбором зразків жовчі й печінки, наступним дослідженням спектру вільних амінокислот методом розподільної паперової хроматографії з нінгідриновим проявленням. Встановлено, що загальний патерн змін у гепатобіліарній системі хворих щурів свідчив про порушення процесів кон'югації жовчних кислот і мітохондріальну дисфункцію, а також виражене блокування використання вільних амінокислот у метаболічних процесах. При цьому, застосування щурам за тетрацикліндукованого жирового гепатозу фосфоліпідовмісної терапії активізувало використання внутрішньопечінкового резерву амінокислот, залучених до кон'югації жовчних кислот і антиоксидантного захисту, зокрема сірковмісних представників та сумарної фракції гліцину/серину/глутаміну. Визначено, що у жовчі цих щурів також зменшувався вміст гліцино- і тауриновмісних фракцій амінокислот за одночасного зростання рівня сумарних фракцій аланіну/тирозину/треоніну й аргініну/орнітину/лізину, що підтверджує актуалізацію в печінці процесів детоксикації із залученням вільних амінокислот. Виявлено, що застосування фосфоліпідної фракції молока зменшувало прояви порушення в проміжному обміні амінокислот та сприяло відновленню вмісту лейцино- і валіновмісних фракцій у жовчі й печінці хворих щурів. Встановлені закономірності мають практичну цінність для лабораторної діагностики та доклінічної оцінки гепатопротекторних засобів, оскільки амінокислотний профіль гепатобіліарної системи може бути використаний в якості чутливого індикатору тяжкості ушкодження печінки та як критерій у визначенні ефективності терапії

Ключові слова: хроматографія; фракції амінокислот; печінка; жовч; коригувальна терапія; тетрацикліну гідрохлорид; лабораторна діагностика

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Assessment of the effect of a biologically active preparation on the development of laboratory animals

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Abstract. Investigation of the effects of newly developed drugs containing several compounds on laboratory animals is an important step in scientifically establishing their effective dosage and safety. The purpose of this study was to investigate the effect of the new drug "Imun-depo" on the development of laboratory animals (white mice), their haematological parameters, and the viability of *Tetrahymena pyriformis* ciliate cells. The study material was female white laboratory mice of improved convential type in the amount of 60 animal units (30 units each in the control and experimental groups). The drug was administered to white laboratory mice intragastrically at a dose of 1.0 mL/animal from 14 days of age for 60 days. A set of methods was used to assess the state of the internal environment in the vivarium, the quality of drinking water, the dynamics of body weight and internal organs, behavioural responses of mice, haematological indicators of their body, and the viability of *Tetrahymena pyriformis ciliates*. It was found that the indicators of the internal environment of the vivarium and drinking water were within the limits of regulatory requirements. It was found that the drug at a dose of 1.0 mL/animal had an effect on body weight gain, while the weight of the thymus, thyroid gland, kidneys, liver, and spleen did not change. It was proved that in the blood of white laboratory mice, the haemoglobin content, the number of red blood cells, and the haematocrit level increased (within the reference values). Moreover, the indicators of the leukocyte formula of the mice's blood did not change. In the blood serum, there was an increase (within the reference values) in the content of globulins, total protein, alanine aminotransferase activity, and a decrease in total cholesterol. According to the effect of the "Imun-depo" preparation on the viability of *Tetrahymena pyriformis* ciliate cells, the absence of toxic effects was noted. Scientifically based results of the effect of the "Imun-depo" preparation on the body of white laboratory mice are necessary for further experimental studies with the use of the drug to productive animals as a feed additive to the main diet

Keywords: white mice; keeping conditions; body weight dynamics; morphology and biochemistry of blood; *Tetrahymena pyriformis* ciliates; "Imun-depo" preparation

Introduction

The use of newly created biologically active preparations, which include a number of substances, requires preclinical studies on various types of laboratory animals. The effectiveness of complex drugs on a living organism depends on the amount of each substance in their composition and the possible synergistic effect. The degree of exposure to drugs was assessed by indicators of body weight dynamics, behaviour, haematological parameters of laboratory animals, and also depended on the action of each of the components of the preparation. Therefore, it is extremely important to determine the optimal dose of multicomponent drugs and the first stage is preclinical studies. Biologically active compounds in the composition of such drugs, first of all, have an immunomodulatory effect on the body, the degree of influence of which depends on the dosage of the drug, the state of health of laboratory animals, their age, and sex.

Relevance in the study of the effect of multicomponent biologically active drugs lies in the possible prevention of immunodeficiency and the development of immune responses in the body, which depend on a balanced supply of nutrients to the body. Even a slight deficiency of certain nutrients leads to a violation of immune competence in the body.

Micro- and macronutrients, vitamins, and amino acids have an important regulatory effect on immune mechanisms (Islam & Bellah, 2026). Carotenoids – natural pigments synthesised by plants, microorganisms, and fungi – play an important role in maintaining homeostasis and reactivity of the body (Bohn *et al.,* 2023). Considerable attention of researchers was focused on investigating the effects of carotenoids and vitamin A on adipose tissue metabolism, which determines the balance between energy storage (lipogenesis) and its release (lipolysis). B.V. Aygun *et al.* (2026) found that retinoic acid, β-cryptoxanthin, zeaxanthin, and lycopene contribute to reduced adipogenesis and lipogenesis, inhibit the expression of the peroxisome proliferator-activated gamma receptor (PPARγ), CCAAT/enhancer binding protein (C/EBPα), and sterol regulatory element binding protein 1C (SREBP1-c). As a result, there is a decrease in adipocyte hypertrophy, systemic inflammation, and an increase in mitochondrial activity in cells.

M.S. Abou El-Fetouh *et al.* (2023), when conducting preclinical studies on female albino rats, found that zinc in the composition of the drug affects the functional ability of the immune system, which is manifested by an immunostimulating effect on immunocompetent cells in the body. M.S. Hameed & A.I.A. Al-Ezzy (2024) proved the immunomodulatory activity of vitamins C and E – sodium selenite in studies on laboratory mice intoxicated with sodium nitrate. Sodium selenite and vitamin C have been found to be powerful antioxidants and affect the improvement of kidney function. The effect of drugs on the body, which include sodium selenite, depends on the dose, the size of the compounds of substances, and the combined effect with other biologically active substances. L. Li *et al.* (2024) found that the use of microencapsules with selenium and protein in mice did not lead to toxic effects on the body. Weight gains and growth of organs in mice of the experimental group were observed in comparison with the control group. In addition, the positive effect of selenoprotein was less accumulation in the liver and kidneys. Instead, the use of sodium selenite without proteins led to an increase in selenium content in the kidneys, liver, and other organs. M.K. Basher *et al.* (2024) found that due to the toxic effects of sodium arsenite on the body of female mice, the use of sodium selenite caused a protective effect against haematotoxic, biochemical changes, and toxic effects on organs.

One of the biologically active substances in the body is a water-soluble micronutrient – riboflavin (vitamin B_2), which is considered a precursor of nucleotides, synthesising protein structures of the inner membrane of mitochondria, forming the respiratory chain for the synthesis of ATP (energy) through oxidative phosphorylation, bind to enzymes through complex mechanisms, providing structural stability during the processes of dissimilation and catabolism. E.R. da Silva-Araújo *et al*. (2025) concluded that riboflavin is involved in the regulation of dissimilation and catabolism, ensuring cell growth and development, and the development of proteins, lipids, carbohydrates, and nucleic acids. S. Gomathi *et al*. (2026) proved in experimental studies on female rats whose diet was deficient in riboflavin, weight loss, and developmental delay in their offspring, including weight loss and increased mortality after weaning.

Thus, the study of drugs that include compounds that create an immunomodulatory effect, participate in the processes of energy metabolism, help reduce inflammation, fat deposition, and thereby reduce cholesterol in the body of laboratory mice and rats is an important stage of preclinical studies to obtain appropriate conclusions about their possible further use in productive animals, considering the optimal dose, sex, and age of animals. Thus,

the purpose of this study was to investigate the effect of the "Imun-depo" preparation on the growth and development of white laboratory mice, morphological and biochemical parameters of blood, and the viability of *Tetrahymena pyriformis* ciliates.

Literature Review

Biotechnological, pharmacological, and medical methods are widely used to assess the functional modulation of immune system components. The process of correction of the immune system can be used both in clinical practice and in basic research, the purpose of which is to investigate potential targets for modulating immunity, the body's response to the introduction of certain compounds, and the state of homeostasis. But there are a number of problems in immunomodulation that require the development of new effective and safe therapeutic compounds for the body (Strzelec *et al.*, 2023). S.M. Elhusseiny *et al.* (2022) conducted experimental studies on rats and proved the immunomodulatory activity of aqueous extracts from edible mushrooms of the family *Basidiomycota* that contain carotenoids, enzymes, minerals, proteins, tocopherols, alkaloids, lectins, terpenoids, and volatile compounds. The immunomodulatory effect was confirmed by the results of an increase in the number of lymphocytes, white blood cells, nitric oxide, lysozyme activity, and cytokines in the plasma of experimental animals. R.R. Goel *et al*. (2021) noted that from the list of known endogenous cytokines, interferons involved in the regulation of the immune response are among the most therapeutically useful in the treatment of various diseases. Interferons can be formed by functional units of the body in response to a foreign protein, and participate in cell growth, followed by an immunomodulatory effect.

An important role in immunomodulation belongs to the compound beta-carotene, which is a source of vitamin A, is involved in the regulation of the immune response by restoring the intestinal microbiome. H. Kuang *et al.* (2022) in an experimental study on mice found that had clinical allergic symptoms that beta-carotene is likely to lead to normalisation of intestinal microflora by increasing the number of beneficial bacteria, such as *Clostridiaceae* and reduce pathogenic bacteria, such as *Streptococcaceae*. A. Eroglu *et al.* (2018) pointed out that some of the important carotenoids are α-carotene, β-carotene, and β-cryptoxanthin, which act as precursors (provitamins) of vitamin A, have antioxidant and immunological properties, and serve as proxy biological markers of overall health. In addition, F. Zhou *et al.* (2020) found that retinoic acid, as the most active and biologically available form of vitamin A, can affect the reduction of triglyceride and cholesterol esters in cell cultures and in the body of mice in experimental studies. K.M.M. Koriem & M.S. Arbid (2018) found that an experimentally created favism in an experiment with rats (an acute form of haemolytic anaemia) suppressed blood proteins, and treatment of rats with β-carotene stopped blood cell damage and protein suppression. Furthermore, haematological parameters stabilised and the level of cholesterol in the blood serum of sick rats decreased.

Lipid metabolism and reduction of inflammation in the body depends on the dose and form of administration of drugs with selenium, preclinical studies of which are important for further use in mammals. M.L. Ojeda *et al.* (2022) investigated the effect of low doses of selenite and selenium nanoparticles on adipose tissue deposition, associated insulin secretion, and GPx1, IRS-1, and FOXO3a expression in immature rats. They found that when administered orally to rats with water of different doses of selenite, low levels of supplements contributed to increased adipogenesis, through the pathway of pancreatic peptide hormone signalling

and modulation of LCN2, while the introduction of a small amount of selenite prevented fat accumulation, through reduced pancreatic peptide hormone signalling and promoted cell renewal, reducing the inflammatory process. D. Kumar *et al*. (2025) showed that both low and high doses of sodium selenite applied to laboratory mice resulted in a significant ($p < 0.05$) decrease in haemoglobin concentration and cell mass volume, increased levels of aspartate aminotransferase, alanine aminotransferase, urea, and creatinine, which indicated toxicity to the liver and kidneys. An increased level of lipid peroxidation, enzymes, and a state of oxidative stress were established during repeated oral administration of similar doses of sodium selenite to rats. The micronucleus analysis revealed potential changes that indicated genotoxicity and chromosomal damage. Thus, the results showed the importance of compliance with the administration of certain specific doses of sodium selenite.

R. Li *et al.* (2025) found that arginine is one of the most metabolically adaptive amino acids that can adapt metabolism to environmental influences and changes in the internal environment of the body, providing an energy supply of muscle tissue, protein metabolism, and wound healing. T.S. Fung *et al.* (2025) noted that L-arginine is a key precursor for the synthesis of nitrogen-containing metabolites and promotes the elimination of excess nitrogen (urea) from the body, cellular signalling, and the accumulation of cellular energy. The body does not synthesise enough of this amino acid and the exchange of cellular energy depends on its exogenous intake. Therefore, it is important that animal feeding diets are balanced in the amount of arginine to maintain the health of the body, because its deficiency can contribute to the progression of diseases.

Dosage of drugs and their components remains the main task for achieving a positive effect on the body. Y. Wang *et al.* (2025) proved that a micronutrient such as copper is an important trace element for the animal body, excessive consumption of which leads to the accumulation of copper ions in tissues and organs, and has a toxic effect. Along with this, M.Y. Abbas *et al.* (2025) conducted a study on male white mice using low- and high-dose copper oxide nanomaterials and measured the amount of shaped blood elements and serum lipids, and the activity of liver and kidney enzymes. A high dose of copper oxide led to significant fluctuations in the content of white blood cells, haemoglobin and the number of red blood cells, which led to the conclusion that copper nanoparticles are toxic to the body when using different doses of the substance.

Thus, the analysis of scientific material presented in literature sources indicates that now the study of the effect of biologically active compounds on the body of experimental animals is widely used and is extremely relevant. Furthermore, the dose of the test substance, the age period of the animals used in the experiment, the degree of action of substances on the integral and haematological parameters of the body are considered.

Materials and Methods

The study was conducted in 2025 based on the immunological laboratory of the Bila Tserkva National Agrarian University and the Department of Animal and Food Hygiene named after Prof. A.K. Skorokhodko at the National University of Life and Environmental Sciences of Ukraine, based on the tasks set for scientific research on the topic: "Scientific and practical substantiation for the use of phytogenic feed additives in the production of meat from productive animals" (State registration number 0124U005020). To conduct an experimental study, a new "Imun-depo" preparation was used, which included the following substances: sodium selenite,

potassium selenite, riboflavin, total interferon (dry), L-arginine, zinc sulphate, copper sulphate, 0.25% beta-carotene solution in oil.

The study used 60 animal units of female (body weight at the beginning of the study was 11.0 g) white laboratory mice (*Mus Musculus L*), belonging to the conventional type (*Minimal diseases*). Two groups of laboratory animals were created: a control group (30 units) and an experimental group (30 units). In laboratory mice of the experimental group, the "Imun-depo" preparation at a dose of 1.0 mL/animal was injected through the oral cavity into the stomach using a metal probe. The experiment lasted for 60 days, starting from the 14-day age of white mice.

The experimental study was conducted in four stages. At the first stage, the conditions of keeping, feeding, and giving water to white laboratory mice were investigated. The study of indicators of the internal environment (microclimate) of the room was conducted three times during the research period. The main diet of the control and experimental groups of laboratory mice was formed from fresh crushed wheat and combined feed. Laboratory animals were provided with water from a nipple drinker. Indicators of drinking water for laboratory mice were within the limits of sanitary standards (Order of the Ministry of Health of Ukraine No. 400, 2010) (Table 1).

Table 1. Water quality and safety indicators

Indicators	Unit of measurement	DSanPiN 2.2.4-171-10	Actually
Organoleptic parameters			
Odour (at t = 20°C)	points	⩽ 2	1.7 ± 0.02
Turbidity	mg/dm^3	⩽ 1.0	0.6 ± 0.02
Colour	degrees	⩽ 20.0	15.3 ± 0.07
Taste and aftertaste	points	⩽ 2	1.5 ± 0.17
Physicochemical parameters			
Hydrogen index	pH units	6.5-8.5	6.6 ± 0.04
Overall hardness	mmol/dm^3	⩽ 7.0	6.2 ± 0.24
Chlorides	mg/dm^3	⩽ 250	150.0 ± 4.8
Nitrates	mg/dm^3	⩽ 50	16.3 ± 4.87
Nitrites	mg/dm^3	⩽ 0.5	0.4 ± 0.03
Manganese	mg/dm^3	0.1	0.09 ± 0.002
Sulphates	mg/dm^3	⩽ 250	27.6 ± 1.8
Total Iron	mg/dm^3	⩽ 0.2	0.04 ± 0.01
Epidemic safety indicators			
Common coliforms	CFU/100 cm^3	absent	-
Intestinal helminths	Cells, eggs, larvae, in 50 dm^3	absent	-

***Source**: compiled by the authors*

The room in which the white laboratory mice were kept was typical, built of brick, one-story, the place was well lit by the sun and was located on a flat area. Natural and artificial lighting was used – fluorescent lamps. The flow of fresh air into the room for keeping mice was carried out through a door and a transom. The standards for keeping white laboratory mice in metal cages and the physical indicators of the internal environment of the room in which the laboratory mice were kept met the requirements for sanitary and hygienic condition (SOU 85.2-37-736:2011, 2011) (Table 2). To assess the conditions of keeping white laboratory mice, the following were measured: room temperature

(mercury thermometer, degrees Celsius); relative humidity, % (psychrometric hygrometer VIT-2 (Ukraine)); air velocity, m/s (ball catathermometer (Ukraine)); carbon dioxide concentration, % (gas analyser WT8807 WINTACT (China)); ammonia and hydrogen sulphide, mg/m^3 (Wintact WT8823 gas analyser (China)); illumination, lux (digital lux meter photometer HS1010 (China)). The study of indicators of the internal environment of the room was carried out three times – before the start of the study and on day 30 and day 60.

Table 2. Indicators of the internal environment in the room for keeping laboratory mice

Indicators	Sanitary and hygienic standards	Actual indicator
Temperature, ^{0}C	20-24	22.4 ± 1.13
Relative humidity, %	55	55.6 ± 2.14
Air velocity, m/s	0.3	0.28 ± 0.03
Concentration of CO_2, %	0.15	0.15 ± 0.009
Concentration of NH_3, mg/m^3	10	0.07 ± 0.02
Concentration of H_2S, mg/m^3	5	1.6 ± 0.2
Illumination, lux (1 m above the floor level)	200	198.2 ± 0.35
Photoperiod, h (light: darkness)	12:12	12:12
Air exchange (times/hour)	10-15	11

***Source**: compiled by the authors*

During the second stage of the experiment, the effect of the "Imun-depo" preparation on the behaviour of laboratory mice, the dynamics of body weight, and the weight of internal organs – the liver, thymus, spleen, kidneys and thyroid gland – was investigated. To determine the body weight of the mice, they were weighed on WALCOM LB3002 laboratory scales (Taiwan). Subsequently, the mice were anaesthetised by the intraperitoneal administration of a 2% solution of Xylazine (Netherlands), followed by decapitation using a DCAP guillotine (Germany). The thoracic and abdominal cavities of the mice were then opened using a Surgiwell surgical scalpel (Pakistan) and an Adson-Brown forceps (Pakistan). Internal organs were extracted using an anatomical Adson forceps (Germany) and Asanus MN0654 micro-scissors (Germany). Between extractions, the instruments were washed in physiological saline and dried. The removed organs were placed in Petri dishes filled with physiological saline. Prior to weighing, the internal organs were blotted with filter paper, and the weighing was conducted swiftly to prevent drying of the organs. For weighing, WALCOM LB3002 laboratory scales (Taiwan) were used. Body weight gain in the laboratory mice was compared with the initial body weight at the start of the experiment and at 14, 30, and 60 days of the experiment.

At the third stage of the study, haematological parameters of the body of white laboratory mice were determined. The material for haematological studies was peripheral blood, which was taken on day 60 of the experiment. The following parameters were measured using the DH33 Vet automated haematology analyser (Japan): red blood cell count (RBC, 10^{12}/L), haemoglobin concentration (HGB, g/L), haematocrit level (HCT), white blood cell count (WBC, 10^9/L), neutrophils (NEUT, %), lymphocytes (LYMPH, %), basophils (BASO, %), eosinophils (EO, %), and monocytes (MONO, %). Biochemical parameters of blood serum were evaluated on an automatic biochemical analyser BA-88A Mindray (China). The fourth stage of the

experiment was devoted to the investigation of the effect of the "Imun-depo" preparation on the vital activity of the *Tetrahymena pyriformis* ciliates. A laboratory strain (WH_{14}) was used to conduct the work. Cells of *Tetrahymena pyriformis* ciliates were divided into control and experimental groups, with the number of cells in each group – 10^4 in 1 cm^3 environments. Ciliate cells belonging to the control group were filled with a solution of sea salt with a mass concentration of 0.56%, and the experimental group was filled with a solution of the "Imun-depo" preparation at a dose of 1.0 cm^3 in chemical flasks and placed in a thermostat at a temperature of 37°C. The study was conducted in three repetitions.

The experimental study was conducted in accordance with the ARRIVE recommendations (n.d.) in accordance with Law of Ukraine No. 3447-IV (2006) and in accordance with the requirements of the international principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (1986) and the Order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 (2012). Statistical processing of the results was performed using the table processor "Microsoft Excel". The following were calculated: arithmetic mean and average error (M ± m), confidence level (P) according to Student, and significance criteria – $P < 0.05$; $P < 0.01$; $P < 0.001$.

Results and Discussion

Monitoring of the animal behaviour during experimental studies was a key indicator for identifying adaptive responses to the environment, manifestations of instincts, social interaction, and the ability to perform assigned tasks. Analysis of the parameters of integral behaviour of laboratory mice, such as horizontal and vertical motor activity, "mink reflex", integral activity provides an opportunity to investigate the physiological state of the animal and possible manifestations of anxiety or apathy, the level of activity or stress under the influence of the "Imun-depo" preparation. According to the data obtained (Table 3), no significant changes in body temperature, horizontal and vertical motor activity, integral activity and "mink reflex" between the animals of the experimental and control groups were detected during the use of the "Imun-depo" preparation in white laboratory mice.

Table 3. Indicators of behaviour of laboratory mice after the use of the "Imun-depo" preparation, M ± m, n = 30

Indicators	Experimental group	Control group
Body temperature, °C	38.1 ± 0.36	38.2 ± 1.15
Horizontal motor activity	34.8 ± 1.48	34.5 ± 1.12
Vertical motor activity	18.6 ± 1.2	18.3 ± 1.5
"Mink reflex"	13.6 ± 2.04	13.0 ± 2.02
Integral activity	66.0 ± 1.98	66.3 ± 1.76

***Source**: compiled by the authors*

The body weight of laboratory mice is a key indicator of their health and physiological state. Changes in body weight and internal organs allow analysing the experiment as a whole, critically assessing the keeping conditions, feeding ration, dose of the drug, which may affect the further conduct of the study or reduce its duration. Indicators of body weight and internal organ mass of white laboratory mice after administration of the "Imun-depo" preparation are presented in Table 4.

Table 4. Body weight and internal organ weight of white laboratory mice after administration of the "Imun-depo" preparation, m ± m, n = 30

Indicator	Day of research			
	Beginning of the experiment	14	30	60
Body weight, g	11.0 ± 0.85 11.0 ± 0.64	14.0 ± 0.74 15.0 ± 0.85▲	17.0 ± 0.94 20.0 ± 0.79*	22.1 ± 1.03 25.6 ± 1.08*
Average daily weight gain, mg	–	214.0 ± 1.06 285.0 ± 1.30**	187.5 ± 1.72 312.0 ± 1.56**	170.0 ± 1.94 186.7 ± 1.89**
Thymus weight, mg	–	–	–	31.2 ± 2.89 33.6 ± 2.54
Liver, g	–	–	–	1.12 ± 0.02 1.17 ± 0.03
Spleen, g	–	–	–	0.18 ± 0.04 0.18 ± 0.03
Thyroid gland, mg	–	–	–	4.15 ± 0.02 4.17 ± 0.04
Kidneys, g	–	–	–	0.15 ± 0.06 0.16 ± 0.07

***Note**: * – P < 0.05; ** – P < 0.001 compared to the indicators in the control group mice; ▲ – P < 0.001 compared to the indicator at the beginning of the study. Indicators of the control group are presented in the numerator, the experimental group – in the denominator*
***Source**: compiled by the authors*

As a result of experimental studies, it was found that on the day 14 after administration of the "Imun-depo" preparation in laboratory mice of the experimental group, their body weight increased by 36.3% (P < 0.001) compared to the start of the study, and on day 30 – by 17.6% (P < 0.05), on day 60 – by 15.8% (P < 0.05) compared to the indicators in mice in the control group. The animals actively ate food, were active, and no negative reaction was observed in the mice of the experimental group during intragastric administration of the drug. The average daily weight gain on day 14 increased by 33.2% (P < 0.001), on day 30 – by 66.4% (P < 0.001), and on day 60 of the study – by 9.82% (P < 0.001) compared to the indicator in the control mice. The weight indicators of the studied internal organs of white laboratory mice of the control and experimental groups did not significantly change. According to the physiological state, the studied internal organs corresponded to the physiological norm for sexually mature white laboratory mice.

One of the limiting factors for the body of white laboratory mice, after applying the "Imun-depo" preparation, may be the effect of zinc and copper. On this occasion N.V. Grigorova (2025) pointed out that a deficiency of zinc, magnesium, and copper in the body leads to gradual atrophy of the thymus and to a significant violation of the processes of cellular and humoral immunity. However, as a result of experimental studies, it was found that the introduction of the "Imun-depo" preparation at a dose of 1.0 ml/animal did not cause symptoms of toxic effects on the body of white laboratory mice, due to the combined action of the substances included in the drug. The greatest degree of positive effect on the body of laboratory mice can be created by a 0.25% solution of β-carotene in oil, which is part of the "Imun-depo" preparation. T. Breniere *et al.* (2024) evaluated the effect of tomato supplements of various genotypes and the content of caratinoids on the body condition of experimental mice. The IL6-2 tomato genotype, which

contains a significant amount of β-carotene, was found to reduce obesity in mice and contribute to weight gain.

In the future, the effect of the "Imun-depo" preparation on the indicators of the number of cellular elements in the blood of laboratory mice was determined. The study of the effect of drugs on the blood status of animals plays an important role in predicting possible side effects on morphological and biochemical haematological parameters (Table 5). When evaluating the effect of biologically active drugs, the following risks can be assumed: toxic effects on blood cells and organs, liver and kidney damage, oxidative stress, allergies, gastrointestinal disorders, and overdose. Therefore, the analysis of the effect of the "Imun-depo" preparation is extremely important in conducting preclinical studies.

Table 5. Morphological parameters and leukocyte formula of white mouse blood, M ± m, n = 30

Indicators, measuring units	Control group	Experimental group
Haemoglobin (HGB), g/l	110.56 ± 6.24	127.12 ± 5.02*
Red blood cells (RBC), 10^{12}/l	2.23 ± 0.15	2.85 ± 0.06**
Haematocrit (HCT), %	42.5 ± 1.48	47.9 ± 1.43*
White blood cells (WBC), 10^{9}/l	6.60 ± 0.40	6.80 ± 0.50
Rod neutrophils (NEUT), %	2.90 ± 0.28	3.12 ± 0.19
Segmentonuclear neutrophils (NEUT), %	19.72 ± 1.46	18.63 ± 2.39
Basophils (BASO), %	0.4 ± 0.002	0.4 ± 0.004
Lymphocytes (LYMPH), %	50.0 ± 4.54	51.0 ± 3.22
Eosinophils (EO), %	2.54 ± 0.21	2.12 ± 0.17
Monocytes (MONO), %	5.89 ± 2.38	5.83 ± 1.06

***Note**: * – P < 0.05; ** – P < 0.001 compared to the indicators in the control group mice*
***Source**: compiled by the authors*

According to the obtained morphological parameters of white laboratory blood, it follows that the "Imun-depo" preparation led to the activation of erythrocytopoiesis processes. On day 60 of the study, the concentration of haemoglobin in the blood of mice in the experimental group increased by 15.0% (P < 0.05), and the number of red blood cells – by 27.8% (P < 0.001) compared to mice in the control group. Simultaneously, the haematocrit level increased by 5.4% (P < 0.05) compared to those in the control mice. There was no significant difference in the content of white blood cells, rod-shaped and segmentonuclear neutrophils, basophils, lymphocytes, eosinophils, and monocytes in the blood of mice of the experimental and control groups. Finding out the effect of the "Imun-depo" preparation on the body of laboratory mice, a component attracted attention – copper sulphate, which has bactericidal properties, promotes the digestion of feed nutrients, and improves intestinal function. A.A. Al-Saghee *et al.* (2023) found in an experiment that the addition of copper nanoparticles to rabbits contributed to better feed intake, increased protein content, haematocrit, and haemoglobin levels in the blood compared to other forms of copper. As a result, an increased nutritional value was found when rabbits were fed a diet with added copper.

Thus, the use of the "Imun-depo" preparation at a dose of 1.0 mg/animal intragastrically to white laboratory mice led to the activation of erythrocytopoiesis processes and the maintenance of myeloid and lymphoid chains of haematopoiesis of cells, and also indicated the absence of viral or bacterial diseases, stress, immunodeficiency or the effect of toxins on the body of mice during the study. Along with

morphological parameters of blood, important markers that can be used to confirm the level of metabolic transformations in the body, including in laboratory mice during the experiment, are biochemical parameters of blood serum (Table 6).

Table 6. Morphological parameters and leukocyte formula of white mouse blood, M ± m, n = 30

Indicators, measuring units	Control group	Experimental group
Total protein (T-Pro), g/L	56.3 ± 1.23	60.8 ± 1.06**
Albumins (ALB), %	47.2 ± 3.18	54.0 ± 2.10
Globulins (Glob), %	42.8 ± 0.34	49.6 ± 0.62***
Alanine aminotransferase (ALT), IU/L	24.6 ± 0.6	26.3 ± 0.4*
Aspartate aminotransferase (AST), IU/L	44.8 ± 1.2	45.0 ± 0.03
Total cholesterol, mmol/L	2.56 ± 0.14	2.01 ± 0.12*

***Note**: * – P < 0.05; ** – P < 0.001 compared to the indicators in the control group mice*
***Source**: compiled by the authors*

As can be seen from the table, the effect of using the "Imun-depo" preparation at a dose of 1.0 mg/animal led to an increase in the blood serum of white laboratory mice in total protein by 8.0% (P < 0.01) and globulin by 6.8% (P < 0.001) compared to the indicators in the control mice. It can be assumed that an increase in the content of total protein and globulin (within the physiological norm) are the main factors for increasing the energy of growth and development and increasing the activity of humoural immunity in mice of the experimental group. Therefore, it can be assumed that it is the content of sodium selenite and potassium in the composition of the "Imun-depo" preparation that can lead to an improvement in the state of immunity in laboratory mice. J. Zhang *et al.* (2021) determined the immunomodulatory effects of selenium-rich soy peptides (Se-SPep) in an experiment on mice with cyclophosphamide-induced immunosuppression. They found that injecting selenium into mice maintained proper body weight, total protein levels, albumin, and white blood cells, and stimulated the production of interleukin-2, interferon-gamma, and nitric oxide. These results confirmed that Se-SPep is an effective immunomodulator.

In addition, the content of L-arginine in the composition of the "Imun-depo" preparation affected the improvement of immunity, especially during the period of intensive growth and development of the body. The use of supplements in productive animals containing arginine improved the functional state of the body in other studies. In particular, B. Tan *et al.* (2009) showed that arginine supplementation in pig feeding resulted in a 6.5% (P < 0.05) increase in body weight and skeletal muscle content in the carcass – by 5.5%, while reducing the fat content in the carcass by 11% (P < 0.01). The increase in pig body weight occurred with an increase in protein, glycogen, and fat content in the longest back muscle by 4.8, 42, and 70% (P < 0.05), respectively. Based on the results of the study, researchers concluded that arginine supplements to feeding pigs contributed to weight gain while reducing the accumulation of fat in the carcass. The longest back muscle, which is the most commercially valuable, accumulated protein and other nutrients. A.O. Oso *et al.* (2017) found that feeding arginine supplements to turkeys of different ages and doses affected an increase in the number of red blood cells (P < 0.001), lymphocytes (P < 0.001), and basophils (P < 0.001), total

protein, globulin concentrations, and a decrease in serum enzyme levels. Thus, it was experimentally confirmed that the addition of arginine to feed fattening turkeys led to an increase in haematocrit volume, an improvement in serum parameters, and an increase in the mass of the thymus, spleen and a decrease in the number of salmonella in the small intestine.

In the current study, a significant difference between the amount of albumins in the blood serum of mice of the control and experimental group was not established, although there was some increase in them within the reference values, which may indicate the regulation of oncotic pressure and the transfer of lipids, hormones, and drugs in the bloodstream. The "Imun-depo" preparation also affected the activity of enzymes of the transferase class. It was found that in laboratory mice of the experimental group, the activity of alanine aminotransferase increased by 6.9% ($P < 0.05$), within the physiological norm, compared with the indicator in mice of the control group. However, there was no significant difference in the activity of aspartate aminotransferase between the indicators in the control and experimental mice. An increase in the enzymatic activity of alanine aminotransferase in the blood serum of laboratory mice within the physiological norm indicated activation of the body's energy and plastic needs.

The content of total cholesterol in the blood serum of mice in the experimental group decreased by 21.5% ($P < 0.05$) compared to the indicator in mice in the control group. It is possible to explain the significant decrease in the content of total cholesterol in the blood serum of mice of the experimental group, based on the fact that the composition of the "Imun-depo" preparation includes the essential amino acid L-arginine. B. Tan *et al*. (2012) found that L-arginine is a precursor of nitric oxide and affects the regulation of the metabolism of fatty acids, proteins, amino acids at the cellular level with the emergence of DNA transcription and translation processes, with the stimulation of lipolysis and activation of fatty acid oxidation to carbon dioxide and water. These processes are also associated with the regulation of metabolic pathways to optimise the formation of ATP and the use of energy substrates (glucose, fatty acids) towards reducing the content of triacylglycerols. Therefore, arginine supplementation affected the reduction of fat in subcutaneous tissue, omentum, and around internal organs in diabetic rats and obese rats in the experiment.

In addition, the content of 0.25% β-carotene solution in oil in the "Imun-depo" preparation can help reduce the cholesterol content in the blood serum of laboratory mice. A.M. Abd El-Hady *et al*. (2022) found that a supplement containing β-carotene and zeaxanthin was applied to productive animals (broiler chickens) – *Spirulina platensis*, has lipid-lowering properties. In addition, an increase in the number of red blood cells, haemoglobin, white blood cells, and lymphocytes in the bird's blood was observed ($P < 0.05$) compared to the control indicators. N. Melnikov *et al*. (2022) proved in male mice that vitamin A and carotenoids are involved in the regulation of adipose tissue metabolism and inflammation. The concentration of cholesterol and triglycerides in the blood plasma of mice fed excess fat and supplemented with β-carotene decreased by 30% and 28% compared to mice fed a diet high in fat and supplemented with vitamin A. Thus, the presence of β-carotene in the feed, rather than vitamin A, led to a decrease in the number of monocytes in the bloodstream, which indicated a decrease in inflammation in the body and indicators of fat metabolism in blood plasma.

A rather urgent scientific problem was the investigation of the influence of the environment on the vital activity of both the whole organism and its individual components: functional systems, organs, tissues, and cells.

Conducting research to confirm the degree of environmental impact on the body is a rather complex methodological process, which creates problems for high-performance assessment of the toxicity of numerous chemicals (Sun *et al.*, 2026). One way to solve this problem is to conduct experiments on cell cultures. Their aetiology is limited to a specific organ or tissue, and the range of functions of these cells is quite narrow and specific. Therefore, the use of *Tetrahymena pyriformis*, a representative of protozoa, is unique for such studies. Morphologically, it is a cell, but physiologically, it is a complete organism. These cells are easy to use and do not require special costs for maintaining the vital activity of the culture and conducting research. In addition, due to their short life span and high reproductive capacity, they allow tracking changes in physiological processes over several generations in a short time and are very sensitive to toxins (Yu *et al.*, 2024). The current study monitored the vital activity of *Tetrahymena pyriformis* ciliates under the action of the "Imun-depo" at a dose of 1.0 cm^3 (Table 7).

Table 7. Effect of the drug "Imun-depo" on the vital activity of Tetrahymena pyriformis ciliates, M ± m, n = 3

Observation via:	Control group	Experimental group	Number of dividing cells
1 min	30.60 ± 1.13	30.65 ± 1.10	-
30 min	30.60 ± 1.25	30.59 ± 1.16	-
1 h	30.60 ± 1.10	30.64 ± 1.18	-
2 h	30.50 ± 1.18	30.71 ± 1.12	-
3 h	30.50 ± 1.15	34.15 ± 1.50	8.00 ± 0.06
4 hours	30.60 ± 1.17	34.20 ± 1.60	8.00 ± 0.05
5 h	30.50 ± 1.16	34.29 ± 1.70	8.00 ± 0.06
6 h	30.60 ± 1.23	35.62 ± 1.14*	10.00 ± 0.05
12 h	30.50 ± 1.22	35.92 ± 1.17*	12.00 ± 0.04
24 h	30.60 ± 1.33	35.84 ± 1.14*	14.00 ± 0.09
48 h	30.42 ± 1.26	35.58 ± 1.17*	14.00 ± 1.03
72 hours	30.78 ± 1.32	35.87 ± 1.13*	16.00 ± 1.09
96 h	30.20 ± 1.12	35.17 ± 1.03*	16.00 ± 1.10
120 h	30.40 ± 1.09	35.19 ± 1.24*	16.00 ± 1.08
144 h	30.50 ± 1.22	35.28 ± 1.14*	16.00 ± 1.11
168 h	30.40 ± 1.19	35.18 ± 1.04*	16.00 ± 0.92
192 h	30.20 ± 1.16	35.14 ± 1.32*	16.00 ± 0.84

***Note**: * – P < 0.05 compared to the control group*

***Source**: compiled by the authors*

Consistent with the results obtained, the "Imun-depo" preparation did not cause any deviations from the normal growth and development of the *Tetrahymena pyriformis* ciliates. Three hours after the start of the experiment, active division of ciliate cells of the control and experimental groups was observed. A significant increase in the number of ciliate cells began at 6 hours in the study and lasted until the end of the study – up to 192 hours. Thus, after 6 hours of the experiment, the number of ciliate cells in the bacteriological dish with the drug increased by 16.4%, at 12 hours – by 17.8%, at 24 hours – by 17.1%, at 48 hours – by 17.0%, at

72 hours – by 16.5%, at 96 hours – by 16.4%, at 120 hours – by 15.9%, at 144 hours – by 15.7%, at 168 hours – by 15.7%, and at 192 hours – by 16.3% ($P < 0.05$) compared to the indicators of ciliate cells of the control group.

Investigating the functional parameters of the *Tetrahymena pyriformis* ciliates, it was found that the cells assigned to the control and experimental groups moved in a straight line and energetically. During the entire follow-up period, no slowing of cell growth was detected, which would indicate a decrease in their vital activity due to the presence of toxic substances in the environment. There were no changes in the nature of movement, cell morphology, or the appearance of abnormal shapes. The paramecium cells were densely clustered, moving actively and in a straight line. They were oval and elongated in shape, with a moderate amount of filling. No cell death was observed during the study period. All this gives grounds to assert the harmless effect of the "Imun-depo" preparation at a dose of 1.0 cm^3 per living cell. Slowing down the process of ciliate division with increasing exposure time indicated a time dependence of the stimulating effect of the drug. Thus, according to the results of the study, it was established that the "Imun-depo" preparation activated the vital processes of the ciliary infusoria, based on the increase in dividing cells from 3 to 192 hours of the study. The drug did not have a mutagenic effect on protozoan cells. In a similar study of another immunomodulatory drug, "Biomagn", researchers O.M. Chechet *et al*. (2021) also found no toxic effects on infusoria culture of *Tetrahymena pyriformis* within 30 minutes, with 100% preservation of cell vital activity. The behaviour of this cell population can be an evaluation criterion for an immunostimulating drug, which is important for studying the environment. physiological and medical aspects of the sensitivity and stability of living systems to anthropogenic factors.

Thus, the preclinical experimental study showed that the "Imun-depo" preparation has a positive effect on the physiological state of white laboratory mice, stimulating their growth and development without negatively affecting important haematological and morphological parameters while maintaining 100% survival of mice during the experiment. Simultaneously, the use of the drug did not reveal toxic or mutagenic effects on the cells of *Tetrahymena pyriformis*, which confirms its safety and potential for further use in veterinary and biomedical research. These results open up prospects for the use of "Imun-depo" preparation as an effective and safe immunomodulator in various fields of science.

Conclusions

The effect of the drug "Imun-depo" at a dose of 1.0 mL/animal on body weight and internal organs, morphological and biochemical parameters of the blood of white laboratory mice, and on the vital activity of *Tetrahymena pyriformis* ciliates from 14 to 60 days of the experiment were investigated. The parameters of the internal environment in the room for keeping laboratory mice and the indicators of drinking water for their drinking were evaluated and full compliance with the approved standards was established. Administration of the "Imun-depo" preparation at a dose of 1.0 mL/animal into the stomach of laboratory mice of the experimental group contributed to an increase in their body weight compared to mice of the control group, observed from day 14 (by 36.3% ($P < 0.001$)), on day 30 (by 17.6% ($P < 0.05$)) and up to day 60 of the experiment (by 15.8% ($P < 0.05$)). Furthermore, the average daily weight gain of white laboratory mice in the experimental group on day 14 of the experiment increased by 33.2% ($P < 0.001$), on day 30 – by 66.4% ($P < 0.001$), and on day 60 – by 9.82% ($P < 0.001$) compared to the control. On day 60 of the experiment, a

significant difference in the weight of internal organs of white laboratory mice of the experimental and control groups was not determined. The drug helped to stimulate the development of red blood cells in the body of experimental mice, which was manifested by an increase (within the physiological norm) in the number of red blood cells by 27.8% ($P < 0.001$); an increase in the concentration of oxygen-carrying protein in the body – haemoglobin by 15.0% ($P < 0.05$), and the percentage volume of shaped elements – haematocrit by 5.4% ($P < 0.05$). The effect of the drug on the body of white laboratory mice of the experimental group led to an increase in the total protein content by 8.0% ($P < 0.01$) and globulin by 6.8% ($P < 0.001$) (within the physiological norm), which indicated an increase in the activity of humoural immunity. An increase in the enzymatic activity of alanine aminotransferase in the blood serum of laboratory mice by 6.9% ($P < 0.05$) was established, which indicated stimulation of catabolism and anabolism processes in the body, and activation of enzymatic and hormonal regulation and energy charge of cells. Instead, the total cholesterol content decreased by 21.5% ($p < 0.05$) in the blood serum of white laboratory mice in the experimental group. During the study of the vital activity of the *Tetrahymena pyriformis* ciliates, no toxic effects of the drug "Imun-depo" have been established. In further studies, it is planned to conduct an experiment on the use of the "Imun-depo" preparation with food for productive animals to find out the effect of the drug components on maintaining the physiological status of the body and preventing the risks of morbidity.

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Conflict of Interest

None.

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Оцінка впливу біологічно активного препарату на розвиток організму лабораторних тварин

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Анотація. Дослідження дії на організм лабораторних тварин новостворених препаратів, що містять у своєму складі декілька сполук, є важливим етапом наукового обгрунтування їх ефективної дози і нешкідливості. Метою цієї роботи було вивчити вплив нового препарату «Імун-депо» на розвиток організму лабораторних тварин (білих мишей) та їх гематологічні показники, а також життєздатність клітин інфузорії *Tetrahymena pyriformis*. Матеріалом дослідження слугували самки білих лабораторних мишей поліпшеного ковенціального типу у кількості 60 голів (по 30 голів у контрольній і дослідній групі). Препарат білим лабораторним мишам вводили внутрішньошлунково у дозі 1,0 мл/гол. з 14 добового віку впродовж 60 діб. Застосовували комплекс методів для оцінки стану внутрішнього середовища у віварію, якості питної води, динаміки маси тіла та внутрішніх органів, поведінкових реакцій мишей, гематологічних показників їх організму та життєздатності інфузорії *Tetrahymena pyriformis*. З'ясовано, що показники внутрішнього середовища у віварію і питної води, якою напувались миші, знаходились

у межах нормативних вимог. Встановлено, що препарт у дозі 1,0 мл/гол. впливав на прирости маси тіла, а маса тимусу, щитоподібної залози, нирок, печінки та слезінки не змінювались. Доведено, що у крові білих лабораторних мишей збільшувався (у межах референтних значень) вміст гемоглобіну, кількість еритроцитів і рівень гематокриту. При цьому, показники лейкограми крові мишей не змінювались. У сироватці крові відбулося збільшення (у межах референтних значень) вмісту глобулінів, загального білка, активності аланінамінотрансферази та зменшення – загального холестерину. За дією препарату «Імун-депо» на життєздатність клітин інфузорії *Tetrahymena pyriformis* констатували відсутність токсичного впливу. Науково обгрунтовані отримані результати впливу препарату «Імун-депо» на організм білих лабораторних мишей є необхідними для подальшого проведення експериментальних досліджень із застосуванням препарату продуктивним тваринам як кормової добавки до основного раціону

Ключові слова: білі миші; умови утримання; динаміка маси тіла; морфологія і біохімія крові; інфузорія *Tetrahymena pyriformis*; препарат «Імун-депо»

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Autonomic regulation of heart rate in sows during the farrowing period

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Abstract. Contemporary research demonstrates the significant influence of the autonomic nervous system on the adaptation and productivity of farm animals. The farrowing period in sows is particularly critical, as changes in autonomic tone determine the course of parturition stress and the rate of recovery. The aim of the study was to investigate the dynamics of variational pulsometry

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parameters in sows with normotonic, sympathicotonic and vagotonic autonomic nervous system tone before farrowing, as well as one day and five days after parturition stress. The experiment was conducted on fifteen sows of the Large White breed, divided into three groups according to the level of the stress index. For each animal, one day before farrowing, and on the first and fifth days after farrowing, one hundred consecutive intervals between heartbeats were recorded using an electrocardiograph. The mean interval duration, interval mode, mode amplitude, range of variation, vegetative balance index and stress index were calculated. It was found that prior to farrowing, vagotonic animals exhibited the highest heart rate variability, whilst sympathicotonic animals exhibited the lowest. One day after parturition stress, a decrease in the mean duration of intervals and an increase in the stress index were noted in all groups; however, the fastest recovery of parameters was observed in vagotonic sows. On the fifth day after farrowing, the parameters in sows of the normotonic group approached baseline values, whereas in animals with increased sympathetic tone, the stress response persisted. Thus, the use of variational pulse wave analysis to assess the tone of the autonomic nervous system prior to farrowing allows for the identification of the most stress-resistant sows and the implementation of targeted supportive measures in the first days after farrowing. This approach will contribute to improving piglet survival, shortening the recovery period for sows and increasing the economic efficiency of pig farming

Keywords: variational pulsometry; autonomic nervous system; parturition stress; adaptive mechanisms; pigs

Introduction

Under conditions of the intensification of pig production and increased requirements for animal productivity and welfare, the study of physiological mechanisms of adaptation in sows during critical periods of ontogenesis has become particularly relevant. Farrowing represents one of the most stressogenic stages of the reproductive cycle, accompanied by considerable neuroendocrine load and capable of significantly affecting both the condition of the maternal organism and the viability of the offspring. Individual differences in the functional state of the autonomic nervous system determine the variability of responses to parturition stress and the rate of post-farrowing recovery; however, these aspects remain insufficiently considered in livestock practice. Thus, the application of methods for assessing autonomic regulation of heart rhythm may allow the prediction of stress resistance in sows and the optimisation of management decisions aimed at improving the efficiency and sustainability of production.

The autonomic nervous system (ANS) plays a decisive role in the formation of adaptive responses in the organism of sows under various stress factors, particularly during farrowing. The ANS consists of two antagonistic branches – the sympathetic and the parasympathetic nervous systems – which, through neuroendocrine interactions, respond rapidly to external and internal stimuli. K. Steinerová *et al*. (2025) noted that under resting conditions parasympathetic tone predominates, maintaining a lower heart rate and promoting energy conservation. L. Stovbetska *et al*. (2021) reported that with the onset of stress, particularly acute pain or emotional stress during labour contractions, the sympathetic nervous system becomes activated:

the secretion of catecholamines (adrenaline and noradrenaline) increases and cortisol is released into the bloodstream. M.E. Lagoda *et al.* (2022) and N. Manu *et al.* (2021) indicated that this mechanism mobilises energy resources, increases heart rate and arterial pressure; however, an excessively pronounced sympathetic response may be accompanied by impaired uterine perfusion, prolonged labour and even acute foetal hypoxia. R. Lemery (2024), in a historical review of the development of the concept of cardiac autonomic regulation, demonstrated how ideas about sympathetic and parasympathetic interaction evolved gradually from early anatomical studies to modern multidisciplinary approaches.

Analysis of heart rate variability (HRV) is a widely accepted non-invasive method for assessing the state of the ANS in animals and is recognised as one of the most informative approaches for determining indicators of the functional state of the ANS in farm animals (Ishaque *et al.*, 2021). A decrease in HRV indicators within the low-frequency (LF) and high-frequency (HF) spectra indicates the predominance of sympathetic activity, whereas the predominance of parasympathetic activity is reflected in an increase in the HF component and in the overall SDNN index (standard deviation of NN intervals) (Tiwari *et al.*, 2021). Considering that parturition stress in sows is accompanied by abrupt changes in autonomic tone and may significantly influence piglet survival and the economic efficiency of production (Von Borell & Raoult, 2024), further investigation of these processes is necessary. According to J. Calderón-Amor *et al.* (2024), chronic stress during pregnancy in sows, resulting from unfavourable microclimatic conditions or hierarchical interactions, is associated with suppression of HRV and elevated cortisol concentrations long before farrowing, which negatively affects subsequent reproductive performance. Complicated farrowing may prolong labour due to impaired coordination of uterine contractions: high levels of catecholamines reduce the effectiveness of oxytocin and hinder the movement of piglets through the birth canal (Buckley *et al.*, 2023). Existing studies by Ukrainian researchers are mainly focused on investigations of young pigs (Todoryuk *et al.*, 2023). However, issues related specifically to comparative analysis during the farrowing period remain insufficiently studied. Despite numerous investigations conducted in different animal species, information concerning sows with different baseline ANS tone in the context of parturition stress and post-farrowing recovery remains limited. It is therefore necessary to determine how differences in the initial ANS tone (normotonia, sympathicotonia and vagotonia) influence the recovery of animals.

Thus, based on the analysis of the available literature, there was a need to conduct studies comparing the dynamics of HRV in sows with normotonic, vagotonic and sympathicotonic ANS tone during critical periods of pregnancy and after farrowing. Such research will allow the development of scientifically substantiated methods for the selection and management of animals with the highest potential for stress resistance. The aim of the study was to determine the characteristics of the dynamics of key indicators of variational pulsometry in sows with different baseline ANS tone before farrowing, as well as on the first and fifth days after parturition stress.

Materials and Methods

The study was conducted during 2024-2025 at the Department of Vertebrate Physiology and Pharmacology of the National University of Life and Environmental Sciences of Ukraine. Experimental procedures involving animals were carried out in accordance with the fundamental principles of bioethics and complied

with Law of Ukraine No. 3447-IV (2006), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (1986), Directive 2010/63/EU (2010), Law of Ukraine No. 249 (2012), and the ARRIVE (n.d.) guidelines for reporting experiments involving live animals.

The research was carried out on 15 Large White sows (second-third farrowing, aged 2.5-3.5 years, body weight 180-210 kg), selected from a total of 40 animals using a preliminary analysis of the stress index (SI) according to Baevsky's method modified for animals (Todoryuk *et al.*, 2023). The selected animals were divided into three groups (n = 5): normotonic – NT (SI 80-100 conventional units), sympathicotonic – ST (SI > 150 conventional units) and vagotonic – VT (SI < 50 conventional units).

The study material consisted of short-term electrocardiogram recordings (up to 5 min) obtained one day before farrowing and on the first and fifth days after farrowing, using a Heaco 300G MDT electrocardiograph (manufacturer: HEACO, UK) with a tape speed of 50 mm/s. Key indicators of heart rate variability were calculated based on the filtered intervals: Mean RR (mean R-R interval), Mo (mode), AMo (amplitude of mode), variability range (Δx), and the vegetative balance index (VBI):

$$VBI = AMo/\Delta x, \quad (1)$$

where AMo – the mode amplitude; Δx – the difference between the maximum and minimum mode values (s).

And also the SI stress index:

$$SI = AMo/(2 \times Mo \times \Delta x), \quad (2)$$

where AMo – mode amplitude; Mo – a variational pulsometry parameter; Δx – the difference between the maximum and minimum values of the mode (s).

Statistical analysis of the results was performed using MS Excel. For each parameter, the mean and standard deviation were calculated, and the results were presented as M ± SD. Prior to conducting a comparative analysis, the distribution of the samples was assessed for conformity to a normal distribution. The significance of differences between the mean values in the experimental and control groups was determined using Student's t-test for independent samples. A difference was considered statistically significant at levels: $P < 0.05$, $P < 0.01$, and $P < 0.001$.

Results and Discussion

Summary indicators of variational pulse rate in sows with different baseline autonomic nervous system tone were determined prior to farrowing, as well as on the first and fifth days following parturition stress. The results of the study presented in Table 1 made it possible to analyse both the baseline intergroup differences in the autonomic regulation of heart rate and the dynamics of the autonomic nervous system's response to farrowing and subsequent recovery. Analysis of the table indicates significant changes in the integral indices of stress and autonomic balance in the early post-farrowing period, as well as varying rates of normalisation of these indices depending on the animals' initial autonomic status.

Table 1. Indicators of variational pulsometry in sows (M ± SD; n = 5)

Indicators HRV	ANS Tone		
	Normotonic	Vagotonic	Sympathicotonic
Before farrowing			
Mean RR (s)	0.81 ± 0.01	1.02 ± 0.01^^^	0.68 ± 0.01^^^

Table 1. Continued

Indicators HRV	ANS Tone		
	Normotonic	Vagotonic	Sympathicotonic
Before farrowing			
Mo	0.81 ± 0.01	1.03 ± 0.01^^^	0.68 ± 0.01^^^
AMo	27.2 ± 2.2	17.4 ± 0.6^^^	40.6 ± 1.4^^^
Δx (s)	0.19 ± 0.01	0.25 ± 0.01^^^	0.09 ± 0.01^^^
VBI (a.u.)	140.1 ± 10.0	68.5 ± 0.8^^^	466.0 ± 27.5^^^
SI (a.u.)	86.6 ± 6.7	33.4 ± 0.5^^^	343.2 ± 23.0^^^
1 day after farrowing			
Mean RR (s)	0.76 ± 0.01***	0.80 ± 0.01***^^^	0.62 ± 0.01***^^^
Mo	0.76 ± 0.01***	0.80 ± 0.01***^^^	0.62 ± 0.01***^^^
AMo	37.8 ± 1.3***	38.8 ± 2.5***	49.0 ± 0.9***^^^
Δx (s)	0.10 ± 0.01***	0.13 ± 0.01***^^	0.08 ± 0.01^^
VBI (a.u.)	389.0 ± 44.3***	296.4 ± 22.0***^	660.8 ± 57.4**^^^
SI (a.u.)	257.3 ± 29.7***	185.5 ± 14.8***^	533.3 ± 46.9***^^^
5 days after farrowing			
Mean RR (s)	0.79 ± 0.01*	0.85 ± 0.01***^^^	0,665 ± 0.005^^^
Mo	0.79 ± 0.01*	0.86 ± 0.01***^^^	0.666 ± 0.006^^^
AMo	34.0 ± 1.5**	35 ± 2.2***	42.0 ± 1.1^^^
Δx (s)	0.16 ± 0.01***	0.17 ± 0.01***	0.11 ± 0.01^^^
VBI (a.u.)	213.7 ± 13.1***	210.1 ± 16.4***	414.7 ± 49.7^^^
SI (a.u.)	135.2 ± 7.8***	122.9 ± 10.0***	311.0 ± 36.6^^^

***Note**: 1. Mean RR – mean R-R interval duration (s); Mo – mode; AMo – amplitude of the mode; Δx – difference between the maximum and minimum values of the mode (s); VBI – vegetative balance index (units); SI – stress index (units). 2. Statistically significant differences compared with the previous study period: *** – P < 0.001; ** – P < 0.01; * – P < 0.05. 3. Statistically significant differences compared with pigs with normotension: ^^^ – P < 0.001; ^^ – P < 0.01; ^ – P < 0.05*

***Source**: authors' own work*

Table 1 shows that in sows with balanced sympathetic and parasympathetic regulation of heart rhythm (normotonia, NT) before farrowing, the mean values of the following indicators – R-R intervals (Mean RR), R-R mode (Mo), mode amplitude (AMo), R-R mode range (Δx), vegetative balance index (VBI) and stress index (SI) – indicated the absence of signs of excessive autonomic load. In vagotonic sows (VT) before farrowing, Mean RR increased by 26.0% (P < 0.001) compared with NT animals. This indicates a slowing of heart rhythm under the influence of increased parasympathetic activity. At the same time, Mo increased by 27.2% (P < 0.001) compared with NT animals, confirming a shift of the most frequent R-R interval towards prolongation. In contrast, AMo decreased by 36.0% (P < 0.001) compared with NT animals, indicating a wider distribution of intervals and increased variability. Δx increased by 31.6% (P < 0.001) compared with NT animals, suggesting greater diversity of cardiac rhythms. VBI decreased by

51.1% (P < 0.001) compared with NT animals, indicating the predominance of parasympathetic tone. SI decreased by 61.4% (P < 0.001) compared with NT animals, reflecting a substantial reduction in autonomic stress at rest.

In sows of the ST group before farrowing, Mean RR decreased by 16.2% (P < 0.001) compared with NT animals. Mo also decreased by 16.0% (P < 0.001) compared with NT animals. However, AMo increased by 49.3% (P < 0.001) compared with NT animals, indicating a narrowed distribution of intervals and predominance of a single mode. Δx decreased by 52.6% (P < 0.001) compared with NT animals, reflecting lower diversity of R-R intervals. VBI increased by 232.7% (P < 0.001) and SI by 296.8% (P < 0.001) compared with NT animals, indicating pronounced sympathetic load and a high level of autonomic stress at rest. In sows of the NT group one day after farrowing, Mean RR decreased by 6.2% (P < 0.001) compared with the pre-farrowing value, indicating an acceleration of heart rhythm due to activation of the sympathetic nervous system. Mo also decreased by 6.2% (P < 0.001) compared with pre-farrowing values, confirming a shift of the modal R-R interval towards shortening. AMo increased by 39.0% (P < 0.001) compared with pre-farrowing values, indicating strengthening of the dominant interval and reduced diversity of rhythms. Δx decreased by 47.4% (P < 0.001) compared with pre-farrowing values, demonstrating a reduction in the overall variability of intervals. VBI increased by 177.6% (P < 0.001) and SI by 197.2% (P < 0.001) compared with pre-farrowing values, indicating a shift in autonomic balance towards sympathetic tone and reflecting a high level of autonomic stress.

In sows of the VT group one day after farrowing, Mean RR decreased by 21.6% (P < 0.001) and Mo by 22.3% (P < 0.001) compared with pre-farrowing values, confirming a shift of the most frequent R-R interval towards shortening. AMo doubled, indicating the strengthening of one dominant interval and reduced diversity. Δx decreased by 48.0% (P < 0.001) compared with pre-farrowing values, demonstrating a reduction in the overall variability of R-R intervals. VBI increased fourfold (P < 0.001) and SI increased fivefold (P < 0.001) compared with pre-farrowing values, indicating a shift in autonomic balance towards the dominance of the sympathetic nervous system. In sows of the ST group one day after farrowing, Mean RR and Mo decreased by 8.8% (P < 0.05) compared with the pre-farrowing values. In contrast, AMo increased by 20.7% (P < 0.001) compared with the pre-farrowing values, reflecting an increased contribution of the modal interval and a simultaneous narrowing of the variability spectrum. Δx decreased by 11.1% compared with the pre-farrowing values, indicating reduced diversity of intervals. VBI increased by 41.8% (P < 0.01) and SI by 55.4% (P < 0.001) compared with the pre-farrowing values, indicating predominant activation of the sympathetic branch of the autonomic nervous system during the first day after farrowing.

From the first to the fifth day after farrowing in the NT group, Mean RR increased by 4% (P < 0.05), indicating partial recovery of the initial heart rate. At the same time, AMo decreased by 10% (P < 0.01) and Δx increased by 60% (P < 0.001), reflecting an increase in variability. Meanwhile, VBI decreased by 45% (P < 0.001) and SI by 47% (P < 0.001). In sows of the VT group, Mean RR increased by 6% (P < 0.001) and Δx by 31% (P < 0.001). However, AMo decreased by 10% (P < 0.001), indicating greater diversity of intervals. VBI decreased by 29% (P < 0.001) and SI by 34% (P < 0.001). In sows of the ST group, Mean RR increased by 7%, indicating a gradual reduction of excessive sympathetic activity. AMo decreased by 14%, while Δx increased by 38%. VBI decreased by 37% and SI by 42%, indicating a gradual reduction of sympathetic dominance.

Thus, a similar pattern of variational pulsometry dynamics was observed in all studied

groups of sows during parturition stress and recovery after farrowing: before farrowing, ANS tone determined the baseline levels of variability (lowest stress in VT, moderate in NT, and highest in ST). After farrowing (day 1), all groups demonstrated a sharp increase in SI and VBI indices together with a decrease in Δx and Mean RR, indicating activation of the sympathetic branch and a high level of autonomic stress. By the fifth day, heart rate variability began to recover gradually: Mean RR and Δx approached their initial values, AMo decreased, and SI and VBI declined, reflecting restoration of autonomic balance and a reduction in the stress response to a level closer to the baseline. Figure 1 presents measurements of 100 consecutive R-R intervals (in seconds) for one sow in each group (normotonic – blue, vagotonic – green, sympathicotonic – red) at three time points: before farrowing, on the first day, and on the fifth day after farrowing.

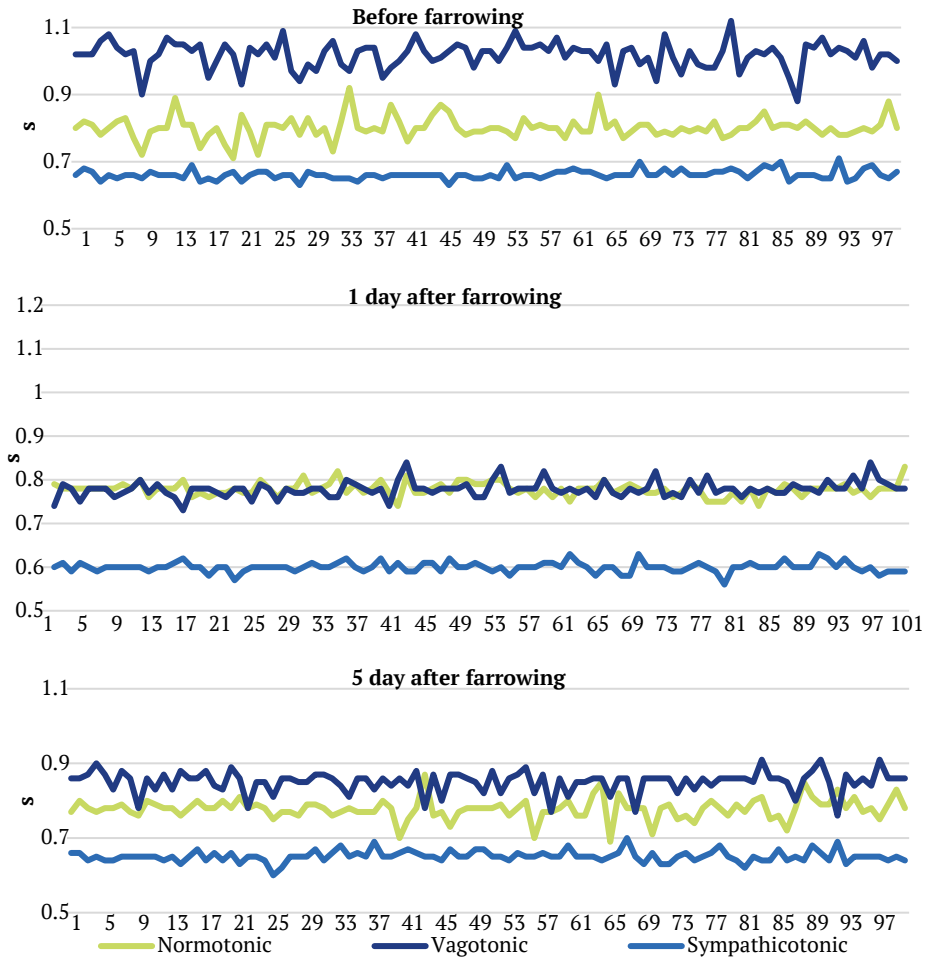

Figure 1. Cardiointervalogram of sows with different parameters of autonomic regulation, R-R, s (n = 1)

***Source**: authors' own work*

Before farrowing, sows in the VT group showed wide fluctuations around 1.00 s (peaks up to 1.10 s and a minimum of about 0.90 s), indicating high interbeat variability under the influence of parasympathetic dominance. In sows of the NT group, moderate fluctuations were observed around 0.80 s (range approximately 0.75-0.90 s), whereas in sows of the ST group a narrow clustering of intervals within 0.65-0.70 s was detected, indicating low variability associated with the predominance of sympathetic tone. On the first day after farrowing, shifts towards shorter intervals were observed in all three curves. In ST sows, the interval remained the most "compressed" (approximately 0.60-0.65 s), whereas in sows of the NT and VT groups the curves converged around 0.75-0.80 s with smaller fluctuations, demonstrating acute sympathetic activation in all groups. On the fifth day after farrowing, the cardiointervalogram curve of VT sows expanded again to 0.85-0.95 s, indicating recovery of parasympathetic activity. In NT sows, fluctuations partially recovered (approximately 0.70-0.85 s), whereas in ST sows the intervals remained relatively "compressed" (0.60-0.70 s), indicating a slower return to baseline variability. These cardiointervalograms illustrate the influence of the initial tone of the autonomic nervous system on the level of heart rate variability and on the rate of its recovery after farrowing stress.

Figure 2 presents the distribution of R-R intervals in sows of the three experimental groups with different ANS tones before farrowing: vagotonic – the green curve – show a peak interval frequency around 1.02 s with a wide distribution "tail" (maximum ≈12%), indicating high variability due to dominant parasympathetic influence; normotonic – the blue curve – demonstrate an average interval of approximately 0.78 s with a smoother and more symmetrical distribution (maximum ≈14%), reflecting balanced sympathetic-parasympathetic regulation; sympathicotonic – the red curve – are characterised by a narrow peak around 0.67 s with the highest recurrence percentage (≈18%), indicating rigid "clustered" interval grouping and low variability under sympathetic dominance.

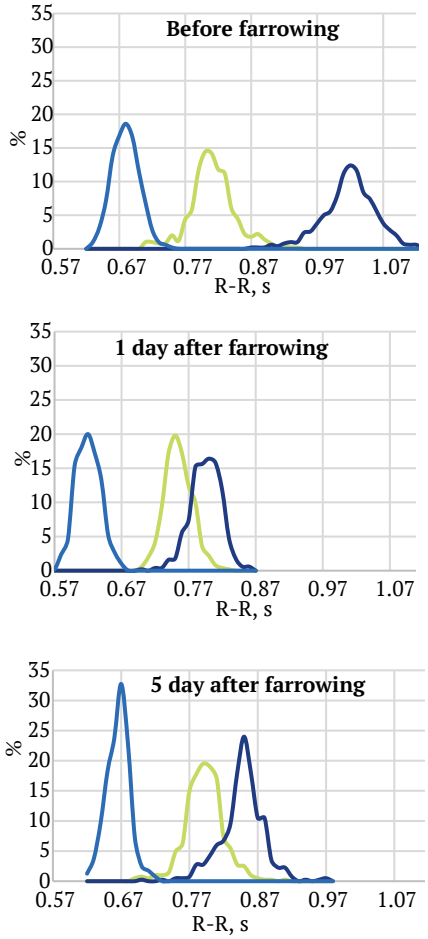

Figure 2. Variation graph of R-R interval repetition (s) in pigs with different ANS tone (M; n = 5)

***Note**: yellow line – normotonic; dark blue line – vagotonic; light blue line – sympathicotonic*

***Source**: authors' own work*

On the graph of the R-R interval distribution on the first day after farrowing, the red curve (ST sows) was narrowly concentrated around ~0.62 s with a peak of about 20%, indicating the

persistence of rigid "clustered" interval contraction under dominant sympathetic activity. The blue curve (NT sows) had a peak value of approximately 0.76 s with a peak of about 20% and a slightly wider yet fairly symmetrical distribution, reflecting moderate variability during the acute stress response. The green curve (VT sows) was shifted towards approximately 0.80 s with a peak of about 16% and showed the most extended distribution among all groups, indicating relatively high R-R interval variability even in the presence of enhanced autonomic activation following parturition stress.

The fifth day after farrowing was characterised by a further shift and widening of the R-R interval distributions in sows of all groups. In sympathicotonic animals, the intervals remained narrowly clustered around ~0.665 s with the highest peak percentage (~32%), indicating persistent rigid sympathetic tone. In normotonic sows, the peak interval was approximately 0.79 s with a more uniform but still relatively narrow distribution (~19%), reflecting moderate variability during the recovery period. In vagotonic animals, the widest distribution was observed, with a maximum around 0.85 s (~24%), indicating a well-expressed parasympathetic recovery response and a gradual restoration of high heart rate variability.

Analysis of the R-R interval distributions in sows with different ANS tones showed that the baseline autonomic status determines the initial level of variability: vagotonic animals had the widest distribution and the longest intervals, normotonic animals had intermediate values, and sympathicotonic animals had the narrowest distribution. On the first day after farrowing, sows in all experimental groups demonstrated a shift of the peaks towards shorter R-R intervals and a sharp "clustered" contraction of the distributions, reflecting activation of the sympathetic branch and high autonomic stress. By the fifth day after farrowing, the distributions gradually widened and approached their initial values: the greatest variability was again observed in vagotonic sows, moderate variability in normotonic animals, whereas sympathicotonic sows still retained a relatively rigid "clustered" interval structure. Such dynamics demonstrate a universal response to parturition stress, followed by a gradual reduction of sympathetic load and restoration of autonomic balance in sows during the first five days after farrowing.

Thus, this study presents the first comprehensive analysis of variational pulsometry in sows with different baseline tones of the autonomic nervous system during the periods before and after farrowing. The obtained results revealed a clear correlation between the baseline balance of sympathetic and parasympathetic tone and the cardiovascular response to parturition stress, supporting hypotheses regarding the role of autonomic regulation in post-parturition recovery.

Firstly, the baseline HRV indicators reflect the classical distribution according to autonomic tone: in VT sows, an increased mean duration of R-R intervals and wide variability were observed, correlating with enhanced parasympathetic activity (Todoryuk *et al.,* 2023). In NT sows, a balance between both branches of the ANS was maintained, whereas in ST sows the sympathetic branch predominated, with a rigidly "compressed" spectrum of R-R intervals corresponding to lower adaptive flexibility, as reported by A. Youssef *et al.* (2020). These findings are consistent with the work of C.J. Byrd *et al.* (2020), which indicated that high sympathetic activity is associated with a reduced range of variability in mammals during stress.

Secondly, on the first day after farrowing, sows in all groups demonstrated a significant increase in the stress index (SI) and the vegetative balance index (VBI), as well as a decrease in Mean RR and Δx, indicating activation of the

sympathetic branch and a high level of stress response. Such rapid changes correspond to the phenomena of "parturition shock" in mammals described by F.T. Spradley (2019), in which sympathetic predominance promotes mobilisation of physiological resources and thermoregulation. Similar observations were reported in the studies of W. Sipos *et al.* (2013), where in pigs during parturition stress the stress index and vegetative balance index increased almost twofold within the first 12 hours after farrowing. According to L. Stovbetska *et al*. (2021), acute pain stress and hormonal changes during farrowing cause a sharp shift towards sympathetic dominance: sows exhibit tachycardia, reduced heart rate variability, and elevated levels of adrenocorticotropic hormone and cortisol.

Thirdly, by the fifth day after farrowing, HRV indicators partially returned towards baseline levels: Mean RR and Δx approached their initial values, while AMo decreased, indicating recovery of parasympathetic activity. According to C.J. Byrd *et al.* (2020), after farrowing the intensity of sympathetic activity gradually declines and the parasympathetic nervous system becomes capable of regulating homeostasis: in sows respiratory rate and cardiac intervals normalise, uterine perfusion improves, and milk let-down becomes more efficient. However, in the present study, sympathicotonic sows showed the greatest delay in recovery, with VBI and SI values remaining markedly elevated compared with NT and VT animals even on the fifth day. This finding is consistent with the concept that a baseline sympathicotonic tone reduces adaptive capacity to repeated stress stimuli (Lipsitz *et al.,* 1997). As noted by L. Stovbetska *et al.* (2021), insufficient transition to parasympathetic recovery may lead to complications such as delayed uterine involution, increased risk of postpartum metritis, and deterioration in milk quality. Conversely, facilitating a rapid shift towards parasympathetic dominance correlates with a better onset of lactation and improved survival of newborn piglets.

In veterinary science, ANS tone is regarded as a key factor determining stress resistance and productivity in animals. Scientific literature describes in detail the anatomical and functional relationships between the ANS and central brain structures and their influence on cardiac regulation (Ten Donkelaar *et al.,* 2020). V. Todoryuk *et al*. (2023), in experiments on piglets, found that vagotonic individuals adapt better to environmental changes during early ontogenesis, whereas sympathicotonic animals demonstrate increased levels of stress reactions. V.O. Danchuk *et al.* (2024) also drew attention to changes in biochemical markers (total protein and its fractions) in sows with different ANS tones during pregnancy and farrowing, emphasising the influence of autonomic status on the physiological condition of animals during critical periods.

As the results of the present study demonstrated, assessment of adaptive changes in the ANS of sows during farrowing should include HRV monitoring both before and directly during parturition. This conclusion is supported by C.J. Byrd *et al.* (2020), who indicated that data collection using ECG telemetry enables the determination of spectral indicators such as LF and HF, the LF/HF ratio, and, shortly after farrowing, the RMSSD (root mean square of successive differences). A decrease in RMSSD and HF during the contraction phase indicates intense sympathetic activation, which can be moderated by the administration of antispasmodics and analgesics to reduce pain load. M.D. Ghezzi *et al*. (2024) emphasised the importance of combining HRV analysis with infrared thermography to record real-time changes in ANS tone and to predict physiological stress before visible clinical signs appear. K.E. Speer *et al.* (2024) also noted that the inclusion of

hormonal markers, particularly cortisol, provides a deeper understanding of the mechanisms of adaptation in homeothermic organisms. Furthermore, the authors emphasised that most responses arising in animals under the influence of stress factors are mediated by the autonomic nervous system, and that a comprehensive assessment of HRV parameters combined with thermographic and endocrine markers increases the accuracy of early stress diagnostics. Such a multidisciplinary approach creates a basis for the development of predictive models for assessing stress reactivity and for the timely implementation of corrective measures under production conditions.

Thus, determination of ANS tone using variational pulsometry, as demonstrated in the present study, may serve as a practical indicator of stress resistance in sows prior to farrowing and may help predict their ability to recover rapidly afterward. This is particularly important for highly intensified livestock production systems, where reducing the recovery period after farrowing contributes to increased piglet survival and improved sow productivity. Farrowing stress in sows is accompanied by a universal activation of the sympathetic branch of the autonomic nervous system, resulting in pronounced changes in variational pulsometry parameters regardless of the initial autonomic status. At the same time, the type of autonomic regulation influences both the magnitude of the stress response and the rate of post-farrowing recovery of cardiovascular regulation, which was reflected in different dynamics of the stress index and vegetative balance indicators.

The greatest adaptive potential was demonstrated by vagotonic sows, whereas sympathicotonic animals exhibited prolonged autonomic tension. The obtained data confirm the feasibility of using variational pulsometry as an integral tool for assessing stress resistance and predicting adaptive capacity in sows during the farrowing period. The expected outcome of such studies will be the development of validated predictive models for evaluating stress resistance in pigs, which will improve the accuracy of early diagnosis of maladaptive conditions, optimise housing and management technologies, and enhance productive performance during different physiological periods.

Conclusions

According to the obtained results, a clear temporal dynamics of variational pulsometry parameters was identified in Large White sows with different baseline tones of the autonomic nervous system during the pre-farrowing period and the early post-farrowing period. A step-by-step analysis of heart rate variability was conducted in normotonic, vagotonic and sympathicotonic animals one day before farrowing, as well as on the first and fifth days after parturition stress using the method of variational pulsometry. It was established that the baseline autonomic status determines the initial level of heart rate variability, the intensity of the stress response to farrowing, and the subsequent rate of recovery.

The highest adaptive potential was demonstrated by vagotonic sows, whereas sympathicotonic animals were characterised by a prolonged persistence of autonomic tension. On the first day after farrowing, a pronounced activation of the sympathetic branch of the autonomic nervous system was recorded in all groups, accompanied by an increase in the stress index and the vegetative balance index, reflecting the mobilisation of the organism's regulatory resources. In particular, in normotonic sows the VBI increased by 177.6% ($P < 0.001$) and the SI by 197.2% ($P < 0.001$), while in vagotonic animals the VBI increased fourfold and the SI fivefold ($P < 0.001$), indicating acute sympathetic activation regardless of the initial autonomic tone. By the fifth day after farrowing, partial restoration of autonomic balance was observed, the degree of which depended on the type of

autonomic regulation: in normotonic sows the SI decreased by 47% ($P < 0.001$) and the VBI by 45% ($P < 0.001$) compared with the first day, whereas in sympathicotonic animals, even after a 42% decrease, the SI remained at the level of 311.0 ± 36.6 conventional units, indicating a slower normalisation of regulatory mechanisms.

The obtained data allow heart rate variability to be considered an integral indicator of stress resistance and adaptive capacity in sows during the critical period of farrowing and early post-farrowing recovery. The limitations of the study are associated with the small sample size ($n = 5$ in each group) and the absence of long-term monitoring beyond the fifth day, which may affect the assessment of longer-term adaptive processes. A promising direction for further research is the integration of heart rate variability indicators with hormonal, behavioural and productivity markers in order to develop comprehensive predictive models of stress resistance in pigs during different physiological periods.

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Conflict of Interest

None.

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Автономна регуляція серцевого ритму свиноматок у період опоросу

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Анотація. Сучасні дослідження демонструють значний вплив вегетативної нервової системи на адаптацію та продуктивність сільськогосподарських тварин. Особливо критичним є період опоросу в свиноматок, коли зміни вегетативного тонусу детермінують перебіг родового стресу та швидкість відновлення. Метою дослідження було вивчити особливості динаміки параметрів варіаційної пульсометрії у свиноматок із нормотонічним, симпатикотонічним та ваготонічним тонусом вегетативної нервової системи до опоросу, а також через добу та п'ять діб після родового стресу. Експеримент проведено на п'ятнадцяти свиноматках великої білої породи, розподілених на три групи за рівнем індексу напруги. Для кожної тварини за одну добу до опоросу, на першу та п'яту добу після опоросу реєстрували сто послідовних інтервалів між серцевими скороченнями за допомогою електрокардіографа. Розраховували середню тривалість інтервалів, моду інтервалів, амплітуду моди, варіаційний розмах, індекс вегетативного балансу та індекс напруги. Встановлено, що до опоросу ваготонічні тварини мали максимальну варіабельність серцевих інтервалів, симпатикотонічні – найнижчу. Через добу після родового стресу в усіх групах відзначено зменшення середньої тривалості інтервалів та збільшення індексу

напруги, однак найшвидше відновлення параметрів спостерігалося у ваготонічних свиноматок. На п'ятий день після опоросу показники у свиноматок нормотонічної групи наблизились до вихідних значень, тоді як у тварин з підвищеним симпатичним тонусом стресова реакція зберігалася. Отже, використання варіаційної пульсометрії для оцінки тонусу вегетативної нервової системи перед опоросом дозволяє виявити найбільш стресостійких свиноматок і запровадити цільові підтримуючі заходи у перші доби після опоросу. Такий підхід сприятиме підвищенню виживаності поросят, скороченню періоду відновлення свиноматок та підвищенню економічної ефективності свинарства

Ключові слова: варіаційна пульсометрія; вегетативна нервова система; родовий стрес; адаптивні механізми; свині

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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).

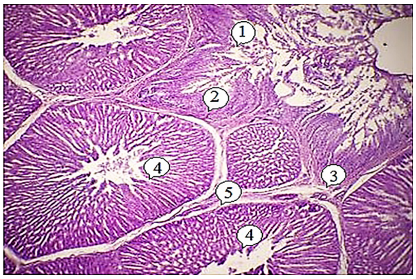

Figure 1. Mucous membrane of the glandular part of the stomach of a day-old turkey

***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).

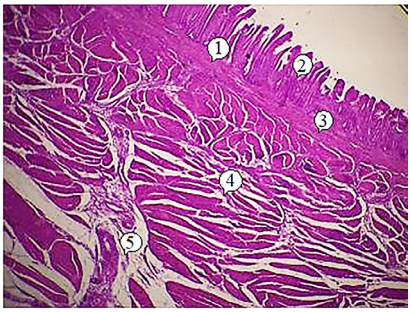

Figure 3. Muscular part of the stomach of a day-old turkey
***Note**: 1 – mucous membrane; 2 – tubular glands of the lamina propria; 3 – submucosa; 4 – bundles of smooth muscle cells of the muscular coat; 5 – loose fibrous connective tissue with blood vessels. Stained with haematoxylin and eosin, ×80*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

phoid 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).

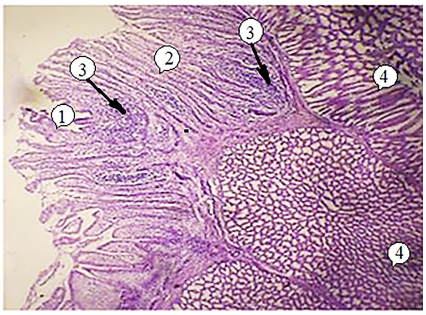

Figure 4. Diffuse lymphoid tissue in the glandular part of the stomach of a day-old turkey

***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 ± 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*

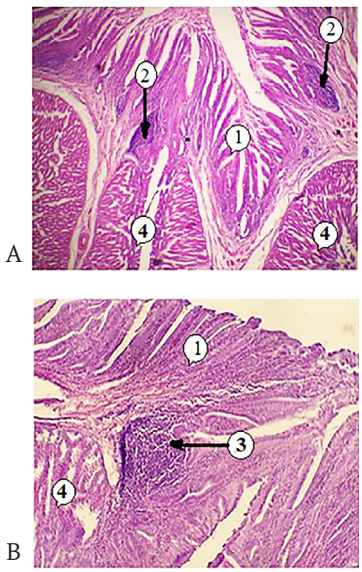

Figure 5. Glandular part of the stomach of 10-day-old turkeys of the control (A) and experimental (B) groups
***Note**: 1 – tubular glands of the lamina propria; 2 – foci of diffuse lymphoid tissue; 3 – pre-nodule; 4 – lobules of deep glands of the submucosa. Stained with haematoxylin and eosin, ×80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

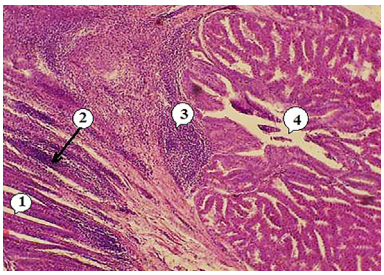

Figure 6. Primary lymphoid nodule in a lobule of a deep gland of the glandular part of the stomach of a 10-day-old turkey from the experimental group
***Note**: 1 – tubular glands of the lamina propria; 2 – foci of diffuse lymphoid tissue; 3 – primary lymphoid nodule; 4 – lobule of a deep gland of the 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).

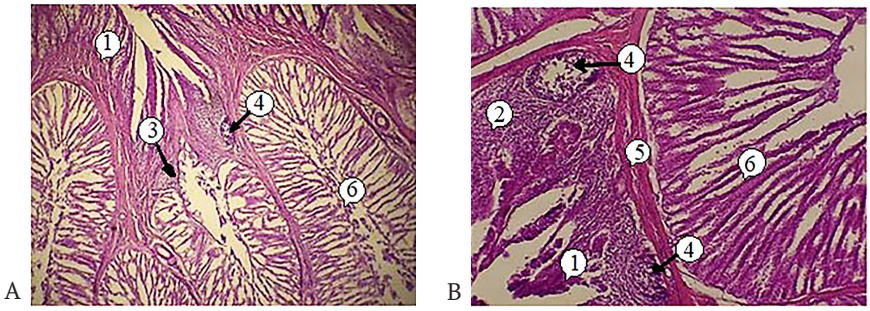

Figure 7. Glandular part of the stomach of 20-day-old turkeys of the experimental group (A, B)
***Note**: 1 – tubular glands of the lamina propria; 2 – foci of diffuse lymphoid tissue; 3 – primary lymphoid nodule; 4 – secondary lymphoid nodules; 5 – muscularis mucosae; 6 – lobules of deep glands of the 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).

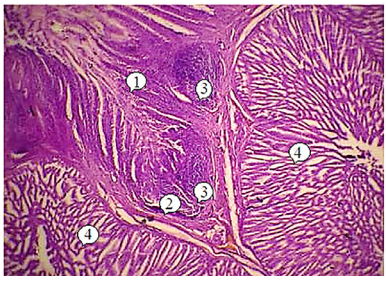

Figure 8. Glandular part of the stomach of a 20-day-old turkey from the control group
***Note**: 1 – tubular glands of the lamina propria; 2 – lamina propria of the mucous membrane; 3 – primary lymphoid nodules; 4 – lobules of deep glands of the submucosa. Stained with haematoxylin and eosin, ×80*
***Source**: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

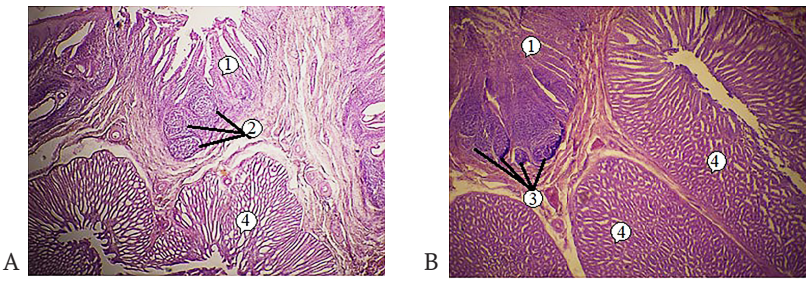

Figure 10. Clusters and chains of lymphoid nodules in the lamina propria of the mucous membrane of the glandular part of the stomach of turkeys from the experimental group at 40 days (A) and 50 days of age (B)

***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.

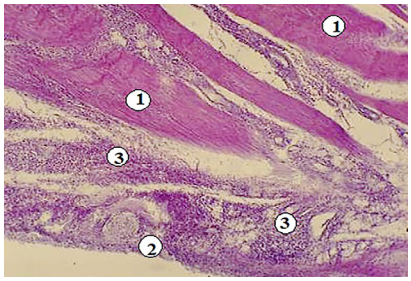

Figure 11. Diffuse lymphoid tissue in the muscular part of the stomach of a domestic turkey from the experimental group at 40 days of age

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

***Source**: authors' own work*

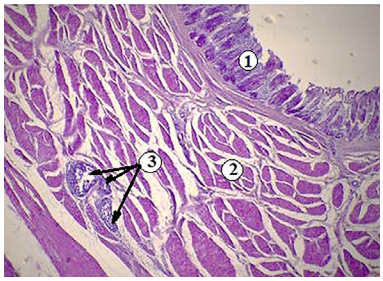

Figure 12. Secondary lymphoid nodules in the muscular part of the stomach of a 50-day-old turkey from the experimental group

***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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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

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