Theoretical and Applied Veterinary Medicine

ISSN 2663-1156 (print)
ISSN 2663-1164 (online)
Theoretical and Applied Veterinary Medicine, 10(3), 21–26
doi: 10.32819/2022.10013

Original research

# Topography and dynamics of spleen and lymph nodes' morphometric parameters in rabbits

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Received: 24 October 2022
Revised: 31 October 2022
Accepted: 15 November 2022

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**Abstract.** The meat productivity of animals, including rabbits, depends on many decisive factors, the main of which are the quality and type of feeding, housing conditions, the use of intensive rearing protocols and breed affiliation. Now in industrial rabbit breeding, improved hybrid crosses are preferred, which, in terms of their quality characteristics, are significantly dominated by the usual domestic rabbit breeds. Therefore, it is important to have a balanced model with species and age characteristics of the syntopy and morphology of internal organs, in particular hemo- and lymphopoiesis, in this animal species. There have been established main anatomical, topographic and morphometric parameters of the spleen and visceral lymph nodes (cranial mesenteric and cranial mediastinal) for rabbits of the meat productivity direction from the age of 1 day old, 10-, 20-, 30-, 60- and 90-day-old. Each age group consisted of 6 rabbits. All experimental animals were clinically healthy, not vaccinated and not treated against ecto- and endoparasites. The features of the topographic location of the organs were described, as well as length and width parameters, and absolute and relative masses were determined. It was identified that the topography of the spleen and lymph nodes corresponds to the generally accepted species principles for the placement of these organs in rabbits. A relationship has been established between a sharp increase in the dynamics of organ morphometric parameters and the rapid development of a hybrid cross in the period from birth to reaching 90 days of age. Organs' morphometric parameters in general correlate with age.

**Keywords:** secondary organs of hemo- and lymphopoiesis; morphogenesis; structural and functional organization; organs' absolute and relative mass; organs' length and width; rabbit breeding

# Топографія і динаміка морфометричних параметрів селезінки та лімфатичних вузлів кролів

**Анотація.** М'ясна продуктивність тварин, в тому числі кролів, залежить від низки вирішальних факторів, основні з яких – якість і тип годівлі, умови утримання, застосування схем інтенсивного вирощування та породна приналежність. Нині в промисловому кролівництві надають перевагу вдосконаленим гібридним кросам, які за своїми якісними характеристиками значно переважають звичні породи свійських кролів. Тому важливо мати виважену модель з видовими і віковими особливостями синтопії та морфології внутрішніх органів, зокрема гемо- і лімфопоезу, у цього виду тварин. Встановлені основні анатомо-топографічні та морфометричні параметри селезінки і вісцеральних лімфатичних вузлів (краніального брижового та краніального середостінного) для кролів м'ясного напрямку продуктивності від добового,10-, 20-, 30-, 60- та 90-добового віку. Кожна вікова група складалась з 6 кролів. Всі піддослідні тварини були клінічно здорові, не вакциновані та не піддавалися обробці від екто- та ендопаразитів. Описані особливості топографічного розташування відносно органів, а також визначені параметри довжини і ширини, абсолютної та відносної маси. Визначено, що топографія селезінки та лімфатичних вузлів відповідає загальноприйнятим видовим принципам розміщення цих органів у кролів. Морфометричні параметри органів в цілому взаємо корелюються з віком. Встановлений взаємозв'язок між різким збільшенням динаміки морфометричних показників органів та швидкісним розвитком гібридного кросу у період від народження до досягнення 90-добового віку. Морфометричні параметри органів в цілому взаємо корелюються з віком.

**Ключові слова:** вторинні органи гемо- і лімфопоезу; морфогенез; структурно-функціональна організація; абсолютна і відносна маса органів; довжина і ширина органів; кролівництво

**Cite this article**: Myroshnychenko, I. I., & Lieshchova, M. A. (2022). Topography and dynamics of spleen and lymph nodes' morphometric parameters in rabbits. *Theoretical and Applied Veterinary Medicine*, 10(3), 21–26. doi: 10.32819/2022.10013

## Introduction

Peripheral (secondary) organs of hemo- and lymphopoiesis are anatomically separate organs that can effectively capture and accumulate foreign agents to initiate an adaptive immune response (Sapin, 2006; Parker & Makori, 2018). These specialized lymphoid organs include the lymph nodes, spleen, and mucosal-associated lymphoid tissue. Together they form a highly complex and diverse system that maintains the relationship between antigen-presenting and immunocompetent cells, which contributes to the formation of long-term immunity (Randall et al., 2008).

Each secondary organ has a unique set of components to capture and identify antigens in all areas of the body. Thus, the lymph nodes are located in strategic places and form a protective network for the detection of interstitial antigens, while the spleen plays a role in maintaining the antigenic purity of the blood (Nishikawa et al., 2003; Dunajevs'ka et al., 2021).

Morphologically, lymph nodes and the spleen are organized parenchymal organs, the structure of which is determined by their performance of an immune protection specific function, in particular, the interaction of immunocompetent cells with antigens, their identification with the subsequent development of the appropriate specific immune response, clonal expansion, differentiation and accumulation of reverse products due to the corresponding reaction (Sapin et al., 2008; Shapkin, 2009; Cupedo et al., 2011).

Lymph nodes are externally limited by a connective tissue capsule, the parenchyma is formed by lymphoid tissue, divided into many functional zones between which there are lymphatic sinuses. In their functioning, they are characterized as a kind of prefabricated filtration lymph collectors, in which microorganisms or their parts are retained, in addition, they act as a centre for the immunocompetent cells interaction in the process of a specific immune response, they synthesize antibodies-immunoglobulins (Sapin et al., 2008). The spleen is an unpaired, mononodous parenchymal organ of haematopoiesis and immune defence (Dunajevs'ka, 2016). It initiates and supports most immune responses of both cellular and humoral nature, innate or acquired immunity, while controlling the quantitative and qualitative composition of blood and lymph (Prasolova et al., 2004).

It is known that the immune system is one of the most important homeostatic systems of the body, which determines vitality, health, productivity and adaptive capabilities (Randall et al., 2008; Klein 2008). For the organs of this system, in particular, the lymph nodes and spleen, patterns of growth and development are unique in many respects. They are characterized by early laying in embryogenesis, almost complete morpho-functional maturity, sufficient for the implementation of definitive functions (proliferation of various lymphocyte clones and immunoglobulin synthesis) before the moment of birth and early age-related involution (Sapin, et al., 2008). Also, one of the main morphogenesis regularities of these organs is a sharp increase in morphometric characteristics, which is accompanied by increased differentiation of lymphoid tissue in the early postnatal period, as the newborn organism enters the relatively sterile conditions of intrauterine development into an environment with many antigens (Gavrylin, 2000; Gavrylin et al., 2017).

Well-known that the growth of somatic systems of the body is closely interconnected with the organs of hemopoiesis and lymphopoiesis. This is usually manifested in a ratio change of the stromal and parenchymal components of the latter, the degree of development and spatial arrangement and cytoarchitectonic of the lymphoid parenchyma functional zones (T-dependent and B-dependent). By identifying and monitoring these changes, it is possible to establish the state and course of antigenic processes in the body and to find out the effect of various factors on the immune system and general processes of the body's development as a whole (Voloshin et al., 2006; Klein, 2008). It should be noted that the intensity of growth and development of the body in combination with the organs' morpho-functional state of hemo- and lymphopoiesis in meat-producing animals are often the direct cause of a decrease in their immune status (Gavrylin, 2000; Gavrylin & Gibert, 2016).

It is noted that the spleen and lymph nodes during the neonatal period of ontogenesis have minimal capabilities to respond to antigens, which are compensated by colostral immunity (Gavrylin & Lieshchova, 2008). Later, precisely thanks to active antigenic stimulation, alternate differentiation and transformation of parenchymal components of these organs occur, with subsequent activation of the immunological reactivity of the organism itself (Lieshchova, 2007; Moldavskaya & Dolin, 2007; Gavrylin et al., 2018).

Several publications have been devoted to the study of the morphogenesis patterns of hematopoiesis and lymphopoiesis secondary organs in rabbits during ontogenesis, but they were conducted on wild animals or animals of ordinary breeds (Jeklova et al., 2007; Gavrylin & Nikitina 2017). The peculiarities of the development of these organs in animals of hybrid crosses, which are characterized by the rapid growth of all somatic systems, are insufficiently covered in the scientific literature.

A classical morphological study, in particular on the research of hematopoietic and lymphopoietic organs morphogenesis, involves a clear sequence of experimenting to determine the state of the organ at all levels of its structural organization, starting with the organ and ending with the submicroscopic and molecular level (Maslianko & Venhryn, 2004; Panikar et al., 2015). Initial studies usually begin with the definition of topographic features and macromorphometric parameters, which is what this work is dedicated to.

The purpose of the study was to establish the features of the topography, macrostructure and dynamics of the spleen and some lymph nodes' morphometric characteristics in rabbits of the meat productivity direction during the early postnatal period of ontogenesis.

## Materials and methods

All manipulations with experimental animals used for scientific purposes were carried out following the «European Convention for the Protection of Vertebrate Animals used for Experimental or other Scientific Purposes» (Strasbourg, France, March 18, 1986, ETS No. 123) and the Law of Ukraine "On protection of animals from cruel treatment» (Kyiv, February 21, 2006, No. 3447-IV). The research protocol was reviewed and agreed upon by the Local Ethics Committee of the Faculty of Veterinary Medicine of the Dnipro State Agrarian and Economic University (Dnipro, Ukraine).

Morphological studies were carried out in the Department of Animals' Anatomy, Histology and Pathomorphology of the Dnipro State Agrarian and Economic University. The research material was the spleen and visceral lymph nodes (cranial mediastinal, cranial mesenteric) from newborn, 10-, 20-, 30-, 60-, and 90-day-old rabbits of the Hyplus hybrid cross, obtained from private farm animals (total number of animals, that were studied - 36, 6 heads in each age group). The dynamics of the rabbits' body weight during the study period were as follows: newborns – 74.8 g; at the age of 10 days – 184.3 g; 20 days – 354.2 g; 30 days – 607.3 g; 60 days – 2262.5 g, and upon reaching the 90th day of age – 3137.5 g. From birth to reaching 3 months of age, the total weight of animals increased by 3060 g on average.

The conditions of keeping the animals corresponded to zoohygienic standards, the rabbits were constantly provided with high-quality balanced feed (feeding twice daily) with constant access to fresh drinking water. Previously, the studied animals were not given prophylactic vaccinations and treatments against ecto- and endoparasites.

During a full pathological autopsy, was established the topography

of the rabbit's spleen and lymph nodes. Linear measurements (length, width) of each organ were determined using a centimetre ruler with a division value of 1 mm. The absolute weight of the organs was determined on an analytical balance (Metrinco AB224, China) with an accuracy of 0.0001 g. The organs' relative weight was calculated compared to the animals' body weight.

Statistical processing of digital data was carried out by univariate analysis of variance and a box plot. In the table and diagrams, the data are presented in the form of x ± SD. The probability of a difference in values between groups was calculated using the Student's t-test ($p < 0.05$) after checking the normality of the sample distribution.

**Results**

In rabbits, the spleen closely adheres to the dorsal surface of the stomach, is located between its rear edge and the left kidney, and is suspended on the omentum, occupying the entire front part of the left iliac area. Anatomically, the visceral surface of the spleen is slightly concave in the direction of the stomach. The lower edge is blunt and directed ventrally and backwards, and the sharp upper edge is directed dorsally and forward. Its ventral end is placed in the direction down and forward to the left costal arch, and the dorsal end is up and back to the spine. The spleen has a soft consistency, and its shape is characterized by significant variations: elongated, rounded, pointed, or in the form of a drumstick. The colour of the organ varies depending on the intensity of blood supply and the age of the animals: bright red (from birth to 10 days of age), dark red (from 20 to 30 days of age), and from brown-red to dark purple (from 60 to 90 days old). The spleen's hilum is located on its visceral surface, it is in this area that significant thickening of the organ is observed (Fig. 1).

Visceral lymph nodes (LN) in rabbits are compact, dense organs that are mostly collected in regional centres in the corresponding areas. Their shape varies depending on the location and age of the animals, it can be oval, round, bean-shaped, or oval-elongated. The lymph nodes' colour during the postnatal period of ontogenesis also has variations. From birth to 10 days of age - mainly light grey or pale yellow, from 20 to 30 days of age – pale grey or light pink, after reaching 90 days of age - pale pink or yellow. Anatomically, lymph nodes of rabbits have two surfaces: a convex (area of entry of afferent lymphatic vessels) and a portal depression (area of exit of efferent lymphatic vessels with blood vessels) (Fig. 2.)

The cranial mediastinal lymph node is located on the dorsal (left) and ventral (right) surfaces of the brachiocephalic trunk in the region of the right and left cranial vena cava walls. Organs are surrounded by fatty tissue and covered by connective tissue and muscles.

The cranial mesenteric LN is located in a large amount of adipose tissue, indirectly, in the exit area of the cranial mesenteric artery from the aorta.

In one-day-old rabbits, the average value of the cranial mesenteric and cranial mediastinal LN length is minimal and is 1.06 mm and 1.17 mm, and the width is 0.81 mm and 0.58 mm, respectively, with the median values located close to the average value (Fig. 3, 4). The lymph node absolute mass in animals of this age is also minimal and does not exceed 0.011 g and 0.013 g. The lymph node relative mass in animals of this age is 0.016 and 0.018%, respectively (Table).

For the spleen of one-day-old rabbits, the length and width average values were 1.03 mm and 0.18 mm, respectively (Figs. 5, 6). The organ absolute mass in rabbits of this age is 0.030 g, which was 0.044%, relative to the body weight of the animals (Table).

Upon reaching the age of 10 days, the length of lymph nodes increased by 2.1 times in the cranial mesenteric one and by 1.5 times in the cranial mediastinal one, compared to the previous age group. The width parameters of these organs increased uniformly by 1.3 times (Fig. 3, 4). The absolute mass of both lymph nodes increased, but significantly only in the cranial mediastinal one (by 92%). The relative mass of lymph nodes, on the contrary, decreased by 1.2 and 1.3 times, respectively (Table).

Starting from the 10-day-old in the spleen, the length and width average values were located near the medians, the increase of these parameters relative to the previous age period was 2.3 and 1.8 times, respectively (Fig. 5, 6). Indicators of the spleen mass were probably increasing, so the absolute mass increased 4 times, and the relative mass increased 1.4 times (Table).

At the age of 20 days, a sharp and reliable increase in the lymph nodes and spleen macromorphometric parameters was noted. The mean values of the lymph node length parameters were located next to the medians, continuing a proportional increase, in the cranial mesenteric one - by 1.5 times, and in the cranial mediastinal - by more than twice. In this age period, the interquartile ranges are the smallest, and the median and mean values are practically the same for the parameters of the LN width. Compared to the previous age group, the lymph node width increased uniformly, almost 2 times.

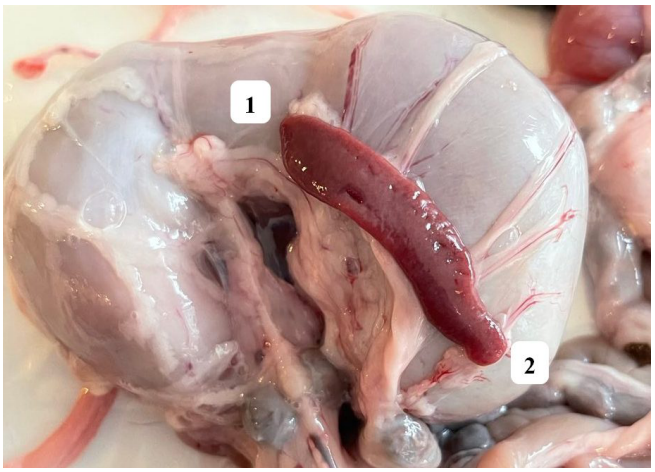


**Fig. 1.** Spleen topography of a 90-day-old rabbit:
1 – dorsal edge; 2 – ventral edge. Macro preparation, mm.

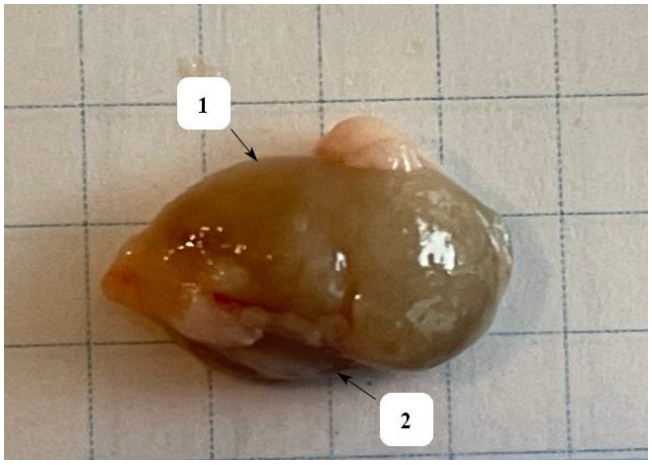


**Fig. 2.** Cranial mesenteric lymph node aged 90 days-old:
1 – convex surface, 2 – hilum cavity. Macro preparation, mm.

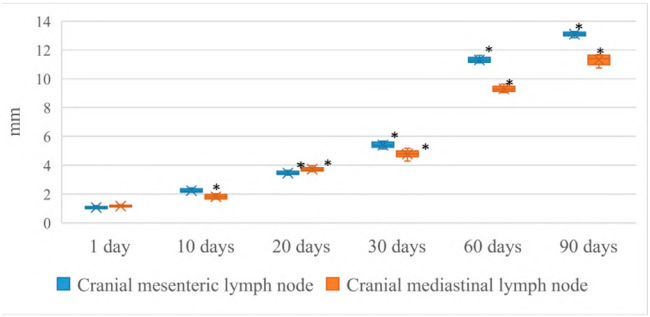


**Fig. 3.** Length dynamics of the rabbit visceral lymph nodes from day one to 90 days of age, mm, n = 36.
*Note*: *– indicated samples that are significantly different relative to the previous age group ($p > 0.05$)

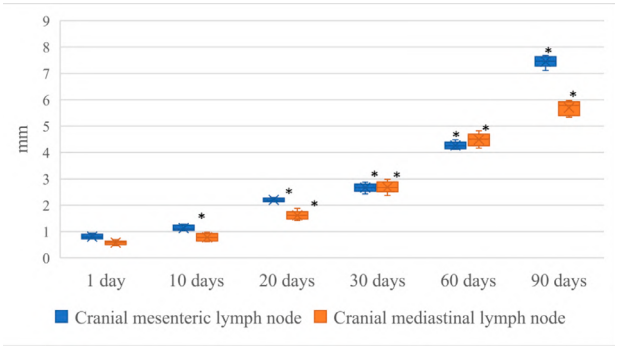


**Fig. 4.** Width dynamics of the rabbit visceral lymph nodes from day one to 90 days of age, mm, n = 36.
*Note*: *– indicated samples that are significantly different relative to the previous age group ($p > 0.05$)

The weight indicators of the lymph nodes reliably were increased by the age of 20 days. Thus, the absolute mass increased by 61 and 24%, and the relative mass by 1.2 and 1.8 times, respectively (Table). The length and width median values of the spleen were located close to the average value, which was expressed in the increase of the corresponding parameters by 1.1 and 1.2 times, compared to the previous age period (Fig. 5, 6). The absolute mass of the spleen at this age increased by 33%, while the relative mass, on the contrary, decreased by 1.4 times (Table).

When the rabbits reached 30 days of age, the parameters of LN length medians were close to the average values, while in the cranial mesenteric there was a 1.5 times increase and in the cranial mediastinal - only 1.2 times. As for the parameters of the LN width, in this age period, they reach parity, which is determined in the smallest interquartile ranges, the medians and average values of which coincide, and the numerical values were reflected by a 1.2 times increase in the cranial mesenteric LN, and by 1.6 times in the cranial mediastinal LN, which is significantly less compared to the previous age group (Fig. 1, 2). The absolute mass of lymph nodes increases, but not reliably. The relative mass of the cranial mediastinal one, on the contrary, decreased, and the cranial mesenteric one increased by 2.2 times (Table). The average values of the spleen length and width parameters were placed close to the medians, which in the digital value was reflected in increased indicators by 1.4 times, compared to the previous age group (Fig. 5, 6). The weight indicators of the spleen increased sharply and reliably, so the absolute weight increased by 206%, and the relative weight increased by 1.8 times (Table).

In 60-day-old rabbits, an increase in parameters, compared to the previous age period, was found in the cranial mesenteric and cranial mediastinal lymph nodes more than twice. At the same time,

**Table** – Visceral lymph node and spleen absolute and relative mass of one to 90-day-old rabbits, (x ± SD, n = 36)

| Organ | Age, days | Absolute mass, g | Relative mass, % |
|---|---|---|---|
| Cranial mesenteric | 1 | 0.011 ± 0.003 | 0.016 ± 0.002 |
| | 10 | 0.023 ± 0.004* | 0.013 ± 0.001 |
| | 20 | 0.037 ± 0.004* | 0.010 ± 0.001* |
| | 30 | 0.136 ± 0.021* | 0.022 ± 0.002* |
| | 60 | 0.273 ± 0.065* | 0.012 ± 0.002 |
| | 90 | 0.558 ± 0.089* | 0.018 ± 0.003 |
| Cranial mediastinal | 1 | 0.013 ± 0.002 | 0.018 ± 0.001 |
| | 10 | 0.025 ± 0.003* | 0.014 ± 0.0006* |
| | 20 | 0.085 ± 0.008* | 0.024 ± 0.001* |
| | 30 | 0.124 ± 0.019* | 0.020 ± 0.002* |
| | 60 | 0.211 ± 0.028* | 0.009 ± 0.001* |
| | 90 | 0.328 ± 0.064* | 0.010 ± 0.001 |
| Spleen | 1 | 0.030 ± 0.005 | 0.044 ± 0.011 |
| | 10 | 0.120 ± 0.025* | 0.063 ± 0.007* |
| | 20 | 0.160 ± 0.020 | 0.045 ± 0.004 |
| | 30 | 0.490 ± 0.035* | 0.081 ± 0.002* |
| | 60 | 0.730 ± 0.069* | 0.032 ± 0.002* |
| | 90 | 1.100 ± 0.103* | 0.035 ± 0.002 |

*Note*: *– indicated samples that are significantly different relative to the previous age group ($p > 0.05$)

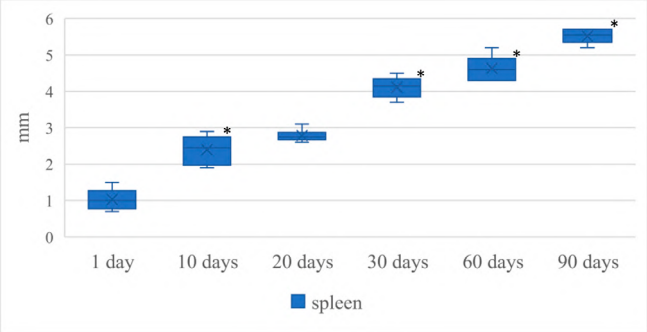


**Fig. 5.** Length dynamics of the rabbit spleen from day one to 90 days of age, mm, n=36.
*Note*: *– indicated samples that are significantly different relative to the previous age group ($p > 0.05$)

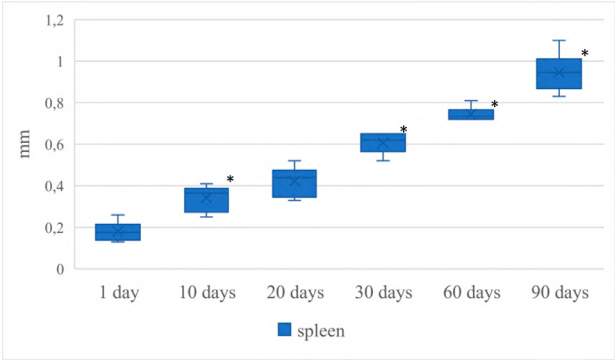


**Fig. 6.** Width dynamics of the rabbit spleen from day one to 90 days of age, mm, n=36.
*Note*: *– indicated samples that are significantly different relative to the previous age group ($p > 0.05$)

the median and average values were almost identical. The width parameters of the nodes correlated with each other, and the medians and average values practically coincided. Relative to the previous age period, the width and length of both lymph nodes grew evenly (Fig. 3, 4). The absolute mass of both lymph nodes increased, and the cranial mesenteric one reliably doubled. The relative mass of LN decreased, probably by 2.2 times in the cranial mediastinal one. At 60 days old, the increase in the parameters of spleen length and width relative to the previous age period was 1.1 and 1.2 times, respectively, while the median values were close to the average value (Fig. 5, 6). The spleen's absolute mass reliably increased by 34%, and the relative mass decreased by 2.5 times (Table).

With reaching the slaughter age (90 days in rabbits of precocious crosses), the length and width average values of the lymph nodes were close to the medians, while in the cranial mesenteric LN, the parameters increased by 1.1 and 1.7 times, and in the cranial mediastinal one by 1.2 and 1.2 times. The absolute mass of lymph nodes increased at 90 days of age (by 104 and 55%), and the relative mass increased by 1.4 and 1.1 times, however, this increase was unreliable (Table). The median and average values of the spleen length and width parameters in rabbits were practically the same, and relative to the previous age period, they increased (Fig. 5, 6). The spleen absolute and relative mass increased, but unreliably.

## Discussion

In the modern world, rabbits are increasingly considered not only from the point of view of an object for conducting scientific experiments but also as a precocious source of meat products. That is why there is a problem with their breeding within the boundaries of large farms that are engaged in the raising of hybrid crosses. One of the most pressing issues is maintaining the high immune status of these animals with the most intensive breeding technology. This is impossible without a thorough and comprehensive study of the immunomorphology of the hemopoietic and lymphopoietic organs, in particular, their morphogenesis in the period of postnatal adaptation (Vakulenko & Ochkovs'ka 2007; Savchuk & Demchuk, 2009).

Comparing the obtained results with previous studies conducted on domestic rabbits (Dănacu et al 2013; Willaert 2022) confirmed that the spleen of hybrid crosses does not differ significantly, having small sizes, an elongated flat shape, which varies depending on the age and body weight of the animals. In the scientific literature, there are also reports that in certain cases rabbit's spleen has a tongue-like shape or a tetrahedral shape (Grigorev & Moljanova, 2009), but in our research, such a shape of the spleen was not found in any animal. Hristov et al. (2006), Dimitrov et al. (2012) and Huynh & Berry (2017) reported the possibility of dividing the spleen into two unequal parts in the hilum area, which was also not confirmed by our studies. Vishnevskaya & Abramova (2015) and Ikegami et al (2016) have noted that spleen indicators such as the size, colour, consistency and shape are variable, in most cases, even within the same animal species, and depend on the functional load, the condition of the surrounding organs and the age of the studied animals.

Information about the topographical location of the rabbits' spleen is mostly similar. Its placement is described in the area of the middle third of the abdominal cavity with a shift to the caudo-medial surface of the abdominal wall. In the dorsal part, it is closed to the duodenum and reaches the pancreas, and latero-ventrally – to the jejunum (Hristov et al., 2006; Ryabikina et al., 2009; Gazizova et al., 2014; Dunajevs'ka, 2016).

Data on the topography of rabbit lymph nodes mostly refer to individual somatic nodes or their lymph centres (Dunne et al., 2003; Jeklova et al., 2007; Șeicaru, 2016). They are described as separate formations located in the direction of lymphatic vessels branching, which have a constant localization that does not change from birth to the end of life. Regarding visceral lymph nodes, the literature describes in detail the topography of gastric lymph nodes and the mesenteric lymph centre (Gazizova et al., 2014; Șeicaru, 2016; Gavrylin & Gibert, 2016). It is noted that these are separate groups located in adipose tissue at the roots of the corresponding arteries. Our research confirms they do not coalesce in rabbits, unlike other animal species.

## Conclusions

The topography of the cranial mediastinal and cranial mesenteric lymph nodes in hybrid cross rabbits corresponds to the general anatomical principles of these organs' localization in this mammalian species. Visceral lymph nodes of precocious crosses rabbits do not differ anatomically and topographically from rabbits of ordinary breeds. They are anatomically separated from each other within their groups, placed compactly, and do not form conglomerates.

The spleen in rabbits is flat and elongated, its topographic location is constant and does not change with age. A slight change in size and shape during postnatal ontogenesis is associated with the growth and formation of internal organs.

The morphometric parameters of the lymph nodes in Hyplus hybrid cross rabbits during the postnatal period are characterized by a gradual increase with maximum peaks at 20 days of age for the cranial mediastinal and 30 days – for the cranial mesenteric lymph node. In the spleen, the foremost indicators of an increase in morphometric parameters are 10-day and 30-day age. The largest indicators of the relative mass of these organs correspond to the maximum growth of their absolute mass.

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