УКРАЇНСЬКИЙ ЧАСОПИС ВЕТЕРИНАРНИХ НАУК

Видавець:
Національний університет біоресурсів і природокористування України

Рік заснування: 2010

Рекомендовано до друку та поширення
через мережу Інтернет Вченою радою
Національного університету біоресурсів і природокористування України
(протокол № 2 від 23 вересня 2025 р.)

Рішення Національної ради України
з питань телебачення і радіомовлення № 1795,
протокол № 31 від 21.12.2023 р.
Ідентифікатор медіа - R30-02299.

Журнал входить до переліку наукових фахових видань України

Категорія «А». Галузь наук – Ветеринарні,
Спеціальність H6 (211) - Ветеринарна медицина
(Наказ Міністерства освіти і науки України від 8 липня 2025 р. № 986)

Журнал представлено у міжнародних наукометричних базах даних, репозитаріях та пошукових системах: Google Scholar, Національна бібліотека України імені В. І. Вернадського, BASE, CAB Abstracts and Global Health (CABI), Polska Bibliografia Naukowa (PBN), Ulrichsweb, Dimensions, University of Oslo Library, University of Hull Library, SOLO – Search Oxford Libraries Online, European University Institute, Leipzig University Library, Cambridge University Library, UCSB Library, German Union Catalogue of Serials, OUCI (Open Ukrainian Citation Index), WorldCat, Фахові видання України, EBSCO, Litmaps

Адреса редакції:
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https://veterinaryscience.com.ua/uk

UKRAINIAN JOURNAL OF VETERINARY SCIENCES

Publisher:
National University of Life and Environmental Sciences of Ukraine

Year of foundation: 2010

Recommended for printing and distribution
via the Internet by the Academic Council
of National University of Life and Environmental Sciences of Ukraine
(Minutes No. 2 of September 23, 2025)

Decision of the National Council
of Television and Radio Broadcasting of Ukraine No. 1795,
Minutes No. 31, dated 21.12.2023.
Media identifier - R30-02299.

The journal is included in the list of professional publications of Ukraine
Category "A".
Specialty: 0841 – Veterinary
(Order of the Ministry of Education and Science of Ukraine
of July 8, 2025, No. 986)

The journal is presented international scientometric databases, repositories and scientific systems: Google Scholar, Vernadsky National Library of Ukraine, BASE, CAB Abstracts and Global Health (CABI), Polska Bibliografia Naukowa (PBN), Ulrichsweb, Dimensions, University of Oslo Library, University of Hull Library, SOLO - Search Oxford Libraries Online, European University Institute, Leipzig University Library, Cambridge University Library, UCSB Library, German Union Catalogue of Serials, OUCI (Open Ukrainian Citation Index), WorldCat, Professional publications of Ukraine, EBSCO, Litmaps

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UKRAINIAN JOURNAL OF VETERINARY SCIENCES

2025. Volume 16, No. 3. P. 9-25

Received: 15.05.2025 Revised: 06.08.2025 Accepted: 23.09.2025

DOI: 10.31548/veterinary3.2025.09

UDC: 636.92.09:616-018:602.9

Microscopic changes in experimentally damaged rabbit bone following intravenous administration of stem cells

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Abstract. The search for methods and means of stimulating reparative osteogenesis is a pressing issue in modern veterinary traumatology that requires further study, and the use of stem cells

Suggested Citation:
Savchuk, T., Malyuk, M., Kharkevych, I., Bokotko, R., & Paramonova, Yu. (2025). Microscopic changes in experimentally damaged rabbit bone following intravenous administration of stem cells. *Ukrainian Journal of Veterinary Sciences*, 16(3), 9-25. doi: 10.31548/veterinary3.2025.25.

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is considered one of the most promising approaches in this area. The study aimed to investigate microscopic changes in experimentally damaged bone tissue of rabbits under the influence of intravenously administered allogeneic mesenchymal stem cells on reparative osteogenesis. Mesenchymal stem cells were obtained from rabbit bone marrow and cultured in a nutrient medium according to standard protocols. The injury was simulated using a 2.5 mm diameter surgical drill on the medial surface of the middle third of the tibial diaphysis. After 24 hours, the animals in the experimental group were given a single intravenous injection (into the jugular vein) of 3.5 × 106 stem cells. The defect in the animals in the control group healed naturally. Histological studies were performed on days 3, 7, 14, 21, 28 and 42 of the experiment. The samples were stained with haematoxylin-eosin and examined under a microscope. Intravenous administration of allogeneic mesenchymal stem cells to animals stimulated regenerative processes and accelerated the stages of reparative osteogenesis at the site of injury. The study determined that in the experimental group, already on the third day, there were no blood clots and bone tissue fragments in the damage area, and the growth of fibrous connective tissue and intensive osteogenesis were recorded. The formation of bone callus and consolidation of bone tissue proceeded faster than in the control group. By day 21, the bone marrow had already acquired a normal structure, while in the control group, a similar result was observed only on day 28. Almost complete restoration of the defect after the introduction of stem cells was observed on day 28, while in the control group, a similar result was achieved only on day 42. The results of the study can be used to stimulate reparative osteogenesis and further study the processes of the influence of stem cells on the restoration of damaged tissues

Keywords: reparative osteogenesis; bone tissue; bone callus; bone marrow; allogeneic mesenchymal stem cells

Introduction

One of the key problems in veterinary surgery remains the improvement of methods for treating bone fractures in animals. Serious damage to bone tissue can threaten the life of an animal or impair the functionality of bone structures. Despite significant progress in this area, the mechanisms that promote accelerated reparative osteogenesis have not yet been sufficiently studied. A promising direction in bone tissue regeneration is the transplantation of allogeneic mesenchymal stem cells (MSCs) obtained from bone marrow or adipose tissue. This method has been successfully used to stimulate the regeneration of other tissues (Uğurbaş *et al.*, 2021).

A successful solution to this problem requires mastery not only of the techniques of connecting and fixing bone fragments, but also a basic awareness of the dynamics of changes occurring during reparative regeneration. As noted by L. Impieri *et al.* (2024), successful restoration of damaged bone requires proficiency in methods of stimulating osteogenesis and means of avoiding complications. According to Y. Zhang *et al.* (2024), autologous bone tissue and alloplastic materials are widely used to treat bone tissue damage. Despite their advantages, practicality and popularity, these methods are not universal due to several significant issues. The main problems include the risk of nerve damage, the occurrence of pain syndrome, the development of infections, and the limited availability of donor material.

At the same time, V. Kutsevlyak & O. Lyubchenko (2024) proposed an alternative approach that involves the use of biologically active substances and stem cell transplantation to regenerate damaged bone tissue. J. Zou *et al.* (2023) proved that mesenchymal stem cells, due to the ease of their isolation and cultivation, have proven to be a particularly promising source for the treatment and regeneration of bone defects, as well as for maintaining bone tissue homeostasis. A substantial prerequisite for the successful functioning of mesenchymal stem cells in osteogenesis is the selection of an adequate cell culture medium. Studies conducted by W. Pan *et al.* (2024) and T.L. Savchuk *et al.* (2025) demonstrated that autologous cell injections are already being used for various pathologies and may be a promising alternative for accelerating the healing process of bone fractures.

Stem cells are increasingly applied for bone tissue regeneration. F. Li *et al.* (2022) proposed innovative strategies for restoring lost bone tissue using MSCs, growth factors, and special bone tissue scaffolds. This approach aims to overcome the shortcomings of traditional treatments, such as the use of autologous grafts or artificial bone substitutes. In addition, M. Murayama *et al.* (2024) demonstrated that a matrix based on hydroxyapatite-tricalcium phosphate populated with allogeneic mesenchymal stem cells provides effective repair of femoral bone defects in large dogs. At the same time, the use of immunosuppressive therapy was not necessary.

R.D. Calixto *et al.* (2023) demonstrated the ability of injected stem cells to actively migrate to the bone defect area and differentiate into specific cells, such as osteoblasts and chondrocytes. In turn, studies by Y. Guo *et al.* (2024) demonstrated that in the bone microenvironment, allogeneic MSCs can originate from the peripheral walls of stromal vessels located on the surface of trabecular bone and fill interfibrillar spaces for tissue regeneration. According to A.M. Theodosaki *et al.* (2024), MSCs can stimulate cell proliferation and angiogenesis, reducing inflammation, and producing significant amounts of bioactive molecules involved in tissue regeneration processes.

Therefore, the use of cell therapy methods to restore the structure and functions of damaged tissues in animals is becoming increasingly relevant. Modern orthopaedics emphasises the use of stem cells, which creates prospects for solving the problem of stimulating reparative osteogenesis. The study aimed to evaluate microscopic changes in the course of reparative processes in experimentally damaged bone tissue and the stimulating effect of intravenously administered allogeneic mesenchymal stem cells on it.

Literature Review

Musculoskeletal injuries in animals are quite common, and their nature depends significantly on the mechanism of occurrence, severity, and treatment methods. Bone tissue can be damaged both by external factors, such as trauma, and by internal processes in the body, such as tumour formation. According to H. ElHawary *et al.* (2021), the body of an animal is not capable of independently regenerating critically large bone defects. Bone tissue repair is often accompanied by impaired consolidation of bone fragments, as noted by researchers A.I. Alford *et al.* (2021). F. Shen & Y. Shi (2022) identified that difficulties in regenerating damaged bone can cause delayed union, non-union of fragments, or even the formation of false joints.

In modern medicine, various methods of clinical and tissue therapy are becoming increasingly relevant, demonstrating potential in the treatment of complex wounds and injuries. As noted by S. Avnet *et al.* (2021), many of these cases cannot be effectively treated using traditional methods. According to O. Wittig *et al.* (2016), cell biotechnologies are notable, as they create new opportunities, particularly in

the regeneration of bone tissue in conditions of its deficiency. M. Jayankura *et al.* (2021) suggest local implantation of allogeneic bone marrow MSCs as a treatment option for animals with delayed union or non-union of damaged bones, which promotes the replacement of defective or missing osteoblastic cells. Mesenchymal stem cells are a type of cell with a characteristic immunophenotype that has the ability to differentiate into cells of various organs and tissues. This process depends on microenvironmental factors, as noted by A.O. Luby *et al.* (2019) and Y. Ren *et al.* (2021). According to studies by V. Venkataiah *et al*. (2021), the use of MSCs demonstrates significant advantages, as it minimises the risks associated with the immunological and microbiological safety of the recipient.

Since the early 2000s, the therapeutic potential of mesenchymal stem cells has received considerable attention, as evidenced by O. Riester *et al.* (2020) and R.R. Bokotko *et al.* (2021), in an analysis of their effect on various pathological processes in the body. Experimental studies by M.P. Benavides-Castellanos *et al.* (2021) suggest that mesenchymal stem cells from bone marrow and adipose tissue have comparable potential for bone formation in vitro and in vivo. A study conducted by A.M. Dimarino *et al.* (2013) on rats demonstrated that MSCs can promote complete graft-to-bone union. Accordingly, bone grafts should be implanted with bone tissue stimulators or pre-treated with stem cells to ensure regeneration.

S.P. Bruder *et al.* (1998) determined that the use of a porous ceramic scaffold saturated with mesenchymal stem cells promoted a faster regenerative process in dogs with segmental femoral defects compared to cases where a scaffold without cells was used. In turn, E. Kon *et al.* (2000) demonstrated the positive effect of implanting a porous hydroxyapatite carrier enriched with MSCs on the course of the regenerative process in sheep. The use of MSCs in the process of xenogeneic matrix transplantation contributed to the suppression of the foreign material rejection reaction, as indicated in the study by F.M. Elahi *et al.* (2020). In addition, the results of C.R. Harrell *et al.* (2021) demonstrate that stem cells can facilitate the intracellular recurrent response of the transplant and effectively reducing the risk of rejection in kidney transplantation in rats.

Following M. Ullah *et al.* (2019), Intravenous administration of MSCs within the first 24 hours after femoral fracture in mice promoted significant accumulation of these cells in the injury site after 7 days. This mechanism is key to the regeneration process, as it indicates the involvement of progenitor cells, which significantly contribute to bone tissue healing. In addition to their high differentiation potential, MSCs are also characterised by potent immunomodulatory and angiomodulatory properties, as highlighted by R. Otsuka *et al.* (2020) and Y. He *et al.* (2022).

An analysis of the current state of treatment of animals with bone regeneration disorders has shown that, despite a significant number of studies, there are still controversial and unresolved issues. Among them, the question of improving the process of reparative osteogenesis is relevant. In this regard, there is a need to introduce new effective methods that would improve the conditions for bone tissue regeneration. Therefore, research into the properties of animal stem cells and their use in experimental bone tissue damage is a substantial and relevant area that will contribute to the development of scientifically sound and effective approaches to cell therapy in veterinary medicine.

Materials and Methods

The experimental study was conducted during 2021-2022 at the educational and scientific laboratory "Centre for Cell Technologies in Veterinary Medicine", which operates based on the Department of Veterinary Surgery named after

Academician I.O. Povazhenko at the National University of Life and Environmental Sciences of Ukraine (NULES), Kyiv, Ukraine. Thirty-six three-month-old chinchilla rabbits, each with an average body weight of 3 kg, were used in the study. The conditions of keeping and feeding were the same for all animals and fully complied with the standard requirements for this species. The use of rabbits in experiments was conducted in compliance with the requirements of Directive 2010/63/EU (2010), Law of Ukraine No. 3447-IV (2006) and the permission of the bioethics committee of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine (Protocol No. 80-1 of 27 October 2020).

Mesenchymal stem cells were obtained from the bone marrow aspirate of the femur of clinically healthy donor rabbits. The extracted cell mass was cultured in a standard medium consisting of 80% DMEM (Gibco™, USA) and 20% calf foetal serum (Sigma, USA), with the addition of 10 µl/cm^3 of antibiotic-antimycotic medium (VioWest, France). Cell isolation and cell material manipulation procedures were performed in a Class II biological safety cabinet (ESCO). Cultivation was conducted in a CO2 incubator (HERA CELL, Germany) at 37°C and 5% CO_2 concentration. At the same time, MSCs settled, adhering to the surface of Petri dishes and spreading out. The suspended culture of haematopoietic cells was removed, after which only those cells that had adhesive properties were further cultivated. Cell passage was performed at a ratio of 1:2 (from one Petri dish to two) at a seeding density of 5 x 10^4 cells/cm^2. Microscopic analysis of the culture was performed using a PrimoVert inverted microscope (Germany). Cells were harvested at the third passage, counted in the obtained substrate, and doses were prepared for administration (Fig. 1).

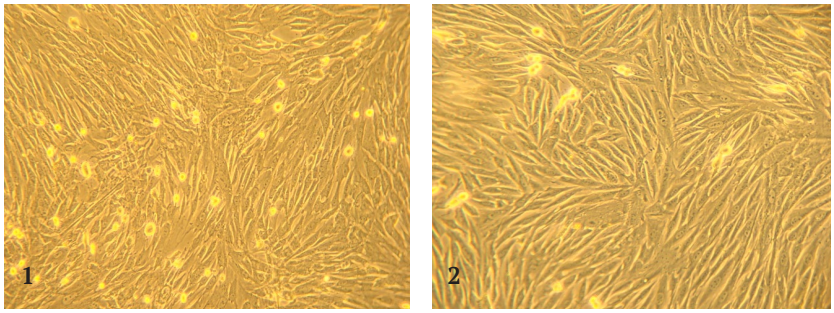

Figure 1. Unstained live culture of mesenchymal stem cells from rabbit bone marrow
***Note**: 1 – zero pass; 2 – third pass. ×100*
***Source**: compiled by the authors*

Two groups of animals were formed for the experiment: a control group and an experimental group (18 animals in each). At the initial stage of the study, animals in both groups were artificially subjected to mechanical damage to the tibia. To minimise trauma to healthy structures, the damage was created in the middle third of the tibial diaphysis from the medial surface in the form of a rounded defect using a 2.5 mm diameter surgical drill. The operation was performed under general anaesthesia (Zoletil at a dose of 0.05 mg/kg of animal weight). Before this, infiltration anaesthesia was performed in surgical intervention with a 0.5% solution of Novocaine. Before the start of the operation, the 2 × 2 cm surgical field was vibrated and treated twice with a 5% iodine solution to ensure sterility. All stages of the surgical

intervention were performed in accordance with the rules of asepsis and antisepsis. The wound was sutured after forming a defect 0.5 mm deep and 2.5 mm in diameter, and the animal was brought out of anaesthesia. After modelling the experimental bone tissue damage, the animals in the experimental group were administered a single intravenous (into the jugular vein) injection of 3.5×10^6 allogeneic mesenchymal bone marrow stem cells in 0.5 mL of phosphate-buffered solution using a syringe. The animals in the control group did not receive treatment, and wound healing occurred naturally.

From each group, three animals were selected on days 3, 7, 14, 21, 28, and 42 of the experiment to obtain bone tissue samples, which were used for histological analysis. The selected tibia samples were labelled and fixed in a 10% aqueous solution of neutral formalin for 7 days. After that, the tissue was decalcified in a 5% nitric acid solution for 72 hours. Pieces 2-3 mm thick were cut from the decalcified bone, washed with tap water, dehydrated in solutions of ethyl alcohol with increasing concentration, and sealed with celluloidin.

For histological analysis, 7-9 μm thick sections were prepared using a microtome and stained with Karatsy's haematoxylin and eosin. The prepared specimens were examined under a Micros MSI 100 LED microscope (Germany). The histological sections were evaluated considering the surface structure of the bone, the characteristics of the newly formed tissue, and the presence and location of cellular elements in the damaged area.

Results and Discussion

The studies showed that on the third day of the experiment, in contrast to the control group (Fig. 2), no clots with blood cell elements were found in the experimental group animals in the created defect or in the adjacent bone marrow. In addition, no bone tissue fragments formed as a result of bone defect modelling were recorded. At this stage of observation, the defect itself was filled with reticular tissue (Fig. 3).

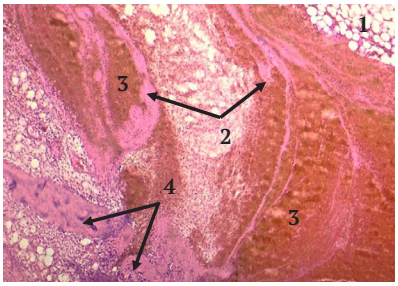

Figure 2. Condition of the defect on the third day of the experiment in animals of the control group
***Note**: 1 – bone marrow; 2 – fibrin strands; 3 – blood clots; 4 – bone fragments. Haematoxylin, Karatsi, and eosin, ×100*
***Source**: compiled by the authors*

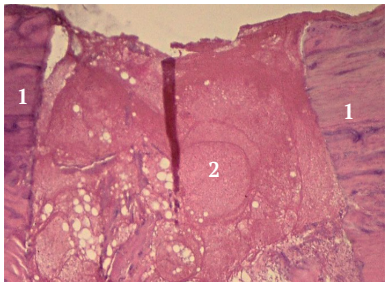

Figure 3. Condition of the defect on the third day of the experiment in animals of the experimental group
***Note**: 1 – bone tissue around the defect; 2 – reticular tissue at the site of the defect. Haematoxylin, Karatsi and eosin, ×50*
***Source**: compiled by the authors*

The bone tissue located near the defect site in the experimental group animals had a typical microscopic structure characteristic of healthy bone. In the defect area itself, an active process of fibrous connective tissue formation was observed, which served as a kind of basis for further regeneration (Fig. 4). New foci of bone tissue

appeared in the structure of the bone marrow, indicating the activation of reparative mechanisms. However, this newly formed tissue had not yet reached the full morphological maturity characteristic of tubular bones. Osteocytes within this tissue were arranged chaotically, indicating the initial stage of new structure formation. In addition, the orientation of the newly formed osteons remained unregulated, which is natural for the early stages of bone regeneration.

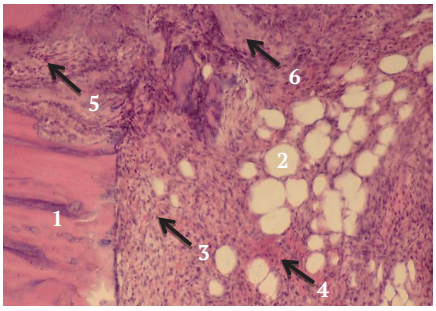

Figure 4. Condition of the defect on the third day of the experiment in animals of the experimental group
***Note**: 1 – bone tissue on the side of the defect; 2 – newly formed bone tissue; 3 – accumulation of red blood cells; 4 – reticular tissue; 5 – fibroblast proliferation; 6 – proliferation of fibrous connective tissue. Haematoxylin, Karatsi, and eosin, ×100*
***Source**: compiled by the authors*

The processes of fibrous connective tissue growth and bone tissue formation in the damaged bone marrow area are activated by the intravenous administration of allogeneic mesenchymal stem cells. This procedure not only contributes to a significant increase in the mechanical strength of the tibia as a whole, but is also significant in compensating for the mechanical weakness caused by the defect. Thus, on day 3 after intravenous administration of allogeneic mesenchymal stem cells, the first phase of reparative regeneration was observed, manifested as the onset of the third stage of inflammation proliferation. In contrast, only the first and second stages of the inflammatory process, including alteration and vascular reaction with exudation, were observed in the control group animals. The data obtained are consistent with the findings of studies conducted by R. Otsuka *et al.* (2020) and T. Duangchan *et al.* (2021), which showed that the introduction of mesenchymal stem cells contributes to the manifestation of their immunomodulatory properties and affects the course of the inflammatory process that occurs when bone tissue is damaged.

On the 7th day of the experiment, the animals in the experimental group showed significant defect repair, which differed significantly from the animals in the control group (Fig. 5). In the defect area, partial filling with bone tissue was observed, extending to the entire depth of the affected area. In addition, an active process of osteogenesis was detected, as evidenced by a significant number of fibroblasts and osteoblasts, which demonstrated high activity, producing structural elements necessary for the formation of bone tissue. The dynamics of these cells emphasise enhanced regeneration in the damage zone (Fig. 6).

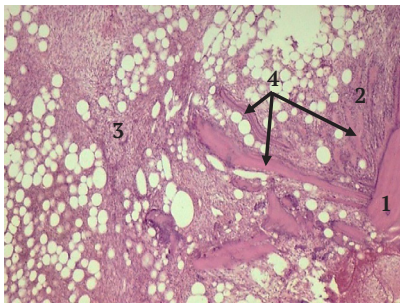

Figure 5. Condition of the defect on the 7th day of the experiment in animals of the control group
***Note**: 1 – bone tissue on the side of the defect site; 2 – proliferation of fibrous connective tissue in the bone marrow on the side of the defect site; 3 – proliferation of fibrous connective tissue in the bone marrow opposite the defect site; 4 – newly formed bone tissue. Haematoxylin, Karatsi, and eosin, ×50*
***Source**: compiled by the authors*

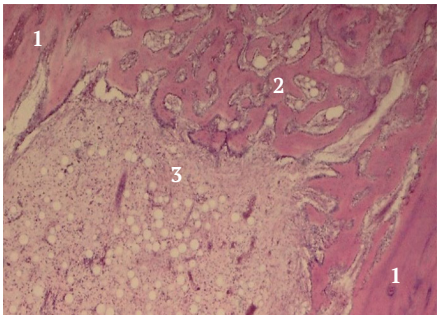

Figure 6. Condition of the defect on the 7th day of the experiment in animals of the experimental group
***Note**: 1 – bone wall; 2 – newly formed bone tissue; 3 – proliferation of fibrous connective tissue in the bone marrow. Haematoxylin, Karatsi, and eosin, ×50*
***Source**: compiled by the authors*

In the area of the created bone marrow defect, active formation of fibrous connective tissue was observed, along with a pronounced process of osteogenesis. On the outside of the bone in the damaged area, small foci of bone formation could be seen, which were beginning to develop. At the same time, the osteon plates of the compact bone layer were in the early stages of differentiation, demonstrating a low level of structural maturity. On the outer side of the bone, in the defect area, active formation of bone callus was observed, consisting mainly of newly formed cartilage tissue. Within this tissue, there were occasional thin strands of fibrous connective tissue, which were insignificant in terms of quantity and structural density (Fig. 7). In addition, the initial formation of bone tissue was already noticeable, indicating the gradual restoration of the damaged area and the start of regeneration processes.

Similar results were recorded in studies conducted by S.P. Bruder *et al.* (1998) and S. Poliwoda *et al.* (2022), where a ceramic carrier enriched with mesenchymal stem cells was used. At the same time, the study showed that in animals of the experimental group, after the introduction of stem cells, the process of cartilage and bone callus formation had already begun. Thus, on day 7, the animals in the experimental group showed the second phase of reparative regeneration differentiation. At the same time, the animals in the control group showed only the third stage of the inflammatory process, proliferation, which was accompanied by active growth of fibrous connective tissue in the defect area and a significant increase in osteogenesis.

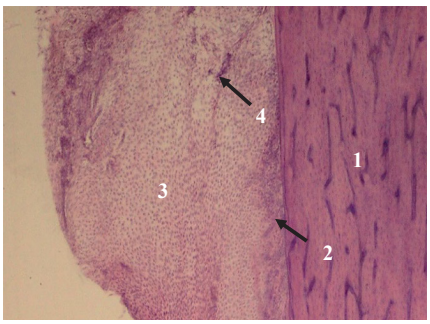

Figure 7. Bone callus on the 7th day of the experiment in animals of the experimental group
***Note**: 1 – bone in the defect area; 2 – newly formed bone tissue; 3 – newly formed cartilage tissue of the bone callus; 4 – fibrous connective tissue. Haematoxylin, Karatsi, and eosin, ×50*
***Source**: compiled by the authors*

On the 14th day of the experiment, the defect area in the experimental group animals was completely covered with newly formed bone tissue. According to the results of microscopic analysis, this tissue was characterised by a structure similar to cancellous bone (Fig. 8), which differed significantly from the condition in the control group animals (Fig. 9). In addition, active growth of fibrous connective tissue was observed in the bone marrow area, accompanied by an intense process of osteogenesis. Thanks to these regenerative changes, new bone tissue was formed in the

specified area, demonstrating a high level of regenerative capacity.

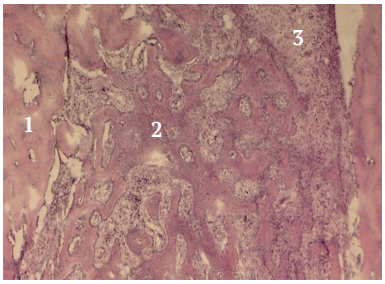

Figure 8. Condition of the defect on the 14th day of the experiment in animals of the experimental group
***Note**: 1 – bone wall; 2 – proliferation of fibrous connective tissue; 3 – newly formed bone tissue. Haematoxylin, Karatsi, and eosin, ×50*
***Source**: compiled by the authors*

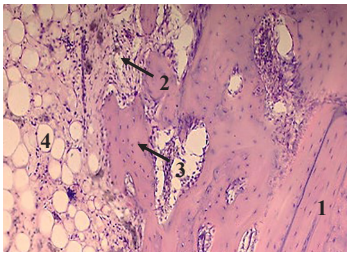

Figure 9. Condition of the defect on the 14th day of the experiment in animals of the control group
***Note**: 1 – newly formed bone tissue; 2 – fibrous connective tissue; 3 – osteogenesis in bone marrow; 4 – bone marrow. Haematoxylin, Karatsi, and eosin, ×100*
***Source**: compiled by the authors*

On the surface of the newly formed bone tissue in the defect area in the experimental group, bone callus formation was observed. The bone callus was characterised by the presence of a thick layer of dense fibrous connective tissue (Fig. 10). Inside the bone callus, a fairly active process of osteogenesis was observed, indicating intensive regeneration and formation of bone structures in the studied area.

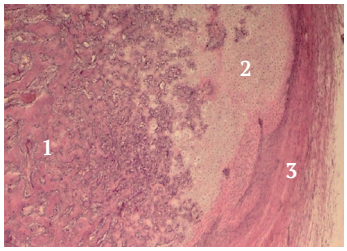

Figure 10. Bone callus on the 14th day of the experiment in animals of the experimental group
***Note**: 1 – dense fibrous connective tissue; 2 – cartilage tissue; 3 – bone tissue. Haematoxylin, Karatsi, and eosin, ×50*
***Source**: compiled by the authors*

Thus, on day 14, the animals in the experimental group underwent the third phase of reparative regeneration reorganisation, while the animals in the control group underwent the second stage of this process differentiation with active formation of tissue-specific structures and cell differentiation in the damage area. Studies by Y. Ren *et al.* (2021) demonstrated similar changes with the use of mesenchymal stem cells for the regeneration of bone tissue defects in dogs. In subsequent works, R.D. Calixto *et al.* (2023) and Y. Guo *et al.* (2024) confirmed the ability of stem cells to undergo osteogenic differentiation, which is central in the process of bone defect consolidation during this period.

On the 21st day of the study, the defect site in the experimental group animals was filled with newly formed bone tissue. The structure of this tissue was similar to the microscopic structure of compact bone, where osteoid-like formations were already visible, indicating an active phase of regeneration (Fig. 11). Microscopic examination of the bone callus revealed that its structure corresponded to the characteristic features of compact bone. The outer layer of the callus was covered with dense fibrous connective tissue, which provided additional protection and insulation from the external environment.

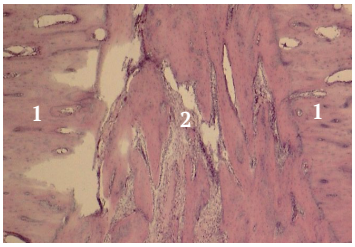

Figure 11. Condition of the defect on the 21st day of the experiment in animals of the experimental group
***Note**: 1 – bone tissue around the defect; 2 – newly formed bone tissue. Haematoxylin, Karatsi and eosin, ×50*
***Source**: compiled by the authors*

Compared to the control group animals (Fig. 12), the newly formed bone tissue showed a higher degree of maturity. This was evidenced not only by the presence of isolated large irregularly shaped cavities, but also by the formation of new osteons, which is a characteristic feature of mature bone structure. In addition, numerous areas of increased calcium salt concentration were observed in the newly formed bone matrix, indicating active processes of mineral saturation of the tissue.

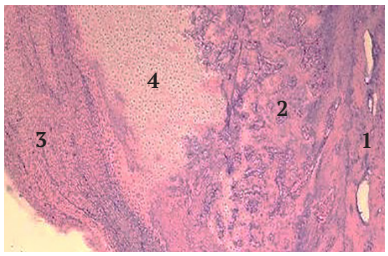

Figure 12. Condition of the defect on the 21st day of the experiment in animals of the control group
***Note**: 1 – newly formed bone tissue; 2 – inner part of the bone callus; 3 – outer part of the bone callus; 4 – large round cells. Haematoxylin, Karatsi and eosin, ×50*
***Source**: compiled by the authors*

According to the author's position, which is consistent with the data from studies by M.P. Benavides-Castellanos *et al.* (2020) and W. Katagiri *et al.* (2022), there was an evident intensification of bone matrix calcification processes under the influence of allogeneic mesenchymal stem cells, which stimulated reparative osteogenesis. The structure of the bone marrow in the defect area was similar to the microscopic structure of intact bone marrow. There were isolated areas of newly formed bone tissue, and the level of cell proliferation in these areas was relatively low (Fig. 13).

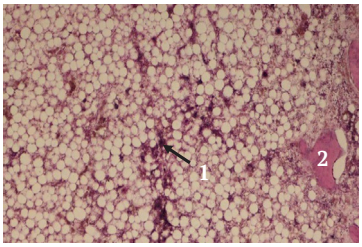

Figure 13. Bone marrow at the site of the defect on the 21st day of the experiment in animals of the experimental group
***Note**: 1 – cell proliferation; 2 – bone tissue centre. Haematoxylin, Karatsi, and eosin, ×100*
***Source**: compiled by the authors*

Thus, on day 21, the animals in the experimental group transitioned to the fourth phase of reparative regeneration remodelling. At that time, the animals in the control group were still in the third phase of this process, which involves the reorganisation of tissue structures and their gradual mineralisation. On day 28 of the experiment, the defect site in the experimental group animals was filled with compact bone tissue, the structure of which resembled the typical structure of compact bone. At the same time, its microscopic organisation remained somewhat disordered, and the channels remained quite wide (Fig. 14). Compared to the animals in the control group (Fig. 15), the bone callus in the experimental group was significantly smaller and showed signs of hardening

to bone. At the defect site, the newly formed bone tissue showed signs of greater maturity. The study noted that the surface of the bone callus was covered with a thick layer of dense fibrous connective tissue. Such changes may indicate a significant acceleration of bone tissue formation in the defect area due to the introduction of allogeneic MSCs.

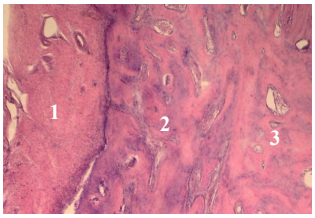

Figure 14. Condition of the defect on the 28th day of the experiment in animals of the experimental group
***Note**: 1 – dense fibrous connective tissue; 2 – bone callus; 3 – newly formed bone tissue at the site of the defect. Haematoxylin, Karatsi and eosin, ×50*
***Source**: compiled by the authors*

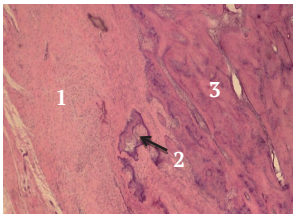

Figure 15. Condition of the defect on the 28th day of the experiment in animals of the control group
***Note**: 1 – dense fibrous connective tissue; 2 – bone callus; 3 – newly formed bone tissue at the site of the defect. Haematoxylin, Karatsi and eosin, ×50*
***Source**: compiled by the authors*

Microscopic changes in bone tissue structure in animals of the experimental group, observed on day 28 of the experiment, indicate a transition to the fifth phase of reparative regeneration. This stage is characterised by the completion of recovery processes, which include not only the return of tissue to its original form, but also the restoration of the functional properties of the bone structure. Similar early morphological changes at this stage may be an indicator of the activation of regeneration mechanisms, in particular the migration of stem cells to the site of bone damage and their influence on the process of osteogenesis, as noted by M. Ullah *et al.* (2019) and A. Theodosaki *et al.* (2024). At the same time, only the fourth phase of the regenerative process, which emphasises tissue remodelling, was observed in the control group animals.

On day 42 of the study, no bone callus was observed in the experimental group animals, unlike in the control group animals (Fig. 16). Newly formed bone tissue was observed in the defect area, which was already approaching the typical structure of compact bone. However, there were still some cavities in its structure, but their number was insignificant (Fig. 17). During the observation period, no other significant microscopic changes were found in the animals of the experimental group. At the same time, the animals in the control group underwent the fifth phase of reparative osteogenesis, which was characterised by the completion of the process: restoration of the structure and functional capacity of bone tissue.

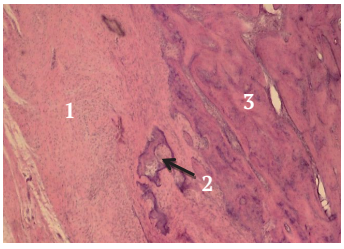

Figure 16. Condition of the defect on the 42nd day of the experiment in animals of the control group
***Note**: 1 – intact bone tissue on the side of the defect site; 2 – newly formed bone tissue; 3 – bone marrow; 4 – capillary hyperemia. Haematoxylin, Karatsi and eosin, ×50*
***Source**: compiled by the authors*

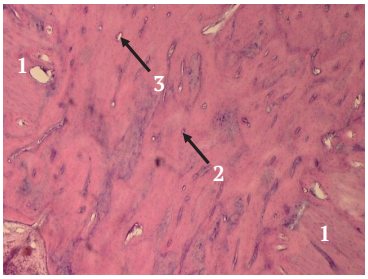

Figure 17. Condition of the defect on the 42nd day of the experiment in animals of the experimental group
***Note**: 1 – bone tissue on the side of the defect; 2 – osteon of newly formed bone tissue; 3 – osteon canal. Haematoxylin, Karatsi and eosin, ×50*
***Source**: compiled by the authors*

In general, the results obtained regarding the effect of allogeneic mesenchymal stem cells on the process of reparative osteogenesis showed a high degree of consistency with the data presented in the works of F. Shen & Y. Shi (2022) and J. Zou *et al.* (2023). These scientific works proved that mesenchymal stem cells can actively migrate to the sites of damage, thereby promoting accelerated restoration of damaged tissue areas and ensuring a more effective and rapid healing process. The current study provides a detailed analysis of the microscopic changes accompanying the process of bone tissue regeneration and investigates the effect of intravenous administration of allogeneic mesenchymal stem cells on the corresponding dynamics. The results indicate that the use of allogeneic MSCs accelerates the process of reparative osteogenesis, which highlights their therapeutic potential in the restoration of damaged bone tissue.

Conclusions

Histological studies conducted by experimentally modelling the pathological process in the tibia through mechanical damage with defined parameters can be used to track in detail the stages of reparative regeneration of bone tissue. In addition, they provided reliable data on the effectiveness of intravenously administered allogeneic mesenchymal stem cells as a stimulator of reparative osteogenesis.

The study established that on the third day of the study, no blood clots or bone tissue fragments were observed in the bone tissue of the experimental group animals after intravenous administration of allogeneic MSCs in the created defect and in the adjacent bone marrow, unlike the animals in the control group. Intensive formation of new bone tissue and active growth of fibrous connective tissue in the created defect in the animals of the experimental group began on the third day of the experiment, while similar processes in the control group were recorded only on the seventh day. The process of cartilage callus formation in the experimental group began much more quickly and could be observed on the 7th day of the experiment, while in the control group, this process was delayed, appearing only on the 14th day of the study. The formation of bone callus in the experimental group was recorded on the 14th day of the experiment, while in the control group, the same process started much later and was recorded only on the 21st day of the experiment, which showed a significant difference in the rate of tissue regeneration. Bone marrow regeneration in the experimental group was established on the 21st day, while in the control group, similar changes were recorded only on the 28th day of the study.

Intravenous administration of allogeneic MSCs demonstrated the ability to significantly activate regenerative processes and accelerate the stages of reparative osteogenesis in the damaged area. Under these conditions, defect repair was practically completed by day 28 of the experiment, whereas in the control group animals, it was completed by day 42. The results obtained open up prospects for the introduction of new effective methods of

stimulating reparative osteogenesis, as well as creating a basis for more in-depth research into the effect of stem cells on the regeneration of damaged tissues. Accordingly, a promising direction for further scientific research is the use of allogeneic mesenchymal stem cells for the treatment of various clinical cases of bone tissue damage in animals.

Acknowledgements

None.

Funding

The study was not funded.

Conflict of Interest

None.

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Мікроскопічні зміни в експериментально ушкодженій кістці кролів за внутрішньовенного введення стовбурових клітин

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Анотація. Актуальною проблемою сучасної ветеринарної травматології, котра потребує вивчення, є пошук способів та засобів стимулювання репаративного остеогенезу і одним із перспективних підходів у цьому напрямі вважається застосування стовбурових клітин. Метою досліджень було вивчити мікроскопічні зміни в експериментально ушкодженій кістковій тканині кролів за впливу на репаративний остеогенез внутрішньовенно введених алогенних мезенхімальних стовбурових клітин. Мезенхімальні стовбурові клітини отримано з кісткового мозку кролів і культивовано у живильному середовищі згідно зі стандартними протоколами. Моделювання ушкодження виконували хірургічним свердлом діаметром 2,5 мм на медіальній поверхні середньої третини діафіза великогомілкової кістки. Через добу тваринам дослідної групи одноразово внутрішньовенно (у яремну вену) вводили 3,5 × 106 стовбурових клітин. Відновлення дефекту у тварин контрольної групи відбувалось природним способом. Гістологічні дослідження проводили на 3, 7, 14, 21, 28 та 42 добу експерименту. Зразки забарвлювали гематоксиліном-еозином та вивчали під мікроскопом. Внутрішньовенне застосування тваринам алогенних мезенхімальних стовбурових клітин стимулювало регенеративні процеси та прискорювало етапи репаративного остеогенезу в

місці пошкодження. Встановлено, що у дослідної групи вже на 3 добу в зоні ушкодження були відсутні згустки крові і уламки кісткової тканини та зафіксовано розростання волокнистої сполучної тканини й інтенсивний остеогенез. Формування кісткового мозоля та консолідація кісткової тканини проходили швидше аніж у контрольній групі. До 21 доби кістковий мозок набував вже нормальної будови, тоді як у контрольній групі аналогічний результат спостерігали лише на 28 добу. Практично повне відновлення дефекту за введення стовбурових клітин спостерігалося на 28 добу, а у контрольної групи аналогічний результат досягався лише на 42 добу. Результати дослідження можуть бути використані для стимулювання репаративного остеогенезу та подальшого вивчення процесів впливу стовбурових клітин на відновлення ушкоджених тканин

Ключові слова: репаративний остеогенез; кісткова тканина; кісткова мозоль; кістковий мозок; алогенні мезенхімальні стовбурові клітини

UKRAINIAN JOURNAL OF VETERINARY SCIENCES

2025. Volume 16, No. 3. P. 26-44

Received: 26.05.2025 Revised: 19.08.2025 Accepted: 23.09.2025

DOI: 10.31548/veterinary3.2025.26

UDC:619: 612.6:614.9.618.636.09

Morpho-physiological features of endometrial and ovarian remodelling in cows during the oestrous cycle

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Suggested Citation:
Bondarenko, I., Mutlu Yaganoglu, A., Lazorenko, A., Koreneva, Zh., & Karpovskyi, V. (2025). Morpho-physiological features of endometrial and ovarian remodelling in cows during the oestrous cycle. *Ukrainian Journal of Veterinary Sciences*, 16(3), 26-44. doi: 10.31548/veterinary3.2025.26.

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Abstract. The profitability of dairy farming directly depends on the intensification of reproduction indicators, which requires an increase in the percentage of calves per hundred cows and optimisation of milk productivity. Given the need to improve reproductive efficiency, the aim of the study was to identify the morphological and physiological changes in the ovaries and endometrium of cows during the oestrous cycle for the further development of evidence-based methods for correcting sexual cyclicity. Analysis of histological samples revealed a number of important changes. During the oestrus phase, the endometrium shows maximum swelling and blood filling of the arterioles, while the cells of the functional layer become decidual-like due to the active growth of stromal cells. Numerous uterine glands have a convoluted shape and enlarged cavities filled with secretions, while the epithelial cells of the glands become multinucleated with vacuolisation due to the accumulation of glycogen. Primary follicles with cubic-shaped follicular cells are recorded in the ovaries, indicating active replication and remodelling processes, as cubic cells divide faster than flat ones. During proestrus, the covering epithelium of the endometrium becomes wavy, and the uterine glands, although numerous, are located next to a small number of stromal cells, do not contain secretions and have a folded shape. Vascular changes include hyperplasia of the intima of arterioles with narrowing of the lumen and obliteration of thrombotic masses, which is the beginning of the desolation of vascular territories and remodelling of the connective tissue matrix. At this stage, the ovaries contain tertiary follicles without signs of atresia and corpus luteum in the stage of resorption. The metestrus phase is characterised by the formation of large, almost completely structured corpus luteum with large lutein cell nuclei, while the corpus luteum from previous cycles show signs of degradation. During metestrus, the endometrium simultaneously shows signs of hormone-dependent desquamation and active cell proliferation. The practical value of the study lies in the creation of a reliable morphological basis that reveals in detail the mechanism of hormone-dependent changes for the development of evidence-based diagnostic protocols and methods for correcting hormonal status and sexual cyclicity

Keywords: histological changes in the endometrium; ovarian follicles; ovarian reserve; corpus luteum; physiology of the sexual cycle

Introduction

The physiological basis of cows' reproductive capacity is critically important for ensuring stable milk production and economic sustainability of dairy farms. Despite improvements in husbandry and feeding technologies, disorders of the sexual cycle and reproductive function remain one of the key problems in veterinary medicine. A detailed understanding of the morpho-functional dynamics of the ovaries and endometrium throughout the oestrous cycle enables veterinary specialists to substantiate and develop effective methods for the diagnosis, prevention and correction of reproductive disorders, which is a prerequisite for maintaining high herd fertility rates.

According to M. Lamanna *et al.* (2025) and R. Maculan *et al*. (2025), the issue of cattle reproduction, especially highly productive cows, which are characterised by singleton births, a long diestrus period, lactation dominance, and the prospect of infertility, will always remain relevant in both theoretical and practical terms. According to J.B. Santos *et al*. (2025), physiological data on the reproductive capacity of cows,

including physiological and morphological changes in the ovaries and endometrium during the oestrus cycle, also make it possible to scientifically ensure planned livestock growth rates, sufficient milk productivity, and reduced production costs for raising replacement young stock, as they reflect the effectiveness of the technologies used on farms and highlight the economic feasibility of the cattle breeding industry in general.

To address these issues, a scientifically sound basis for the organisation of reproduction is needed, taking into account physiological indicators and the requirements of modern technologies for the development of dairy cattle breeding in Ukraine. The most common technologies for housing cows in Europe are tethered and untethered. According to S. Hassan *et al.* (2024), housing systems that assign service personnel to each specific cow (such as tethered housing) can have a positive effect on productivity. This simplifies individual feeding and ensures timely diagnosis of oestrus, since during the service period the animals are monitored not only by artificial insemination technicians, but also by service personnel, which is critical for restoring optimal ovarian function after calving. At the same time, as indicated by A. Pavlenko *et al*. (2023), loose housing better meets technological requirements but requires clear grouping based on the productivity and physiological condition of cows and complicates oestrus diagnosis due to limitations on the time staff can spend observing the animals.

In addition to timely indication of oestrus, the physiological state of the endometrium during the period of preparation of the body for implantation is extremely important, as it ensures not only nidation but also the further course of pregnancy. According to S.F. Sitko *et al.* (2024), cows with higher genomic fertility potential demonstrate better uterine condition and higher progesterone levels during synchronisation protocols, indicating a close relationship between genetics, hormonal status and morphological readiness of the reproductive organs for implantation.

Thus, the uterus has a multi-level regulatory system based on a hierarchical principle of interaction between the methods of controlling metabolic processes and the morpho-functional transformation of the functional layer of the endometrium, synchronised with the physiological work of the ovaries. which is why the study of the above-mentioned components in preparation for proestrus, oestrus, and zygote implantation is a pressing issue, the solution of which will contribute to the development of new approaches to the correction of reproductive function in cows. Therefore, given the need to improve reproductive efficiency, the aim of the research was to elucidate the morpho-physiological changes in the ovaries and endometrium of cows during the oestrus cycle for the further development of evidence-based methods for the correction of sexual cyclicity. To this end, the physiological and histological features of the morphological restructuring of the ovaries and endometrium of cows during oestrus, pro-oestrus and metoestrus were studied.

Literature Review

The reproductive efficiency of dairy herds is inextricably linked to complex and cyclical morphofunctional changes in the reproductive system. Understanding these dynamics is critical for developing protocols to improve fertility. According to T. Sugiura *et al*. (2018), the uterus is a key organ that exhibits constant morphofunctional changes characteristic of the oestrus cycle. Under the influence of hormonal background, not only the height of the endometrium changes, but also the shape of its cells, the location of the nuclear apparatus, blood vessels and numerous uterine glands necessary

for nourishing the embryo in the early stages of development are transformed.

Research by I.M. Sheldon *et al*. (2006) indicated that among the cells of the cylindrical epithelium of the uterine glands there are cells in a state of mitosis, wandering cells, large bubble-like cells located near the basement membrane and present in the endometrium during all stages of the oestrus cycle, especially when oestrogen levels rise, whereas in atrophic endometrium, "bubble cells" are not found. According to A. Pavlenko *et al*. (2023), throughout the entire oestrus cycle, the uterus is filled with fluid content formed as a result of transudation of various blood serum components, secretion of proteins, carbohydrates and other metabolic products, the synthesis of which is carried out in the cells of the glandular epithelium. Secretion from epithelial cells occurs asynchronously, in an apocrine manner. This structure of the surface epithelium ensures the implantation of the zygote during pregnancy. J.S. Stevenson & S.L. Pulley (2016) pointed out that under the covering epithelium there is a stroma, together with the uterine glands, containing a functional and deeper basal layer, whereas the former does not completely detach during the inhibition phase, and the basal layer, due to which the endometrium is restored during the next oestrus cycle, is preserved in its entirety.

According to P. Saini *et al.* (2019), the surface of the endometrium includes three morphologically and functionally distinct areas: the caruncular, intercaruncular, and glandular areas, in which the ducts of the uterine glands open. The histological specificity of these areas is quite heterogeneous, so the process of placentation in them is completely different. J.S. Stevenson & S.L. Pulley (2016) indicated that the connective tissue of the caruncle contains a large number of cells of the reticuloendothelial system and a well-developed capillary network, and with age and during pregnancy, their number and size increase. According to T. Taktaz *et al*. (2015), the covering epithelium of the caruncular zone undergoes lysis in places of direct contact with the tissues of the embryo, therefore, on the surface of the exposed endometrium, there are isolated islands of epithelium that have a wavy appearance.

M. Zargar *et al*. (2020) suggested considering the uterine gland, the stroma surrounding it, and the adjacent blood vessels as a structural unit of the endometrium. Such a structural unit is a multicellular system, the vital activity of which is ensured by intercellular interaction under the influence of steroid hormones and paracrine factors. The authors believed that the endometrium has a unique blood supply system: these are, first of all, the terminal vessels of the functional layer – spiral arterioles, which change depending on the phenomenon and stage of the oestral cycle, as well as hormonally independent arterioles of the basal layer, which ensure its constant blood supply and whose destruction leads to irreversible uterine infertility. According to A.G. Zhou *et al*. (2018), in addition to creating a nutritious and supportive environment for early pregnancy, the endometrium plays a role in suppressing the mother's immune response to the antigenically foreign foetus. The covering epithelium also secretes substances (steroids, growth factors, enzymes, cytokines, prostaglandins), the function and interaction of which have not been sufficiently studied.

M. Wang *et al.* (2018) noted that the cyclical transformations of the functional layer of the endometrium proceed in accordance with the ovarian cycle in several successive stages. The cyclical activity of the ovaries depends on the formation of two temporary endocrine structures: the mature dominant follicle (Graafian follicle) and the corpus luteum. T.B. Ault-Seay *et al.* (2022) noted that the functional state of the epithelial cells of the uterine glands and

the cellular elements of the endometrial stroma during the oestrus cycle are characterised by heterogeneity. The biological value of the genotypic and phenotypic heterogeneity of a homogeneous cell population in the components of the endometrium lies in ensuring tissue homeostasis, since heterogeneity at all levels of organisation, starting from the molecular level, contributes to the adaptation of cells and tissues to changing environmental conditions by involving reserve structures in the process.

According to E. Tuckerman *et al.* (2010), morphological changes in the components of the endometrium during the oestrus cycle, which occur under the influence of 17b oestradiol and progesterone, allow to distinguish between the early and late stages of proliferation and secretion. Meanwhile, T.B. Ault-Seay *et al.* (2022) indicated that endometrial regeneration begins against a background of low (basal) levels of steroid hormones, and, more importantly, endometrial regeneration is significantly influenced by growth factors, which are polypeptides that interact with cell membrane receptors, initiating gene expression, metabolism, and cell division. According to these scientists, the functional layer of the endometrium is characterised by the connection between structure and function in various phenomena of the cycle: during oestrus, the structural and functional characteristics of the epithelial cells of the uterine glands are typical of growing and multiplying cells, while during ovulation and the inhibition phase, they are typical of cells that perform a secretory function.

According to T. Taktaz *et al.* (2015), it is during the proliferation stage in the glandular crypts that processes occur that demonstrate the growth and reproduction of cellular elements, as evidenced by an increase in nucleoprotein metabolism of epithelial cells of the glands and the accumulation of glycogen. Aerobic glycolysis processes predominate in the endometrial tissue. I.M. Sheldon *et al*. (2006) founded that at the time of ovulation, the maximum amount of proteolytic enzymes accumulates in the endometrium, and decidual transformations begin in the stroma (the cells of the compact layer increase in size, acquire a rounded or polygonal shape, glycogen accumulates in their cytoplasm), significant vascularisation of the stroma is observed, the endometrium transitions from the proliferation phase to the secretion phase, and the secretory activity of the uterine glands is stimulated.

According to M. Wang *et al*. (2018), the physiological reorganisation of the maternal part of the placenta before implantation is the most important prerequisite for physiological pregnancy, and the endometrium must be in a hormone-dependent state of receptivity during the self-regulating period, during which the blastocyst adheres to the functional layer. In the case of fertilisation of the egg, the epithelial layer of the endometrium undergoes degenerative changes: focal proliferation of the connective tissue stroma is observed, resulting in the growth of caruncles with the subsequent formation of septa and crypts on their surface, and hypertrophy of the uterine glands. These changes progress and become a factor determining the nature of placentation (Stevenson & Pulley, 2016). During the equilibrium stage, changes in the endometrium associated with the flourishing and regression of the corpus luteum consist in the disappearance of mitoses, changes in the tortuosity of the spiral arteries, and their location not only in the basal but also in the superficial sections of the functional layer of the endometrium.

P. Saini *et al*. (2019) believed that the division of the oestral cycle into stages and phenomena is rather arbitrary, since a high level of proliferation is maintained throughout the oestral cycle, only the maximum amount of progesterone after ovulation suppresses

proliferative processes in the endometrium and ovaries. It is believed that progesterone production begins after puberty, mainly by the corpus luteum of the ovaries and, to a lesser extent, by the adrenal glands, as well as by the placenta during pregnancy (Nagy *et al.*, 2021). According to E. Labarta *et al*. (2021), the cellular composition of the parenchymal zone of the ovaries has also not been definitively determined. To determine the stage of the oestrus cycle during histological examination of the ovaries, it is important to determine the condition of the tertiary follicles and the preovulatory Graafian follicle against the background of the condition and functioning of the corpus luteum. In addition to the size of the follicles, informative signs of oestrus may include an increase in the number and ratio of atretic and primordial follicles. Also, the presence of a mature, resorbed, or residual corpus luteum is a marker of metoestrus and, in most cases, an indicator of stable, full-fledged oestrus cycles.

According to T. Pedersen & H. Peters (1968) and J.L. Ireland *et al*. (2008), there are small, medium and large follicles, which are in turn divided into groups according to the number of layers of follicular cells of the largest cross-section. Small primordial follicles contain inactive oocytes that are not surrounded by a layer of follicular cells. Medium primordial follicles are oocytes surrounded by a single layer of flat follicular cells. Large primordial follicles can be classified as medium-sized follicles, as they have between 2 and 80 layers of follicular cells, but no visually formed cavity. Large follicles have a fully formed oocyte surrounded by a large number of layers of follicular cells (from 201 to 400), but the follicle cavity is not completely filled with follicular fluid. Preovulatory or antral follicles have a cavity (antrum), and the follicular cells (from 401 to 600) are separated by several layers of follicular fluid. As the follicles grow and develop from primordial to preovulatory, quantitative and qualitative changes occur: the diameter of the oocyte increases, the shape and number of follicular cells change, the theca membrane appears, and its own vascularisation develops (Labarta *et al*., 2021).

The extracellular matrix plays an equally important role, influencing the morphology, proliferation and connection between follicle cells, forming paracrine and endocrine receptors. The basement membrane, which separates the follicular cell layer from the theca cells and externally separates the follicle from the brain matter, also changes. The transition from the primordial stage to the primary follicle stage is an irreversible process. A distinction is made between initial and cyclical follicular metamorphosis. Initial maturation occurs continuously throughout life, starting from birth, and is regulated by local growth factors. Cyclic maturation begins with the onset of sexual maturity, depends on the level of follicle-stimulating hormone (FSH) and the expression of its receptors, and is accompanied by follicular atresia, which is not characteristic of initial maturation (Kuru *et al.*, 2022). T.B. Ault-Seay *et al*. (2022) indicated that the increase in the diameter of a mature follicle occurs mainly due to the thickening of the layer of theca cells, on which receptors for luteinising hormone are formed. The growth of follicles until the appearance of a cavity in them is regulated by the interaction between follicular cells and theca cells, as well as follicle-stimulating and luteinising hormones.

The selection of the dominant follicle occurs at the beginning of metestrus and is caused by a decrease in FSH levels in the blood in response to increased oestradiol synthesis. After ovulation, the basement membrane between the granulosa and theca cells breaks down, the capillary vessels of the theca cell layer grow through the granulosa layer, creating a dense vascular network, and the granulosa cells transform into luteocytes. However, the process

of follicular cell luteinisation can begin without follicle rupture, while ovulation and egg release do not guarantee the physiological development and functioning of the corpus luteum. Structural regression of the corpus luteum begins with the apoptosis of capillary endothelial cells, which leads to the destruction of the capillary barrier (Kuru *et al.,* 2022).

Immune cells play an important role in such important processes as follicular maturation, ovulation, luteogenesis, and luteolysis. According to B.S. Muasa (2010), under physiological conditions, the ovary contains immune cells such as macrophages, neutrophils, eosinophils, cytotoxic T cells, and NK cells. The populations of these cells vary depending on the cycle, increasing sharply before ovulation. According to a study by B. Nagy *et al*. (2021), macrophages do not affect the early stages of follicle growth (primordial and primary), but they promote their growth and development by regulating parenchymal cell proliferation, producing growth factors and cytokines, and at the same time suppressing follicular cell apoptosis. Thus, the cyclical histological remodelling of the ovaries and endometrium must be taken into account when fully assessing the reproductive system of a cow, as well as when choosing a method of stimulation and correction of reproductive function. This is why it is necessary to study in detail the morpho-histological transformation of the endometrium during the oestrus cycle.

Materials and Methods

Methodology for determining structural and morphological changes in the ovaries and endometrium based on the manifestation of the stages of the sexual cycle

The material for the research was samples of ovaries: selected from the middle part (the plane from the curvature to the gate); and endometrium – from the upper third of the uterine horns. Ovarian and endometrial tissue samples were taken from cows culled due to loss of productive qualities, without pathological changes in the reproductive system, aged 3-10 years: during oestrus (n = 5), metoestrus (n = 5), and prooestrus (n = 5). After collection, the tissue material was fixed in a 10% neutral formalin solution. Next, the endometrial fragments were washed in water, dehydrated, cleared in an alcohol-xylene solution, poured into paraffin blocks, and histological sections 4-10 µm thick were prepared on a Shandon Finnesse 325 (Thermo Scientific) rotary microtome. The samples were examined using a binocular light microscope with different magnifications, and digital images of the preparations were obtained using the "ZEN" digital imaging system for "Carl Zeiss" microscopes (Germany) at the Ukrainian-Swedish research centre SUMEYA (Medical Institute of Sumy State University). For review microscopy, histological preparations were stained with haematoxylin-eosin, and for the study of connective tissue structure, with a picrofuchsin mixture according to Van Gieson, using the methods described by L.P. Horalskyi *et al*. (2015).

Method for determining the stages of the sexual cycle in cows

Oestrus was diagnosed in cows that clearly exhibited the immobility reflex at the peak of luteinising hormone secretion. Metestrus (the peak of corpus luteum activity) was diagnosed on the 7^{th}-8^{th} day of the oestrous cycle, since by the 7^{th} day the corpus luteum is formed and hormonally active. The progesterone secreted by the corpus luteum is sufficient for pre-pregnancy preparation of the uterus for embryo implantation. Proestrus was diagnosed on the 17^{th}-18^{th} day of the oestrous cycle in the presence of clinically pronounced hypertrophy and hyperemia of the external and internal genital organs (the beginning of proliferation under the influence of liberins, which enhance FSH

production and indirectly activate the growth of primordial ovarian follicles).

Object and conditions of the study

The research was carried out in the laboratories of Sumy State University (contract No. 87.01.03.11 on the provision of scientific and diagnostic services dated 30 March 2011) and in the laboratory of LLC "Health Diagnostics" in Sumy. Despite the age of the material collected, the methods of histological processing and analysis used remain relevant. The morphological samples obtained are of scientific value as unique primary material that has retained methodological reliability and reproducibility of results. The results have not been published before. The current publication includes updated statistical processing and modern interpretation of data in the context of current scientific knowledge of the 2020s, which provides a new level of understanding of the structural and morphological features of the reproductive system.

For the clinical and experimental studies, meat and dairy breeds (Schwyz and Simmental) and dairy breeds (Holstein and Ukrainian Black-and-White) of cows were selected, aged 3 to 10 years, with a body weight of 400-600 kg. They were kept on dairy farms of various ownership forms in the Sumy region: JSC Breeding Farm "Mykhailivka" of Lebedynskyi District; PE "Vitaliya" of Burynskyi District; LLC AF "Vladana" of Sumy District; LLC AF "Lan" of Sumy District. The experimental farms used tethered and untethered systems for housing cows in typical cowsheds. With tethered housing, cows rested and ate in stalls on tether. Each animal had an individual feeder and drinker. Milking was carried out in stalls on tethers or in milking parlours. With tethered housing, cows moved freely inside the cowsheds and in the exercise yards. Feeders and drinkers were located at the end of the cowsheds. Cows were milked in a separate, specially equipped room.

Experimental studies, animal husbandry, and all manipulations with animals were carried out in accordance with modern methodological approaches, in accordance with the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (1986), 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 digital material obtained in the studies was processed using variational-statistical methods with the use of statistical techniques (STATISTICA 10.0 for Windows) to determine the arithmetic mean (M), the statistical error of the arithmetic mean (m), and the probability of difference (P) between two variation series according to Student's criterion with values $P \leqslant 0.05$; $P \leqslant 0.01$; $P \leqslant 0.001$.

Results and Discussion

Results of the study of histological, histochemical and structural-morphological changes in the endometrium

During the study of structural and morphological changes in the functional layer of the endometrium during the sexual cycle, it was found that at all stages of the oestrus cycle, the endometrium is conditionally divided into basal and functional layers covered with epithelial cells. The stroma contains uterine glands. The surface of the endometrium contains morphologically and functionally different areas: glandular, intercaruncular and caruncular. The glandular area is covered with resistant, intensively secreting epithelium. It is on its surface that the ducts of the uterine glands open.

The caruncular zone contains a complex of decidual tissue capable of lysing microorganisms, inactivating their toxins, and synthesising carbohydrates, lipids, proteins, prolactin, and prostaglandins. These functional features complement the data of M.E. Diessler *et al*. (2023)

and M.J. Ruiz-Magaña *et al.* (2022). In the intercervical zone, the epithelium undergoes temporary degeneration, while the endometrial stroma does not change significantly.

Histological studies of the endometrium of cows conducted by the authors of this work complement and detail the findings of other researchers, in particular in the works of Y.A Amin & H.A. Hussein (2022) and Y.A. Amin *et al.* (2025), since it has been found that during oestrus: a clear division of the functional layer into spongy and compact remains, while the arterioles form maximally dilated and blood-filled clusters with swollen walls (Fig. 1a).

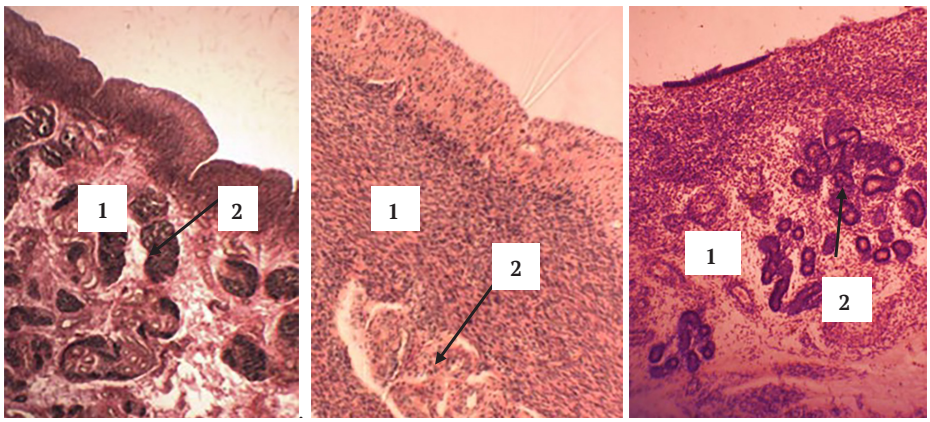

Figure 1. Caruncular zone, functional layer of the endometrium
***Note**: a – oestrus; b – proestrus; c – metestrus; 1 – compact (superficial) layer; 2 – uterine glands. Coloured according to Van Gieson, x 400*
***Source**: developed by the authors*

Mitotic activity of the epithelial cells of the uterine glands has also been detected. Pronounced oedema due to the growth of stromal cells reveals decidual-like elements of the endometrium (cells of the maternal part of the placenta appear). Increased secretory activity of endometrial cells leads to the formation of numerous uterine glands in the form of clusters, due to their elongated and sinuous shape. The latter acquire enlarged cavities and are filled with secretions rich in protein-carbohydrate compounds. The epithelial cells of the uterine glands are of uneven cubic shape, mainly multinucleated with uneven vacuolisation due to the accumulation of glycogen.

Histological examination of the endometrium during proestrus (17-18 days of the sexual cycle) supplemented and detailed the data obtained by Y.A. Amin & H.A. Hussein (2022), M.E. Diessler *et al*. (2023), as it established the presence of a clear division of the functional layer into compact and spongy. The covering epithelium was smooth, gathered in folds, or wavy (Fig. 1b). Numerous formed uterine glands were located in the functional layer of the endometrium next to a small number of stromal cells. The surface layer, on the contrary, contained isolated unformed glands. Located close to each other, the uterine glands did not contain secretions and had a folded shape. There were areas of separation between the stromal cells and the epithelial cells of the glands (Fig. 1b). The endometrial arterioles were dilated and excessively tortuous. There were changes in

the vascular territories due to narrowing of the lumen of the vessels as a result of significant hyperplasia of the intima. There were signs of intravascular stasis, perivascular oedema with loosening around the vascular fibrillar connective tissue elements. Thrombotic masses appeared in the vessels and their obliteration was recorded, with the simultaneous formation of paravasal fuchsinophilic connective tissue fibres, indicating the onset of vascular territory desolation and the development of remodelling of the connective tissue matrix of the endometrium due to replacement by newly formed fibrillar elements.

Histological examination of the endometrium during metestrus (7-8 days of the sexual cycle) showed no clear division of the functional layer into compact and spongy. The covering epithelium was smooth or wavy, partially desquamated in the caruncle areas. The deep layer of the endometrium contained a small number of unformed uterine glands scattered in the stroma; there were almost none in the surface layer (Fig. 1c). There were dystrophic-degenerative changes in the functional layer of the endometrium, in the form of destruction of the compact and spongy layers with significant thinning and disappearance of the clear zoning of the latter. This is associated with hormone-dependent cellular desquamation of the compact and spongy layers of the endometrium. During metestrus, massive apoptotic death was observed in the cells of the functional layer, accompanied by karyopyknosis, karyorhexis, and hyperchromatosis of the cytoplasm. This is due to the development of hypoxic changes in the surface areas of the functional layer as a result of involution and desolation of vascular territories. Along with apoptosis of stromal cells, spindle-shaped cells with mitotic division were found, indicating simultaneous processes of cell proliferation with apoptosis. This may be related to the following fact: during the stage of corpus luteum development, against the background of dystrophic-degenerative changes, regenerative processes occur simultaneously.

Results of the study of histological, histochemical and structural-morphological changes in the ovaries

The study of structural and morphological changes in the ovaries is a widespread practice in scientific research, but the specifics of remodulation changes during the sexual cycle require well-founded additions, since the combined study of the ovaries and endometrium is diagnostically important in defining the stage of the oestrus cycle, because the histology of other organs of the reproductive system of cows is less informative. Histological examination of the ovaries of cows during oestrus revealed that the cortical substance is broad and contains mainly densely packed spindle-shaped connective tissue cells that form elastic fibres. A small number of stromal cells are subject to luteinisation. The medulla of the ovary consists of loose connective tissue that forms a well-developed connective tissue matrix and contains elastic smooth muscle fibres. Along the course of the nerve fibres, groups of polyhedral cells with oxyphilic cytoplasm were visible. A large number of elastic fibres of the medulla surrounded a dense network of ovarian vessels, nerve endings, and specialised cells similar to the interstitial cells of the testes, known as Leydig cells. The protein membrane, consisting of cells of dense connective tissue with admixtures of smooth muscle and elastic fibres, is clearly visible. The protein membrane lies under the covering epithelium (formed by cells of a low single-layer cylindrical epithelium) and the underlying cortical substance (Figs. 2a, 2b). The primordial follicles were ellipsoidal in shape and had no membrane. At the poles of the primordial follicles were flattened follicular cells surrounding a small oocyte.

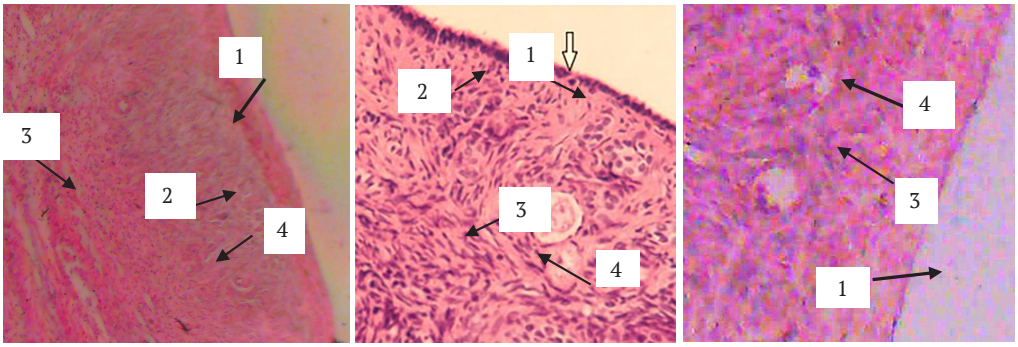

Figure 2. Surface epithelium of the ovary
***Note**: a – oestrus (x 100); b – prooestrus (x 100); c – metoestrus (x 400); 1 – covering epithelium; 2 – protein membrane; 3 – spindle-shaped fibres; 4 – primordial follicle. Staining according to Van Gieson*
***Source**: developed by the authors*

Large tertiary follicles reached 5-8 mm in diameter and appeared under the microscope as large cavities filled with fluid (Fig. 3). The spherical inner layer of the tertiary follicle consisted of 5-6 layers of follicular cells. Some of the tertiary follicles located near the dominant follicle were atretic. The corpus luteum of the previous cycle appeared as wide strips of lutein cells in the stage of degeneration, as evidenced by foamy cytoplasm with large vacuoles. Fibrosis of the corpus luteum cells was observed. There were also corpus albicans without any clear remnants of corpus luteum cells. The latter contained loose connective tissue, intercellular substance with lutein cells located in it, and collagen fibres.

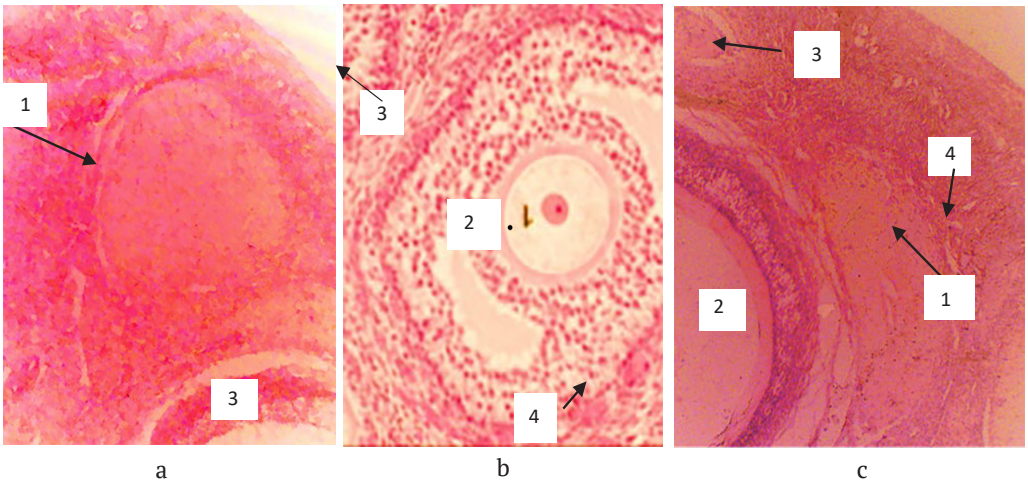

Figure 3. Corpus luteum, secondary and atretic follicles
***Note**: a – metestrus; b – proestrus; c – oestrus; 1 – corpus luteum; 2 – secondary follicle; 3 – atretic follicle; 4 – ovarian vessels. Van Gieson staining, x 400*
***Source**: developed by the authors*

The primary follicles contained a large oocyte surrounded by a membrane and a layer of cubic-shaped follicular cells. The cubic shape of the follicular cells is formed due to the onset of replication, as the latter acquire a cube-like shape in prophase and metaphase. This indicates active metabolic and remodelling changes in the ovary, as cuboid follicular cells divide faster than flat ones, as confirmed by the research of M. Kuru *et al*. (2022). After ovulation, the ovary contains a spherical layer of follicular cells with blood elements, which is a consequence of the ovulated Graafian follicle.

As a result of studying the ovaries during metestrus, data was obtained that clarifies the research of I. Nanas *et al*. (2021), as it was found that the main feature of this phase is the presence of one or more large corpus luteum in the cortical zone. The latter are almost completely structured, but sometimes there are cavities with blood cells in the centre (Fig. 3). The lutein cells had large nuclei with noticeable nucleoli and eosinophilic cytoplasm. Corpus luteum from previous cycles were also present in the ovary, but contained loose connective tissue, intercellular substance, collagen fibres, and sometimes remnants of lutein cells in the stage of degradation. In the ovaries of cows during proestrus (Fig. 3b), small tertiary follicles are observed, smaller in diameter than Graafian follicles. Most of these follicles show no signs of atresia, unlike in the metestrus stage. The corpus luteum is in the stage of resorption, but does not have wide bands of lutein cells, similar to the oestrus stage.

Follicular atresia (Fig. 4) was recorded in the ovaries during proestrus, oestrus and metestrus due to the fact that the follicular cells lost contact with the follicle wall, after which the follicle cavity was filled with fibroblasts. Due to the degeneration of follicular cells and theca cells, so-called hyaline eosinophilic masses are formed, since follicular cell atresia causes the death of the entire follicle, including the oocyte. In addition, atresia is accompanied by follicle resorption, which includes macrophage infiltration, phagocytosis,

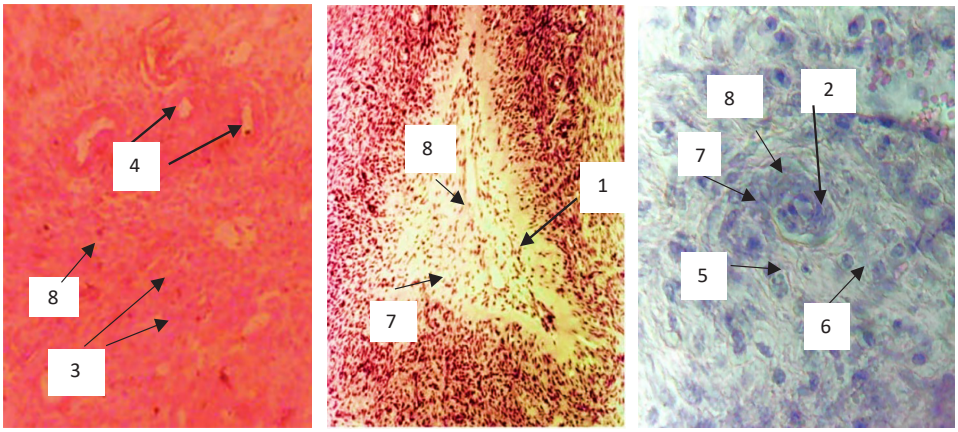

Figure 4. Ovarian follicular atresia

***Note**: a – proestrus, atresia of primary follicles (x 100); b – metestrus, atresia of tertiary follicles (x 400); c – oestrus after ovulation, atresia of secondary follicles (x 1000); 1 – fibroblasts in the follicle cavity; 2 – atresia of the secondary follicle; 3 – hyaline eosinophilic masses; 4 – primary follicle at the stage of atresia; 5 – macrophage; 6 – lymphocyte; 7 – fibrocyte; 8 – fibroblast. a and b – Van Gieson staining; c – haematoxylin and eosin staining*

***Source**: developed by the authors*

migration of fibroblasts from the theca, and collagen accumulation. The above processes are similar to those observed during wound healing (Schultz & Wysocki, 2009).

Cell atresia is a physiological component of tissue homeostasis. There are several types of atresia. In the case of destruction of all layers of follicular cells, "antral (apical) atresia" and basal atresia (Rodgers & Irving-Rodgers, 2010) are diagnosed, characterised by the destruction of the follicular membrane of the follicles closest to the dominant follicle. Apical atresia was recorded during proestrus, oestrus and metestrus (Fig. 4), which are more extensive studies compared to the previously presented data from scientists. Numerous pyknotic nuclei are visible in the cells of the apical layer of follicular cells of primary, secondary and tertiary follicles (Fig. 3c). In the case of destruction of the basal layer of follicular cells, while maintaining the integrity of the inner layer, "basal atresia" is diagnosed, as described by W.M. Noseir (2003), which was recorded during proestrus and oestrus (Figs. 4a, 4c).

These studies necessitated the calculation of the ovarian follicular reserve of cows in tethered and untethered housing systems in a comparative aspect, since this indicator correlates with the data of J.B. Santos *et al.* (2025) with veterinary and sanitary housing conditions and can be used in assessing reproductive capacity. The results of the comparison are shown in Table 1. Tethered cows demonstrated a tendency towards a larger ovarian reserve than untethered cows. The number of follicles in the right ovary was slightly higher than in the left ovary in cows in both housing systems

Table 1. Influence of housing conditions on the average number of follicles in cows' ovaries in comparative terms

Conditions for housing cows	Number of follicles in the ovaries		
	Right ovary	Left ovary	Both ovaries
Tethered housing	9.21 ± 0.27	8.87 ± 0.44	18.08 ± 0.31
Untethered housing	9.09 ± 0.18	8.71 ± 0.36	17.8 ± 0.24

Source: *developed by the authors*

There was a lack of scientific data on the difference in physiological activity between the left and right ovaries of cows in the available literature, but M.N. Purpera *et al.* (2009) claimed that the number of follicles in the right ovary exceeded that in the left ovary, which was confirmed by the current study. The data obtained also confirmed the results of N. Yimer *et al.* (2010), who claimed that the activity of the right ovary is due to better blood supply. A reasonable analysis of the ovarian reserve of tethered and untethered cows allows to conclude that the tethered method has obvious advantages, as it causes a stable tendency towards a greater number of follicles in the ovaries. It can be assumed that the tethered method of housing, which requires the assignment of personnel to care for each cow, has a positive effect on physiological reproduction indicators, since the formation of the main herd involves the participation of service personnel who are personally interested in obtaining the largest amount of high-quality products.

Conclusions

The aim of the study was to investigate morphological and physiological changes in the ovaries and endometrium of cows during the oestrus cycle for the further development of evidence-based methods for correcting sexual cyclicity. Based on the histological and morphological analysis, it was confirmed that the

reproductive organs of cattle are characterised by high plasticity and regenerative qualities. Their functional state is completely hormone-dependent, has autonomous regulation of its own enzyme apparatus, local immune system and is capable of steroidogenesis, which is the result of a regenerative-adaptive tissue response to constant hormonal changes.

In the course of the work, the physiological and histological features of the morphological restructuring of the ovaries and endometrium in the key phases of the cycle were studied in detail. During proestrus, the endometrium showed a folded form of the uterine glands without secretion, and the vessels were replaced by newly formed fibrillar elements. At this time, the ovaries were dominated by tertiary follicles without signs of atresia, while the corpus luteum from previous cycles was in the stage of resorption. The oestrus phase was characterised by maximally dilated and blood-filled arterioles of the endometrium, and the epithelial cells of the uterine glands were multinucleated with pronounced vacuolisation, indicating glycogen accumulation. At the same time, active metabolic and remodelling changes associated with preparation for ovulation were taking place in the ovaries. At the metestrus stage, fully structured corpus luteum were found in the cortical zone of the ovaries, while signs of hormone-dependent desquamation and intense cell proliferation were simultaneously observed in the endometrium.

The results obtained conceptualise the consistency and synchrony of morphological transformations of the endometrium and ovaries, emphasising the critical importance of timely detection and correction of dysfunctions at the tissue level. This holds practical significance for improving artificial insemination protocols and developing differentiated programmes for hormonal support. In view of this, a promising direction for further research involves a detailed analysis of the cyclical dynamics of hormone receptors in different layers of the endometrium, which will allow the creation of precise methods for correcting sexual cyclicity, taking into account the individual sensitivity of animals. Further research will focus on studying the dynamics of biochemical indicators of ovarian tissue during the oestrus cycle, which will allow for the rational correction of the reproductive capacity of cows.

Acknowledgements

None.

Funding

The study was not funded.

Conflict of Interest

None.

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Морфо-фізіологічні особливості ремодуляції ендометрія та яєчників корів за естрального циклу

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Анотація. Рентабельність молочного скотарства напряму залежить від інтенсифікації показників відтворення, що вимагає збільшення відсотку отримання телят на сто корів та оптимізації молочної продуктивності. З огляду на необхідність підвищення ефективності відтворення, метою досліджень було з'ясування морфо-фізіологічних змін яєчників та ендометрія корів протягом естрального циклу для подальшої розробки обґрунтованих методів корекції статевої циклічності. Аналіз гістологічних зразків виявив низку важливих змін. У фазу еструсу ендометрій демонструє максимальну набряклість і кровонаповнення артеріол, тоді як клітини функційного шару набувають децидуалоподібності за рахунок активного росту клітин строми. Численні маткові залози мають звивисту форму та розширені порожнини, наповнені секретом, при цьому епітеліальні клітини залоз стають багатоядерними з вакуолізацією через накопичення глікогену. В яєчниках реєструються первинні фолікули з кубічною формою фолікулярних клітин, що вказує на активні процеси реплікації та ремодуляції, оскільки кубовидні клітини діляться швидше, ніж плоскі. Протягом проеструсу покривний епітелій ендометрія стає хвилястим, а маткові залози, хоча й численні, містяться поруч із незначною кількістю клітин строми, не містять секрету

та мають складчасту форму. Судинні зміни включають гіперплазію інтими артеріол зі звуженням просвіту та облітерацією тромботичних мас, що є початком запустіння судинних територій і ремодуляції сполучнотканинного матриксу. На цій фазі в яєчниках наявні третинні фолікули без ознак атрезії та жовті тіла на стадії розсмоктування. Фаза метеструсу характеризується утворенням великих, майже повністю структурованих жовтих тіл із великими ядрами лютеїнових клітин, тоді як жовті тіла попередніх циклів демонструють ознаки деградації. В ендометрії під час метеструсу одночасно наявні ознаки гормонозалежної десквамації й активної клітинної проліферації. Практична цінність дослідження полягає у створенні надійної морфологічної основи, яка детально розкриває механізм гормонозалежних змін, для розробки обґрунтованих протоколів діагностики та методів корекції гормонального статусу і статевої циклічності

Ключові слова: гістологічні зміни ендометрія; фолікули яєчника; яєчниковий резерв; жовті тіла; фізіологія статевого циклу

UKRAINIAN JOURNAL OF VETERINARY SCIENCES

2025. Volume 16, No. 3. P. 45-62

Received: 13.06.2025 Revised: 22.08.2025 Accepted: 23.09.2025

DOI: 10.31548/veterinary3.2025.45

UDC: 612.357.15; 612.357.32; 616.36-003.826

Milk phospholipids in correction of liver lipid profile in rats with tetracycline-induced fatty hepatosis

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Suggested Citation:
Gryshchenko, V., Pototskyi, A., Veselskyi, S., Danchenko, O., & Illek, J. (2025). Milk phospholipids in correction of liver lipid profile in rats with tetracycline-induced fatty hepatosis. *Ukrainian Journal of Veterinary Sciences*, 16(3), 45-62. doi: 10.31548/veterinary3.2025.45.

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Abstract. The research relevance is determined by the limited information on the molecular mechanisms of fatty hepatosis development in mammals and the identification of effective markers for the diagnosis of this pathology. Timely diagnosis of fatty hepatosis is also relevant for the prevention of dangerous complications, primarily cirrhosis of the liver and hepatocellular carcinoma. Disorders in lipid metabolism are substantial in the pathogenesis of fatty hepatosis. Therefore, the study aimed to establish regular changes in the liver lipid profile in rats with artificially induced fatty hepatosis and the use of milk phospholipids as corrective therapy. For this purpose, thin-layer chromatography was used. The study determined that in the case of artificial reproduction of tetracycline hepatosis in the liver of rats, a deficient level of total lipid fractions is formed. The esterified cholesterol fraction undergoes particularly sharp changes, the content of which decreased by 51.3% compared to the control. Oral administration of a milk phospholipid-based dietary supplement to sick rats prevented disruption of the lipid composition of the liver, which may indicate sufficient efficiency of absorption of the dietary supplement's phospholipids in the intestine and a stimulating effect of its components on their endogenous formation in hepatocytes. In addition, deficient levels of liver phospholipid spectrum indicators were observed in sick animals. A decrease in the total fraction of inositol phosphatide and phosphatidylinositol by 26.6%, phosphatidylserine by 19.9%, sphingomyelin by 18.2%, phosphatidylcholine by 18.3% and phosphatidylethanolamine by 19.6%, and the restoration of their parameters when milk phospholipids were administered to rats. When the dietary supplement was administered to clinically healthy rats, a significant increase in the liver content of all fractions of total lipids and individual phospholipids was observed. Based on the results obtained, it is reasonable to recommend the bioactive supplement "FLP-MD" as a corrective therapy for lipid metabolism disorders in cases of functional liver disorders in animals with fatty hepatosis

Keywords: tetracycline hydrochloride; corrective therapy; thin-layer chromatography; lipid and phospholipid spectra; fatty dystrophy

Introduction

Lipids are a substantial group of organic components that function within the structure of cells. These hydrophobic substances act as a form of energy storage, participate in signal transmission, and their metabolism is regulated at many levels. As described by T. Mousavi (2023), lipids belong to biomolecules that perform plastic (structural), metabolic, and regulatory functions in the bodies of living beings. A. Frydrych *et al.* (2025) noted that the composition of lipids in the body of mammals depends on genetics, gene regulation, and diet. Since all tissues in the animal body have the same genetic composition, the regulation of lipid metabolism enzyme genes is a key factor in changing the lipid profile in different tissues and organs. Mammalian cells contain polar and non-polar lipids. R.G. Parton & K. Simons (2024) reported that cholesterol, its esters, and triacylglycerols constitute most nonpolar lipids, while glycerophospholipids account for approximately 60% of polar lipids in the cell.

Biological membranes are flexible barriers for cells and organelles thanks to lipids. As noted by S. Errico *et al.* (2023), the physical properties of lipids influence membrane processes. Each membrane has a unique structure, composition, and function, and within it, there are microdo-

mains with a unique lipid composition, such as lipid rafts. Lipids create a hydrophobic-hydrophilic environment in which membrane protein function, determining their organisation and orientation. They provide flexibility and specific interactions with other macromolecules. However, determining the functions of lipids is complicated by their diverse properties and involvement at different levels of cell function. M. Murata *et al.* (2025) demonstrated that lipids such as sterols, fatty acids, phospholipids, and triacylglycerols can act as signalling molecules that can actively influence the course of inflammation and intracellular homeostasis indirectly through the structural components of biological membranes and various related metabolic mechanisms.

Since 2015, the knowledge base on lipotoxicity has expanded significantly, and advances in lipidomics analysis have provided new perspectives on lipid profiles and pathophysiological mechanisms associated with chronic inflammation and liver cell damage, as reflected by S. Lobasso *et al.* (2022), C. Garcia *et al.* (2023), and Y. Zhou *et al.* (2023). Lipidomics provides detailed information on various representatives of the lipid class, the characteristics of their structural organisation and biological role in a specific biological environment, including cells, individual organs and tissues, and even the whole organism. This complex research involves the identification, detailed characterisation, and quantitative assessment of thousands of molecular species of lipids in a biological matrix. This relatively new field of research represents a promising approach to obtaining a comprehensive overview of the overall lipid metabolism in a biological system or even at a specific stage of a disease. Y. Zhou *et al.* (2023), as a result of studying liver and blood serum lipids in patients with fatty liver disease, found that lipid metabolism disorders are a key factor in the development of fatty liver disease and that several complex types of lipids, including sphingolipids and glycerophospholipids, are involved in the manifestation of lipotoxicity and the pathogenesis of fatty liver disease.

Scientific and technological progress and the growing pace of synthetic pharmaceutical production increase the burden of xenobiotics on the mammalian body, which negatively affects the structural and functional state of the liver and the entire body. It is necessary to address the characteristics of metabolic disorders and their most sensitive links to the negative effects of certain factors when developing new means for preventive measures and corrective therapy for affected animals. The author's development of a reparative dietary supplement based on natural and safe raw materials of animal origin, namely milk phospholipids, is aimed at the implementation of scientifically sound and effective prevention of numerous disorders in the bodies of animals and their treatment. In general, this is substantial for the implementation of endoecological technology for restorative therapy in clinical veterinary medicine. Therefore, the study aimed to determine the characteristic changes in the lipid and phospholipid profiles of the liver in rats under the toxic effect of tetracycline hydrochloride and the corrective efficacy of milk phospholipids.

Literature Review

Fatty liver disease (fatty hepatosis, steatohepatosis) is a pathology that often manifests as simple steatosis in its early stages and subsequently progresses to steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. An analysis of statistical studies by C. Berardo *et al.* (2020) and N. Pydyn *et al.* (2020) indicated a growing prevalence of fatty liver disease, which, as the researchers noted, is becoming a substantial clinical problem. In veterinary practice, liver damage in animals, in particular fatty

degeneration, is quite common (up to 25% of cases), has various aetiologies and requires the development of appropriate non-invasive diagnostic methods and effective treatment. According to experts J.P. Arab *et al.* (2018) and A.I. Dajani & B. Popovic (2020), the development of fatty hepatosis is associated with impaired lipid metabolism in the liver, which, as an option, may result from the hepatotoxic effects of synthetic drugs, in particular tetracycline antibiotics, as well as the negative impact of genetic, epigenetic and environmental factors that contribute to the progression of fibrosis and the risk of hepatocellular carcinoma.

The development of any pathology in the mammalian body is accompanied by changes at the molecular level. As noted by S. Li *et al.* (2025), this is primarily associated with destructive processes in cell membranes due to interaction with an etiopathogenic factor. The cell plasma membrane is the first to respond to signals from external factors and triggers the intracellular production of corresponding metabolites. In response to various factors, it is capable of effective and rapid restructuring, which is a manifestation of the adaptation of complex biological systems at the cellular level. The physical-dynamic state of the biomembrane, as described by J. Zhang *et al.* (2023), is determined by phospholipids, primarily phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine, which contain the majority of omega-6 and omega-3 polyenoic fatty acids.

The biotransformation of toxic and harmful substances of exogenous and endogenous origin occurs mainly in the liver. Therefore, according to D.O. Melnychuk & V.A. Hryshchenko (2014), hepatopathology is also possible due to the entry of xenobiotics into the body, especially if this phenomenon is chronic. This can manifest as dystrophic changes in individual hepatocytes or as destructive changes in the organ's parenchyma. The latter situation is life-threatening for the animal.

The leading factor in the progression of destructive changes in hepatocytes in fatty hepatosis is the accumulation of lipids in them. S.T. Tan *et al.* (2020) noted that liver samples with fatty hepatosis show noticeable changes in the composition of fatty acids and phospholipids, indicating a disruption in lipid metabolism as a key factor in the pathogenesis and progression of this disease. N. Nikolajevic *et al.* (2024) indicated that altered phospholipid composition and reduced membrane fluidity can lead to liver damage, which further provokes the development of fatty liver disease. The majority of therapeutic agents in the veterinary medicine arsenal are artificially synthesised. They have a rapid effect when used in animals, but at the same time can provoke the development of numerous substantial complications, which can be dangerous in complex clinical situations. As noted by S. Thakur *et al.* (2024), the effect of tetracycline hydrochloride initially manifests itself in the form of fatty degeneration with the accumulation of triacylglycerols in hepatocytes and an imbalance between lipid production and catabolism. J. Mao *et al.* (2024) also mentioned that in high doses, this antibiotic causes a decrease in the activity of mitochondrial beta-oxidation of fatty acids and an increase in the synthesis of endogenous fatty acids, which leads to insufficient incorporation or export of triacylglycerols into low-density lipoproteins.

Recent studies on lipotoxicity and advances in lipid profile analysis, as a leading indicator of pathophysiological mechanisms in fatty liver disease, show a link between lipid metabolism disorders and chronic inflammation and damage to hepatocytes. Phospholipid-containing drugs, particularly those of animal origin (milk phospholipids), are essential in correcting changes in the structural and functional state of hepatocytes in drug-induced hepatopathy.

Materials and Methods

The lipidogram of liver tissue in laboratory rats was studied using the scientific base of the biochemical laboratories of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine and the Educational and Scientific Centre "Institute of High Technologies" of Taras Shevchenko National University of Kyiv from 2023 to the end of March 2025. For the experiment, analogue groups of 32 white laboratory rats with a body weight in the range of 200-225 g were formed. During the experiment, the animals were weighed regularly using ORION OS-0K22 electronic scales (ORION ELECTRONICS LTD, Hungary), which were essential for monitoring changes in body weight and calculating the dose of drugs for oral administration. The inclusion of only male rats in the study is explained by the known sexual dimorphism in the development of fatty hepatosis in mammals (Martin-Grau & Monleon, 2023) and differences in the clinical manifestation of hepatopathology in animals of different sexes.

The laboratory rats used in the experiment were kept in a vivarium under standard conditions. The animals received a standardised diet complete with essential nutrients and had free access to water. Experimental studies involving animals were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (1986) and Law of Ukraine No. 3447-IV (2006). The planned manipulations on laboratory animals were conducted following the main principles of Directive 2010/63/EU of the European Parliament and of the Council (2010) regarding their protection during the experiment.

Artificial reproduction of fatty hepatosis in laboratory rats lasted for 7 days and involved oral intragastric administration of a 4% solution of tetracycline hydrochloride to the test animals using a flexible probe at a dose of 250 mg/kg body weight once a day. Rats in the "Self-rehabilitation" experimental group were administered tetracycline hydrochloride according to the above scheme and left without treatment (n = 8). The animals in the second experimental group, "Correction" (n = 8), were administered a 1% liposomal solution of the biologically active supplement (BAS) "FLP-MD" (corrective therapy) in the same manner, one hour before intragastric administration of the antibiotic for 7 days and for an additional two days in a row. The main components of this dietary supplement are phospholipids obtained from milk. The therapeutic dose of this dietary supplement corresponded to 13.5 mg/kg of body weight of the test animal (Melnychuk & Hryshchenko, 2014). Rats in the "Control" group (n = 8) were given an equivalent volume of distilled water synchronously. At the same time, a third experimental group was formed from clinically healthy animals ("Healthy animals + FLP-MD dietary supplement"), which were administered only the phospholipid-containing dietary supplement daily according to a schedule similar to that of the previous group (n = 8). At the end of the experiment, after euthanasia, liver samples were taken from the animals for further determination of the lipid and phospholipid spectrum content. Liver pieces were ground in a mortar and manipulated according to the method (Veselskyi *et al.*, 2001).

Determination of total lipid content in liver samples. The lipid spectrum components were identified and their concentrations determined in the extract obtained from liver samples. Silufol thin-layer plates (15 x 15 cm, Czech Republic) were used to separate total lipids into fractions by thin-layer chromatography. Filter paper with an extract volume of 40 μdm^3 obtained from liver samples was placed in the chromatographic chamber. For improved saturation and chromatographic separation of

lipid fractions, a mixture of solvents was added: hexane-diethyl ether-acetic acid (7 : 23 : 1). The dry residue of lipids was dissolved in a chloroform-benzene-acetone mixture (1 : 2 : 1) in 20-100 μdm^3 and applied to a pre-marked chromatogram. Within 10-15 minutes, the total lipid fractions of the liver samples under study were separated on the plate. In particular, fractions of free cholesterol, cholesterol esters, triacylglycerols, free fatty acids, and phospholipids were isolated. The corresponding markers from Sigma (USA) were used for their identification. The process of removing the solvent from the chromatogram was conducted in a fume hood. Next, the chromatogram was stained with a 10% solution of phosphomolybdic acid in ethanol using a glass laboratory sprayer. To reveal the lipid fractions, the stained chromatogram was placed in a thermostat at a temperature of 110°C. The quantitative parameters of total lipids in liver samples were assessed using a KO-1M densitometer (Ukraine). The lipid content in liver samples was expressed in mg%.

Determination of phospholipid content in liver samples. In the extract obtained from liver samples, the components of the phospholipid spectrum were identified and their concentrations determined using the method of V.E. Vaskovsky *et al.* (1975). The prepared extract from the homogenate of liver samples was applied to chromatography paper in a volume of 40 μdm^3 from each sample and kept at room temperature until completely dry. Then, the samples of liver extract that had dried on the paper were crushed into small pieces and placed in test tubes, which were hermetically sealed with a cork. The following mixture was used as a solvent for determining five individual phospholipid fractions in the prepared liver samples: chloroform : methanol : water : acetic acid in a ratio of (63 : 25 : 4 : 2) according to the method of M. Kates (1986). The mixture was stirred vigorously and centrifuged for 10 minutes at 3000 rpm. An OPN-8 laboratory centrifuge (Ukraine) was used to centrifuge the resulting mixture. Acetone and butanol were passively evaporated from the supernatant. The resulting extract, which contained phospholipids from liver samples after solvent evaporation, was subjected to analysis.

Chromatographic separation was performed on thin-layer plates "Silufol" (15 x 15 cm, Czech Republic), pre-activated in a thermostat at 110°C for 1 hour. At this point, filter paper was placed in the chromatographic chamber for improved saturation, and a mixture of solvents was poured in: hexane-diethyl ether-acetic acid (7 : 23 : 1). The dry residue of phospholipids was dissolved in a chloroform-benzene-acetone mixture (1 : 2 : 1) in 20-100 μl and applied to a pre-marked chromatogram.

The results of the chromatographic study were statistically processed using the Statistica 5.0 computer program (StatSoft Inc., USA). To determine the statistical significance of the differences between the lipid and phospholipid spectrum indicators, the Student's t-test was used following the methodology of N.B. Filimonova *et al.* (2004). In addition, the Shapiro-Wilk test was used to check the normality of the distribution. The study determined that the results obtained in the experiment were normally distributed. The differences between the two compared indicators from two different samples were considered statistically significant at $P < 0.05$.

Results and Discussion

Chromatographic analysis of the lipid spectrum of liver samples from laboratory rats with artificially induced tetracycline-induced hepatosis identified five fractions of total lipids: phospholipids, free fatty acids, free cholesterol, esterified cholesterol, and triacylglycerols. In the study of total lipid fractions in the liver of diseased rats in the experimental group "Self-rehabilitation", a significant decrease in the content of all studied indicators was noted in the context of modelling fatty hepatosis (Figs. 1, 2).

As noted by I.I. Kovalchuk *et al.* (2025), lipids are synthesised in the liver, and therefore experimentally established patterns of quantitative changes in total lipids may indicate the nature of the functional activity of hepatocytes, in particular their synthetic function.

In particular, the content of total phospholipids in the liver of the experimental

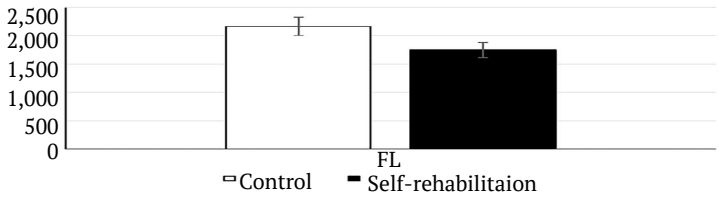

Figure 1. Total phospholipid content (mg/100 g of raw tissue) in liver samples from rats in the experimental group "Self-rehabilitation" with tetracycline-induced fatty hepatosis (M ± m, n = 8)
***Note**: P < 0.05, compared to the values of the corresponding indicator in intact animals in the "Control" group. FL – phospholipids*
***Source**: compiled by the authors*

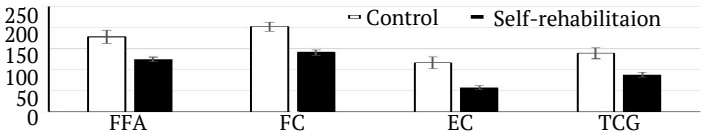

Figure 2. The content of free fatty acids, free and esterified cholesterol, triacylglycerols (mg/100 g of raw tissue) in liver samples of rats in the experimental group "Self-rehabilitation" with tetracycline-induced fatty hepatosis (M ± m, n = 8)
***Note**: P < 0.05, compared to the values of the corresponding indicators in intact animals in the "Control" group. FFA – free fatty acids, FC – free cholesterol, EC – esterified cholesterol, TCG – triacylglycerols*
***Source**: compiled by the authors*

rats in the "Self-rehabilitation" group decreased by 19.2% compared to the control (2,165.73 ± 61.0 mg/100 g of raw tissue) (Fig. 1). This fact is probably associated with a violation of the metabolism of these phosphorus-containing complex lipids due to the toxic effect of the antibiotic on hepatocytes. At the same time, more pronounced changes were observed in the content of free fatty acids, which was characterised by a decrease of 31.2% (in the control 178.11 ± 15.6 mg/100 g of raw tissue), which was possibly caused by disorders in lipid metabolism and their intensive use in maintaining energy balance. The content of free cholesterol in the liver of diseased rats, similarly to previous indicators, decreased significantly by 31.3% compared to the control (202.52 ± 11.0 mg/100 g of raw tissue), which is evidence of a violation of synthetic processes and the predominance of catabolic ones. The parameters of esterified cholesterol showed the greatest changes among the studied lipid profile indicators, namely a decrease of 51.3% compared to the control (116.67 ± 13.8 mg/100 g of raw tissue). This may be due to a deficiency of unsaturated fatty acids (oleic, linoleic), which are intensively used in the esterification reaction. In addition, there are possible changes in the activity of acyl-CoA-cholesterol acyltransferase, which catalyses the process of internal esterification in liver cells and is localised in the membranes of the endoplasmic reticulum, which in turn undergo destructive changes in tetracycline-induced hepatopathology. According to O. Stein & Y. Stein (2005), this may negatively affect the activity of this enzyme and enhance the removal of free cholesterol from hepatocytes. In addition, the enzyme acyl-CoA-cholesterol

acyltransferase is involved in the formation of intracellular cholesterol reserves, which are used by the body to synthesise steroid hormones, bile acids, sex hormones and vitamin D_3. At the same time, the content of triacylglycerols in the liver of rats in this group was 36.2% lower than the control values (139.01 ± 12.8 mg/100 g of raw tissue).

Analysing the results obtained, it is worth noting the existing violation of the quantitative characteristics of total lipids under the action of the xenobiotic drug group tetracycline hydrochloride. When studying the fractions of total lipids in the liver of rats that were given a phospholipid-containing dietary supplement (experimental group II, "Correction"), the content of total phospholipids reached control values, indicating the sufficient corrective effectiveness of the components of this dietary supplement (Fig. 3). At the same time, the content of total phospholipids in the liver of rats in the III experimental group "Healthy animals + dietary supplement "FLP-MD"" increased significantly by 27.1% compared to that in animals in the "Control" group. The level of free fatty acids in the liver of animals in the second experimental group, "Correction", corresponded to the control limits, while in rats in the third experimental group, "Healthy animals + dietary supplement FLP-MD", it exceeded these limits by 19.3%. The free cholesterol levels in rats in the "Correction" and "Healthy animals + FLP-MD dietary supplement" experimental groups did not differ from the control group range. The esterified cholesterol content in the liver of the experimental rats in the "Correction" group remained unchanged, while in the "Healthy animals + FLP-MD dietary supplement" group, it exceeded the control values by 21.3% (Fig. 4).

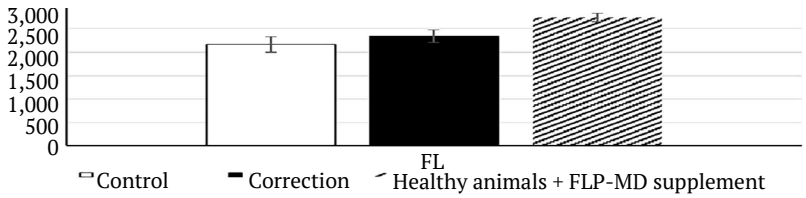

Figure 3. Total phospholipid content (mg/100 g of raw tissue) in liver samples from rats in experimental group II "Correction" and experimental group III "Healthy animals + FLP-MD dietary supplement" with tetracycline-induced fatty hepatosis (M ± m, n = 8)

***Note**: P < 0.05, compared to the values of the corresponding indicator in intact animals in the "Control" group. FL – phospholipids*

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

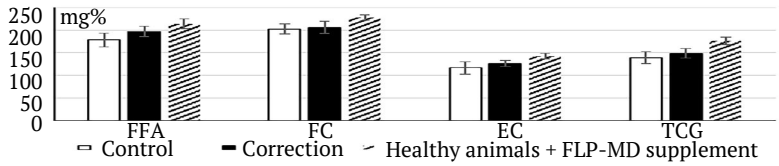

Figure 4. The content of free fatty acids, free and esterified cholesterol, triacylglycerols (mg/100 g of raw tissue) in liver samples from rats in experimental group II "Correction" and experimental group III "Healthy animals + dietary supplement "FLP-MD"" for tetracycline-induced fatty hepatosis (M ± m, n = 8)

***Note**: P < 0.05, compared with the values of the corresponding indicators in intact animals in the "Control" group. FFA – free fatty acids, FC – free cholesterol, EC – esterified cholesterol, TCG – triacylglycerols*

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

According to the results shown in Figure 4, the content of the individual fraction of triacylglycerols in liver samples from rats in the II experimental group "Correction" reached values characteristic of the "Control" group, while in similar samples from the third experimental group, "Healthy animals + FLP-MD dietary supplement", their values even exceeded the corresponding parameters in the control group by 24.8%. The established patterns of changes in the quantitative characteristics of the main fractions of the lipid spectrum of the liver in the experimental animals indicate the pronounced corrective effectiveness of the phospholipid-containing dietary supplement and its hepatoprotective properties in the case of steatogenic effects on the liver of the antimicrobial synthetic drug of the tetracycline series. At the same time, laboratory rats in the corresponding experimental groups retained their appetite, indicating no disturbances in the feeding behaviour of the test animals.

When studying the spectrum of individual phospholipids in liver samples from laboratory rats in the "Self-rehabilitation" experimental group, a deficiency in all the studied fractions was observed (Fig. 5). In particular, the quantitative parameters of the combined fraction of inositol phosphatide and phosphatidylinositol in the liver of these rats (experimental group I, "Self-rehabilitation") showed a significant decrease of 26.6% compared to the control (114.52 ± 8.7 mg/100 g of raw tissue). N.J. Blunsom & S. Cockcroft (2020) noted that the described phospholipids are substantial for the manifestation of signal transduction and are a source of relevant biologically active messengers, diacetylglycerols and polyene arachidonic acid. The established patterns may be the result of increased phospholipase C activity and an indirect increase in the rate of phosphatidylinositol hydrolysis.

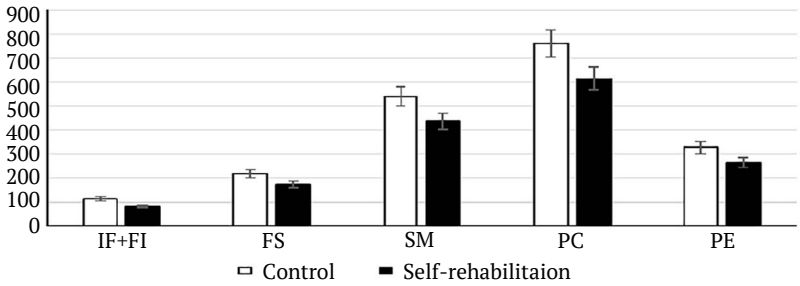

Figure 5. Content of individual phospholipids (mg/100 g of raw tissue) in liver samples of rats in the experimental group "Self-rehabilitation" with tetracycline-induced fatty hepatosis (M ± m, n = 8)
***Note**: P < 0.05, compared with the values of the corresponding indicators in intact animals in the "Control" group. IF+FI – total fraction of inositolphosphatidyl and phosphatidylinositol, FS – phosphatidylserine, SM – sphingomyelin, PC – phosphatidylcholine, PE – phosphatidylethanolamine*
***Source**: compiled by the authors*

At the same time, the phosphatidylserine content in these samples was characterised by a 19.9% decrease compared to the corresponding control (217.67 ± 15.6 mg/100 g of raw tissue). Phosphatidylserine synthase is involved in the formation of this phospholipid. As described by X. Ma *et al.* (2022), the catalytic activity of phosphatidylserine synthase is regulated in the mitochondrial-associated membranes of the endoplasmic reticulum. The reticulum produces phosphatidylserine, which is then transported to the mitochondria or Golgi complex through mitochondrial-associated membranes. In the mitochondria, a certain part of

phosphatidylserine is transformed into phosphatidylethanolamine with the participation of phosphatidylserine decarboxylase, which occurs on the inner membrane of the mitochondria, while the other part is involved in the structure of the mitochondrial membrane. To ensure cell viability, phosphatidylserine must be localised on the inner surface of the plasma membrane. As investigated by B.A. Chua *et al.* (2019), if this phospholipid moves to the outer surface of the bilayer due to the "flip-flop" mechanism, apoptosis may be triggered.

The decrease in sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine levels in liver samples from experimental rats (experimental group I, "Self-rehabilitation") was similar and corresponded to values of 18.2%, 18.3%, and 19.6% lower than these values in experimental rats of the "Control" group (540.66 ± 39.5 mg/100 g of raw tissue, 759.47 ± 55.6 and 328.52 ± 26.1 mg/100 g of raw tissue, respectively). As described by L. Sessa *et al.* (2021), sphingomyelin is a substantial structural component of biological membranes and belongs to the group of sphingolipids, which are capable of influencing the rigidity and compactness of cell membranes through the organisation of two-dimensional domains. Therefore, the established decrease in their content in liver samples from diseased rats may have negative consequences for the lateral structure of membranes. At the same time, the detected decrease in phosphatidylcholine content in liver samples from sick rats in the experimental group "Self-rehabilitation" (Fig. 5) may be associated with the activation of phospholipase D, which, as noted by P. Shyu *et al.* (2019), catalyses its hydrolytic cleavage with the subsequent formation of phosphatidic acid. At the same time, intracellular phosphatidylcholine homeostasis is substantial for ensuring the functional stability of organelles, while a decrease in its content indicates the development of cellular stress, which has been termed "lipid bilayer stress". Thus, a decrease in phosphatidylcholine content triggers cellular adaptive mechanisms to reduce the negative impact on numerous cellular processes through a response to stress.

Thus, when determining the qualitative and quantitative characteristics of the phospholipid composition of the liver in the experimental rats, a deficient level of all studied indicators was established. This fact may indicate a violation of the processes of phospholipid absorption in the intestine, inhibition of their synthesis in hepatocytes and secretion into the blood and bile in conditions of experimental tetracycline-induced fatty hepatosis. At the same time, the most pronounced quantitative changes in the liver of the experimental rats were observed in the total fraction of inositol phosphatide and phosphatidylinositol. According to C.N. Feriod *et al.* (2017), inositol triphosphate is directly related to the development of fatty liver infiltration. In combination with diacylglycerol, this phospholipid is formed from phosphatidylinositol of cell membranes in response to biological signals, in particular pathological ones. At the same time, this secondary messenger influences the course of various physiological processes in the intracellular environment. Based on the fact that inositol triphosphate is directly related to the development of fatty hepatosis, namely the activation of synthesis and deposition of fat droplets in liver cells (Feriod *et al.*, 2017), the effect of the dietary supplement "FLP-MD" prevents the development of fatty degeneration of the liver parenchyma. The study of the quantitative parameters of this fraction of phospholipids in biological material is relevant for clarifying the possible links in its metabolic transformations in clinically healthy and diseased animals. Thus, F.O. Lemos *et al.* (2019) recommended conducting additional studies of the inositol triphosphate-calcium regulatory intracellular

pathway, which in the future will contribute to the formation of pharmacological strategies in the treatment of hepatopathology. At the same time, research addressing each isoform of the intracellular receptors of this secondary mediator is of great significance.

The content of individual phospholipids was also studied in liver samples from experimental rats that received the dietary supplement FLP-MD based on milk phospholipids (experimental group II, "Correction") and in clinically healthy animals that were administered only the dietary supplement FLP-MD (experimental group III, "Healthy animals + dietary supplement FLP-MD") (Fig. 6). A detailed assessment of the phospholipid spectrum of liver tissues in experimental fatty hepatosis and with the use of milk phospholipids reveals existing molecular disorders of intermediate phospholipid metabolism, which is the basis of the pathogenesis of drug-induced hepatopathology and the determination of the characteristics of marker changes that can clarify the hepatoprotective efficacy of the newly created drug.

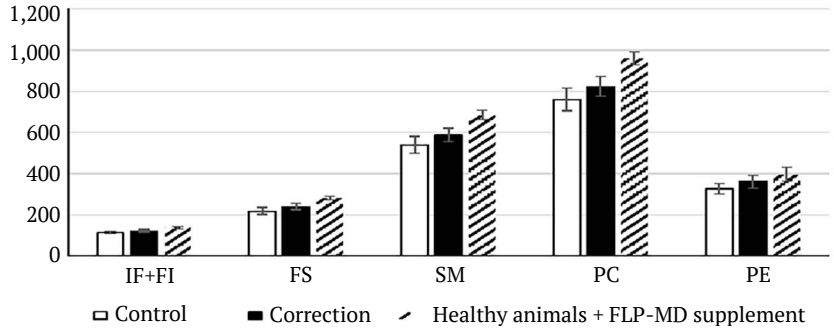

Figure 6. The content of individual phospholipids (mg/100 g of raw tissue) in the liver of rats in experimental group II "Correction" and experimental group III "Healthy animals + dietary supplement "FLP-MD"" for tetracycline-induced fatty hepatosis (M ± m, n = 8)

***Note**: P < 0.05, compared to the values of the corresponding indicators in intact animals in the "Control" group. IF+FI – total fraction of inositolphosphatidyl and phosphatidylinositol, FS – phosphatidylserine, SM – sphingomyelin, PC – phosphatidylcholine, PE – phosphatidylethanolamine*

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

In studying the content of individual phospholipid fractions obtained from the liver of rats in the "Correction" group, no significant changes were observed (Fig. 6). All results were within the control values. This fact proves the effectiveness of the corrective therapy based on milk phospholipids for sick animals. At the same time, the results of the study of these indicators in animals of the III experimental group "Healthy + FLP-MD dietary supplement" showed that the total fraction of inositol phosphatide and phosphatidylinositol exceeded the corresponding values in the control by 21.9% (114.52 ± 8.7 mg/100 g of raw tissue). The content of phosphatidylserine in rats in this group also increased by 27.7% compared to that in animals in the "Control" group (218.56 ± 16.6 mg/100 g of raw tissue). The content of sphingomyelin and phosphatidylcholine in liver samples from rats in the III experimental group "Healthy + FLP-MD dietary supplement" exceeded that in the control group by 25.9 and 25.7%, respectively (540.66 ± 39.5 mg/100 g and 759.47 ± 55.6 mg/100 g of raw tissue). The quantitative indicator of phosphatidylethanolamine in the liver of the experimental rats in the

"Healthy + FLP-MD dietary supplement" group also showed an increase of 24.6% compared to the control group (328.52 ± 26.1 mg/100 g of raw tissue). Thus, when milk phospholipids were administered to both sick rats (experimental group II "Correction") and animals in experimental group III "Healthy + FLP-MD dietary supplement", their positive effect on intermediate metabolism and the studied indicators of the phospholipid spectrum was established. This indicates the ability of milk phospholipids to eliminate the negative effects of the use of anti This indicates the ability of milk phospholipids to eliminate the negative effects of tetracycline hydrochloride antibiotics and their hepatoprotective effect.

The established patterns confirm the presence of significant lipid metabolism disorders in rats with tetracycline-induced fatty hepatosis, which is directly related to the functional state of the liver. The results obtained complement the data of previous studies that dealt with the established patterns of changes in the lipid and phospholipid spectra of blood plasma and the mucous membrane of the jejunum of calves suffering from a toxic form of dyspepsia, in which complications in the form of fatty and granular liver dystrophy were observed. Thus, V.A. Gryshchenko *et al.* (2023), a complete recovery in blood plasma was noted three weeks after the clinical recovery of calves that additionally received a dietary supplement based on milk phospholipids, total lipid content and most phospholipid fractions. Some of them even demonstrated an increase in relation to the control values. In particular, the content of phospholipids increased by 25%, phosphatidylcholine by 24%, phosphatidylethanolamine by 25%, and phosphatidylserine by 25% compared to the control. At the same time, the epithelium of the small intestine mucosa showed stabilisation of both lipid and phospholipid spectrum parameters, with a significant increase in the content of total phospholipids by 5%, phosphatidylcholine by 11% and a decrease in the lipid/protein ratio by 22% relative to control values, which indicated both positive changes in lipid metabolism and a significant improvement in the protein synthesis function of enterocytes under the action of milk phospholipids. In addition, the choleretic effect of phospholipid molecules on the bile-secreting activity of the liver was confirmed. As noted by J.F. Rehfeld (2025), the physiological mechanism of their effect is explained by the following mechanism: the humoral stimulus that causes contraction of the gallbladder, increased flow of hepatic bile and relaxation of the sphincter of the bile duct is the hormone cholecystokinin. One of the main sites of hormone production is the mucous membrane of the duodenum and the proximal part of the jejunum. The intensity of this process is influenced by fatty acids released during the hydrolysis of exogenous lipids. Therefore, the additional intake of milk phospholipids, which are similar in structure to those in the liver parenchyma, into the small intestine stimulates the activity of this hormone.

Thus, under conditions of artificial modelling of tetracycline liver damage in laboratory rats, a significant disturbance in the metabolism of lipids and phospholipids was observed, which may be due to the suppression of their endogenous synthesis, disorders in their absorption in the intestine and delivery to hepatocytes, as well as inhibition of their secretion into bile. The phospholipid components of the dietary supplement are of natural origin and are made from milk. This determines their high bioavailability for the animal organism. When milk phospholipids enter the liver, they primarily stimulate the development of regenerative processes, which have a positive effect on intracellular metabolism and the transport of substances in hepatocytes. This ensures the formation of bile with a complete composition

of the main biologically active components. Experimentally determined patterns of quantitative changes in the lipid and phospholipid spectrum indicators when modelling drug-induced fatty hepatosis in animals made it possible to identify the main marker indicators of the liver lipidogram and to recommend this dietary supplement as a corrective therapy for the development of hepatopathology in animals. It can be effective when using tetracycline antibiotics, as well as for preventing complications such as cholestasis, fibrosis, cirrhosis, liver failure, bilirubin encephalopathy, etc.

Conclusions

A study of total lipid fractions in liver samples from rats with tetracycline-induced fatty hepatosis showed that the most pronounced changes were in the content of esterified cholesterol, which decreased by 51.3% compared to the control. Similar patterns were observed for other lipid fractions, namely: a decrease in the content of phospholipids by 19.2%, free fatty acids by 31.2%, free cholesterol by 31.3% and triacylglycerols by 36.2% compared to the control group, which proves the presence of significant lipid metabolism disorders in the body of rats with tetracycline-induced fatty hepatosis. The use of a dietary supplement based on milk phospholipids in sick rats contributed to the restoration of the lipid spectrum of liver tissues in the experimental rats. When this dietary supplement was used in healthy animals, the content of lipid fractions in the liver of rats showed significant changes towards an increase in the content of some of them. Thus, in liver samples from experimental rats, there was an increase in the content of phospholipids by 27.1%, free fatty acids by 19.3%, esterified cholesterol by 21.3% and triacylglycerols by 24.8% compared to the control. This fact may indicate the sufficient effectiveness of the absorption of phospholipids from the dietary supplement in the intestine and the stimulating effect of its components on the processes of endogenous lipid formation in hepatocytes. Given the pathological disturbances in lipid homeostasis in experimental fatty liver degeneration, a deficiency in phospholipid spectrum indicators was also established: a decrease in the total fraction of inositolphosphatide and phosphatidylinositol by 26.6%, phosphatidylserine by 19.9%, sphingomyelin by 18.2%, phosphatidylcholine by 18.3% and phosphatidylethanolamine by 19.6%, and the high effectiveness of phospholipid-containing dietary supplements in normalising the content of phospholipids in the liver of sick animals. This suggests that the use of milk phospholipids as a dietary supplement is promising for correcting the structural and functional state of the liver and improving phospholipid metabolism in cases of liver damage, in particular, damage caused by tetracycline antibiotics. At the same time, when the dietary supplement was administered to clinically healthy rats, an increase in the content of all studied phospholipid fractions in the liver of rats was observed, in particular, the total fraction of inositolphosphatide and phosphatidylinositol by 21.9%, phosphatidylserine by 27.7%, sphingomyelin by 25.9%, phosphatidylcholine by 25.7% and phosphatidylethanolamine by 24.6% compared to the control. In accordance with the results obtained, it is reasonable to recommend the dietary supplement “FLP-MD” as a means of corrective therapy for the development of fatty hepatosis. In the future, it is planned to consider the issue of determining marker changes in blood plasma protein spectrum indicators in rats with tetracycline liver damage. This area of research is based on the leading role of the liver’s bilirubin synthesis function in the mammalian body.

Acknowledgements

The authors express their sincere gratitude to Associate Professor Yevdokiia Mykolaivna

Reshetnik, Department of Anatomy and Pathophysiology, Educational and Scientific Centre "Institute of Biology and Medicine", Taras Shevchenko National University of Kyiv, for her valuable assistance in conducting the experiment.

Funding

The study was not funded.

Conflict of Interest

None.

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Фосфоліпіди молока у коригуванні ліпідограми печінки у щурів за тетрацикліндукованого жирового гепатозу

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Анотація. Актуальність наукового дослідження полягає в обмеженій інформації з питань молекулярних механізмів розвитку жирового гепатозу в ссавців та визначення ефективних маркерних показників у діагностиці цієї патології. Своєчасне діагностування жирового гепатозу також важливе для запобігання виникнення його небезпечних ускладнень, передусім, цирозу печінки та гепатоцелюлярної карциноми. Важливу роль у патогенезі виникнення жирового гепатозу відіграють розлади у метаболізмі ліпідів. Тому мета цього дослідження полягала у встановленні закономірних змін у ліпідограмі печінки щурів за штучного відтворення жирового гепатозу й застосування фосфоліпідів молока в якості коригувальної терапії. Для цього використовували метод тонкошарової хроматографії. Визначено, що у разі штучного відтворення тетрациклінового гепатозу в печінці щурів формується дефіцитний рівень загальних фракцій ліпідів. Особливо різких змін зазнає фракція естерифікованого холестеролу, вміст якого зменшився на 51,3 % порівняно з контролем. Пероральне введення хворим щурам біодобавки на основі фосфоліпідів молока запобігало порушенню ліпідного складу печінки, що може свідчити про достатню

ефективність засвоєння фосфоліпідів біодобавки в кишечнику та стимулюючий вплив її компонентів на ендогенне їх утворення в гепатоцитах. Крім того, у хворих тварин відмічали формування дефіцитного рівня показників фосфоліпідного спектра печінки. Зокрема, зменшення вмісту сумарної фракції інозитолфосфатиду і фосфатидилінозитолу на 26,6 %, фосфатидилсерину на 19,9 %, сфінгомієліну на 18,2 %, фосфатидилхоліну на 18,3 % та фосфатидилетаноламіну на 19,6 % та відновлення їх параметрів за введення щурам фосфоліпідів молока. У разі застосування біодобавки клінічно здоровим щурам виявляли достовірне зростання в печінці вмісту всіх фракцій загальних ліпідів та індивідуальних фосфоліпідів. У відповідності до отриманих результатів обґрунтовано рекомендувати біоактивну добавку «FLP-MD» в якості засобу коригувальної терапії за розладів метаболізму ліпідів у разі функціональних порушень печінки за жирового гепатозу тварин

Ключові слова: тетрацикліну гідрохлорид; коригувальна терапія; тонкошарова хроматографія; ліпідний і фосфоліпідний спектри; жирова дистрофія

UKRAINIAN JOURNAL OF VETERINARY SCIENCES

2025. Volume 16, No. 3. P. 63-77

Received: 03.06.2025 Revised: 18.08.2025 Accepted: 23.09.2025

DOI: 10.31548/veterinary3.2025.63

UDC 636.09-636.8:614.9

Nutritional effects on mineral metabolism in cats with chronic kidney disease

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Abstract. The relevance of this study arises from the high prevalence of mineral metabolism disorders in cats with chronic kidney disease (CKD), which are accompanied by the development of hypercalcaemia and hyperphosphataemia and have a significant impact on prognosis and quality of life. Accordingly, the aim of the study was to assess the effect of different dietary calcium-to-phosphorus ratios on calcium-phosphorus homeostasis and the overall condition of cats with stage II CKD. The principal research method involved a comparative evaluation of clinical and biochemical parameters in animals depending on diet type, which enabled a comprehensive assessment of mineral metabolism changes. The study included fourteen cats divided into two groups. Group 1 animals received feed with a higher calcium-to-phosphorus ratio (1.71) and restricted phosphorus content, whereas Group 2 cats were fed a diet with a moderate calcium-to-phosphorus ratio (1.33). Over a six-month period, blood biochemical parameters were monitored, including total and ionised calcium, phosphorus, creatinine, urea, and symmetric dimethylarginine concentrations. In addition, the cats' clinical condition, body weight, muscle condition, body condition score, and arterial blood pressure were assessed, all of which remained stable in both experimental groups. It was found that cats in Group 1 showed an increase in calcium levels, while cats in group 2 developed ionised hypercalcaemia. In Group 2 cats, calcium levels remained stable without signs of hyperphosphataemia. Azotaemic markers did not differ significantly between the groups. Based

Suggested Citation:
Umanyets, M., & Tsvilikhovskyi, V. (2024). Nutritional effects on mineral metabolism in cats with chronic kidney disease. *Ukrainian Journal of Veterinary Sciences*, 16(3), 63-77. doi: 10.31548/veterinary3.2025.63.

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on the obtained results, it was established that excessive phosphorus restriction leading to a high calcium-to-phosphorus ratio did not provide additional benefits in controlling phosphataemia but increased the risk of calcium metabolism disturbances. The material of this article holds practical value for veterinary clinicians, as it experimentally confirms the advisability of using diets with a moderate calcium-to-phosphorus ratio at early stages of chronic kidney disease in cats

Keywords: dietary effect; metabolic disorders; secondary hyperparathyroidism; phosphate binders; calcium homeostasis; nephrocalcinosis; renal diet

Introduction

Chronic kidney disease (CKD) is among the most prevalent pathological conditions in cats, significantly affecting both the quality and duration of life. The disease is progressive in nature, characterised by the loss of nephrons and the development of mineral and bone disorders. A key factor in this process is the disturbance of calcium-phosphorus balance, which contributes to the formation of secondary hyperparathyroidism, tissue calcification, and nephrolithiasis, thereby accelerating the progression of renal failure.

According to the European Pet Food Industry Federation (FEDIAF, 2024) guidelines, the recommended calcium-to-phosphorus (Ca:P) ratio for adult cats ranges from 1:1 to 2:1. However, such a broad range does not account for the individual sensitivity of cats with CKD. In practice, high Ca:P ratios combined with excessive phosphorus restriction may provoke the development of ionised hypercalcaemia. M.R. Ehrlich *et al*. (2024) demonstrated that, in cats with hypercalcaemia, transitioning to diets with a Ca:P ratio below 1.4 resulted in normalisation of calcium levels in most cases within 3-20 weeks. Similarly, J. Stockman (2024) emphasised the absence of clearly defined safe limits for Ca:P ratios in current clinical guidelines and the need to revise existing recommendations.

A high Ca:P ratio is also associated with the formation of calcium-containing deposits. W. Zhang *et al*. (2024) showed that populations with elevated Ca:P ratios exhibited a higher prevalence of nephrolithiasis. In turn, E. Maniaki *et al*. (2024) reported that more than one quarter of cats with hypercalcaemia had early-stage CKD, and none of them had been fed renal diets prior to diagnosis – highlighting the influence of nutrition on calcium status.

An important direction in current research is the study of the interactions between calcium and phosphorus and other minerals. P.-K. Tang *et al.* (2024) demonstrated that an increased magnesium content in the diet of cats with CKD stabilises FGF-23 levels and prevents the development of hypercalcaemia. This highlights the complex interrelationships among calcium, phosphorus, and magnesium in maintaining metabolic balance. Meanwhile, findings by M. Krofič Žel *et al.* (2024) indicated that the addition of vitamin E to renal diets did not affect the survival of cats, confirming the priority of mineral control over other dietary interventions. Proteinuria also deserves particular attention as a predictor of CKD progression. M.A. Fidalgo *et al.* (2022) established that the urine protein-to-creatinine ratio (UPC) serves as a valuable clinical marker, since even moderate elevations are associated with the presence and further development of kidney disease in cats. This underscores the necessity of considering proteinuria when evaluating the effectiveness of dietary therapy and the control of the Ca:P ratio.

Therefore, determining the optimal Ca:P ratio in cats with CKD remains an urgent issue in veterinary medicine. Despite existing recommendations, current data indicate the need to refine the safe limits of this ratio, which has direct practical significance for improving dietary therapeutic strategies. Another major gap lies in the lack of long-term studies comparing different approaches to regulating the Ca:P ratio, particularly those combining dietary interventions with phosphate-binding agents, with evaluation of their effects on survival, quality of life, and the incidence of complications. Most available data are based on short- or medium-term observations, which complicates the establishment of consistent clinical recommendations.

In this context, the aim of the study was to evaluate the effect of different dietary calcium-to-phosphorus ratios on the development of hypercalcaemia and the effectiveness of hyperphosphataemia control in cats with stage II chronic kidney disease over a six-month observation period, as well as to determine a safe Ca:P range for long-term dietary therapy under controlled calcium and protein levels. The objectives of the study included analysing the dynamics of ionised and total calcium at different Ca:P ratios, assessing the influence of these ratios on phosphorus levels and the need for phosphate-binding therapy, determining the frequency of hypercalcaemia development depending on dietary profile, and investigating the relationship between the Ca:P ratio and proteinuria indicators as a prognostic marker of CKD.

Literature Review

Chronic kidney disease (CKD) in cats is among the most common and complex pathologies affecting small companion animals. It is characterised by a gradual loss of functional nephrons, progressive azotaemia, and the development of systemic complications. One of the key pathogenetic mechanisms involves disruption of calcium–phosphorus homeostasis due to reduced renal phosphate excretion, alterations in vitamin D metabolism, and activation of the parathyroid axis. Understanding these pathophysiological changes is critically important for accurate diagnosis and effective monitoring of the patient's condition.

As emphasised by J.A. Hokamp & M.B. Nabity (2016), the combined measurement of creatinine and symmetric dimethylarginine (SDMA) allows for a more precise assessment of the glomerular filtration rate, particularly in the early stages of the disease, when creatinine levels may remain within the normal range due to low muscle mass. Additionally, V. Pedrinelli *et al*. (2020) highlighted the importance of a comprehensive diagnostic approach to CKD, including urinalysis with evaluation of specific gravity, proteinuria, and the UPC, which enables the detection of glomerular and tubular damage and helps to predict disease progression rate. L. Hahn & C. Callaband (2022) stressed the diagnostic value of combining laboratory and imaging methods: assessment of proteinuria together with ultrasonographic examination provides a comprehensive evaluation of renal status and reveals morphological changes such as increased parenchymal echogenicity, reduced kidney size, and the presence of cystic formations or calcifications, reflecting chronic disturbances of mineral metabolism. R.F. Geddes *et al*. (2021) demonstrated that measurement of ionised calcium, in contrast to total calcium, serves as a more informative indicator of calcium status, particularly under conditions of hypoalbuminaemia.

The pathophysiological mechanisms underlying tissue mineralisation were described by P.-K. Tang *et al*. (2021), who noted that an increased calcium load against a background of low phosphorus levels may promote the calcification of extraosseous tissues, including nephrocalcinosis and vascular lesions. The

imbalance between calcium and phosphorus underlies the development of renal mineral and bone disorder (RMBD), which encompasses secondary hyperparathyroidism, osteodystrophy, nephrocalcinosis, and vascular calcification. These conditions can accelerate the progression of CKD, especially in the presence of concurrent metabolic disturbances.

Particular attention has been drawn to fibroblast growth factor-23 (FGF-23) as a biomarker of early disturbances in phosphorus metabolism. J. Lin *et al.* (2021) established that serum FGF-23 levels in cats with CKD increase at the early stages of the disease – prior to the onset of hyperphosphataemia – and correlate with disease progression while contributing to reduced calcitriol synthesis. This, in turn, decreases intestinal calcium absorption, stimulates parathyroid hormone secretion, and accelerates the formation of calcium–phosphate deposits, reflecting the key role of FGF-23 in the pathogenesis of renal mineral and bone disorders.

In humans with CKD, FGF-23 also possesses substantial clinical significance. S. Seiler *et al*. (2009) demonstrated that elevated concentrations of this hormone are associated with a higher risk of mortality and more rapid progression of renal insufficiency, independent of serum phosphorus levels – underscoring the universality of this pathophysiological mechanism. Its action includes suppression of calcitriol synthesis, reduction of intestinal calcium absorption, and stimulation of parathyroid hormone secretion, which, when combined with high Ca:P ratios, increases the risk of extraosseous tissue calcification.

One of the key mechanisms involved is the formation of an excessive Ca × P product in the blood, exceeding the solubility limit of calcium–phosphate compounds and promoting their deposition in soft tissues. As noted by J.A. Hokamp & M.B. Nabity (2016), such deposits may occur in the renal parenchyma, myocardium, vasculature, and gastrointestinal tract, impairing the function of affected organs. Hormonal dysregulation further amplifies this process: elevated Ca:P ratios in the context of CKD stimulate parathyroid hormone secretion, leading to secondary hyperparathyroidism, bone resorption, and additional calcium release into the bloodstream. L. Hahn & C. Callaband (2022) demonstrated that this creates a self-perpetuating cycle, whereby increased calcium levels sustain hypercalcaemia and elevate the risk of calcification.

Excessive phosphorus restriction combined with elevated dietary calcium shifts the Ca:P ratio beyond physiologically safe limits. This not only provokes hypercalcaemia but also increases plasma saturation with calcium-phosphate complexes, predisposing to nephrocalcinosis and vascular calcification. J. Stockman (2024) found that such alterations accelerate both structural and functional lesions of renal and extra-renal tissues, worsening the disease prognosis.

L. Hahn & C. Callaband (2022) further noted that cats exhibit greater sensitivity to variations in the Ca:P ratio than dogs, which may be associated with species-specific characteristics of calcium-phosphorus regulation and hormonal control. This underscores the necessity of a species-specific approach to dietary correction. An elevated dietary calcium-to-phosphorus ratio in cats with chronic kidney disease creates conditions for a cascade of pathophysiological changes leading to complications. Control of phosphataemia in cats with CKD remains a cornerstone of disease management, crucial for slowing progression and preventing complications. Dietary therapy with reduced phosphorus content remains the first-line intervention, particularly in the early stages of CKD. It is recommended to use diets containing 0.3-0.6% phosphorus on a dry matter basis and to

maintain the Ca:P ratio near the lower limit of the range recommended by FEDIAF (2024) to avoid excessive calcium loading.

When dietary phosphorus restriction alone is insufficient to achieve target levels, phosphate binders are employed. R.F. Geddes *et al*. (2021) emphasised that the choice of binder type should take into account the patient's current calcium status. Calcium-containing agents, such as calcium acetate or calcium carbonate, are effective in lowering serum phosphate levels but, with long-term use, may increase the Ca:P ratio. This can contribute to hypercalcaemia, particularly in animals receiving low-phosphorus diets. An alternative approach involves the use of non-calcium-containing binders. V.J. Parker (2021) found that sevelamer hydrochloride and lanthanum carbonate effectively controlled phosphorus levels without adding a calcium load. However, the use of sevelamer may reduce the absorption of fat-soluble vitamins, while lanthanum carbonate can accumulate in tissues with prolonged administration, necessitating regular monitoring.

J. Stockman (2024) highlighted the importance of an individualised dietary strategy for cats with CKD. The author stressed that in patients with high Ca:P ratios, preference should be given to non-calcium phosphate binders, and excessive phosphorus restriction combined with elevated dietary calcium should be avoided. The optimal approach involves a combination of moderate phosphorus restriction, a controlled Ca:P ratio, and, where necessary, the use of non-calcium binders, which together minimise the risk of calcification and help maintain a stable course of CKD. J.A. Hokamp & M.B. Nabity (2016) underscored the need for a comprehensive assessment of mineral metabolism incorporating novel biomarkers such as FGF-23 and parathyroid hormone to enable early detection of imbalances before the onset of clinical signs or hyperphosphataemia. However, standardised monitoring protocols for these indicators in cats have not yet been developed.

Materials and Methods

The study was conducted from October 2024 to March 2025 at the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine) and the "Zoolux" Veterinary Clinic (Kyiv, Ukraine). The research involving animals complied with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and the Law of Ukraine No. 3447-IV (2006). All procedures were performed in accordance with the recommendations of the ARRIVE Guidelines 2.0 (n.d.) and did not contravene the provisions of Directive 2010/63/EU of the European Parliament and of the Council (2010). The study protocol was approved by the Bioethics Committee of the National University of Life and Environmental Sciences of Ukraine (Protocol No. 7/2024).

The study involved fourteen domestic cats aged between six and nine years with confirmed chronic kidney disease, diagnosed according to the criteria of the International Renal Interest Society (IRIS, 2023). Animals with concurrent endocrine, cardiovascular, or infectious diseases were excluded from the sample. All cats were patients of the "Zoolux" Veterinary Clinic and remained under the care of their owners throughout the study. They were kept under home conditions with free access to water and were fed diets according to their assigned study group. The cats were divided into two groups – Group 1 and Group 2 – with seven animals in each. Both groups received two types of veterinary diets formulated for cats with CKD, differing in fat and phosphorus content and in the calcium-to-phosphorus ratio, while maintaining a comparable level of crude protein (Table 1).

Table 1. Chemical composition of the dietary feeds used in the study

Parameter	Diet 1	Diet 2
Crude protein (%)	28	27
Crude fat (%)	12	20
Crude fibre (%)	3	2.7
Calcium (%)	0.6	0.6
Phosphorus (%)	0.33	0.45
Ca:P ratio	1.71	1.33
Sodium (%)	0.2	0.3
Potassium (%)	0.8	0.65
Vitamin A (IU/kg)	25,668	24,000
Vitamin D_3 (IU/kg)	1,523	1,800

***Note**: the presented values reflect the content of the main nutrients in the composition of the feeds according to the official data provided by the manufacturers*
***Source**: feed manufacturers' data (Nestlé Purina, 2024; Josera, 2024)*

The calcium content was identical in both diets (0.6%), and the concentrations of vitamin D_3 were comparable (1,523 IU/kg in Diet 1 and 1,800 IU/kg in Diet 2). Dietary phosphorus restriction is a key component of nutritional management in CKD, as it reduces the risk of secondary hyperparathyroidism and prolongs feline lifespan. However, contemporary evidence from P.-K. Tang *et al*. (2021) and R.F. Geddes *et al*. (2021) indicates that the effectiveness of this approach depends not only on the absolute phosphorus level but also on the calcium-to-phosphorus ratio (Ca:P), which determines mineral balance and influences the organism's metabolic stability. Therefore, this study compared diets with different Ca:P ratios under controlled calcium and protein levels, enabling the assessment of the isolated effect of this parameter on the development of hypercalcaemia, hyperphosphataemia, and the overall progression of CKD. This approach provided an objective basis for determining a safe Ca:P range for long-term dietary management.

Throughout the experiment, regular veterinary examinations and monitoring of the cats' clinical condition were performed. The general health status, appetite, body weight dynamics, and arterial blood pressure were assessed. Animals were weighed using a Momert 6551 electronic scale (Momert Ltd., Hungary). Blood pressure was measured using a petMAP graphic II oscillometric tonometer (CardioCommand Inc., USA). A cuff width corresponding to 30-40% of the limb circumference was used, and 5-7 measurements were taken; the median value was recorded for analysis. To reduce stress and minimise the "white-coat effect," all animals received Gabapentin prior to the procedure in accordance with the Cat Friendly Practice® Guide (AAFP, 2023).

Venous blood samples were collected from the jugular vein every three months after an overnight fast (8-12 hours) using a 22G needle under sterile conditions. For haematological analysis, K_2-EDTA tubes were used, while serum was obtained in clot activator tubes for biochemical analysis. After clot formation, serum was separated by centrifugation (10 minutes at 1,500-2,000 g) and stored at 2-8°C for no longer than 24 hours. Samples showing evidence of haemolysis or lipaemia were excluded from further analysis.

In the serum biochemical profile, the concentrations of creatinine, urea, potassium, phosphorus, sodium, chloride, total and ionised calcium, total protein, albumin, and symmetric

dimethylarginine (SDMA) were determined. Biochemical parameters were analysed using an automated biochemical analyser Mindray BS-240 (Mindray Biomedical Electronics Co., China), and SDMA was measured with a veterinary biochemical analyser IDEXX Catalyst One (IDEXX Laboratories Inc., USA). Complete blood counts were performed using an automated haematology analyser Mindray BC-5000 (Mindray Biomedical Electronics Co., China).

Urine samples were primarily obtained by cystocentesis (22G needle, 5-10 ml syringe) under aseptic conditions. When cystocentesis was not feasible, free-catch collection was employed. Urine specific gravity was measured using a veterinary refractometer BRCtech (BRCtech, Poland), calibrated beforehand with distilled water; the result was calculated as the mean of three measurements. Urine sediment was evaluated within 60 minutes of collection (centrifugation for 5 minutes at approximately 400 g; microscopy at ×100 and ×400 magnification).

To assess the body condition of the cats, a combination of a 9-point Body Condition Score (BCS) system and a 4-point Muscle Condition Score (MCS) system was used. The BCS system, proposed by D.P. Laflamme (1997), is widely applied in clinical practice for cats. In particular, C.R. Bjornvad *et al*. (2011) confirmed its high reproducibility and correlation with objective methods for assessing adipose tissue. Muscle mass was assessed using the 4-point MCS scale validated by K.E. Michel *et al*. (2011), which demonstrated a significant correlation with dual-energy X-ray absorptiometry results and is recommended by the World Small Animal Veterinary Association (WSAVA, 2013) as a clinical tool for evaluating the degree of muscle loss.

All procedures were carried out with the utmost attention to animal welfare and stress minimisation. Manipulations were performed in a clinic certified according to the Cat Friendly Clinic standards (ISFM), which ensured a low-stress environment. Statistical analysis of the results was performed using the STATISTICA 7.0 software package (StatSoft Inc., USA). Data were expressed as mean ± standard deviation (M ± SD). The Shapiro-Wilk test was used to assess normality of data distribution. Comparisons between groups were conducted using Student's t-test for normally distributed data or the Mann-Whitney U-test for non-parametric data. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Throughout the study period, the clinical condition of the cats remained relatively stable. During routine examinations, appetite, water intake, and general behaviour were recorded. In most cats, appetite remained satisfactory, with no cases of prolonged anorexia. Water consumption stayed within the normal individual range, without marked polydipsia or decreased thirst. Clinical examination revealed no signs of dehydration, vomiting, or diarrhoea; mucous membranes remained pink and moist. The overall condition of the coat was assessed as satisfactory, without alopecia or visible deterioration in quality. Behaviour and activity levels showed no significant deviations from baseline observations.

Over the course of monitoring, mean systolic and diastolic blood pressure values remained within the physiological range for cats. Individual fluctuations did not exceed clinically relevant limits. Heart rate values also remained stable throughout the experiment. No statistically significant differences were observed between Groups 1 and 2, or compared with baseline measurements (Table 2).

The body weight of cats in both groups at the beginning of the study ranged from 3.4 kg to 5.0 kg. Over the six-month observation period, individual fluctuations of 50-150 g were recorded, which were not statistically significant. Body condition score (BCS) remained

stable at 4/9, and muscle condition score (MCS) at 4/4 in most animals. The dynamics of body weight in both groups are presented in Table 3. As shown by the data, the body weight of the cats remained relatively stable in both groups throughout the six-month period. Individual variations did not exceed clinically meaningful limits and did not result in significant changes in mean values. Both BCS and MCS showed no notable dynamics, indicating that the animals maintained a stable physical condition during the observation period.

Table 2. Systolic and diastolic blood pressure and heart rate in cats of the experimental groups at different observation periods (M ± SD), (n = 7)

Observation period	Group 1			Group 2		
	Baseline	3 months	6 months	Baseline	3 months	6 months
Systolic blood pressure (mmHg)	148.1 ± 7.6	146.9 ± 3.9	145.3 ± 3.9	148.3 ± 8.4	144.7 ± 5.5	148.0 ± 2.4
Diastolic blood pressure (mmHg)	85.4 ± 4.5	82.6 ± 1.6	83.4 ± 2.5	84.0 ± 4.4	82.9 ± 2.0	82.2 ± 2.3
Heart rate (beats/min)	154.9 ± 5.9	155.0 ± 8.5	153.4 ± 5.6	155.0 ± 5.6	152.1 ± 8.3	155.1 ± 6.7

***Note**: data are presented as mean ± standard deviation (M ± SD)*
***Source**: authors' own data*

Table 3. Dynamics of body weight in cats (M ± SD), (n = 7)

Observation period	Group 1	Group 2
Day 1	4.10 ± 0.36	4.23 ± 0.53
3 months	4.06 ± 0.30	4.22 ± 0.55
6 months	4.10 ± 0.29	4.23 ± 0.57

***Note**: data are presented as mean ± standard deviation (M ± SD)*
***Source**: authors' own data*

Evaluation of azotaemic markers (creatinine, urea, and symmetric dimethylarginine) demonstrated relative stability of the parameters throughout the study period. No statistically significant differences were observed between the groups. As shown in the data (Table 4), the mean serum creatinine concentrations in cats of both groups at the beginning of the experiment were within the reference range. During the course of the study, a slight decrease in serum creatinine levels was noted in cats at the third month, which was likely associated with adaptation of the animals to the study conditions and stabilisation of dietary intake. By the sixth month, creatinine levels remained largely unchanged compared with baseline values.

Table 4. Dynamics of azotaemic markers in cats (M ± SD), (n = 7)

Parameter	Group 1			Group 2		
	Baseline	3 months	6 months	Baseline	3 months	6 months
Creatinine (μmol/L)	192.58 ± 7.62	180.03 ± 9.52	185.55 ± 10.84	186.6 ± 12.5	178.0 ± 8.0	182.4 ± 7.6
Symmetric dimethylarginine (mg/dL)	20.00 ± 0.47	19.50 ± 0.66	19.33 ± 1.02	19.90 ± 1.22	19.38 ± 1.38	20.13 ± 1.25
Urea (mg/dL)	14.17 ± 1.49	11.98 ± 1.57	11.65 ± 1.33	11.48 ± 1.72	10.68 ± 1.38	10.84 ± 1.11
Total calcium (mmol/L)	2.48 ± 0.02	2.56 ± 0.02	2.53 ± 0.02	2.38 ± 0.07	2.41 ± 0.05	2.59 ± 0.12

***Note**: data are presented as mean ± standard deviation (M ± SD)*
***Source**: authors' own data*

Serum urea concentrations decreased in cats of both groups during the first three months of the study and then stabilised. This may reflect a reduced influence of dietary factors (specifically protein content) on azotaemic parameters. Levels of symmetric dimethylarginine (SDMA), considered a more sensitive marker of decreased glomerular filtration rate, remained within physiological limits and showed no significant differences over the observation periods. Minor individual variations within ±1 mg/dL were not statistically significant.

In addition to azotaemic markers, indices of mineral and electrolyte metabolism – key components in the pathogenesis of CKD – were analysed to assess the potential dietary influence on the cats' metabolic status. As shown in Table 5, serum levels of calcium (total and ionised), phosphorus, sodium, potassium, and chloride remained within physiological ranges for the species throughout the observation period. The most pronounced changes during the study were observed in calcium-phosphorus metabolism indicators. In Group 1 cats, serum total calcium levels gradually increased and by the end of the experiment were elevated by approximately 15% compared with baseline values. In some cats, these changes exceeded physiological limits, corresponding to a state of hypercalcaemia. In contrast, in Group 2 cats, calcium levels remained stable, fluctuating within ± 1% – a variation without clinical significance.

Table 5. Dynamics of calcium–phosphorus and electrolyte metabolism indicators in cats (M ± SD), (n = 7)

Parameter	Group 1			Group 2		
	Baseline	3 months	6 months	Baseline	3 months	6 months
Total calcium (mmol/L)	2.48 ± 0.02	2.56 ± 0.05	2.86 ± 0.07	2.38 ± 0.07	2.41 ± 0.05	2.40 ± 0.12
Ionised calcium (mmol/L)	1.30 ± 0.02	1.35 ± 0.06	1.43 ± 0.07	1.26 ± 0.05	1.28 ± 0.05	1.27 ± 0.08
Phosphorus (mmol/L)	1.31 ± 0.08	1.32 ± 0.06	1.29 ± 0.06	1.30 ± 0.20	1.26 ± 0.14	1.28 ± 0.12
Potassium (mmol/L)	4.1 ± 0.22	4.00 ± 0.28	4.11 ± 0.24	3.80 ± 0.24	3.90 ± 0.28	4.00 ± 0.32
Chloride (mmol/L)	117.0 ± 4.2	115.0 ± 4.2	116.0 ± 4.3	117.0 ± 4.1	115.0 ± 3.8	117.0 ± 3.7
Sodium (mmol/L)	155.0 ± 3.3	156.0 ± 2.8	15.0 ± 3.2	156.0 ± 4.0	157.0 ± 3.8	155.0 ± 3.2

***Note**: data are presented as mean ± standard deviation (M ± SD)*
***Source**: authors' own data*

Ionised calcium, representing the biologically active fraction, increased by almost 10% in the serum of cats in Group 1, whereas only minimal fluctuations (not exceeding 2%) were observed in Group 2. This indicated a tendency toward activation of calcium metabolism specifically in the experimental group. Serum phosphorus concentrations in cats of both groups remained within a range close to physiological values (1.26-1.32 mmol/L), without indications of hyperphosphataemia. The Ca:P ratio remained stable, showing no signs of imbalance, which suggested preservation of mineral homeostasis. Serum potassium concentrations in both experimental groups did not increase and remained within reference limits. Sodium and chloride levels in the serum of cats fluctuated within a narrow range, with deviations not exceeding 2-3%, indicating no evidence of hypo- or hypernatraemia or chloraemia. Overall, analysis of mineral and electrolyte parameters in the cats revealed no statistically significant changes during the six-month observation period and no differences between the groups. These findings indicate the absence of any disturbances in calcium-phosphorus

or electrolyte homeostasis in the cats over the course of the study.

Urine analysis in cats included assessment of the UPC and urine specific gravity (USG). Throughout the observation period, UPC values in both groups remained within physiological limits (< 0.2), with no evidence of proteinuria. Individual fluctuations did not exceed clinically relevant ranges and showed no trend toward progressive increase. Urine specific gravity ranged from 1.030 to 1.050, reflecting normal renal concentrating ability, and no signs of isosthenuria were detected. No statistically significant differences between experimental groups were found for these parameters. Thus, the results of urine analysis confirmed the stability of renal functional status and the absence of any adverse dietary effects during the study period.

Throughout the six-month observation period, the clinical condition of the cats remained stable: appetite, water intake, behaviour, and coat appearance showed no notable changes. Blood pressure and heart rate were within physiological ranges, with no signs of arterial hypertension or hypotension. Body weight, body condition score (BCS), and muscle condition score (MCS) remained stable, indicating preservation of overall somatic status.

Analysis of azotaemic markers (creatinine, urea, and SDMA) in the serum of cats from both experimental groups confirmed the absence of progressive renal dysfunction. Serum sodium, potassium, and chloride levels remained within physiological limits, showing no evidence of electrolyte imbalance. Urinalysis parameters also demonstrated no significant changes: the urine protein-to-creatinine ratio remained below the threshold for proteinuria, and urine specific gravity values indicated preserved renal concentrating ability.

Based on the obtained results, it was established that lower calcium-to-phosphorus (Ca:P) ratios were associated with a tendency toward stabilisation of calcium metabolism, whereas cats in the group with higher Ca:P ratios more frequently exhibited hypercalcaemia, including that of the ionised fraction. Similar observations were reported by M.R. Ehrlich *et al*. (2024), who described normalisation of serum calcium levels in cats with CKD following a dietary transition to lower Ca:P ratios. Conversely, J. Stockman (2024) noted that excessive restriction of dietary phosphorus, leading to elevated Ca:P ratios, may provoke the development of hypercalcaemia even at early stages of the disease. The findings of the present study are consistent with these observations and indicate that controlling the Ca:P ratio is a critical factor in the management of feline CKD.

The observed tendencies can be explained in terms of calcium-phosphate complex supersaturation. Exceeding the solubility product of Ca × P increases the risk of calcium deposition in renal and extra-renal tissues. As highlighted by J.A. Hokamp & M.B. Nabity (2016), this mechanism underlies nephrocalcinosis and vascular calcification in chronic kidney disease. In the present study, cats with higher Ca:P ratios exhibited more pronounced alterations in calcium balance, which may reflect a latent increase in the risk of calcification.

From a pathophysiological perspective, a higher calcium-to-phosphorus (Ca:P) ratio may enhance intestinal calcium absorption, which is particularly relevant in patients with CKD, where the regulatory mechanisms of this process are already impaired. In healthy animals, compensatory mechanisms involving parathyroid hormone (PTH) and fibroblast growth factor 23 can partially maintain calcium levels within the normal range; however, in CKD, their effectiveness is reduced. This creates a predisposition to the development of hypercalcaemia even in response to relatively minor dietary fluctuations, which may explain why serum calcium levels in some experimental cats exceeded

physiological limits, whereas in others they remained stable. Thus, the absence of progressive hyperphosphataemia in the experimental cats indicates the effectiveness of the applied dietary therapy and may contribute to slowing the development of complications.

Particular attention should be given to FGF-23, which is considered an early biomarker of phosphorus imbalance. Although it was not measured in the present study, published data suggest that an increase in FGF-23 levels may precede the marked changes observed in the experimental animals. Therefore, even with stable serum phosphorus concentrations in the cats, subtle shifts in phosphorus homeostasis mediated by FGF-23 cannot be excluded.

An additional factor may be the variability of individual physiological responses among animals, whereby hypercalcaemia resolves in some cats following the transition to a diet with a lower calcium-to-phosphorus (Ca:P) ratio, while others show only a partial or absent response. This finding indicates the multifactorial nature of the processes involved. In particular, individual variations in vitamin D metabolism, differences in sensitivity to FGF-23, and the baseline condition of renal tissue – determining the organism's adaptive capacity – may all play contributory roles.

The stability of the clinical condition observed in the present study aligns with the findings of D.P. Machado *et al*. (2022), who confirmed that dietary modifications can slow the progression of CKD. The absence of proteinuria is consistent with the observations of M.A. Fidalgo *et al*. (2022), who emphasised the prognostic value of the urine protein-to-creatinine ratio in cats with CKD. Similarly, V. Pedrinelli *et al*. (2020) noted that survival in affected animals depends not only on disease stage but also on dietary composition, particularly protein content and the calcium-to-phosphorus ratio.

Particular attention should be paid to the selection of phosphate binders. R.F. Geddes *et al*. (2021) reported that calcium-containing preparations effectively reduce serum phosphate concentrations but may simultaneously increase the Ca:P ratio and promote hypercalcaemia. As an alternative, V.J. Parker (2021) recommended the use of non-calcium-based binders such as sevelamer and lanthanum carbonate. According to the results obtained in the present study, it was confirmed that excessive restriction of dietary phosphorus in combination with a high Ca:P ratio should be avoided, particularly in animals at increased risk of tissue calcification.

Thus, even with comparable dietary calcium and vitamin D levels, it is the magnitude of the Ca:P ratio that determines the direction of changes in calcium balance. Lower Ca:P ratios were associated with more stable calcium levels, whereas higher ratios increased the risk of ionised hypercalcaemia. These findings confirm the clinical importance of monitoring the Ca:P ratio in the dietary management of cats with CKD. In light of the obtained data, it is advisable to integrate Ca:P ratio monitoring into the standard follow-up protocol for feline CKD. At the onset of dietary therapy, attention should be given to maintaining values near the lower limit of the FEDIAF (2024) recommended range. Measurement of total and ionised calcium, phosphorus, and calculation of the Ca × P product should be performed after 2-4 weeks of diet initiation and subsequently every 8-12 weeks, in accordance with the recommendations of the International Renal Interest Society (2023).

In cases where ionised calcium levels increase despite "normal" serum phosphorus concentrations, it is advisable to review the dietary formulation by reducing the calcium-to-phosphorus (Ca:P) ratio and, where appropriate, to prioritise the use of non-calcium-based phosphate binders. Concurrent monitoring of the

UPC and USG should be performed as indicators of glomerular and tubulointerstitial injury, alongside regular blood pressure assessment to minimise the risk of extra-renal complications. It is also essential to account for potential pre-analytical variability in ionised calcium measurement – particularly the influence of sample pH – and to maintain stable conditions for collection and transport in order to avoid misinterpretation of results.

When evaluating the effectiveness of dietary therapy, simultaneous monitoring of UPC, USG, and arterial pressure provides valuable information regarding renal function and systemic stability. Particular attention should be paid to the accuracy of ionised calcium measurement, given its sensitivity to pH changes, which necessitates standardised sampling and handling procedures. In cats predisposed to hypercalcaemia, a temporary and moderate relaxation of dietary phosphorus restriction may be appropriate, provided that the Ca:P ratio remains within controlled limits. This approach can help to stabilise calcium-phosphorus balance and minimise the risk of calcification.

Conclusions

In this six-month clinical study involving cats with chronic kidney disease, comprehensive dietary management contributed to the stabilisation of the animals' clinical condition. Throughout the observation period, appetite, behaviour, hydration status, and cardiovascular parameters remained within physiological limits. Body weight, body condition score, and muscle condition score demonstrated nutritional stability. Azotaemic markers – creatinine, urea, and symmetric dimethylarginine – showed no statistically significant changes, confirming the absence of CKD progression in most animals under appropriate dietary support.

The most pronounced differences were observed in calcium-phosphorus metabolism. Cats receiving a diet with a higher calcium-to-phosphorus (Ca:P) ratio (1.71) exhibited an increase in total calcium from 2.48 ± 0.02 to 2.86 ± 0.07 mmol/L (+ 15%) and ionised calcium from 1.30 ± 0.02 to 1.43 ± 0.07 mmol/L (+ 10%), corresponding to hypercalcaemia in some individuals. Conversely, in cats fed a diet with a lower Ca:P ratio (1.33), calcium concentrations remained stable (2.38-2.40 mmol/L for total calcium and 1.26-1.27 mmol/L for ionised calcium) without clinically significant deviations. These findings indicate that even with identical dietary calcium content, the Ca:P ratio may be a decisive factor influencing the risk of hypercalcaemia in cats with CKD. The results support the necessity not only of dietary phosphorus restriction – a key element of CKD therapy – but also of optimising the Ca:P ratio in therapeutic diets. The balance between these macrominerals affects the progression of mineral metabolism disorders associated with CKD and thus influences long-term outcomes.

The main limitations of this study were the small sample size (n = 14) and the relatively short observation period (six months). The study duration was chosen for ethical reasons, as prolongation could have increased the risk of clinically significant hypercalcaemia in cats receiving the higher Ca:P diet. Nonetheless, these factors may have limited the ability to detect longer-term dietary effects. Despite these limitations, the findings demonstrate that the Ca:P ratio – even with equivalent calcium content and comparable vitamin D levels – can influence calcium homeostasis and has clinical significance in dietary formulation for cats with CKD.

Future research should aim to establish optimal target Ca:P ranges for different CKD stages and to standardise the monitoring of mineral metabolism biomarkers. Incorporation of FGF-23 as a biomarker for personalised dietary management appears promising, as its monitoring could help detect subclinical phosphorus imbalance before the onset of overt hyperphosphataemia,

thereby improving precision in Ca:P correction and reducing the risk of tissue calcification.

Acknowledgements

None.

Funding

The study was not funded.

Conflict of Interest

None.

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Нутріціологічний вплив на мінеральний обмін у котів за хронічної хвороби нирок

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Анотація. Актуальність дослідження зумовлена значною поширеністю порушень мінерального обміну в котів із хронічною хворобою нирок, що супроводжуються розвитком гіперкальціємії та гіперфосфатемії й суттєво впливають на прогноз і якість життя тварин. У зв'язку з цим, мета дослідження була спрямована на оцінку впливу різного співвідношення кальцію до фосфору в раціоні на показники кальцієво-фосфорного гомеостазу та загальний стан котів із хронічною хворобою нирок другої стадії. Провідним методом дослідження слугувала порівняльна оцінка клінічних і біохімічних показників у тварин, залежно від типу дієти, що дозволило комплексно визначити зміни мінерального обміну. У дослідженні використано 14 котів, яких поділено на дві групи. Тварини групи 1 отримували корм із вищим співвідношенням кальцію до фосфору (1,71) та обмеженим вмістом фосфору, тоді як коти групи 2, отримували дієту з помірним співвідношенням кальцію до фосфору (1,33). Впродовж шести місяців контролювали біохімічні показники крові: рівень загального та іонізованого кальцію, фосфору, креатиніну, сечовини та симетричного диметиларгініну. Додатково оцінювали у котів клінічний стан, масу тіла, м'язову кондицію, вгодованість та артеріальний тиск, які залишалися стабільними у тварин в обох дослідних групах. Встановлено, що у котів групи 1 відзначалося підвищення рівня кальцію, а у котів групи 2 сформувалася іонізована гіперкальціємія. У котів групи 2 рівень кальцію залишався стабільним, без ознак гіперфосфатемії. Показники азотемічних маркерів істотно не відрізнялися між групами котів. За отриманими результатами встановлено, що надмірне обмеження фосфору з формуванням високого співвідношення кальцію до фосфору не забезпечило додаткових переваг у контролі фосфатемії, але підвищувало ризик порушень кальцієвого обміну. Матеріал статті становить практичну цінність для ветеринарних лікарів-клініцистів, оскільки експериментально підтверджено доцільність використання дієт із помірним співвідношенням кальцію та фосфору на ранніх стадіях хронічної хвороби нирок у котів

Ключові слова: дієтичний вплив; порушення метаболізму; вторинний гіперпаратиреоз; фосфат-біндери; кальцієвий гомеостаз; нефрокальциноз; ниркова дієта

UKRAINIAN JOURNAL OF VETERINARY SCIENCES

2025. Volume 16, No. 3. P. 78-93

Received: 06.06.2025 Revised: 14.08.2025 Accepted: 23.09.2025

DOI: 10.31548/veterinary3.2025.78

UDC 636.7.09:616-089:616.366

Surgical treatment of the domestic dog (*Canis familiaris*) for gallbladder mucocele (a clinical case study)

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Suggested Citation:
Kulida, M., Nicpoń, J., Mazurkevych, T., Solonin, P., & Gorkava, I. (2024). Surgical treatment of the domestic dog (*Canis familiaris*) for gallbladder mucocele (a clinical case study). *Ukrainian Journal of Veterinary Sciences*, 16(3), 78-93. doi: 10.31548/veterinary3.2025.78.

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Abstract. The relevance of this study is determined by the rapid increase in cases of gallbladder mucocele in dogs, a condition characterised by the accumulation of excessive mucin and bile within the gallbladder. This pathology causes partial or complete extrahepatic biliary obstruction through the spread of bile-saturated mucus into the common bile ducts, which can be life-threatening. Accordingly, this study aimed to identify the clinical symptoms and effective diagnostic and treatment methods for animals with gallbladder mucocele. A clinical case of gallbladder mucocele was examined in a Chihuahua that developed hepatobiliary insufficiency. The sequence of diagnostic procedures, including laboratory tests and ultrasound examination, as well as the surgical treatment, is presented. The condition in the affected dog manifested as non-specific clinical signs such as frequent vomiting, anorexia, and lethargy. The most common physical examination findings included abdominal pain, jaundice of the skin and visible mucous membranes, and hyperthermia. The biochemical markers of this condition in the affected dog included elevated serum concentrations of total bilirubin and increased activities of alkaline phosphatase, alanine aminotransferase, and gamma glutamyltransferase. Ultrasound imaging was used to visualise changes in the gallbladder tissue. The diagnosis of gallbladder mucocele was established based on the presence of characteristic stellate or finely striated bile patterns. The condition was differentiated from biliary sludge by the absence of gravity-dependent bile movement. Ultrasound examination revealed no specific changes in the thickness or appearance of the gallbladder wall. Based on the diagnostic findings, cholecystectomy was selected as the surgical treatment method for affected dogs. The sterility of the bile sample in bacteriological testing indicated the non-infectious nature of the mucocele. The results obtained have significant practical relevance for both researchers and practising veterinary surgeons, contributing to the improvement of treatment protocols for animals with gallbladder mucocele

Keywords: hepatobiliary pathology; cholecystitis; cholecystectomy; haematological indicators; miniature breeds

Introduction

The digestive system is one of the largest systems in the body and comprises organs responsible for food intake, propulsion, mechanical and chemical processing, absorption of nutrients essential for the organism, and the elimination of undigested residues. According to T.M. Suprovych *et al*. (2024), diseases of the digestive system in dogs account for 64.7% of all internal non-contagious animal diseases. Such disorders may arise from inadequate diet quality or feeding regime, poor housing and management conditions, infectious or parasitic diseases, poisoning, or dysfunction of other organs and systems.

The digestive system consists of the alimentary canal and accessory organs, including the liver. The liver participates in metabolic processes and performs storage, filtration, and detoxification functions. These activities are ensured by hepatic cells and the organ's specific connection to the circulatory system, whereby oxygen is supplied via the hepatic artery passing through the hepatic portal, while the portal vein carries blood from the digestive organs for filtration and metabolic exchange. In the study by J.L. Gookin (2025), it was noted that liver cells, or hepatocytes, produce bile, which enters the bile ducts and, during intervals between

digestive processes, accumulates in a specialised storage reservoir – the gallbladder – whose primary function is bile storage. Within the gallbladder, bile may become concentrated up to tenfold. Through the secretion of epithelial acid, it becomes acidified and modified before entering the gastrointestinal tract via the major duodenal papilla, where mucin and immunoglobulins are added.

K. Azuma *et al*. (2024) reported a rapid increase in the number of extrahepatic biliary pathologies in veterinary medicine. According to R. Fujiwara *et al*. (2025), gallbladder mucocele and cholelithiasis are the most common gallbladder diseases in dogs; however, A. Noguchi *et al*. (2023) noted that these conditions are relatively rare in cats. The formation of gallbladder mucocele in dogs is characterised by excessive secretion of thick mucus by the gallbladder epithelium. Hyperplastic and inflammatory conditions may lead to obstruction or rupture of the gallbladder, followed by the development of bile peritonitis, whereas neoplastic forms are rare and often asymptomatic.

As established by K. Jana-Pitre & G.S. Hennig (2025), the clinical signs of gallbladder mucocele in patients are often non-specific and include vomiting, lethargy, fever, anorexia, diarrhoea, and polyuria-polydipsia. On physical examination, animals typically exhibit abdominal pain upon palpation and jaundice. For an accurate diagnosis, in addition to the animal's medical history and clinical examination findings, S. Renaud *et al*. (2025) recommended considering the results of complete blood count and biochemical analyses (including elevated serum liver enzyme activity and hyperbilirubinaemia), along with the characteristic ultrasound appearance of the affected area. According to S.H. Lee *et al*. (2025), during ultrasonographic examination, gallbladder mucus does not move with changes in the patient's position. The bile becomes concentrated and extends linearly from the gallbladder wall towards the lumen, with the peripheral area appearing anechoic and the centre of the mucus mass demonstrating high echogenicity, forming characteristic stellate or finely striated bile patterns.

As reported by J.L. Gookin (2025), the treatment of dogs with gallbladder pathology depends on the diagnosis and may include medical therapy (such as hepatoprotective agents and antibiotics), adherence to a special diet to support the liver and biliary system, and, in severe cases, surgical intervention. Surgical options include stone removal, gallbladder drainage, or complete excision (cholecystectomy). Surgical intervention in dogs with gallbladder mucocele remains the therapeutic gold standard, with cholecystectomy recognised as the current standard and recommended treatment method.

Thus, the digestive system ensures nutrient absorption and the elimination of metabolic byproducts, and its dysfunction often results in internal non-contagious diseases in dogs. The liver plays a crucial role in this system by filtering blood and producing bile, which accumulates in the gallbladder. Among gallbladder pathologies, the most common are mucocele and cholelithiasis, both of which may lead to obstruction or inflammation. Their diagnosis is based on clinical, laboratory, and ultrasonographic findings, while treatment involves medical therapy, dietary management, and, in severe cases, surgical intervention – most notably cholecystectomy as the principal method. Therefore, this study aimed to identify prognostic indicators and provide recommendations for the treatment of dogs that had undergone surgical management for gallbladder mucocele.

Literature Review

Among extrahepatic gallbladder diseases in dogs that may result in obstruction or rupture, mucocele is most frequently diagnosed.

This pathology is characterised by progressive accumulation of bile rich in mucin and by hyperplasia of the biliary mucosa. According to S.J. Mehler & P.D. Mayhew (2023), gallbladder mucocele can generally be defined as the accumulation of biliary mucus caused by hypersecretion of mucus due to hyperplasia of the gallbladder wall. Under normal conditions, the gallbladder mucosa is capable of reabsorbing compounds such as water and electrolytes to concentrate the bile. This disorder is associated with high morbidity and mortality, and its pathogenesis remains unclear. Affected dogs have a significantly increased likelihood of concurrent diagnoses of hyperadrenocorticism, hypothyroidism, and hyperlipidaemia.

S.A. Jablonski *et al*. (2024) suggested that chronic accumulation of thickened mucoid bile may lead to delayed gallbladder emptying and the development of conditions such as extrahepatic biliary obstruction, pancreatitis, gallbladder wall distension, necrotising cholecystitis, and subsequent gallbladder rupture. S. Mizutani *et al*. (2017) found that gallbladder mucocele and biliary sludge share the same pathophysiological basis and, rather than being distinct diseases, may represent a continuous pathological process. Thus, biliary sludge may be considered a precursor stage in the development of gallbladder mucocele.

F.I. Hill *et al*. (2022) established that gallbladder mucocele most commonly affects miniature or purebred dogs, including Poodles, Pomeranians, Schnauzers, Bichon Frisés, and Chihuahuas. However, the annual rate of hospital admissions for this condition was not dependent on breed. The findings of F.A. Teixeira *et al*. (2024) demonstrated that small-breed dogs possess a genetic predisposition to the development of mucocele. The authors suggested that hyperplasia of the gallbladder mucosa and disturbances in mucin secretion may have a hereditary origin, and that endocrine disorders such as hypo-adrenocorticism, hypothyroidism, and Cushing's syndrome are frequently concurrent conditions. K. Jana-Pitre & G.S. Hennig (2025) reported that the most common clinical signs observed during physical examination of dogs with gallbladder mucocele include vomiting, anorexia, loss of appetite, lethargy, abdominal pain, jaundice, and fever.

To refine diagnosis in clinical practice, additional diagnostic techniques are widely employed. According to S. Kim *et al*. (2024), ultrasonographic evaluation of pathological gallbladders frequently reveals mucosal hyperplasia. M. Itani *et al*. (2023) noted that magnetic resonance cholangiopancreatography has become a widely recognised non-invasive diagnostic tool for assessing diseases not only of the pancreas but also of the biliary tract. Furthermore, Y. Lee *et al*. (2024) demonstrated that information regarding biliary tract abnormalities, portal hypertension, portal collaterals, and hepatic lobe agenesis – which may occur concurrently with gallbladder agenesis – can be obtained through computed tomography imaging.

Regarding medical management of dogs with gallbladder mucocele, S.A. Jablonski *et al*. (2024) noted that it is associated with a shorter survival time compared with surgical treatment, although it remains a reasonable alternative when surgery is not feasible. According to H. Saunders *et al*. (2017), the foundation of medical therapy involves the use of choleretics and hepatoprotective agents. A low-fat diet is also recommended, particularly for animals with dyslipidaemia, as dietary correction may stimulate bile flow; concurrent endocrinopathies should be appropriately managed.

The principal treatment for gallbladder mucocele in dogs is surgical intervention. When determining the surgical technique to be used for cholecystectomy, histopathological changes in the layers of the gallbladder wall affected by disease should be taken into

account. Elective cholecystectomy in dogs with gallbladder mucocele, as established by S.L. Friesen *et al*. (2021), is associated with a low mortality rate and a low incidence of minor complications. Y. Kim & S. Lee (2025) were the first to apply and describe the use of indocyanine green for intraoperative cholangiography during laparoscopic hepatobiliary surgery, aimed at confirming the patency of the common bile duct in a dog with gallbladder rupture. Their findings indicated that such surgical procedures should be performed by specialists experienced in biliary surgery to minimise complications. B. Sambugaro *et al*. (2022) demonstrated that in emergency cases in dogs, when prolonged anaesthesia is undesirable, the use of extradural anaesthesia is advisable to reduce the need for inhalation anaesthetics and intraoperative opioids, as well as to minimise the stress response. Therefore, due to the typical clinical presentation of gallbladder mucocele, it is likely that this condition may have been previously misdiagnosed. The increasing incidence of the disease establishes it as an emerging syndrome in veterinary medicine, warranting further investigation.

Materials and Methods

The research was conducted from January 2024 to March 2025 in scientific laboratories based at the National University of Life and Environmental Sciences of Ukraine and at the private veterinary clinic SHANTY (Kyiv, Ukraine). The subject of the study was a Chihuahua diagnosed with gallbladder mucocele accompanied by the development of hepatobiliary insufficiency. Blood samples were collected from the lateral saphenous vein of the forelimb for morphological and biochemical analyses to assess the general condition of the patient. The diagnostic value of the blood test results for hepatobiliary pathology was determined by comparing the obtained values with reference ranges.

For the complete blood count, biological samples were collected into tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) and analysed using a veterinary haematology analyser, the Heska Element HT (USA). The parameters measured included red and white blood cell counts, platelet count, haemoglobin concentration, and haematocrit level. For the biochemical analysis, blood samples were stabilised with heparin and then centrifuged for 10-15 minutes at a speed of 1,500-2,500 revolutions per minute. After centrifugation, the supernatant – blood plasma – was collected for the determination of biochemical parameters, including the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), as well as total protein and bilirubin levels. The measurements were carried out using an automatic veterinary biochemical analyser, Fujifilm DRI-CHEM NX600 (Japan).

To visualise pathological changes in the gallbladder, an ultrasound examination of the liver was performed. Abdominal ultrasonography was carried out using the MyLab X7 VET device (Esaote, Italy). Following confirmation of the diagnosis of gallbladder mucocele, surgical intervention was undertaken. Under general anaesthesia (AAHA Anaesthesia and Monitoring Guidelines for Dogs and Cats, 2020), laparotomy was performed to carry out a cholecystectomy. A bile sample obtained during the operation was sent to the laboratory for bacteriological analysis to identify pathogens and determine their antibiotic sensitivity. During the postoperative period, the animal was kept under observation in an inpatient facility for three days. Treatment included analgesia, antibiotic therapy, and hepatoprotective medication. On the third day, a follow-up ultrasound examination was performed.

The research involving the animal complied with the requirements of the European

Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (1986) and the Law of Ukraine No. 3447-IV (2006). All necessary procedures involving the animal were carried out in accordance with the guidelines of the European Parliament and of the Council 2010/63/EU (2010) on the protection of animals used for scientific purposes. Statistical processing of the haematological data was performed using a personal computer with the BAF – Veterinary Medicine software and individual patient dental records. To determine statistical significance, Student's t-test was applied at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

Results and Discussion

An analysis of the medical history of the canine patient revealed that the clinical presentation covered a wide range of symptoms. These included lethargy, general depression, abdominal pain, a characteristic "prayer posture", vomiting containing bile, diarrhoea, and jaundice of the skin and visible mucous membranes. The body temperature was recorded at 39.9°C. Similar symptoms in such cases have been reported by other researchers. In particular, S.J. Mehler & P.D. Mayhew (2023) found that patients with gallbladder mucocele may be asymptomatic; however, most present with vomiting, anorexia, lethargy, and diarrhoea. During physical examination, affected animals exhibit abdominal pain upon palpation and signs of jaundice. Rectal temperature tends to increase in cases of mucocele perforation. Proteinuria has been identified as a common finding associated with gallbladder mucocele, highlighting the need for blood testing to assess renal function.

J.L. Gookin *et al*. (2025) demonstrated that, in addition to the previously mentioned signs, animals with gallbladder mucocele may exhibit a variable range of symptoms, which can manifest as non-specific gastrointestinal disturbances progressing to cholestasis or acute peritonitis. According to the authors, these signs are secondary to gallbladder pain, rupture, infarction, infection, or obstruction of the common bile duct. S.A. Jablonski *et al*. (2024) noted that the clinical signs of this condition are often acute and rather vague, making them indistinguishable from more common differential diagnoses such as pancreatitis or acute gastroenteritis. E. Rogers *et al*. (2020) reported that cholecystitis is frequently diagnosed as a concurrent condition in dogs with gallbladder mucocele.

To assess the general condition of the animal and evaluate the functional state of its internal organs, comprehensive clinical (general) and biochemical blood tests were performed to obtain complete information on the dog's health and establish an accurate diagnosis for appropriate treatment (Tables 1, 2). The biochemical profile revealed marked increases in the activity of alanine aminotransferase – by 3.7-fold, aspartate aminotransferase – by 3.4-fold, alkaline phosphatase – by 2.4-fold, and total bilirubin – by 4.9-fold. Conversely, total protein levels decreased by 9.2%. These results indicate pronounced alterations in both the biochemical and haematological parameters of blood in dogs with hepatobiliary disorders. An increase in transaminase and alkaline phosphatase activity, together with elevated bilirubin levels, confirms the development of cytolytic and cholestatic syndromes, which are typical in hepatic disorders. The reduction in total protein levels indicates suppression of the liver's protein-synthesising function. According to the findings of J.A. Jaffey *et al*. (2022), an elevated concentration of total bilirubin in blood plasma is a negative prognostic indicator; however, it is considered an unreliable biomarker for predicting mortality in dogs with glioblastoma during cholecystectomy.

Table 1. Biochemical parameters of blood serum in a dog with gallbladder mucocele

Parameter	Normal range	Dog with gallbladder mucocele
Glucose, mmol/L	3.4-6	3.93
Bilirubin, μmol/L	0-5	27.21*
Potassium, mmol/L	4-5.6	4.35
Calcium, mmol/L	1.87-2.8	2.25
Phosphorus, mmol/L	0.68-2	1.54
Magnesium, mmol/L	0.8-1	0.84
Alkaline phosphatase, U/L	10-150	439.71*
Alanine aminotransferase, U/L	9-75	180.42*
Aspartate aminotransferase, U/L	5-55	131.14*
Urea, mmol/L	3.5-9.2	4.52
Creatinine, mmol/L	26-120	58.64
Alkaline phosphatase, U/L	10-150	439.72*
Total protein, g/L	5,178	63.51*

***Note**: * indicates values that differ from the normal range*
***Source**: authors' data*

Table 2. Complete blood count of a dog with gallbladder mucocele

Parameter	Normal range	Dog with gallbladder mucocele
Erythrocytes, 10^{12}/L	5.5-8.5	4.90*
Haemoglobin, g/L	120-180	117.1*
Haematocrit, %	37-55	35.2*
Leucocytes, 10^{9}/L	8.5-10,5	15.6*
Platelets, 10^{9}/L	150-500	171.2*
ESR, mm/h	2.0-5.0	20.5*

***Note**: ESR – erythrocyte sedimentation rate; * indicates values that differ from the normal range*
***Source**: authors' data*

The complete blood count revealed an anaemic syndrome, characterised by a 21% reduction in erythrocyte count, a 16% decrease in haemoglobin, and a 16% decline in haematocrit, corresponding to reductions of 1.27, 1.2, and 1.2 times, respectively, compared with the reference values. At the same time, leucocytosis was observed (an increase in leucocyte count by 1.7 times) along with an accelerated ESR, which was 3.1 times higher than normal. The platelet count decreased by 1.3 times, representing a 23.4% reduction compared with the reference range. Changes in the complete blood count (anaemia, leucocytosis, thrombocytopenia, and elevated ESR) indicate systemic disturbances arising from chronic inflammation, intoxication, and hepatic dysfunction, given the liver's central role in metabolism. The concurrent presence of anaemia and thrombocytopenia is particularly significant, as it may suggest the development of hypersplenism syndrome or DIC in severe cases. H. Itoh *et al*. (2022) also demonstrated that gallbladder mucocele is often associated with both acute and chronic inflammation, and haematological abnormalities may indicate partial necrosis of the gallbladder wall, leading to disease progression.

Ultrasound examination of the liver in the studied dog revealed the following findings: the organ was in its typical anatomical position but enlarged, with smooth and well-defined margins that appeared slightly rounded. Echogenicity was reduced, and the structure was homogeneous and finely granular. The intensity of the ultrasound beam was uniform throughout the parenchyma. The vascular pattern was within physiological limits, with no neoplasms detected. Major vessels showed no abnormalities, and regional lymph nodes appeared unaltered. Biliary system: the gallbladder was moderately filled, with a typical shape and echogenic content showing multiple hyperechoic strands indicative of a mucocele (Fig. 1).

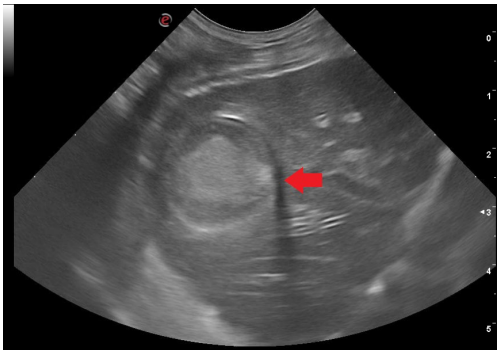

Figure 1. Gallbladder mucocele in a dog
***Note**: the arrow indicates the pathological change in the gallbladder tissue. Numerous hyperechoic strands are visible, occupying approximately 90% of the gallbladder cavity – the so-called "kiwi" type*
***Source**: authors' photo*

The gallbladder wall was diffusely thickened to 3.6 mm, with distinct echogenicity and a clearly defined three-layer structure. The cystic duct was dilated to 3.5 mm, while the wall of the common bile duct appeared hyperechoic. Intrahepatic bile ducts were visualised and found to be dilated. According to the findings of J.A. Jaffey *et al*. (2022), ultrasonographic features of mucocele include the presence of stellate or finely striated bile patterns, differing from biliary sludge by the absence of gravity-dependent movement of bile. The thickness and external appearance of the gallbladder wall on ultrasound are variable and nonspecific. H.J. Piegols *et al*. (2021) reported that gallbladder mucoceles are often detected incidentally during abdominal ultrasonography performed for tumour staging or adrenal gland evaluation in dogs with hyperadrenocorticism. A characteristic ultrasonographic appearance of the gallbladder, in combination with clinical history, physical examination findings, and biochemical blood parameters, facilitates a relatively straightforward diagnosis. However, as demonstrated by R. Fujiwara *et al*. (2025), ultrasonography – although commonly used for diagnosing biliary tract diseases in dogs – provides inferior visualisation of the bile ducts compared with magnetic resonance cholangiopancreatography in cases of hepatobiliary pathology. At the time of diagnosis, S.L. Friesen *et al*. (2021) found that dogs frequently presented with concurrent conditions, including pancreatitis, hyperlipidaemia, corticosteroid excess, hypothyroidism, protein-losing nephropathy, diabetes mellitus, cholestasis, and impaired gallbladder motility.

Following confirmation of the diagnosis, the animal underwent surgical treatment – cholecystectomy, a procedure typically performed in dogs with extrahepatic biliary obstruction, gallbladder mucocele, or gallbladder rupture. The surgical technique involved preparation of the operative field according to standard aseptic procedures: the hair coat was clipped, the skin was washed with antibacterial soap, and the site was disinfected with a betadine solution. The animal was positioned in dorsal recumbency. A ventral midline laparotomy was performed, allowing inspection of the abdominal organs. In this case, the gallbladder wall was firmly adherent to the hepatic parenchyma, and

areas of ischaemia covering approximately 30% of the gallbladder surface showed necrosis. The gallbladder contents were not aspirated, as this could subsequently lead to bile leakage through the wall. According to H.J. Piegols *et al*. (2021), suspension of the gallbladder on a stay suture is possible, although this technique carries a risk of wall perforation. Therefore, dissection of the gallbladder wall began from its fundus, using sterile cotton swabs and gentle rotational movements to avoid trauma to the hepatic parenchyma. The cystic duct and cystic artery were then isolated. Before ligation, catheterisation and flushing of the common bile duct were performed using a sterile 0.9% sodium chloride solution. Three ligatures of absorbable suture material (polyglycolic acid) were applied. The abdominal cavity was subsequently irrigated with a warm sterile 0.9% sodium chloride solution. After cholecystectomy, bile was collected for bacterial culture, and the external surface of the removed gallbladder was examined (Fig. 2).

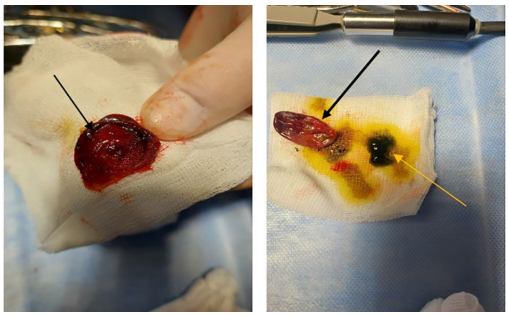

Figure 2. Dog's gallbladder after removal
***Note**: the black arrows indicate the excised gallbladder with a necrotic wall; the yellow arrow shows the gallbladder contents*
***Source**: authors' photo*

Examination of the excised gallbladder revealed pathological changes in its wall, characterised by areas of necrosis and haemorrhage resulting from tissue distension under increased pressure. According to I. Nagao *et al*. (2023), the underlying cause of this pathological condition may be abnormal mucus secretion rather than its overproduction. Alterations in the motility and fluidity of the biliary tract are increasingly identified in dogs and, as noted by J.A. Jaffey *et al*. (2022), are associated with the formation of biliary sludge – bile of higher viscosity caused by the slow sedimentation of dispersed particles. Consequently, macroscopic distension of the gallbladder walls occurs due to the accumulation of greenish-black gelatinous material, which may extend throughout the biliary tree and induce varying degrees of obstruction of the intrahepatic ducts. When such obstruction develops, gallbladder distension often leads to necrosis of its wall and, ultimately, to rupture followed by peritonitis. S. Mizutani *et al*. (2017) reported that the main components of gallbladder contents in both gallbladder mucocele and biliary sludge are mucins, and that both pathophysiological processes exhibit a low level of bacterial infection within the gallbladder. Therefore, it is plausible that gallbladder mucocele and biliary sludge share the same pathophysiology and, rather than representing separate diseases, may constitute a continuum of the same pathological process. Thus, biliary sludge may be regarded as a precursor stage in the development of gallbladder mucocele. L. Ciammaichella *et al*. (2023) noted that hepatobiliary disorders affecting the bile ducts and gallbladder in animals can present in various forms and share similar clinical signs with hepatic diseases, while also contributing to the development of gastroduodenal ulcers. According to the findings of T.-Y. Kim & Y.I. Oh (2025), chronic cholecystitis and factors such as biliary hyperplasia, mucin hypersecretion, and cystic duct obstruction may lead to fibrosis and calcification of the gallbladder wall, potentially resulting in the development of a porcelain gallbladder – a condition characterised by pronounced calcification and thickening of the

gallbladder wall. M. Galley *et al*. (2022) recommended that dogs undergoing cholecystectomy, owing to the possible effects of gallbladder rupture and biliary infection, should receive abdominal imaging, biliary culture, and empirical preoperative antimicrobial therapy.

In the dog under study, the postoperative period progressed without complications. The animal was monitored continuously in a clinical setting for three days, and an individual pain management plan was implemented (butorphanol at 0.5 mg/kg intramuscularly every 12 hours for three days). Antibiotic therapy, hepatoprotective agents (S-adenosylmethionine at 20 mg/kg once daily), omega-3 supplements, and the probiotic Florentero (Candioli Farmaceutici S.p.A, Italy) were prescribed. The antibiotics of choice were: Cefazolin at 25 mg/kg administered intravenously, slowly, once daily (to prevent bacterial complications); and Metronidazole at 10 mg/kg intravenously once daily (to suppress anaerobic flora). On the third day, the animal showed a gradual recovery of appetite and activity. Follow-up abdominal ultrasonography revealed no evidence of free fluid or secondary inflammation. The overall condition of the animal improved, and body temperature remained within the physiological range (37.9°C).

The obtained results are consistent with the findings of H. Saunders *et al*. (2017), who investigated pharmacological therapy that included the administration of ursodeoxycholic acid (a natural hydrophilic bile acid functioning as a choleretic and hepatoprotective agent) at a dosage of 10-15 mg/kg orally, either once daily or divided into two doses, and S-adenosylmethionine (a natural analogue of cysteine essential for the production of the antioxidant glutathione, thereby acting as a hepatoprotective agent) at 18-20 mg/kg orally, administered once daily on an empty stomach. According to F.A. Teixeira *et al*. (2024), dietary factors are likely to play a crucial role in the pathogenesis, prevention, and even treatment of gallbladder diseases such as mucocele and cholelithiasis. Specific dietary interventions, including the use of omega-3 fatty acids, proteins, and fibre, can significantly influence biliary health. Nutritional recommendations include a balanced diet with adequate levels of vitamins and proteins, particularly methionine and tryptophan, along with a moderate content of fats and cholesterol.

Surgical intervention in the form of cholecystectomy proved to be the only appropriate decision in the given clinical situation. According to S. Renaud *et al*. (2025), delaying surgical treatment may result in gallbladder rupture, peritonitis, and fatal outcomes. The presence of hypocoagulation requires particular attention due to the increased risk of postoperative haemorrhage. The authors recommended the use of vitamin K1, antibacterial therapy, and hepatoprotective agents, which enabled successful stabilisation of the animals' condition. Based on the findings of H. Saunders *et al*. (2017), surgical removal of the gallbladder is essential for full recovery. Dogs with gallbladder mucocele that underwent cholecystectomy and survived the early postoperative period demonstrated an excellent long-term prognosis. Although cholecystectomy is not without risk, the approach to this condition requires reconsideration. The current trend is to postpone gallbladder removal while medical therapy remains effective. However, greater emphasis should be placed on performing cholecystectomy at the time of initial presentation, as the procedure at this stage can be carried out on a structurally intact gallbladder, with minimal wall damage. B. Sambugaro *et al*. (2022) noted that, compared with systemic analgesia, the use of epidural anaesthesia reduced the need for perioperative analgesics and promoted postoperative food intake in dogs that had undergone cholecystectomy. Animals that underwent cholecystectomy for gallbladder mucocele without catheterisation and lavage of

the biliary cavity recovered rapidly, according to M. Rossanese *et al*. (2022). S.A. Jablonski *et al*. (2024) also demonstrated that cholecystectomy is considered the treatment of choice for dogs with gallbladder mucocele, as this condition is characterised by the accumulation of dense, immobile mucus and bile within the gallbladder, and histologically by a hyperplastic gallbladder mucosa forming cystic spaces filled with mucus and papillary projections extending into the lumen, although the aetiopathogenesis of the disorder remains unclear. Further studies are required to elucidate the pathological mechanisms underlying posttraumatic gallbladder disease, gallbladder mucocele, and their potential interrelationship. A clearer understanding of these mechanisms could contribute to earlier disease prediction and improve diagnostic and therapeutic strategies.

The importance of postoperative monitoring should also be emphasised, as dogs that have undergone surgery for gallbladder mucocele, according to E. Rogers *et al*. (2020), often present with concurrent diseases requiring further diagnostic evaluation, such as endocrine testing and lipid profiling. In a study by K. Jana-Pitre & G.S. Hennig (2025) on the increased risk of gallbladder rupture and mortality in small-breed dogs with concurrent hypothyroidism or pancreatitis that underwent cholecystectomy for gallbladder mucocele, it was determined that elective cholecystectomy should be considered in dogs with gallbladder mucocele and concomitant endocrinopathies – particularly hypothyroidism and pancreatitis – to reduce the risk of gallbladder rupture and death. In the presented case, the dog did not exhibit any evident endocrinopathy; however, the owners were advised to continue monitoring the animal's health, with periodic assessment of thyroid and adrenal gland function.

It is important to note that the bacteriological examination of the bile confirmed the sterility of the sample, indicating the non-infectious nature of the mucocele in this patient. This finding suggests that the pathology likely developed as a result of physicochemical alterations in bile composition rather than microbial involvement. Nevertheless, sterility does not eliminate the need for postoperative antibiotic prophylaxis, given the potential risk of bacterial translocation and secondary complications. According to H. Itoh *et al*. (2022), the success rate of treating gallbladder mucocele by cholecystectomy exceeds 80% when surgery is performed before gallbladder wall perforation occurs. However, even following successful surgical intervention, patients require long-term monitoring, as the risk of recurrent metabolic disturbances and cholestasis remains.

Thus, this clinical case highlights the importance of early detection of gallbladder mucocele, particularly in small-breed dogs presenting with non-specific gastrointestinal symptoms. Cholecystectomy, combined with comprehensive therapy, ensured a favourable treatment outcome. Postoperative monitoring plays a crucial role in preventing recurrence or secondary complications.

Conclusions

The presented case demonstrated a typical clinical manifestation of gallbladder mucocele in a Chihuahua, characterised by polymorphic and predominantly non-specific clinical signs associated with hepatobiliary disorders, along with a high likelihood of diagnostic challenges. Based on the medical history and clinical examination, the course of the disease was accompanied by systemic reactions and gastrointestinal disturbances, which complicated differential diagnosis. The results of the general and biochemical analyses revealed significant alterations in both biochemical and haematological blood parameters in the dog with hepatobiliary pathology. Increased transaminase and alkaline phosphatase

activity, together with elevated bilirubin concentration, confirmed the presence of cytolytic and cholestatic syndromes, which are typical of hepatic lesions. A decrease in total protein indicated suppression of the liver's protein-synthesising function. The complete blood count revealed systemic abnormalities arising from chronic inflammation, intoxication, and hepatic dysfunction. In particular, anaemia, leukocytosis, thrombocytopenia, and an increased ESR were observed. A particularly notable finding was the combination of anaemia and thrombocytopenia, which may indicate the development of hypersplenism or DIC in severe cases.

Thus, the comprehensive assessment of biochemical and haematological parameters was of great importance for the diagnosis, monitoring, and prognosis of hepatobiliary diseases in dogs. Ultrasonographic examination proved crucial for establishing the diagnosis, as mucoceles are characterised by the appearance of stellate or finely striated bile patterns and can be distinguished from biliary sludge by the absence of gravity-dependent bile movement. During the treatment of the animal with mucocele, a cholecystectomy was performed, which, in combination with comprehensive therapy, resulted in a favourable clinical outcome, rapid recovery, and the absence of postoperative complications. Bacteriological examination of the bile sample confirmed its sterility, thereby supporting the non-infectious nature of the pathology. The findings indicate that surgical removal of the gallbladder, combined with supportive therapy, promotes restoration of the animal's functional condition and improvement of clinical parameters in cases of mucocele. Further research will focus on refining the diagnostic value of instrumental and endoscopic methods for the early detection of internal organ pathologies in small companion animals.

Acknowledgements

None.

Funding

The study was not funded.

Conflict of Interest

None.

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Хірургічне лікування собаки свійського (*Canis familiaris*) за мукоцеле жовчного міхура (на прикладі клінічного випадку)

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Анотація. Актуальність дослідження зумовлена стрімким зростанням випадків захворювання собак на мукоцеле жовчного міхура, що характеризується накопиченням надлишку муцину та жовчі в жовчному міхурі. Ця патологія спричиняє часткову або повну позапечінкову біліарну обструкцію шляхом поширення слизу, насиченого жовчю, в загальні жовчні протоки і, що може бути небезпечним для життя тварин. У зв'язку з цим, мета роботи була спрямована на встановлення клінічних симптомів, ефективних методів діагностики та лікування тварин з мукоцеле жовчного міхура. Розглянуто клінічний випадок мукоцеле жовчного міхура у собаки породи чихуахуа, із розвитком гепатобіліарної недостатності. Наведено послідовність діагностичних заходів, включно з лабораторним аналізом та ультразвуковим обстеженням, а також опис хірургічного лікування. Встановлено, що ця патологія проявлялась у хворої собаки неспецифічними клінічними симптомами, такими як часте блювання, анорексія та млявість. Під час фізикального огляду тварин найпоширенішими симптомами були: біль в ділянці черевної порожнини, іктеричність шкіри та видимих слизових оболонок, гіпертермія. Біохімічними маркерами цієї хвороби

в хворої собаки були підвищення в сироватці крові концентрації загального білірубіну, активності лужної фосфатази, аланінамінотрансферази та гамма-глутамілтрансферази. Зміни у тканинах жовчного міхура візуалізували з використанням методу ультразвукового дослідження тварин. Діагностували мукоцеле жовчного міхура на підставі появи характерних зірчастих або дрібнопосмугованих жовчних візерунків. Диференціювали цю патологію від біліарного сладжу за відсутністю руху жовчі, залежного від сили тяжіння. На ультразвуковому дослідженні товщина та зовнішній вигляд стінки жовчного міхура не мали специфічних змін. За результатами дослідження обрали хірургічний метод лікування хворих собак – холецистектомію. Стерильність зразка жовчі при бактеріологічному дослідженні вказувала на неінфекційний характер мукоцеле. Отримані результати мають важливе прикладне значення як для науковців, так і для практикуючих ветеринарних лікарів, що сприятиме удосконаленню протоколу лікування тварин за мукоцелє жовчного міхура

Ключові слова: гепатобіліарна патологія; холецистит; холецистектомія; гематологічні показники; мініатюрні породи

UKRAINIAN JOURNAL OF VETERINARY SCIENCES

2025. Volume 16, No. 3. P. 94-106

Received: 30.05.2025 Revised: 11.08.2025 Accepted: 23.09.2025

DOI: 10.31548/veterinary3.2025.94

UDC 619:617.5

Pinworm infections in red squirrels at the urban-forest interface

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Abstract. The growing overlap between urban and forest environments increases the risk of cross-species parasite transmission, making the study of parasitic fauna in wild mammals highly relevant. The aim of this research was to investigate the prevalence and host specificity of oxyurid nematodes in Eurasian red squirrels (*Sciurus vulgaris*) inhabiting forested park areas of Polissia, Ukraine. It was analysed 191 faecal samples collected between September and December 2023 using an integrated approach that combined innovative monitoring methods, including video surveillance in the squirrels' natural habitat, systematic sample collection, and detailed

Suggested Citation:
Feshchenko, D., Zghozinska, O., Romanishina, T., & Lakhman, A. (2024). Pinworm infections in red squirrels at the urban-forest interface. *Ukrainian Journal of Veterinary Sciences*, 16(3), 94-106. doi: 10.31548/veterinary3.2025.94.

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microscopic examination of helminth egg morphology. Additionally, the YOLOv5 computer vision model was utilized for automated analysis of video recordings, enabling precise identification of individual squirrels. Three species of parasites from the superfamily *Oxyuroidea* were identified in this study. The squirrel-specific species *Trypanoxyuris sciuri* was the most prevalent, detected in 70.6% of squirrels (12 out of 17 individuals; 95% CI: 44.04-89.69). Parasites typically associated with murids were less common: *Syphacia obvelata* was found in 11.8% of squirrels (2 out of 17; 95% CI: 1.46-36.44), and *Aspiculuris dinniki* in 5.9% (1 out of 17; 95% CI: 0.15-28.69). To further investigate these species, *S. obvelata* eggs were cultured under laboratory conditions, allowing for detailed characterization of L1 larvae morphology, while *A. dinniki* failed to develop, confirming its specific host requirements. These findings highlighted the high prevalence of T. sciuri among squirrels and indicated the potential transmission of *S. obvelata* and *A. dinniki* within their populations. The practical value of this study lies in expanding the understanding of helminth diversity and host-parasite interactions in transitional urban-forest ecosystems. The obtained results can be used to improve veterinary and sanitary measures, prevent parasitic diseases, and develop monitoring strategies aimed at preserving the health of wild populations and minimising risks to domestic animals and humans

Keywords: wildlife parasites; gastric nematodes; Syphacia; pinworms in squirrels

Introduction

With the expansion of urban areas and the reduction of natural habitats, the intensity of contact between wild mammals and animals living in close proximity to humans has increased. This creates favourable conditions for the circulation of parasitic agents and their potential transmission between different host species. One of the important directions in veterinary research is the monitoring of helminth infections in wild animal populations as part of the epizootic surveillance system. Helminths belonging to the family *Oxyuridae* attract particular attention due to their ability to rapidly adapt to new hosts and their potential involvement in interspecies transmission of pathogens. Timely detection of oxyurid nematodes in wild mammals, particularly in red squirrels (*Sciurus vulgaris*), is of great importance for veterinary parasitology, as it allows the assessment of potential risks of forming natural foci of parasitic infections.

In recent years, attention has increasingly focused on the interactions between parasites and their hosts under conditions of urbanisation. A. Karvonen *et al*. (2020) demonstrated that the order and timing of co-infections can significantly influence the progression of parasitic processes: an initial infection may modify the host's immune response, either enhancing or suppressing subsequent infections. This mechanism helps explain the complexity of clinical manifestations observed in mixed helminth infections, which are common among wild mammals. A similar dynamic was described by G. Zilio & J.C. Koella (2020), who showed that the sequence of infections can affect the outcome of competition between helminth species, which is important for predicting the course of parasitoses and the development of immune memory.

Studies of behavioural aspects of parasitism in squirrels indicate that even in the absence of pronounced clinical signs, infected animals may exhibit notable behavioural changes. F. Santicchia *et al*. (2020) found that an invasive

nematode transmitted between species suppresses activity and social interactions in red squirrels, potentially affecting their viability and reproductive success. Comparable results were obtained by C. Romeo *et al*. (2021), who demonstrated that infection with Strongyloides robustus reduces the survival of red squirrels – a classic example of a "spillover" effect, where an invasive species transmits a parasite harmful to native fauna. K. Hamill (2023) emphasised that parasites may act as indirect biomarkers of population stress, reflecting disruptions in animal homeostasis under anthropogenic pressures.

From an epizootological standpoint, numerous studies have shown that rodents, particularly synanthropic species, can serve as persistent reservoirs of parasites hazardous to wild mammals. M.M. Islam *et al*. (2020) identified *Syphacia obvelata* as the most prevalent nematode species among urban rodent populations, while J. Jarošová *et al*. (2020) reported that the same species frequently occurs in pet and laboratory rodents, from which it may spread into wild populations. A. Grandón-Ojeda *et al*. (2022) demonstrated that co-infections are a typical feature of urban populations of Rattus and Mus, and that such complex parasitic assemblages contribute to the greater stability of parasite communities. In this context, *Sciurus vulgaris*, which often interacts both with humans and small rodents, may represent an intermediate link in the transmission chain of helminths. Additionally, F.R. Lima *et al*. (2024) highlighted the clinical aspect of treating oxyurid nematode infections in primates, emphasising that prompt diagnosis and anthelmintic therapy remain key components of preventive health management even in natural ecosystems.

Summarising the findings of these studies, it may be concluded that interspecies transmission of parasites among wild mammals is a widespread phenomenon with potentially serious veterinary implications. However, most available data refer to Western Europe, while information on wild animal parasites in Eastern Europe, particularly in Ukraine, remains scarce. The role of oxyurid nematodes in squirrel populations adapted to urban environments, as well as the mechanisms of possible host-switching between squirrels and rodents, are still insufficiently understood. In view of this, it was hypothesised that in forest-park areas of a moderately urbanised city within the Polissya region, red squirrels could serve as reservoir hosts for various parasites, including certain nematodes of the family *Oxyuridae capable* of host switching. The aim of the study was to determine the species composition of parasites in this population and to assess infection prevalence within a landscape shaped by both natural and anthropogenic factors.

Materials and Methods

The study was conducted from September to December 2023 in forest-park areas within the city of Zhytomyr, northern Ukraine (50°16′N, 28°40′E), where stable populations of the Eurasian red squirrel (*Sciurus vulgaris*) are present. A combination of behavioural observation, non-invasive sample collection, and laboratory parasitological analysis was employed to ensure ethical and reproducible data acquisition. Four fixed feeding stations were established to attract squirrels for consistent monitoring (Fig. 1), while computer-assisted video analysis was used to identify individuals and document activity patterns.

The experiment was designed and implemented in two distinct stages. The preparatory phase (two months) involved regularly providing food (walnuts, raw sunflower seeds, spruce and pine cones) to the squirrels 2-3 times per week. Video surveillance was also implemented to allow squirrels to habituate to human presence. To facilitate faeces sample collection, the soil within a 2-meter radius around the feeders was covered with geotextiles (Mathis *et*

al., 2018). The second phase of the experiment, which was experimental in nature, lasted 60 days. The area was inspected daily, and faeces from squirrels (n = 191) were collected. Data on squirrel activity and abundance were recorded with a Stealth Cam G42 (Stealth Cam Inc., USA) video recorder, which remained operational throughout the experiment. The camera was mounted at a height of 1.5 m to provide coverage of the feeder and the surrounding area. The camera settings included 10 MP resolution and night mode with infrared illumination. It automatically detected movement, resulting in the collection of over 2,000 images and videos over 60 days. Frames with clear images of the squirrels were selected for analysis.

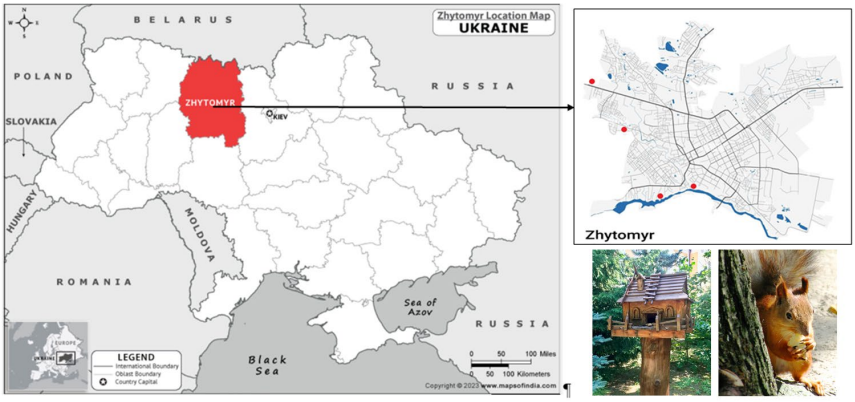

Figure 1. Cartographic representation of the experiment
***Note**: top right: red dots indicate the locations of the feeding stations; below: feeder design (left) and a squirrel during feeding (right)*
***Source**: created by the authors*

Data processing was carried out using the YOLOv5 computer vision model to detect squirrels in the frames. Individual identification was performed using the DeepLabCut neural network, which analysed key features, including fur pattern and colour, tail shape and length, and body size. Based on these parameters, a digital profile was created for each squirrel, and identification was carried out using a re-identification algorithm to prevent data duplication. Algorithmic accuracy was validated through manual verification of 10% of randomly selected data, showing full concordance (Bochkovskiy, 2020). Individual monitoring of the squirrels allowed determination of the number of infested animals through subsequent parasitological analysis.

Quantitative parasitological analysis of faeces (performed within 12 hours of collection) was conducted using the McMaster method (Ministry of Agriculture, Fisheries and Food, 1986). Qualitative analysis was carried out through flotation, with results recorded as "present/absent" (Gerwin *et al*., 2017; World Health Organization, 2019). Microscopy was performed using a KERN OBE digital microscope (KERN, Germany) at magnifications of ×40, ×100, and ×600. Eggs were cultivated in laboratory conditions within a thermostat, and placed in Petri dishes at a temperature range of 23-27°C for 28-30 days, with developmental progression examined every two days. Egg development was monitored microscopically, and the stage of embryonic development was

assessed based on the morphology of the embryo. The taxonomic position of the nematodes was determined according to the current systematics of helminths and specialised identification guides (World Health Organization, 2019; Lynnggaard *et al*., 2020).

All procedures in this study were conducted in accordance with the principles of animal welfare and international ethical standards for the use of animals in research. Ethical approval for this study was obtained from the Ethics Committee of Polissia National University (Approval No. 57, dated 29 June 2023). The research complied with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and adhered to the quality management principles outlined in DSTU EN ISO/IEC 17025:2019 (2019). No animals were captured, restrained, or subjected to invasive procedures during the study. All observations and faecal sampling were carried out non-invasively, using field feeders and passive video monitoring. The research design ensured minimal disturbance to the animals and their habitats, maintaining full conformity with ethical and biosafety regulations.

Statistical analyses were performed to assess spatial differences in infection parameters and to verify potential associations between squirrel infestation rates and the locations of feeding stations. The dependence of oxyurid infection prevalence on feeder sites was evaluated using Pearson's chi-square (χ^2) test. Differences in the number of *Trypanoxyuris sciuri* eggs among samples collected from different feeding stations were analysed using the non-parametric Kruskal-Wallis test owing to non-normal data distribution. Infection prevalence was expressed as the percentage of infected individuals, with 95% confidence intervals (CIs) calculated according to the Clopper-Pearson method. The intensity of infection was presented as the arithmetic mean ± standard error of the mean (S.E.M.). All statistical procedures were performed using R version 4.3.1 (R Core Team, 2023), with the significance threshold set at $p < 0.05$.

Results and Discussion

During the 60-day observation period, four feeding stations were regularly visited by 17 individual red squirrels, each of which was recorded at least three times. The most active individuals were observed visiting almost daily, with up to three squirrels occasionally present simultaneously. The parasitological examination of the collected faecal samples revealed the presence of helminths belonging to the superfamily *Oxyuroidea*. The obtained results (Table 1) demonstrated distinct infection patterns and notable spatial variability among the study sites. Differences in infestation rates across the feeding stations indicate that local environmental conditions and host behavioural activity may play an important role in shaping the transmission dynamics of oxyurid nematodes in semi-urban squirrel populations.

Table 1. Squirrel feeder visits and fecal parasitological analysis

Feeder No.	Squirrels (total)	Fecal samples collected	Total infected fecal samples	Infected squirrels			Total infected squirrels
				Trypanoxyuris sciuri	*Syphacia obvelata*	*Aspiculuris dinniki*	
1	4	59	49	4	2	-	4
2	7	39	11	2	-	-	2
3	3	46	38	3	-	-	3
4	3	47	36	3	-	1	3

***Source**: created by the authors*

Infestation of red squirrels with oxyurids varied significantly depending on feeder location $\chi^2 = 10.12$, p-value = 0.0176), indicating significant differences between study points. At Feeder 1, the highest prevalence of *Trypanoxyuris sciuri* was observed, with 100% of the squirrels infected. Additionally, 50% of the individuals also had a simultaneous infestation of *Syphacia obvelata*. At Feeders 3 and 4, all squirrels (100%) were also infected with *Trypanoxyuris sciuri*. However, a single case of co-infestation with *Aspiculuris dinniki* was recorded at Feeder 4. Feeder 2 exhibited the lowest infestation rate, with only 28.6% of squirrels infected with *Trypanoxyuris sciuri*, and no other oxyurid species were found at this site.

No significant differences in the number of *Trypanoxyuris sciuri* eggs were found in faecal samples (n = 134) from squirrels (n = 12) collected at different locations in the city (P = 0.71). In general, the most common species in the urban squirrel population was *Trypanoxyuris sciuri*, with an infestation rate of 70.59% of all individuals (Table 2). The species *Syphacia obvelata* and *Aspiculuris dinniki*, which are less common in squirrels, were characterized by low infestation intensity in a small number of individuals.

Table 2. Prevalence and mean intensity of oxyurid infection in squirrels, n = 17

Species	Prevalence (%) with 95% CI		Mean intensity ± S.E.M.	
Trypanoxyuris sciuri	70.59	(44.04-89.69)	75.99	**± 3.72**
Syphacia obvelata	11.76	(1.46-36.44)	23.91	**± 1.32**
Aspiculuris dinniki	5.88	(0.15-28.69)	12.5	**± 2.9**

***Source**: created by the authors*

Following the summarised prevalence data presented in Table 2, detailed morphological examination and laboratory cultivation of the detected oxyurid eggs were carried out to confirm species identification and assess developmental viability. Microscopic analysis focused on the distinguishing structural features of the three helminth species, while incubation under controlled laboratory conditions provided additional information on their embryonic development and survival potential.

The morphology of *Trypanoxyuris sciuri* eggs was consistent with species-specific diagnostic characteristics. The eggs were symmetrical and oval, measuring up to 70 µm in length, with a smooth, transparent shell and regular contours. No distinct surface ridges, grooves, or polar thickenings were observed (Fig. 2). These features fully corresponded to the description of *T. sciuri* as a host-specific oxyurid of the red squirrel. Given the well-documented laboratory model status of this species, experimental incubation was not performed, and species identification was based on morphological criteria alone.

Figure 2. External morphology of Trypanoxyuris sciuri egg (×450)
***Source**: photo taken by the authors*

Eggs of *Syphacia obvelata,* reaching up to 60 µm in length, were oval with slightly flat-

tened poles and a thin, smooth shell exhibiting faint transverse striations (Fig. 3). The subtle texture gave the surface a finely lined appearance characteristic of this species. Laboratory cultivation of *S. obvelata* eggs under controlled conditions revealed active embryonic development, with up to 90% viability observed throughout the incubation period. A typical feature of the L1 larvae was the oval anterior end without a vesicle and an oesophagus with a distinct bulb and well-defined cuticular plates (Abdel-Gaber *et al.*, 2018) (Figs. 4-5).

Figure 3. Eggs of Syphacia obvelata at the initial stage of incubation (×450)
***Source**: photo taken by the authors*

Figure 4. The anterior end of Syphacia obvelata at the L1 stage (×600)
***Source**: photo taken by the authors*

Figure 5. Morphology of the esophageal bulb in Syphacia obvelata L1 larvae. (×600)
***Source**: photo taken by the authors*

Eggs of *Aspiculuris dinniki* (Figs. 6-7) were similar in general shape to those of other members of the genus, with a smooth, transparent shell and a clearly visible embryo in the early stages of development. Cultivation did not yield positive results. During the first few days, germ cell division was observed, indicating initial egg activity and favourable conditions for development. However, by days 4-5, the developmental process abruptly stopped, and despite further observation, no viable larvae were obtained. This suggests species-specific sensitivity to cultivation parameters or limited adaptability to artificial incubation conditions.

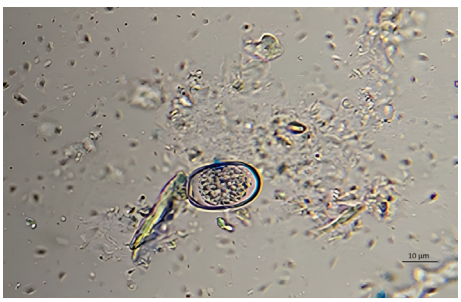

Figure 6. Egg of Aspiculuris dinniki at the onset of incubation (×400)
***Source**: photo taken by the authors*

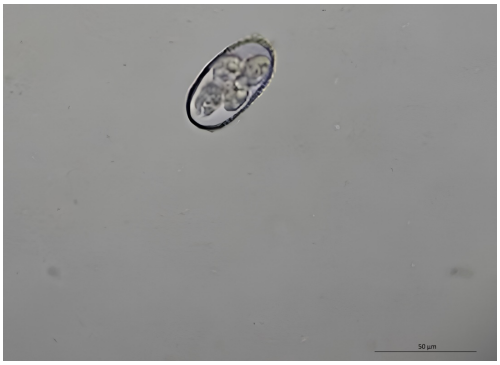

Figure 7. Egg of Aspiculuris dinniki 24 hours after the start of incubation (×400)
***Source**: photo taken by the authors*

Three species of helminths from the superfamily *Oxyuroidea* were identified in the analysed squirrel faecal samples. A total of 191 faecal samples were analysed, of which 134 (70.16 %) contained oxyurid eggs. Infestation of red squirrels with oxyurids varied significantly depending on feeder location ($\chi^2 = 10.12$, $P = 0.0176$), indicating significant differences between study points. The uneven distribution of infected individuals across locations may be due to local differences in ecological conditions (e.g., proximity to a river, lower urbanization), squirrel population density, and closeness to residential areas. The location with the highest infection rate was situated near a private sector, where synanthropic micromammals – natural hosts of some detected nematodes – are potentially present. However, no direct ecological or quantitative assessment was carried out during this study, and the proposed explanations should be regarded as hypothetical. Future research should consider the use of trapping surveys or genetic identification of potential parasite reservoirs in the environment.

The pinworm *Trypanoxyuris sciuri*, a species characteristic of the Eurasian red squirrel, was identified as the dominant helminth in the examined population. Its prevalence confirms the high degree of host adaptation and effective transmission within the population. The absence of significant differences ($P = 0.71$) in egg numbers across faecal samples from different urban sites suggests that infestation intensity is not influenced by local environmental factors. This pattern is consistent with the view that *T. sciuri* exhibits strong host specificity and well-developed mechanisms for maintaining stable transmission cycles within squirrel populations (Santicchia *et al*., 2020; Romeo *et al*., 2021). Such intra-population persistence may be supported by frequent social contact among individuals and overlapping feeding territories, both of which facilitate passive transmission through contaminated substrates.

Of particular note are the rare occurrences of two atypical oxyurid species, *Syphacia obvelata* and *Aspiculuris dinniki*, detected in two and one individuals, respectively, from distinct feeder sites. These findings likely reflect occasional spillover infections originating from murid rodents inhabiting the same urban parks. Similar cross-infections were reported by J.M. Behnke *et al*. (2015), who demonstrated that parasite dispersal in rodents is often linked to overlapping habitats and interspecific contact zones, and by K.E. Galbreath *et al*. (2019), who emphasised that shared ecological niches promote the exchange of parasitic fauna among sympatric mammal species. The detection of *S. obvelata* in squirrels may therefore indicate the existence of environmental reservoirs enriched with parasite eggs, possibly resulting from contamination of feeding areas or shared nesting materials.

The occurrence of *S. obvelata* in non-typical hosts has also been documented in previous research. S. Chawla *et al*. (2015) identified this nematode as a common parasite of murid rodents such as *Mus musculus* and *Apodemus sylvaticus*, while S. Koirala *et al*. (2016) demonstrated its ability to survive under favourable

conditions in a wider range of rodent hosts. The present observation of viable egg development in laboratory culture is consistent with the findings of R. Abdel-Gaber (2016), who described the morphological and genetic adaptability of *S. obvelata*, and P.M. Gerwin *et al*. (2017), who confirmed the species' capacity to persist under experimental conditions. Together, these studies highlight the ecological flexibility of *S. obvelata* and its potential to establish temporary infections in secondary hosts when interspecific transmission routes are available.

In contrast, *Aspiculuris dinniki* is typically associated with arvicoline rodents, particularly the grey vole (*Microtus nivalis oseticus*), as reported by M. Leblanc *et al.* (2014). Its detection in squirrels may represent either accidental ingestion of infective eggs or an early stage of host-range expansion. However, the unsuccessful cultivation of *A. dinniki* eggs observed in the present study may be explained by the species' limited physiological tolerance. V.O. Yevstafieva *et al*. (2020) demonstrated that oxyurid egg development is highly dependent on environmental parameters such as temperature, humidity, and aeration. Even minor deviations from optimal conditions can inhibit embryogenesis, as reflected by the cessation of cell division in the cultured samples. These findings suggest that *A. dinniki* exhibits lower environmental plasticity than *S. obvelata*, reflecting its adaptation to more stable and specific microhabitats.

The detection of murid-associated nematodes in red squirrels highlights the ecological permeability of urban environments. According to C. Lynnggaard *et al*. (2020) and K.E. Galbreath *et al*. (2019), urbanisation promotes increased overlap among synanthropic and wild rodent populations, creating opportunities for parasite transmission across species barriers. The sporadic appearance of *S. obvelata* and *A. dinniki* in squirrels thus supports the hypothesis of limited but ecologically relevant cross-species infections. Given the high mobility of red squirrels and their frequent use of anthropogenic feeding sites, such incidental parasitism may have epidemiological significance, particularly in densely populated urban landscapes.

Overall, the results confirm that *T. sciuri* remains the dominant helminth species in urban red squirrel populations, maintaining stable transmission independent of location. The presence of atypical oxyurids suggests occasional host-switching events, likely facilitated by habitat overlap with murids. These findings align with broader observations on the effects of urbanisation on parasite ecology, which emphasise both increased exposure to environmental infective stages and the emergence of new host – parasite relationships. Further molecular analysis of the detected species would help clarify the extent of such interspecific transmission and its potential role in the evolution of urban parasite communities.

Conclusions

Over a 60-day observation period, 191 faecal samples were collected from individually identified squirrels. Parasitological examination revealed three nematode species from the superfamily *Oxyuroidea*: *Trypanoxyuris sciuri*, *Syphacia obvelata*, and *Aspiculuris dinniki*. The dominant parasite, *T. sciuri*, was found in 70.6% of examined individuals and demonstrated a uniform distribution across the study area, indicating stable transmission within the squirrel population. The detection of *S. obvelata* and *A. dinniki* in a small number of squirrels represents rare and potentially transient infections, suggesting possible spillover from murid rodents that share the same urban habitats. Laboratory cultivation confirmed the high viability of *S. obvelata* eggs under controlled conditions, while *A. dinniki* development ceased at early embryonic stages, reflecting differences in ecological tolerance between the two species.

The study demonstrates that urban red squirrels can serve as sentinel hosts for monitoring parasitic agents circulating at the wildlife-human interface. The presence of atypical oxyurid species in this population highlights the permeability of ecological boundaries in urban ecosystems and underscores the need for continued veterinary surveillance of wild mammals living in proximity to humans. Future research should focus on molecular identification of parasite isolates to clarify host–parasite relationships and potential routes of interspecies transmission. Expanding the geographical coverage of sampling and assessing immunological responses in infected squirrels would provide a more comprehensive understanding of helminth dynamics in urbanised environments.

Acknowledgements

The author acknowledges the use of the OpenAI language model ChatGPT, which assisted in refining the conceptual framework and digital workflow of the experimental design, as well as in improving the clarity and readability of the manuscript.

Funding

The study was not funded.

Conflict of Interest

None.

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Інфекції гостриками у рудих вивірок на межі міста та лісу

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Анотація. Зростання взаємопроникнення міських і лісових екосистем підвищує ризик міжвидової передачі паразитів, що визначає актуальність вивчення паразитофауни диких ссавців. Метою дослідження було з'ясувати поширеність та видовий склад оксіюрідних нематод у популяції рудих вивірок (*Sciurus vulgaris*), які мешкають у лісопаркових зонах Полісся (Україна). Було проаналізовано 191 зразок фекалій, зібраних у період з вересня по грудень 2023 року, із застосуванням інтегрованого підходу, що включав відеоспостереження у природному середовищі, систематичний відбір проб і мікроскопічне дослідження морфології яєць гельмінтів. Для автоматизованої ідентифікації вивірок використовували модель комп'ютерного зору YOLOv5. У результаті виявлено три види паразитів із надродини Oxyuroidea. Найпоширенішим був *Trypanoxyuris sciuri*, типовий для вивірок, який зафіксовано

у 70,6 % тварин (12 із 17 особин; 95 % CI: 44,04–89,69). *Syphacia obvelata* виявлено у 11,8 % (2 із 17; 95 % CI: 1,46–36,44), а Aspiculuris dinniki – у 5,9 % (1 із 17; 95 % CI: 0,15–28,69). Культивування яєць *S. obvelata* у лабораторних умовах дало змогу описати морфологію личинок L1, тоді як *A. dinniki* не розвивалась, що підтвердило її специфічні вимоги до хазяїна. Ці результати підкреслили високу поширеність T. sciuri серед білок і засвідчили потенційну передачу *S. obvelata* та *A. dinniki* в популяціях цих тварин. Практична цінність роботи полягає у розширенні знань про різноманіття гельмінтів і міжвидові зв'язки паразитів у перехідних міського-лісових екосистемах. Отримані результати можуть бути використані для вдосконалення ветеринарно-санітарних заходів, профілактики паразитарних захворювань і розробки стратегій моніторингу, спрямованих на збереження здоров'я диких популяцій і мінімізацію ризиків для свійських тварин та людини

Ключові слова: паразити дикої природи; шлункові нематоди; Syphacia; гострики у білок

УКРАЇНСЬКИЙ ЧАСОПИС ВЕТЕРИНАРНИХ НАУК

Науковий журнал

Том 16, № 3. 2025

Заснований у 2010 р. Виходить чотири рази на рік

Оригінал-макет видання виготовлено
у відділі науково-технічної інформації
Національного університету біоресурсів і природокористування України

Відповідальний редактор:
Н. Шевченко

Підписано до друку 23 вересня 2025 р. Формат 70*100/16
Умов. друк. арк. 8,8
Наклад 100 прим.

Адреса видавництва:
Національний університет біоресурсів і природокористування України
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UKRAINIAN JOURNAL OF VETERINARY SCIENCES

Scientific journal

Volume 16, No. 3. 2025

Founded in 2010. Published four times per year

The original layout of the publication is made in the Department of Scientific and Technical Information of National University of Life and Environmental Sciences of Ukraine

Managing Editor:
N. Shevchenko

Signed for print of September 23, 2025. Format 70*100/16
Conventional printed pages 8.8
Circulation 100 copies

Publisher's address:
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
Tel.: (044) 527-82-42
E-mail: info@veterinaryscience.com.ua
https://veterinaryscience.com.ua/en