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The influence of a prebiotic on the development of laboratory animals

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Abstract. Prebiotics contribute to improved animal health; however, their beneficial effects on the body depend on the composition and dosage of the preparation, as well as the age and species of the animals. This study aimed to examine the effects of a new complex prebiotic, Bio-active,

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on the physiology of white mice and rabbits. The experiment involved 40 white laboratory mice and rabbits. The prebiotic was administered at a dosage of 1.2 g per animal. Standard methods were used to assess the microclimate, the quality of tap water used for drinking, growth dynamics, haematological parameters, and microscopic examination of caecal mucosa smears (in rabbits). The microclimate parameters and water quality indicators met the requirements of current regulatory standards. Administration of the prebiotic to white laboratory mice resulted in a gradual increase in body weight and average daily weight gain throughout the study period. In rabbits, body weight, absolute and relative growth rates increased, contributing to improved survival rates and growth performance. It was demonstrated that the inclusion of the prebiotic in animal feed led to higher red blood cell counts, increased haemoglobin concentration, and elevated levels of total protein and globulins. The prebiotic also influenced the quantity and ratio of Gram-positive and Gram-negative microorganisms in the caecum of rabbits. Specifically, the number of Gram-negative microorganisms decreased by 17.7% ($P < 0.05$), while Gram-positive microorganisms increased by 19.4% ($P < 0.05$). The pH of the caecal content in the experimental group was 6.5, compared to 7.1 in the control group, indicating the restoration of functional capacity in the large intestine. Based on the results obtained, the components of the prebiotic at a dosage of 1.2 g per animal were found to have a positive effect on body weight gain, haematological parameters, and the quantitative composition of the large intestinal microbiota. These outcomes are significant for rearing healthy livestock and producing high-quality food products

Keywords: white mice; rabbits; housing conditions; growth performance; haematological parameters; microorganisms; caecum

Introduction

A relevant strategy in contemporary animal husbandry is the utilisation of promising alternative immunostimulants, which can help to reduce the overuse of antibiotics in livestock farming. Adopting this approach addresses a key aim of the One Health concept, which is to prevent the spread of dangerous zoonotic diseases, produce safe and high-quality food products, and adhere to animal welfare standards.

J. Jing *et al.* (2023) pointed out that prebiotics are nutrients beneficial to human health that are fermented by microorganisms, leading to specific alterations in their composition. In essence, prebiotics are substances that nourish the gut microbiome, thereby regulating its population to favour the production of postbiotics and facilitating the removal of harmful metabolites and exogenous substances. Consequently, for the animal body, particularly during periods of growth and development or when certain diseases arise, the inclusion of prebiotic supplements in the basic diet is essential. The authors concluded that the ongoing development of novel research methods, such as sequencing and artificial intelligence, alongside a more thorough investigation into the mechanisms of action of probiotics, prebiotics, and postbiotics, will further their application in relevant sectors of animal product production.

The application of prebiotics in animal husbandry and the production of high-quality, safe food is crucial for human health. In this context, A. Ballini *et al.* (2023) and V.O. Oluwatobi *et al.* (2024) established that the human gut microbiota contains over 500 species of bacteria and constitutes 95% of the total number of cells in

the body. The authors highlighted that disruptions in the composition of the gut microbiota, resulting from poor diet, obesity, and a sedentary lifestyle, lead to dysfunction in numerous organs and systems within the body. A consequence of such disturbances is a reduction in the body's overall resistance to infectious diseases.

Given the proven beneficial effects of prebiotics, they are now widely used in the rearing of productive livestock (Shkromada *et al*., 2024). Developers of new formulations are particularly focused on the component composition, dosage, and in-depth investigations into the effects of both individual components and their combined action. For instance, amino acids are frequently included in the composition of novel prebiotic preparations, as the process of amino acid catabolism is vital for controlling the metabolism of various biological processes and regulating their mechanisms. In this regard, M. Beaumont *et al.* (2022) demonstrated that amino acids can modulate the composition of the gut microbiota, which influences the oxidation of amino acids and the production of metabolites in the intestine. The ability of bacteria to generate amino acid metabolites in the gut promotes the growth of beneficial microorganisms that can outcompete pathogenic ones. Consequently, research in this area has led to the introduction of the term "aminobiotics", which function as prebiotics within the body.

I. Ishii & M. Bhatia (2023) noted that in the human and animal body, proteins are synthesised from at least 20 of the hundreds of amino acids found in nature. Of particular importance are nine essential amino acids, which are not synthesised by the body's cells and must be obtained through diet. The authors highlighted polymers composed of a specific type of amino acid, known as an α-amino acid, which forms proteins.

The composition of prebiotics can also include live microorganism cultures. Consequently, the role of probiotic cultures in regulating animal and human health is continuing to evolve. D. Guzmán-Escalera *et al*. (2025) indicated that the action of metabolites from lactic acid fermentation leads to an increase in short-chain fatty acids, resulting in an anti-inflammatory effect. Furthermore, the action of bioactive peptides, also metabolites of lactic acid fermentation, leads to antihypertensive, antithrombotic, antioxidant, and antimicrobial effects. Additionally, L.L.C. De Jesus *et al.* (2024) identified several plasmids in *L. delbrueckii* that reduce the spread of antibiotic resistance genes, and this type of bioactive peptide metabolite from lactic acid fermentation has a lower propensity to act as a pathogen.

This research aimed to evaluate the impact of the prebiotic Bio-active at a dosage of 1.2 g per animal on the growth and development of white mice and rabbits. This will involve determining the changes in body weight, internal organs, haematological parameters, and the quantitative composition of the caecal microflora in rabbits.

Literature Review

A primary requirement for the component composition of feed supplements for productive livestock, particularly rabbits, remains the inclusion of natural substances that can serve as alternatives to synthetic drugs. K. El-Sabrout *et al.* (2023) found that rabbit farming is currently facing a feed deficit due to intense competition within the animal husbandry sector, climate change, and wartime conditions. Consequently, the development and application of natural feed additives as substitutes for antibiotics in rabbit nutrition is of critical importance for raising healthy animal populations.

When administering prebiotics with varying component compositions to productive animals, investigating the state of the intestine is a priority. It is well-established that the intestine is one of the most vital organs, responsible for

the absorption of water and nutrients essential for maintaining the physiological functions of the animal body. A key function of the intestine is its barrier role, which is necessary for the elimination of harmful substances such as toxins, allergens, and pathogens. This was highlighted by K. Kobayashi *et al.* (2023), who demonstrated using an epithelial model of the small intestine that both live and heat-treated *L. bulgaricus* 2038 and *S. thermophilus* 1131 improved the condition of the intestine in cases of intestinal barrier dysfunction by modulating the integrity of the apical tight junction (TJ) protein in Caco-2 cells.

J. Jing *et al.* (2023), based on the findings of their research, elucidated the key mechanisms and positive impacts of prebiotics used to restore the homeostasis of the gut microbiota. Y. Zhang *et al.* (2023) noted that the functional capacity of the gut microbiome influences the extent to which nutrients from feed are absorbed, the development or progression of various diseases, and the body's ability to maintain protective functions against opportunistic and pathogenic microorganisms. Similarly, Y.P. Silva *et al.* (2020) demonstrated that the gut microbiome affects the functioning of organs and systems within the body, such as the nervous and immune systems, by acting through microbial signalling and metabolites, including short-chain fatty acids (SCFAs). Research by W. He *et al.* (2024) has shown that feeding mice with lyophilised powder made from fermented whey can regulate the quantity and ratio of the gut microbiota and improve the condition of the experimentally damaged nervous system in mice. This is attributed to the presence of surface receptors for neurotransmitters in the taxa that constitute the gut flora. Consequently, the functions of the central nervous system are closely interconnected with the activity of the intestine.

The digestion of feed and detoxification of substances within the intestine is possible through the action of specific types of microflora present in quantities that ensure an optimal balance in the large intestine. I.A. Biben *et al.* (2021) reached this conclusion and established that the species composition of the gut microflora influences the properties of lymphoid tissue, which is predominantly located in the large intestine. V.V. Kunovsky *et al.* (2024) established that commensal microorganisms regulate the connection between the human gut microbiota and the central nervous system. The gut-brain axis (GBA) comprises a bidirectional communication pathway between the central and enteric nervous systems, linking the emotional and cognitive centres of the brain with peripheral intestinal functions. Furthermore, S.A. Burmei & N.B. Boyko (2024) demonstrated that probiotic preparations should not exhibit any inhibitory effects on commensal microorganisms, and various types of biopreparations (pre-, pro-, syn-, and metabiotics) are employed to restore and correct the microbiota of the digestive tract. This approach forms the foundation for the development of nextgeneration probiotics, which promises to be a significant advancement in the production of new-generation biopreparations.

Vitamins A, E, and B complex are important constituents of prebiotic preparations. M. Asadi *et al.* (2024) highlighted the wide range of metabolic pathways initiated in the animal body through the use of B vitamins, which act as cofactors. When vitamin B complex was administered via injection to pregnant goats, a reduction in the incidence of metabolic diseases was observed, along with a significant increase ($P < 0.05$) in the red blood cell count, haemoglobin concentration, haematocrit level, and overall antioxidant status of their offspring.

Y. Shastak & W. Pelletier (2024) demonstrated that the fat-soluble vitamins A and E play a crucial and interconnected role in various biological processes. They also noted that a deficiency in these vitamins (hypovitaminosis) can lead to severe consequences for the animal

body, including impaired vision, reduced immunity, stunted growth, decreased reproductive capacity, and the development of nervous system disorders. The authors concluded that feed supplements containing vitamins A and E in innovative liquid formulations can prevent the development of diseases and improve the general health and productivity of animals.

M. Koprivica & A. Miljković (2024) investigated the biochemical functions of vitamin E and its effects on various bodily systems, highlighting that a deficiency in this lipophilic compound can lead to serious health consequences, while excessive consumption may cause hypervitaminosis. Simultaneously, the researchers pointed to the potent antioxidant effect and pharmacokinetic properties of alpha-tocopherol. Furthermore, they explained the significant influence of the vitamin on the immune, cardiovascular, and nervous systems, on skin health, and its role in the prevention of carcinogenesis.

Concurrently, researchers focused on the effects of B vitamins on the body. C. Munteanu *et al.* (2024) found that vitamin B_1 is essential for the body as a cofactor in carbohydrate metabolism, nucleotide synthesis, and the production of nicotinamide adenine dinucleotide. Moreover, the important role of vitamin B_2 in maintaining cellular energy balance, growth and development, and metabolism has been established. Vitamin B_2 achieves this function as a component of two primary coenzymes: flavin mononucleotide and flavin adenine dinucleotide, which are crucial components that facilitate biochemical intracellular reactions. Thus, based on an analysis of the literature, it can be concluded that the administration of pro- and prebiotics to animals, containing lactic acid bacteria, amino acids, and vitamin preparations, has a positive impact on blood composition, the state of the gut microbiota, strengthens the central nervous system, enhances immunity, and contributes to increased animal productivity.

Materials and Methods

The experimental studies were conducted throughout 2024 in the research laboratory of immunology of agricultural animals at Bila Tserkva National Agrarian University and the laboratory of anaerobic infections at the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine. The experiment involved 40 white laboratory mice (*Mus musculus* L.), specifically improved conventional animals (*Minimal diseases*) that were free from pathogenic microflora and housed with barrier elements within an improved conventional system. The experiment also included 40 young rabbits of the Grey Giant breed. The laboratory animals (white mice and rabbits) were divided into a control group (20 animals) and an experimental group (20 animals).

In the experiment, white laboratory mice aged between 14 and 60 days were used. The prebiotic Bio-active was administered at a dose of 1.2 g per animal, once daily for 30 days, and the study continued for an additional 30 days after the prebiotic administration ceased. Rabbits were fed the prebiotic at a dose of 1.2 g per animal from 45 to 90 days of age. The diet for the white mice consisted of crushed wheat grain and compound feed, while the rabbits were fed legume hay (80 g), fodder beet (110 g), and compound feed (80 g) per animal. Drinking water for the experimental animals was provided through nipple drinkers.

The investigated prebiotic comprised the metabolic products of the lactic acid bacterium *Lactobacillus bulgaricus delbrueckii*, adsorbed onto zeolite, along with the following amino acids: aspartic acid – 33.77 mg/%, glutamic acid – 10.51 mg/%, glycine – 10.59 mg/%, and phenylalanine – 4.01 mg/%; and the following vitamins: B_1 – 0.13 μg/g, B_2 – 0.17 μg/g, and B_{12} – 0.0012 μg/g; vitamin A – 0.627 μg/g, and vitamin E – 3.0 μg/g.

During the initial phase of the study, the housing conditions for the white laboratory

mice and rabbits were established. Measurements of microclimate parameters within the vivarium were taken three times throughout the experiment. The vivarium of Bila Tserkva National Agrarian University is situated 1,500 metres from the city of Bila Tserkva. The vivarium grounds are landscaped with greenery. The underground groundwater level was more than 2 metres below the foundation's lowest point. The experimental animals were housed in the vivarium following current regulations (VNTP-APK-05.07..., 2007).

To evaluate the microclimate parameters within the experimental animal housing, the following were determined: temperature (°C), using a TFA 12300802 mercury thermometer (Germany); relative humidity (%), using a VIT-2 psychrometric hygrometer (Ukraine); air velocity (m/s), using a globe katathermometer (Ukraine); the concentration of harmful gases (carbon dioxide, %; ammonia and hydrogen sulphide, mg/m^3), using a UG-2 gas analyser (Ukraine); and artificial illumination (lux), using a WT81B lux meter (Wintact, Japan).

The determined microclimate parameters in the vivarium (Table 1), where the white laboratory mice were housed, were within the sanitary and hygienic standards (SOU 85.237736:2011..., 2011). Ventilation was natural – through open doors and a window (or transom). The quality indicators of the water provided to the animals met the requirements of the current State Sanitary Standards – DSanPiN 2.2.4-171-10 (State sanitary norms..., 2010), as evidenced by the data presented in Table 2.

Table 1. Microclimate indicators in the vivarium for housing white laboratory mice

Parameter	Sanitary and hygienic norms	Actual indicator
Temperature, °C	20-24	22.50 ± 1.48
Relative humidity, %	55	53.80 ± 1.54
Air velocity, m/s	0.3	0.22 ± 0.03
CO_2 concentration, %	0.15	0.13 ± 0.01
NH_3 concentration, mg/m^3	10	0.08 ± 0.06
H_2S concentration, mg/m^3	5	0.006 ± 0.001
Illumination, lux (1 m from the floor)	200	197.32 ± 6.89
Photoperiod, hours (light: dark)	12:12	12:12
Air exchange rate (ACH)	10-15	12

***Source**: authors' development*

Table 2. Organoleptic, physicochemical and epidemiological safety indicators of tap water for white laboratory mice

Parameter	Unit of measurement	DSanPiN 2.2.4-171-10	Actual indicator
Organoleptic indicators			
Odour (at t = 20 °C)	points	⩽ 2	1.3 ± 0.1
Turbidity	mg/dm^3	⩽ 1.0	0.4 ± 0.1
Colour	degrees	⩽ 20.0	6.8 ± 0.1
Taste and aftertaste	points	⩽ 2	1.4 ± 0.4
Physicochemical indicators			
Hydrogen index	pH	6.5–8.5	6.9 ± 0.1
Total hardness	mmol/dm^3	⩽ 7.0	6.8 ± 0.2

Table 2. Continued

Parameter	Unit of measurement	DSanPiN 2.2.4-171-10	Actual indicator
Physicochemical indicators			
Chlorides	mg/dm³	⩽ 250	196.0 ± 5.6
Nitrates	mg/dm³	⩽ 50	15.0 ± 3.2
Nitrites	mg/dm³	⩽ 0.5	0.4 ± 0.02
Manganese	mg/dm³	0.1	0.03 ± 0.01
Sulphates	mg/dm³	⩽ 250	38.4 ± 2.5
Total iron	mg/dm³	⩽ 0.2	0.08 ± 0.003
Epidemiological safety indicators			
Total coliforms	CFU/100 cm³	absent	absent
Intestinal helminths	Cells, eggs, larvae in 50 dm³	absent	absent

***Source**: authors' development*

The vivarium where the rabbits were housed was a standard, single-storey building constructed from white brick, located on level ground with good natural sunlight. The control and experimental groups of rabbits were kept in four wire mesh cages (10 animals in each). Each cage was equipped with hay racks, trough feeders, and a trough drinker. The examination of individual physical parameters of the microclimate in the vivarium (Table 3), where the rabbits were housed, indicated that they met the sanitary and hygienic requirements of the document – Departmental Standards for Technological Design of Enterprises of Animal Husbandry and Rabbit Breeding (VNTP-APK-05.07, 2008). Lighting in the room was both natural and artificial – fluorescent lamps were used. Ventilation in the animal housing was natural – through open doors and a window (or transom). The measured indicators of the water (Table 4) provided to the control and experimental groups of rabbits throughout the experiment met the requirements of the current State Sanitary Standards – DSanPiN 2.2.4-171-10 (State sanitary norms..., 2010).

Table 3. Microclimate parameters in the vivarium for housing rabbits

Parameter	Sanitary and hygienic norms	Actual indicator
Temperature, °C	15-20	20.0 ± 1.15
Relative humidity, %	55	52.4 ± 1.36
Air velocity, m/s	0.3	0.26 ± 0.01
CO_2 concentration, %	0.15	0.10 ± 0.03
NH_3 concentration, mg/m³	10	0.82 ± 0.02
H_2S concentration, mg/m³	5	0.007 ± 0.001
Illumination, lux (1 m from the floor)	200	187.26 ± 2.57
Photoperiod, hours (light: dark)	12:12	12:12
Air exchange rate (ACH)	10-15	10-15

***Source**: authors' development*

Table 4. Organoleptic, physicochemical, and epidemiological safety indicators of tap water for rabbits

Parameter	Unit of measurement	DSanPiN 2.2.4-171-10	Actual indicator
Organoleptic indicators			
Odour (at t = 20 °C)	points	⩽ 2	1.6 ± 0.1
Turbidity	mg/dm^3	⩽ 1.0	0.2 ± 0.04
Colour	degrees	⩽ 20.0	5.3 ± 0.1
Taste and aftertaste	points	⩽ 2	1.6 ± 0.2
Physicochemical indicators			
Hydrogen index	pH	6.5–8.5	7.2 ± 0.3
Total hardness	mmol/dm^3	⩽ 7.0	5.9 ± 0.8
Chlorides	mg/dm^3	⩽ 250	200.3 ±7.9
Nitrates	mg/dm^3	⩽ 50	13.6 ± 6.9
Nitrites	mg/dm^3	⩽ 0.5	0.2 ± 0.1
Manganese	mg/dm^3	0.1	0.02 ± 0.01
Sulphates	mg/dm^3	⩽ 250	42.3 ± 5.2
Total iron	mg/dm^3	⩽ 0.2	0.07 ± 0.01
Epidemiological safety indicators			
Total coliforms	CFU/100 cm^3	absent	absent
Intestinal helminths	Cells, eggs, larvae in 50 dm^3	absent	absent

***Source**: authors' development*

During the second stage of the study, the impact of the prebiotic on the dynamics of growth and development, as well as the survival rate of white laboratory mice and rabbits, was assessed. Body weight gain in the laboratory mice and rabbits following prebiotic administration was determined by measuring body weight at the start and end of the study, and by calculating the average daily weight gain using standard calculation methods. To further investigate the effects of the studied prebiotic on the white mice, the mass of internal organs – thymus, kidneys, thyroid gland, liver, and spleen – was determined using standard procedures.

The third stage of the study examined the effect of the prebiotic on haematological parameters in white laboratory mice and rabbits. For the haematological analyses, peripheral blood from white laboratory mice was collected by decapitation on the 60th day of the experiment. Prior to this, the white mice were anaesthetised with 2% xylazine (Netherlands) and then decapitated using a guillotine. In rabbits, blood samples were collected from the marginal ear vein on the 90th day of the study, before the morning feed.

A full blood count of the experimental animals was conducted using an HTI MicroCC-25 Plus automated haematology analyser (USA). The parameters determined were: red blood cell count (RBC, 10^{12}/L), haemoglobin concentration (HGB, g/L), haematocrit (HCT), white blood cell count (WBC, 10^9/L), neutrophils (NEUT, %), lymphocytes (LYMPH, %), basophils (BASO, %), eosinophils (EO, %), and monocytes (MONO, %). Biochemical parameters of the blood serum of the experimental animals were determined using an AS-120 automated biochemical analyser (Japan) and a test system from Global Scientific (USA).

During the fourth stage of the study, the effect of the prebiotic Bio-active on the quantitative composition of the intestinal microflora in rabbits was analysed. Rabbits were culled

at 90 days of age (DSTU 4293:2004, 2004), and smears were prepared from the caecal mucosa onto microscope slides and stained using the Gram method. Subsequently, the number of Gram-negative and Gram-positive microorganisms was counted (Laukens *et al.*, 2016).

The studies involving laboratory animals were conducted following the requirements of the General Ethical Principles of Animal Experiments adopted by the First National Congress on Bioethics (Kyiv, 2001) (On the protection…, 2021) and in compliance with the international principles outlined in the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (European Convention, 1986) and the Law of Ukraine "On the Protection of Animals from Cruelty" (Law of Ukraine No. 3447-IV, 2006).

All obtained numerical data were statistically processed using the Microsoft Excel software package. The following were calculated: the arithmetic mean and its error (M ± m), the level of statistical significance (P) using Student's t-test, and the significance criteria were defined as: $P < 0.05$; $P < 0.01$; $P < 0.001$.

Results and Discussion

Research into the effects of preparations, particularly the prebiotic Bio-active (which contains a range of components aimed at maintaining natural resistance, growth energy, blood condition, and gut microbiome), on the bodies of laboratory animals should commence with an analysis of body weight and internal organ dynamics. The body weight trends of the experimental animals can indicate whether the condition of their organs is within the physiological norm. The study of body weight and internal organ dynamics in white laboratory mice is presented in Table 5.

Table 5. Body weight and internal organ mass in white laboratory mice after prebiotic feeding, M ± m, n = 20

Parameter, unit of measurement	The day of the study			
	1	14	30	60
Animal body weight, g	11.0 ± 0.85 11.0 ± 0.64	14.0 ± 0.74 15.8 ± 0.85▲	17.0 ± 0.94 20.0 ± 0.79*▲	22.1 ± 1.03 25.6 ± 1.08*▲
Average daily weight gain, mg	–	214.12 ± 1.06 341.10 ± 0.85**	186.52 ± 1.72 260.45 ± 2.01**	167.86 ± 1.86 188.56 ± 1.90**
Thymus mass, mg	–		–	30.0 ± 3.0 34.0 ± 2.3
Liver mass, g	–	–	–	1.14 ± 0.04 1.18 ± 0.05
Spleen mass, g	–	–	–	0.19 ± 0.03 0.19 ± 0.02
Thyroid gland mass, mg	–	–	–	4.0 ± 0.05 4.0 ± 0.06
Kidney mass, g	–	–	–	0.14 ± 0.07 0.14 ± 0.03

Note: *numerator – control group; denominator – experimental group; *P<0.05; **P<0.001, compared to the control group. ▲P<0.001, compared to the indicator at the beginning of the study; 1 – start of the experiment*
Source*: authors' development*

According to the results presented in Table 5, by day 14, the body weight of the white mice had increased by 43.6% ($P < 0.001$) compared to the body weight of the mice at the start of the study. Furthermore, the average daily weight gain in the mice had increased by 59.3% ($P < 0.001$) compared to the animals in the control group. By day 30 of the study, the body weight of the white mice had increased by 17.65% ($P < 0.05$) compared to the control

group, and by 81.82% (P < 0.001) compared to the body weight at the start of the study. The average daily weight gain in the mice had increased by 39.64% (P < 0.001) compared to the control group. By day 60 of the study, the body weight in the white mice had increased by 15.84% (P < 0.05) compared to the control group, and by 132.73% (P < 0.001) compared to this indicator at the start of the study. The average daily weight gain in the white mice had increased by 12.33% (P < 0.001) compared to the control group. Throughout the entire study period, the animals actively consumed both the feed from the basic diet and the feed supplemented with the prebiotic. The behaviour of the experimental animals was normal – they were mobile and active. Table 5 indicates that there was no statistically significant difference in the mass of the studied internal organs of the laboratory mice between the experimental and control groups.

As the studied prebiotic is a complex preparation containing live cultures of lactic acid bacteria, amino acids, and vitamins A, E, and B complex, its effect on the animals' bodies depends on the symbiotic influence of these components. Specifically, C. Li *et al.* (2024) demonstrated that synbiotics regulate metabolism, reduce insulin resistance, and positively influence body weight dynamics in mice. The current study established that the prebiotic Bio-active also promoted growth and development in Grey Giant rabbits, with a 100% survival rate (Table 6).

Table 6. Growth parameters in experimental rabbits, M ± m, n = 20

Parameter, unit of measurement	Control group	Experimental group
Number of animals, heads:		
♦ at the start of the study	20	20
♦ at the end of the study	20	20
Survival rate, %	100	100
Live weight of animals, kg:		
♦ at the start of the study	0.965 ± 0.068	0.983 ± 0.057
♦ at the end of the study	2.185 ± 0.024	2.461 ± 0.024
Absolute weight gain of the entire group, kg	1.220	1.478
Relative weight gain per animal, %	126.4	150.3
Growth energy, g	40.6 ± 1.93	49.2 ± 1.64*
Morbidity, %	–	–
Condition of the coat	Normal	Normal

Note: **P < 0.01, compared to the control group*
Source*: authors' development*

According to the body weight measurements taken at the beginning and end of the study, no statistically significant difference was found between the groups. However, the absolute weight gain in all rabbits in the experimental group was 21.15% higher than that of the control group, and the relative weight gain per rabbit in the experimental group was 23.9% higher compared to the control group. It was found that the growth energy of rabbits in the experimental group increased by 21.2% (P < 0.01) compared to the animals in the control group. Thus, the daily feeding of the prebiotic Bio-active at a dose of 1.2 g per animal for 45 days contributed to improved survival, metabolism, and accelerated growth, energy and development in Grey Giant rabbits.

The positive outcomes observed with the use of the prebiotic in rabbits were dependent on the meticulous control of both the

preparation dosage and the feeding ration. In this regard, Y. Shastak & W. Pelletier (2023) demonstrated that research should focus on determining the optimal periods for adjusting feeding rations and the dosage of supplements. Furthermore, considering that the prebiotic contains biologically active components, including vitamin A, scientists highlighted its recognised properties as a potent antioxidant, essential for animals to maintain their productivity levels and immunity.

One of the key stages of the study was the evaluation of the blood condition of the experimental animals. After all, the blood parameters of animals can reveal the functional state of the body, allow for the timely detection of signs of inflammation, and also confirm that the control group of animals consisted of healthy white mice (which naturally maintain a relatively constant blood composition) and rabbits. Therefore, it is very important to determine the dynamics of blood parameters in both white laboratory mice and rabbits during the feeding of the studied prebiotic. The results of its effect on the morphological parameters and leukogram of the blood of white mice are presented in Table 7.

Table 7. Complete blood count in white laboratory mice, M ± m, n = 20

Parameter, unit of measurement	Control group	Experimental group
Red blood cells (RBC), 10^{12}/L	7.98 ± 0.15	8.49 ± 0.12*
Haemoglobin (HGB), g/L	89.0 ± 3.16	98.0 ± 3.02*
White blood cells (WBC), 10^{9}/L	7.60 ± 0.40	7.50 ± 0.50
Band neutrophils (NEUT), %	0.54 ± 0.21	0.78 ± 0.36
Segmented neutrophils (NEUT), %	21.76 ± 1.65	19.43 ± 2.86
Basophils (BASO), %	0.30 ± 0.01	0.28 ± 0.02
Lymphocytes (LYMPH), %	55.0 ± 4.12	57.43 ± 3.02
Eosinophils (EO), %	2.98 ± 0.24	2.45 ± 0.16
Monocytes (MONO), %	5.90 ± 2.45	5.60 ± 1.02

Note: **P < 0.05, compared to the control group*
Source: *authors' development*

Table 7 shows that in the blood of white laboratory mice, the red blood cell count increased by 6.4% (P < 0.05), and the haemoglobin concentration by 10.1% (P < 0.05) compared to the control group. As the obtained research results indicate, the preparation activates erythropoiesis processes in the bodies of white laboratory mice. Furthermore, its administration for 30 days, at a dose of 1.2 g per animal, does not suppress the myeloid and lymphoid lineages of blood cell formation. The effect of the preparation continues up to 60 days of age in the laboratory mice. The biochemical parameters of the blood serum in white laboratory mice are presented in Table 8.

Table 8. Biochemical indicators of blood serum in white laboratory mice, M ± m, n = 20

Parameter, unit of measurement	Control group	Experimental group
Total protein (T-Pro), g/L	63.0 ± 2.20	70.0 ± 2.06*
Albumins (ALB), %	55.0 ± 3.18	57.0 ± 2.10
Globulins (Glob), %	8.10 ± 0.64	11.0 ± 0.82**
Alanine aminotransferase (ALT), U/L	45.38 ± 2.46	46.23 ± 2.67

Table 8. Continued

Parameter, unit of measurement	Control group	Experimental group
Aspartate aminotransferase (AST), U/L	124.61 ± 5.32	127.08 ± 6.89
Total cholesterol, mmol/L	2.14 ± 0.3	2.12 ± 0.2

Note: **P < 0.05; **P < 0.01, compared to the control group*
Source*: authors' development*

The research results showed that under the influence of the prebiotic, the total protein content in the blood serum of white mice increased by 11.1% (P < 0.05) and globulins by 1.4 times (P < 0.01) compared to the control group. It can be suggested that the increase in total protein and globulin levels in the blood serum of the laboratory mice is are key factor in enhancing the growth energy and development of the animals. Thus, the obtained research results indicate an increase in the activity of the humoral immunity component in the experimental group of animals.

The increase in total protein content in the blood serum of white laboratory mice can also be attributed to the additional intake of proteinogenic amino acids present in the studied preparation. In particular, Y. Kamei *et al.* (2020) found that amino acids, acting as biological regulators, stimulate the anabolism of muscle proteins and enhance resistance in animals. Furthermore, no statistically significant difference was found between the activity levels of aspartate aminotransferase and alanine aminotransferase in the blood serum of mice in the experimental and control groups.

Based on the results of the cholesterol content study in the blood serum of the laboratory mice, it can be noted that the changes in metabolism in the experimental group animals occurred due to anabolic processes, as no statistically significant difference was observed for this parameter between the experimental and control groups of mice. However, S. Ghosh *et al.* (2020) demonstrated that the addition of prebiotics to the diet of mice led to a decrease in serum cholesterol levels, irrespective of the animals' body weight changes.

Determining the blood condition of rabbits in the experiment is crucial for a timely response to changes in the functioning of the animals' bodies. Specifically for rabbits, the blood condition can indicate the presence of bacterial, viral, fungal, or parasitic infections, disturbances in the functioning of the gastrointestinal tract, and consequently, a weakening of the immune system. The effect of the prebiotic on the morphological and biochemical parameters of rabbit blood is presented in Table 9.

Table 9. Haematological indicators in experimental rabbits, M ± m, n = 20

Parameter, unit of measurement	Control group	Experimental group
Red blood cells (RBC), 10^{12}/L	4.85 ± 0.14	5.42 ± 0.16*
Haemoglobin (HGB), g/L	112.43 ± 4.12	125.32 ± 3.56*
ESR, mm/hour	4.12 ± 0.50	3.90 ± 0.30
White blood cells (WBC), 10^{9}/L	6.80 ± 0.40	6.56 ± 0.10
Total protein (T-Pro), g/L	59.0 ± 1.15	65.0 ± 2.16*
Albumins (ALB), %	29.40 ± 2.12	30.0 ± 1.84
Globulins (Glob), %	29.6 ± 1.12	35.0 ± 1.91*

Table 9. Continued

Parameter, unit of measurement	Control group	Experimental group
Alanine aminotransferase (ALT), U/L	8.20 ± 0.21	9.80 ± 0.14
Aspartate aminotransferase (AST), U/L	24.0 ± 0.98	28.0 ± 0.64
Total cholesterol, mmol/L	1.96 ± 0.03	1.90 ± 0.02

Note: **P < 0.05, compared to the control group*
Source*: authors' development*

The results of the experimental studies (Table 9) established that after feeding the rabbits with the prebiotic, activation of the erythropoiesis process was observed, as evidenced by an increase in the number of red blood cells in the blood by 11.7% P < 0.05), an increase in haemoglobin concentration by 11.5% (P < 0.05), total protein by 10.2% (P < 0.05), and globulins by 1.2 times (P < 0.05) compared to the control group. It is worth noting that this may be the main factor in increasing the growth energy and development of rabbits. No statistically significant difference was found for the remaining parameters. All investigated parameters were within the reference values.

N.B. Abduljabbar *et al.* (2024) found that adding a prebiotic – algae powder – to the diet of rabbits increased (P < 0.05) in body weight, blood haemoglobin content, red blood cell count, total protein concentration, albumin, and high-density lipoproteins, alongside a decrease (P < 0.05) in white blood cell count and cholesterol concentration. Similar results were obtained by A. Mohammed (2023), who found that adding enzymes to rabbit feed increased the red blood cell count (P < 0.05) and haemoglobin content. In turn, M. Sobri *et al.* (2019) demonstrated that the particle size of fibre in a feed supplement had a significant effect (P < 0.05) on the number of white blood cells, eosinophils, and neutrophils in the blood of post-weaning rabbits. The data from these mentioned studies align with the results of the current experiment. The increase, within the physiological range, of enzymatic activity indicators after feeding the studied prebiotic suggested the absence of liver damage and necrosis, with a moderate stimulation of metabolism.

According to the results of the study on the effect of the prebiotic on the concentration of total cholesterol in the blood serum of rabbits, it was noted that the changes in metabolism in the experimental animals occurred due to anabolic processes, as no statistically significant difference was observed for this parameter between the experimental and control groups of animals. Therefore, the prebiotic contributed to the activation of the energy and structural needs of the experimental group rabbits and stimulated the main metabolic pathways of their functioning.

The studied prebiotic contains the lactic acid bacterium *Lactobacillus bulgaricus delbrueckii*, which inhibits the proliferation of opportunistic and pathogenic microflora and can create conditions for lowering the pH in the animals' intestines. Therefore, this paper presents the results of an evaluation of the effect of the lactic acid bacterium *Lactobacillus bulgaricus delbrueckii*, in combination with other bioactive components of the Bio-active prebiotic, on the percentage composition of the microflora in the caecum of experimental rabbits. According to the results of the performed microscopy of smears (Table 10) prepared from the caecal mucosa of rabbits in the experimental group, it was found that the total number of Gram-positive microorganisms increased by 19.4% (P < 0.05), while Gram-negative microorganisms, conversely, decreased by 17.7% (P < 0.05) compared to the control group.

Table 10. Microscopy of smears from the caecal mucosa of rabbits, M ± m, n = 10

Parameter, unit of measurement	Control group	Experimental group
pH value	7.1 ± 0.2	6.5 ± 0.2
Gram-positive microflora, %	48.0 ± 5.0	67.4 ± 2.6*
Gram-negative microflora, %	39.0 ± 4.6	21.3 ± 1.3*
Ratio of Gram-negative to Gram-positive	1:1.23	1:3.16

Note: **P < 0.05, compared to the control group*
Source*: authors' development*

Furthermore, the Gram-positive microorganisms were observed as thick, long rods, while the Gram-negative ones were small, thin, and long rods with granularity. Coccal forms of Grampositive bacteria (streptococci, staphylococci, and micrococci) were also observed in the microscope's field of view. In the caecal microbiome of rabbits in the experimental group, the proportion of Gram-positive microflora predominated over Gram-negative microflora, which is a physiological phenomenon for the large intestine, ensuring a balanced structural and energy metabolism.

Table 10 shows that there was no statistically significant difference in the pH value of the caecal contents of rabbits between the experimental and control groups. However, in the rabbits of the experimental group, the pH of the caecal contents was 6.5 (slightly acidic). In contrast, in the rabbits of the control group, the pH value corresponded to a neutral reaction (pH 7.1). In this regard, S.H. Abu Hafsa *et al.* (2022) demonstrated that the pH value of the rabbit caecum is within a range that ensures the activity of digestive enzymes in the large intestine and improves digestion and nutrient absorption. The authors found that the pH value of the caecal mucosa ranged from 6.0 to 6.6. If the pH value is too high (greater than 7.5), it will affect the activity of digestive enzymes and lead to the proliferation of pathogenic bacteria, which may provoke the onset of diarrhoea in rabbits. The appropriate pH range of the intestine is maintained, as noted by A.B. Negussie *et al.* (2022), by the epithelial cells of the large intestine. These cells establish osmotic gradients, which are key for retaining fluid and maintaining the level of secretion, as well as regulating cell death. Furthermore, S. Li *et al.* (2023) found that the ratio of fibre content in the diet of rabbits affects the pH value in the caecum. This influences the immunomodulatory function of the intestine and the level of nutrient absorption.

During the experiment, it is important to adhere not only to a specific dose of the preparation but also to the age periods of the experimental animals, which are associated with the transition to a different type of feed. Therefore, to investigate the quantity of cecal microbiota, rabbits aged 45 days were used, that is, during the weaning period and the transition to a plant-based diet. S.M. Leite *et al.* (2024) emphasised that after weaning rabbit kits (from 40 days of age) from the mother, a series of physiological changes occur in the gastrointestinal tract depending on the type of feed. T. Read *et al.* (2023) also indicated that from the 43rd day of life in rabbits consuming solid feed, the structure and interrelationships within the gut microbiome change. Furthermore, E. Cotozzolo *et al.* (2021) demonstrated that the greatest diversity of the microbiome is found in the caecum and throughout the large intestine of rabbits. In particular, clarifying the quantity and species-specific characteristics of the rabbit gut microbiota will help improve their health and contribute to increased productivity.

The establishment of the complex effect of the components of the studied prebiotic on the ratio of microflora in the rabbit caecum proved to be positive. It can be suggested that due to the presence of the lactic acid bacterium strain *Lactobacillus bulgaricus delbrueckii* in the studied prebiotic, such a ratio of microflora in the large intestine was maintained, which contributed to the preservation of growth, development, and overall health indicators in rabbits. K. Song *et al.* (2024) noted that one of the representatives of the beneficial gut microflora involved in the restoration of microflora is the lactic acid bacterium strain – *Lactobacillus delbrueckii* subsp. *Bulgaricus*. The authors demonstrated the beneficial effect of this strain on the experimentally damaged mucosa of the large intestine in mice, as well as the restoration of the intestinal microbiota and the improvement of the intestinal condition. C. Zhao *et al.* (2021) found that supplementing the feed of lactating rabbits with *B. subtilis*, *B. Licheniformis*, and *S. cerevisiae* increases the populations of beneficial gut bacteria, improves nutrient digestibility, enhances fermentation in the caecum, increases feed conversion, and promotes body weight gain. Thus, supplements with live bacterial cultures in rabbit feed are an alternative to antibiotics and significantly affect the improvement of productivity, growth, prevention and treatment of intestinal diseases, as well as increasing animal immunity.

Overall, the positive effect of the studied prebiotic on the bodies of white laboratory mice and rabbits was manifested as a successful symbiotic action of the lactic acid bacterium *Lactobacillus bulgaricus delbrueckii* together with amino acids and vitamins. J.E. Nettleton *et al.* (2021) demonstrated that the prevention and treatment of mice with prebiotics, probiotics, and synbiotics altered the species composition of their gut microbiota and led to improved intestinal function. In this context, A. Bevilacqua *et al.* (2024) presented the updated definition of prebiotics by a consensus panel of the International Scientific Association for Probiotics and Prebiotics (ISAPP): “prebiotics are substrates that are selectively utilized by host microorganisms conferring a health benefit”.

Thus, the complex prebiotic, when fed to white laboratory mice and Grey Giant rabbits, contributed to an increase in body weight, red blood cell count, haemoglobin and globulin levels, and liver enzyme activity, as well as a decrease in cholesterol levels. Furthermore, the percentage composition of Gram-positive microflora in the caecum of rabbits predominated over Gramnegative microflora. The validity of the experimental studies was confirmed by the indicators of the microclimate and the drinking water provided to the laboratory animals (white mice and rabbits), which met the requirements of the current regulatory documents. The presented research results confirm the need to consider the age of the experimental animals, the type of their feed, and the dosage of the prebiotic. This scientific approach is important for the further practical application of the obtained results, for maintaining animal health, and for reducing mortality rates.

Conclusions

The conducted study has revealed the positive effect of the components of the Bio-active prebiotic at a dose of 1.2 g per animal on the bodies of white laboratory mice and rabbits. It was established that the microclimate parameters in the vivarium where the white mice and rabbits were kept, as well as the indicators of drinking water provided to them, met the requirements of the current regulatory documents. Feeding the Bio-active prebiotic at a dose of 1.2 g per animal contributed to an increase in the body weight of white mice in the experimental group compared to the control group throughout the

entire study period (day 14 by 43.6% ($P < 0.001$); day 30 by 17.65% ($P < 0.05$); day 60 by 15.84% ($P < 0.05$)), as well as compared to the indicators at the beginning of the study (day 14 by 43.6% ($P < 0.001$); day 30 by 81.82% ($P < 0.001$); day 60 by 132.73% ($P < 0.001$)). During the experiment, the average daily weight gain of white mice in the experimental group also increased (day 14 by 59.3% ($P < 0.001$); day 30 by 39.64% ($P < 0.001$); day 60 by 12.33% ($P < 0.001$)). The mass of the studied internal organs (thymus, liver, spleen, thyroid gland, and kidneys) in the laboratory mice did not change significantly between the experimental and control groups of animals. The studied prebiotic at a dose of 1.2 g per animal positively influenced the increase in body weight, absolute and relative weight gain in rabbits, which improved survival rates and growth energy. The prebiotic contributed to the activation of erythropoiesis in the bodies of the experimental white mice and rabbits. In the blood of white mice, the red blood cell count and haemoglobin concentration increased by 6.4% ($P < 0.05$) and 10.1% ($P < 0.05$), respectively, and in the blood of rabbits – by 11.7% ($P < 0.05$) and 12.6% ($P < 0.05$), respectively. The effect of the prebiotic on the bodies of the experimental animals was expressed in an increased activity of the humoral immunity component, which manifested in an increase in the blood serum of white mice in the content of total protein by 11.1% ($P < 0.05$) and globulins by 1.4 times ($P < 0.01$), and in rabbits – by 10.2% ($P < 0.05$) and 1.2 times ($P < 0.05$), respectively. The activity of aspartate aminotransferase and alanine aminotransferase, and the cholesterol content in the blood serum of white mice and rabbits in the experimental and control groups, remained unchanged. A positive effect of the studied prebiotic on the composition of the caecal microbiome of rabbits in the experimental group was established, which contributed to an increase in the total number of Gram-positive microorganisms by 19.4% ($P < 0.05$) and a decrease in Gram-negative ones by 17.7% ($P < 0.05$). The effect of the preparation at a dose of 1.2 g per animal on the bodies of white laboratory mice and rabbits contributed to an increase in the indicators of growth and development of the body, and the functional capacity of the large intestine, which affects the maintenance of animal health.

The obtained results of the experimental study may be useful for clarifying the complex effect of the supplement components on the bodies of laboratory animals. In the future, further research will be devoted to determining the species composition of the large intestine microbiome in rabbits.

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Conflict of Interest

None.

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Вплив пребіотику на розвиток лабораторних тварин

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Анотація. Пребіотики поліпшують стан здоров'я тварин, але їх позитивна дія на організм залежить від складу та дози препарату, віку та виду тварин. Мета роботи полягала у дослідженні дії на організм білих мишей і кролів нового комплексного пребіотику «Біо-актив». У досліді використовували 40 голів білих лабораторних мишей і кролів. Пребіотик згодовували піддослідним тваринам у дозі 1,2 г/голову. Застосували класичні методики для оцінювання мікроклімату, показників якості водопровідної води для напування тварин, динаміки приростів, гематологічних показників, мікроскопії мазків слизової оболонки сліпої кишки (у кролів). Встановлено, що параметри мікроклімату і показники якості води відповідали вимогам чинних нормативних документів. Згодовування пребіотику білим лабораторним мишам призводило до поступового збільшення маси тіла та середньодобових приростів упродовж всього періоду дослідження. У кролів зростала маса тіла та величина абсолютного і відносного приростів, що сприяло покращенню збереженості та енергії росту тварин. Доведено, що у разі додавання до кормів у годівлі тварин пребіотику збільшувалася кількість еритроцитів, вміст гемоглобіну, загального білка та глобулінів. Виявлено вплив пребіотику на кількість грампозитивних і грамнегативних мікроорганізмів та їх співвідношення у сліпій кишці кролів. При

цьому, кількість грамнегативних мікроорганізмів зменшувалася на 17,7 % ($P < 0,05$), а грампозитивних збільшувалася на 19,4 % ($P < 0,05$). Величина pH вмісту сліпої кишки кролів дослідної групи відповідала значенню 6,5 проти 7,1 у контролі, що свідчило про відновлення функціональної здатності товстого відділу кишечнику. За отриманими результатами дослідження можна констатувати позитивний вплив компонентів пребіотику у дозі 1,2 г/голову на прирости маси тіла, гематологічні показники та кількісний склад мікробіому товстого відділу кишечнику, що важливо для вирощування здорового поголів'я тварин і виробництва якісних харчових продуктів

Ключові слова: білі миші; кролі; умови утримання; інтенсивність росту; гематологічні показники; мікроорганізми; сліпа кишка

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Effects of probiotic feed supplement on antibiotic resistance of *E. coli* cultures in puppies

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Abstract. The purpose of this study was to evaluate the sensitivity of *Escherichia coli* to antibiotics in the context of supplementing the diet of three-month-old French bulldog puppies with a probiotic as a biologically active additive. The study employed standard clinical, microbiological, and bacteriological methods, specifically, the disk-diffusion method to assess the sensitivity of *Escherichia coli* cultures to antibiotics and the general bacteriological contamination of faeces samples of puppies of one control group and two experimental groups of puppies that received a probiotic preparation as a feed supplement to the main diet. The study of the effects of the probiotic on the intestinal microflora of puppies found that the use of the probiotic significantly influenced the level of bacterial contamination of the stool samples of the experimental groups. The results of determining the sensitivity of *Escherichia coli* culture to 14 antibiotics were provided. The sensitivity of the culture to the selected antibiotic was determined by the size of the diameter of the growth retardation zones of microorganisms. The findings of the study revealed that the cultures were reliably susceptible to fosfomycin, kanamycin, and azithromycin. The addition of a probiotic symbiotic preparation based on *Bacillus subtilis* and *Bacillus licheniformis* to the diet of the dogs had a significant effect on sensitivity in 78.6% of combinations of conditions and antibacterial substances. Considering the current findings of analogous studies of the preparation on poultry and data on the improvement of biosynthetic

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processes in the digestive tract, indicators of poultry productivity, as well as an increase in the weight of individuals and the intensity of metabolic processes in the body, there are significant prospects for further research in this area and a more detailed study on the use of probiotic therapy in healthy puppies. The findings of the present study can be useful for veterinarians, scientists, and kennel workers of the Kennel Club of Ukraine, the Canine Service of the Armed Forces of Ukraine, and other law enforcement agencies that directly maintain service dogs

Keywords: resistance; *Escherichia coli*; sensitivity of bacteria; probiotic therapy; probiotic

Introduction

Antibiotics were one of the most significant discoveries of the 20th century, saving millions of lives from infectious diseases. The high selective pressure resulting from the proliferation and misuse of antibiotics over the years has led to the development of antimicrobial resistance to many agents (Saha & Sarkar, 2021). Numerous interrelated factors in healthcare and agriculture contribute to the development of antimicrobial resistance through various mechanisms of drug resistance. This was largely facilitated by the spread of infection due to the uncontrolled use of antimicrobial drugs in livestock feed. The prevalence of antimicrobial resistance has reached unprecedented levels worldwide, becoming a silent pandemic that threatens global public health and requires immediate intervention (WHO, 2016). Infections brought on by bacteria resistant to antibiotics have few viable therapeutic choices. This results in serious economic repercussions as well as a marked rise in morbidity and death. According to M. Salam *et al.* (2023), the lack of discovery and availability of new antibiotics to treat life-threatening infections caused by resistant pathogens is in stark contrast to the demand. A. Parmanik *et al.* (2022) noted that the rapid spread of antibiotic-resistant pathogens requires the search for natural antibacterial agents. Studying the epidemiology, detection, and clinical management of drug-resistant bacteria is vital for monitoring, diagnosing, and treating drug-resistant bacterial colonies in hospital-acquired and community-acquired outbreaks (Van Camp *et al.*, 2020). Y. Zhu *et al.* (2022) highlighted the clinical implications of antimicrobial resistance in bacteria, focusing on its significant threat to public health. The researchers detailed the mechanisms by which bacteria develop resistance, including genetic mutations and horizontal gene transfer, and emphasised the challenges in treating infections caused by multidrug-resistant strains. The researchers also discussed the global burden of antimicrobial resistance, stressing the urgent need for coordinated efforts in antibiotic stewardship, infection prevention, and the development of novel antimicrobials.

According to L. Zhou *et al.* (2024), probiotics, prebiotics, and synbiotics are increasingly being used in modern animal feeding regimens as effective alternatives to antibiotics, with significant benefits for gut health and overall performance. Probiotics enhance the population of beneficial gut bacteria, regulate microbiota, reduce harmful bacteria, and improve digestion and nutrient absorption, contributing to better feed conversion ratios, growth rates, and carcass yields. Prebiotics, such as mannan oligosaccharides, provide substrates for beneficial bacteria, further supporting gut health and microbial balance. Synbiotics, combining prebiotics and probiotics, have shown even greater efficacy, improving broiler performance more

effectively than either additive alone. Together, these feed additives improve the microbial composition of the gastrointestinal tract, boost resistance to harmful bacteria, and enhance metabolic functions, making them powerful tools for sustainable animal production.

T. Uddin *et al.* (2021) noted that novel approaches like whole genome sequencing, bacteriophage therapy, monoclonal antibodies, and quorum-quenching mechanisms offer promising alternatives. One particularly fascinating insight is the resurgence of bacteriophage therapy, which predates antibiotics and is now being revisited as a targeted and environmentally friendly solution to combat resistant bacterial strains. This analysis underscores the urgency of innovation and collaboration to address the antimicrobial resistance crisis and protect global health. According to C. Brives & J. Pourraz (2020), phage therapy, an alternative to treating bacterial infections using bacteriophage viruses, has been around for over a century. Despite gaining support over the past 15 years from researchers who have utilised it as a promising treatment in the face of rising resistance, this treatment has been challenging to develop.

According to O. Shkromada *et al.* (2022), modern feeding strategies based on the use of probiotic preparations are aimed at reducing the colonisation or population of pathogenic bacteria in the intestines of animals and increasing the amount of beneficial microflora for example in piglets. At the same time, the ban on the use of antibiotics as animal growth stimulators and their limited use for preventive or therapeutic purposes led to an increase in the use of probiotic preparations in compound feed (Yue *et al.*, 2020). The range of probiotics available on the market is growing every year. Moreover, the newly created categories of probiotics differ from each other in terms of properties and mode of action. In the countries of Europe, Asia, and in Ukraine, probiotics are widely used as an alternative to antibiotics in the poultry industry, particularly for growing broiler chickens and the subsequent production of meat and poultry slaughter by-products. The use of probiotic preparations in poultry organisms increases the stimulation of biosynthetic processes in the digestive tract and increases poultry productivity, including livestock preservation, poultry growth, a reduction in the amount of time that poultry are fed, an increase in the weight of poultry carcasses and offal, and improved metabolic processes in the body of grill chickens (Bohatko, 2023). The probiotic is used as a biologically active additive to feed poultry, pigs, cattle, and rabbits to form the normal microflora of gastrointestinal diseases, stabilising the body's defences, increasing productivity, and preserving livestock (Tian *et al.*, 2021). According to A. Hasan *et al.* (2022), the use of additives in the early spring feeding of bee families helps to increase the wax-making activity of bees, as well as honey productivity and honey collection in the spring-summer period. Notably, there is no data that would detail the results of the use of probiotics in puppies. In this regard, the study of the effect of probiotics on the dog population was conducted. Thus, the purpose of the present study was to investigate the effects of probiotics on the antibiotic resistance of the microflora of healthy puppies at varying dosages of the drug.

Materials and Methods

Preliminary analysis of literary sources necessitated the study of the effects of the drug "Subtiform" on the body of the bird, which improved the bird's overall output and the activation of biosynthetic processes in the digestive system. The use of probiotics contributed to the improvement of metabolic processes in the body, the preservation of livestock, the growth of poultry, and at the same time, the reduction of the duration of poultry feeding,

the increase in the weight of the carcass and offal of broiler chickens (Bohatko, 2023). The experiment was conducted in October-November 2024, based on the nursery in Bila Tserkva, Ukraine. The study was conducted in dogs of the French bulldog breed (n = 15). Groups were formed from dogs of the same age and weight characteristics (puppies aged 3 months). The biochemical indicators were weighed and analysed before the start of the experiment and weekly. The dose per dog was adjusted according to the baseline age, weight, and final weight at the end of the study. Probiotic "Subtiform" (TC U 10.9-30165603-027:2023, Ukraine) is a preparation of a symbiotic nature, containing bacteria of the genus *Bacillus subtilis* and *Bacillus licheniformis* in the amount of 2.5×10^9 CFU/g and a filler-dry milk whey. For dogs, the dosages of the probiotic in the experimental groups were as follows: 0.5 g per 10 kg of live weight and 2.0 g per 10 kg of live weight. Probiotic was not used in the control group. The duration of the study was 30 days. Sampling was performed in individuals during sedation with the drug "Madison" subcutaneously, in the amount of 0.3 mL per 10 kg of live weight, faeces were collected directly from the rectum, in sterile gloves, and in sterile containers that were sent to the Control and Testing Laboratory of Food and Agricultural Products of the Kharkiv Regional Consumer Union (Kharkiv, Ukraine) in refrigeration units. The weight of each sample was 10 g.

The sensitivity of the cultures isolated from the samples to antibiotics was determined according to the method of determining the sensitivity of microorganisms to antibacterial drugs (Harkavenko *et al.*, 2015). The general level of tank contamination and the presence of microorganisms of the genus *Proteus* were determined following the methodological recommendations for the bacteriological analysis of animal feed (ISO 4831:2006). Enteropathogenic *Escherichia coli* in 1 g was determined according to the method of DSTU 8680:2016. The level of colonisation of the medium by enterococci was carried out by the general principles and methodical instructions of DSTU 8534:2015. All the studies were conducted following the European Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (1986) and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006). Statistical processing was performed using the software Statistica 6.0 and Microsoft Excel.

Results and Discussion

The study of the effects of the probiotic on the intestinal microflora of puppies found that the use of the probiotic significantly influenced the level of bacterial contamination of the stool samples of the experimental groups (Table 1).

Table 1. Effects of the probiotic on the total bacterial contamination of the samples

	Control		Experimental group 1		Experimental group 2	
Total bacterial contamination	before	2.43×10^8 $\pm0.12\times10^8$ CFU	before	2.17×10^8 $\pm0.11\times10^8$ CFU	before	2.46×10^8 $\pm0.12\times10^8$ CFU
	after	2.48×10^8 $\pm0.12\times10^8$ CFU	after	$^*1.91\times10^8$ $\pm0.09\times10^8$ CFU	after	$^*2.15\times10^8$ $\pm0.11\times10^8$ CFU

Note: ** – the difference is significant at $P<0.05$; CFU – colony-forming unit*
Source*: developed by the authors of this study based on the findings*

General examination showed that before the start of probiotic therapy, organisms of the genus *Proteus* were detected in three samples, and no causative agents of diplococcal

infection were isolated from the material. On the other hand, it was not possible to establish the presence of microorganisms of the genus *Proteus* and isolate the causative agents of diplococcal infection in the samples isolated for the 4th week of the experiment. The presence of enteropathogenic *Escherichia coli* was established in all samples.

It was found that even the use of a lower dosage of the drug helped to significantly reduce the number of bacteria in the samples. The effectiveness of the drug in the experimental group with a greater concentration of the drug in the feed was lower compared to the indicators of the experimental group 1 on the 4th week of the study. Despite this, the indicators of both groups were significantly lower than the values obtained in the control group. Such a regularity may suggest the absence of a cumulative effect from the use of probiotics as a biological supplement to the main diet of dogs. However, this implies the possibility of using the probiotic as an auxiliary component to increase the effectiveness of empiric therapy with antimicrobial drugs. It is known that the use of probiotics allows making the treatment of sick individuals more qualitative, safer, and economically profitable (Pentylyuk, 2006; WHO, 2016). This was confirmed by analogous studies of Ukrainian scientists, which indicated that the use of probiotic preparations in the composition of granulated compound feed contributed to increasing the productivity of young pigs, reducing feed costs by 0.15-0.25 energy feed units (Hutsol *et al.*, 2023). Other scientists, such as P. Markowiak & K. Śliżewska (2017), also obtained analogous findings.

Notably, the obtained findings may also be related to the possibility of reducing the spread of antimicrobial resistance and other undesirable phenomena from the use of antimicrobial drugs. In this regard, the effect of probiotics on the sensitivity of *E. coli* cultures to antibiotics was investigated (Table 2).

Table 2. Sensitivity of selected cultures of *E. coli* to antibiotics

Variant name of antibiotic	1st week			4th week		
	Control	Experimental group 1	Experimental group 2	Control	Experimental group 1	Experimental group 2
Levofloxacin	24±1.20	24.2±1.21	*20.8±1.04	21.8±1.09	*16.6±0.83	*24.0±1.20
Cefepime	26.4±1.32	26.6±1.33	26.8±1.34	27.4±1.37	*22.0±1.10	27.0±1.35
Gentamicin	24.2±1.21	*21.0±1.05	23.2±1.16	23.8±1.19	23.4±1.17	23.0±1.15
Norfloxacin	24.0±1.20	24.0±1.20	25.0±1.25	25.4±1.27	*21.2±1.06	25.0±1.25
Ceftriaxone	23.4±1.17	21.8±1.09	*21.0±1.05	21.8±1.09	21.8±1.09	20.0±1.0
Ofloxacin	24.6±1.23	25.4±1.27	25.6±1.28	24.6±1.23	24.8±1.24	25.0±1.25
Kanamycin	22.4±1.12	21.8±1.09	23.4±1.17	25.0±1.25	*22.0±1.10	*20.0±1.0
Nitrofurantoin	22.0±1.10	23.8±1.19	*26.6±1.33	26.8±1.34	*20.0±1.0	*20.0±1.0
Azithromycin	23.8±1.19	24.4±1.22	25.2±1.26	26.0±1.30	*21.2±1.06	*20.0±1.0
Chloramphenicol	23.8±1.19	*26.6±1.33	24.4±1.22	23.8±1.19	25.8±1.29	25.0±1.25
Doxycycline	3.8±0.19	*8.2±0.41	*10.8±0.54	10.0±0.5	10.2±0.51	10.0±0.50
Cefazolin	2.0±0.10	*9.0±0.45	*11.0±0.55	11.2±0.56	*13.4±0.67	10.0±0.50
Fosfomycin	–	*1.8±0.09	*2.4±0.12	2.4±0.12	*–	*–
Enrofloxacin	2.2±0.11	*2.0±0.10	*–	–	*18.0±0.90	–

Note: ** – the difference is significant at P < 0.05*
Source*: developed by the authors based on the research*

The significant differences were established in indicators of sensitivity to antibiotics of *E. coli* cultures isolated from the intestines of puppies at the beginning of the study

and the end of the experiment. Table 2 lists the names of 14 antibiotics whose diameters of growth zones were the largest: cultures that formed growth zones with a diameter of 1-10 mm were considered weakly sensitive, 10-20 mm – sensitive, and over 20 mm – highly sensitive. The absence of zones of growth retardation indicated the resistance of *Escherichia coli* to the antibiotic.

The indicators of sensitivity of *E. coli* to fluoroquinolone antibiotics (levofloxacin, norfloxacin, and enrofloxacin), as well as to doxycycline, a representative of the tetracycline group of antibiotics, are significant, as the issue of the development of resistance to them is quite acute since the phenomenon is developing quite rapidly. The sensitivity of the cultures to the action of the representative of nitrofurans, nitrofurathion, was significantly higher in experimental group 2 at the beginning of the study, but these indicators significantly decreased after long-term use of the probiotic. This was also confirmed by the findings of other scientists who used a multi-strain probiotic to treat the consequences and symptoms of gastroenteritis, where the improvement occurred as early as on the 7th day of using the drug (Molina *et al.*, 2023). B. Han *et al.* (2024) demonstrated an analogous effect for *Lactobacilli*, which could be a good choice to improve the gut health and immune functions of cats, being associated with the lipid mechanism of cats. The effect of the probiotic on the sensitivity of microorganisms to kanamycin, which is typically active against *Escherichia coli*, and fosfomycin, which rarely develops resistance due to its unique mechanism of action, was expected (O'Brien, 1997; Nunan, 2017). The effects of probiotic on the sensitivity of selected cultures to chloramphenicol, which has a bacteriostatic effect, was analogous, although it was characterised by the rapid development of resistance of pathogenic microorganisms to it, including due to the natural origin of the antibiotic.

As a result of the study, the sensitivity to antibiotic drugs fosfomycin, kanamycin, and azithromycin increased significantly. As for the increased sensitivity to the latter, it was related to the macrolide adsorption site and, accordingly, the synergistic effect of the antibiotic with the symbiotic probiotic. Notably, the sensitivity of *Escherichia coli* stayed high under the action of antibiotics in 78.6% of cases. Thus, the use of probiotics contributed to the formation of fairly high resistance indicators in puppies, even during a fairly short period of use.

Conclusions

During the long-term use of the probiotic, the indicators of the total contamination of the stool samples of the French bulldog puppies significantly decreased, while the indicators of the sensitivity of the *Escherichia coli* culture to the antibiotics fosfomycin, kanamycin, and azithromycin increased, which is associated with the synergism of the action of the probiotic and antibacterial substances. It is known that the use of probiotics allows making the treatment of sick individuals more qualitative, safer, and economically profitable. Furthermore, such an effect on microbiological indicators, specifically, the reduction of microbiological contamination, can be achieved even during a short period of use of the supplement. In addition, the indicators of sensitivity of cultures selected according to bacteriological methods of research suggest the significant synergism in the action of probiotics and antibiotics, which is currently quite significant in the global strategy of combating the emergence of antibiotic resistance in pathogenic microorganisms. Reducing the level of contamination of faeces is a prerequisite for reducing the probability of the developing a more stable immunity and own intestinal microbiota. It also allows reducing

potentially dangerous risks for human health, other animals, and the environment. Such features of the effect of the studied probiotic will be of great interest to specialists in the kennels of the Ukrainian Kennel Service and structures where the well-being of animals is not only a generally accepted area of work, but also a basis for the national security of the country.

Analogous studies testify to the effects of probiotics on metabolic processes, weight gain, and biochemical indicators, which gives grounds for further research. Building on these findings, future studies could focus on exploring the long-term effects of probiotics on broader aspects of animal health, including their role in modulating immune responses and enhancing resistance to various environmental pathogens. Investigating the specific mechanisms underlying the synergistic effects of probiotics and antibiotics could provide valuable insights for optimising combined therapeutic approaches. Additionally, further studies are required to assess the effects of probiotics on metabolic functions, growth performance, and the prevention of antibiotic-resistant infections in various breeds and age groups.

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Conflict of Interest

None.

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Вплив пробіотичної кормової добавки на антибіотикорезистентність культур *E. coli* у цуценят

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Анотація. Метою дослідження було оцінити чутливість *Escherichia coli* до антибіотиків у контексті додавання пробіотика як біологічно активної добавки до раціону тримісячних цуценят французького бульдога. Робота виконана за допомогою загальноприйнятих клінічних, мікробіологічних та бактеріологічних методів дослідження, зокрема дисково-дифузійного методу, з метою оцінки чутливості культур кишкової палички до антибіотиків та загальної бактеріологічної забрудненості зразків калу цуценят однієї контрольної групи та двох експериментальних груп цуценят, що отримували в якості кормової добавки до основного раціону пробіотичний препарат. Під час дослідження впливу пробіотика на мікрофлору кишечнику цуценят було встановлено, що використання пробіотика достовірно впливало на рівень бактеріальної забрудненості зразків калу цуценят експериментальних груп. Наведено результати визначення чутливості культури кишкової палички до 14 антибіотиків. За розміром діаметра зон затримки росту мікроорганізмів визначали чутливість культури до обраного антибіотика. Результати досліджень засвідчили, що достовірно високочутливими культури були до фосфоміцину, канаміцину та азитроміцину.

Додавання до раціону собак пробіотичного симбіотичного препарату на основі *Bacillus subtilis* і *Bacillus licheniformis* мало суттєвий вплив на чутливість у 78,6 % комбінацій умов та антибактеріальної речовини. Зважаючи на сучасні результати аналогічних досліджень препарату на поголів'ї птиці та дані щодо поліпшення біосинтетичних процесів у травному тракті, показники продуктивності птиці, а також збільшення ваги особин та інтенсивність обмінних процесів в організмі, існує значна перспектива подальших досліджень у цьому напрямі та перспектива більш детального вивчення питання використання пробіотикотерапії у здорових цуценят. Дослідження може стати корисним для ветеринарів, науковців та працівників розплідників Кінологічної спілки України, Кінологічної служби ЗСУ та інших силових структур, що безпосередньо утримують службових собак

Ключові слова: резистентність; кишкова паличка; чутливість бактерій; пробіотикотерапія; пробіотик

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Forensic veterinary examination of animal bodies injured by glass fragments

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Abstract. Pre-trial investigation of offences against animal health and life is impossible without the use of specialised knowledge by law enforcement agencies or the court, specifically veterinary and technical knowledge, the perfect form of which is a comprehensive forensic veterinary examination with the study of the instruments of injury: materials, substances, and products. In such cases, the subject of the forensic examination raises questions for the forensic experts to resolve regarding the nature, location, severity, and type of instrument that could have caused a certain injury to the animal's body. Considering the above, the purpose of the present study was to substantiate and develop an algorithm for forensic detection and identification of glass fragments removed from the bodies of injured animals. The study employed a set of modern research methods, including radiographic, ultrasonographic, visual, microscopic, physicochemical, X-ray fluorescence, and statistical analysis, which revealed new data on the informativeness of radiography, and

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ultrasonography as non-invasive methods of detecting foreign objects in the bodies of dead dogs, as well as the possibility of identifying the entire instrument of injury by individual fragments (glass fragments). Thanks to the findings obtained using an integrated approach to detect and identify foreign objects removed from the bodies of injured animals during forensic veterinary examinations, a series of significant issues were resolved: their presence in the body of animals, their attribution to inorganic silicate glass with a detailed description of its chemical composition, and identification of the instrument of injury by its individual parts were confirmed. This study will positively influence the efficiency and effectiveness of forensic examination of animals affected by severe injuries caused by foreign objects removed from their bodies. At the same time, this comprehensive approach allows increasing the degree of validity and objectivity of the expert's opinion as a means of proof in categorical proceedings and expands the evidentiary capabilities of pre-trial investigation bodies and the court

Keywords: comprehensive forensic examination; identification of materials; dogs; instrumental and microscopic examinations; puncture wound; piercing and cutting instruments; animal cruelty

Introduction

The relevance of the subject under study is that forensic veterinary examination (FVE) is actively developing in Ukraine, as well as in many countries of the world. Its problematic issues and ways of solving them have been analysed by many researchers, including the authors of the present study. A. Rebollada-Merino *et al*. (2020) and R. Munro (2022) emphasised that the principal task of the forensic science is to establish the facts and circumstances of the offence related to the determination by the forensic veterinary expert of the damage caused to the health and life of the animal. The forensic veterinary expert determines the nature of the damage caused to the animal based on the application of special veterinary knowledge, by conducting a comprehensive study of material and materialised objects as carriers of information, using relevant means (methods) to solve the identification, diagnostic, and situational tasks of the FVE. Considering the above, according to L. Reese *et al*. (2020) and M. Connor *et al*. (2021), law enforcement agencies and the court must use specialised knowledge to clarify the facts and circumstances of the case during the pre-trial investigation or judicial investigation of animal cruelty as a shameful phenomenon against public morality. D. Kolodin & A. Kolodina (2023) and O. Pirgo (2024) noted that in Ukraine, liability for this offence is prescribed by criminal and administrative Ukrainian legislation, specifically, Article 299 of the Criminal Code of Ukraine and Article 89 of the Code of Administrative Offences of Ukraine.

I. Yatsenko & O. Parilovskyi (2020) proved how animal cruelty is accompanied by the infliction of bodily harm and mutilation to animals. The researchers argued that the qualification of the offence of animal cruelty should factor in the opinion of a forensic veterinary expert on the nature and severity of bodily injuries, as well as the causal relationship between the injuries identified by the expert and the health disorder, injury, or death of the animal. J. Cooper & M. Cooper (2021) and Yu. Fedyk & I. Besaha (2023) proved that forensic examination is a scientifically based means of proof, while the opinion of a forensic expert is evidence in court. R. Rosa & R. Buckley (2024) found that it

is essential to establish the type of instrument that caused the injury, specifically, sharp instruments, when investigating offences against animal health and life. Therewith, A. Davros *et al*. (2023) established the nature of injuries in animals caused by blunt objects. Foreign objects that can be found in the wound channel of an injured animal are also of substantial relevance (Lee *et al*., 2022).

Despite great strides in forensic veterinary examination, the information available in scientific sources on the algorithm for forensic detection and identification of foreign objects removed from the bodies of injured animals relates mainly to legal aspects and assessment of the nature of animal injuries. At the same time, the scientific literature has not yet described an algorithm for forensic veterinary examination of animals injured by sharp objects, nor has it tested the use of supplementary technical and instrumental examinations of injured animals (X-ray, ultrasonographic), nor methods of identifying foreign objects. Therefore, certain aspects of the substantiation and development of an algorithm for detecting and identifying foreign objects removed from the bodies of injured animals are quite problematic, understudied, and must be addressed. The purpose of the present study was to identify the principal factors for conducting a forensic veterinary examination, to describe the injuries inflicted on animals, and to identify foreign objects removed from their bodies.

Literature Review

Many researchers have addressed the problem of forensic detection and identification of foreign objects removed from animal bodies in case of injuries of various kinds. Thus, T. Saul *et al.* (2016) highlighted that the situations when sharps injuries occur may include social violence, accidents, hunting, veterinary care, religious rituals, etc. Accurate documentation and examination of these injuries, as emphasised by A. Van Neer *et al*. (2021), J. Rosa *et al*. (2022), may suggest the instrument of injury, the relative position of the animal and its offender, and the force with which the injury was inflicted. Thus, M. Skrypka *et al*. (2023) revealed the pathogenesis and detailed the pathomorphological signs of spinal cord injury with a sharp object in a domestic cat. J. Love (2019) proposed the use of light microscopy, microcomputer scanning, scanning electron microscope and epifluorescence microscopy, which together can help to identify the instrument that caused a particular injury. According to J. Voss *et al.* (2021), open injuries carry the risk of contamination by foreign bodies. Therefore, an accurate clinical examination is the first step in detecting foreign bodies in both human and animal bodies, but is not sufficient to identify their type, which requires further investigation. Therefore, the choice of a suitable imaging method that is a useful tool for finding foreign bodies is crucial for their detection and analysis.

Scientific research shows the capabilities of various imaging methods for detecting foreign bodies in soft tissues. A. Ayalon *et al.* (2021) and M. Blondel *et al.* (2021) substantiated the feasibility of using various research methods in the forensic examination of animals with injuries, including plain radiography, computed tomography, ultrasonography, magnetic resonance imaging, etc. R. Corzo & E. Steel (2020) and R. Corzo *et al.* (2021) argued the possibility of determining the nature of foreign objects by their different chemical composition, including glass fragments. In addition, A. Alfuraih *et al.* (2022) proposed using such research methods to detect plant particles in the pig's body, E. Palazzo *et al.* (2018) – signs of wound metallisation, etc.

Computed tomography is essential for determining the location of foreign bodies and the relationship with the surrounding structures,

as well as the depth of the injury. However, magnetic resonance imaging is of little use for detecting foreign bodies. Furthermore, J. Del Cura *et al.* (2020) confirmed the effectiveness and efficiency of ultrasonography for the detection and removal of various foreign bodies (plant, metal, and glass objects, etc.), although the researchers emphasised that the signs of ultrasound examination depend on the type and shape of the foreign body. However, all foreign bodies are echogenic. F. Bosma *et al.* (2022) illustrated the possibility of visualising a foreign body with a woody structure in the soft paravertebral tissues and spinal canal of a dog using computed tomography. A. Kashani-Carver *et al.* (2023) reported the diagnosis of an intralenticular foreign body in a dog, and K. Hellbach *et al.* (2018) reported the successful intraoperative removal of a foreign body in a 4-month-old puppy from the cranial cavity under ultrasound guidance.

Undoubtedly, the scientific achievements of the above-mentioned scientists have contributed to solving some problems in the study of foreign objects removed from animal tissues, including glass fragments, which may originate from the instrument of animal injury in case of animal cruelty and may also become material evidence in criminal proceedings, but have not attracted the proper attention of forensic veterinarians. There are no theoretical developments or extensive empirical studies of the problem of detecting and identifying foreign objects of various origins from animal bodies. Therefore, to summarise, the issue of detection and identification of foreign objects and particles in animal wounds is quite problematic and therefore requires further investigation and testing in forensic practice.

Materials and Methods

The study was conducted in 2023-2024 at the Laboratory of Forensic and Military Research of the National Scientific Centre "Mykola Bokarius Institute of Forensic Expertise" of the Ministry of Justice of Ukraine (Kharkiv) in several stages. At the first stage, a forensic veterinary examination of injured animals was performed following the Methodology of forensic veterinary examination of a living animal (Yatsenko & Parilovskyi, 2023). Radiographic examinations were performed using a digital X-ray machine "Diagnostic X-RAY UNIT (model: PXM-408T PLUS)" (China). Ultrasonographic examinations were performed using a Chison CBit 9 device with a Chison linear transducer (China).

At the second stage, foreign objects found in the wounds of dogs were examined following the Forensic Examination of Glass and Glass Products (2016) and the Forensic Examination of the Material of Glass Products Installed on Vehicles (2016). For the purpose of laboratory research, the following were used: polymer scale ruler for photography (Ukraine); microscope MBS-9, magnification 4.5-63$^{\times}$ (Ukraine); microscope KERN OZP 558 with camera KERN ODC-82/ODC-83 (Ukraine); metal measuring ruler LRI 300 (Ukraine); caliper ШЦ-I-125-0.5 (Ukraine), measuring range 0-250 mm, $\Delta = \pm 0.1$ (Ukraine); energy X-ray spectrometer SER-01 (ElvaX Pro) (Ukraine); polarising microscope Ulab XP-501 LM 19095M013 (Ukraine); digital camera Canon Power Shot A3100IS (12.1 megapixels) (Ukraine). The external examination and study of glass fragments was performed visually in daylight and artificial light and in the field of view of the MBS-9 microscope (Ukraine) (magnification up to 63$^{\times}$, reflected light), KERN OZP 558 microscope with KERN ODC-82/ODC-83 camera (Ukraine) and Ulab *XP*-501 LM 19095M013 polarising microscope (Ukraine).

To establish the absence of the effect of double refraction of glass fragments removed from dog wounds, the latter were placed in the focus of a polarising microscope Ulab *XP*-501

LM 19095M013 (Ukraine). Examination in polarised light revealed the absence of the pleochroism effect, which indicated the amorphous structure of the fragments removed from the wounds of the dogs and the object seized at the scene of the offence. To determine the inorganic nature of the glass fragments, they were treated with solvents (dichloroethane, carbon tetrachloride) and sulphuric acid, including heating.

The elemental chemical composition of the shards provided for the study was determined using the X-ray fluorescence analysis (XRF) method. The XRF method is based on the dependence of X-ray fluorescence intensity on the concentration of an element in the sample. When a sample is irradiated with an X-ray flux, characteristic fluorescence radiation is generated, which is proportional to the concentration of the chemical element in the object. The radiation is decomposed into a spectrum using crystal analysers, and then its intensity is measured using detectors and counting electronics. Mathematical processing of the spectrum enables its quantitative and qualitative analysis. An energy X-ray spectrometer CEP-01 (ElvaX Pro) (China) was used to determine the quantitative and qualitative composition of the fragments (objects No. 1-4). The spectrometer is designed to measure the mass fraction of chemical elements in samples of various origins.

The following technical conditions were met during the measurements: range of detected elements from *Na* (Z = 11) to *U* (Z = 92); accelerating voltage of 60 kV; maximum current of the X-ray generator of 1000 μA; quantitative analysis algorithm – method of fundamental parameters and empirical calibrations; type and serial number of the X-ray tube Rh122594. To improve the measurement accuracy, brown layers were removed from the surface of the objects under study with an aqueous solution of ethyl alcohol only to the extent necessary for the study.

For X-ray fluorescence analysis, the fragments (objects No. 1-4) were placed in the working chamber of the spectrometer in the focus of the X-ray tube and subjected to analysis. All animal manipulations were performed following the principles regulated by 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 "On the Protection of Animals from Cruelty" (2006). Digital data were processed using the statistical method on a personal computer using Microsoft Excel. The assessment of statistical reliability was determined by the Student's coefficient at three levels of probability: $P < 0.05$, $P < 0.01$, $P < 0.001$.

This study was based on the conclusions of forensic examinations conducted in criminal proceedings for animal cruelty, when foreign objects, including glass fragments, were removed from the bodies of dead animals. In this case, the forensic veterinary examination included examination of the injured animals and the recovered foreign objects, which were glass fragments, and their identification by a set of features. To solve these problems, a comprehensive forensic veterinary examination of materials, substances, and products was performed.

Results and Discussion

Theoretical and methodologicalprinciples of forensic veterinary research

The expert tasks that can be solved by the forensic expert commission include identification (identification by individual parts, identifying object) of the entire object (identified), diagnostic (determination of the relation of foreign objects to inorganic silicate glass, determination of the chemical composition of glass fragments), and situational (mechanism of damage formation; the possibility of damage formation found in an animal under the established conditions and circumstances of

the offence). The subjects of a comprehensive forensic veterinary and materials, substances and products examination are a commission of forensic experts in the expert specialities 18.1 "Veterinary research" and 8.5 "Research of glass, ceramics, and products made of them". The objects of forensic examination are injured animals and glass fragments.

It should be emphasised that during a comprehensive forensic veterinary examination with the study of materials, substances, and products, the subject of its appointment may raise the following issues for resolution by forensic experts, which are formulated for the first time in the author's version, specifically:

♦ What is the nature of the injuries and their location?

♦ What is the mechanism, sequence (order) of infliction of the injuries?

♦ What is the severity of each injury?

♦ Were the injuries caused to the animal life-threatening at the time of their infliction, and if so, what caused their danger?

♦ How old are the injuries?

♦ Is it possible to determine by the nature of the injury to the animal's body what type of tool could have caused the injury and whether the injury was caused by a tool analogous to the one provided for examination (knife, axe, pitchfork, etc.)? (This is decided by a comprehensive forensic veterinary and trace evidence examination).

♦ Are there any signs suggesting that the injury was caused by repeated exposure to a sharp object?

♦ What is the sequence in which the injuries were inflicted?

♦ Was the injury caused by one or more instruments?

♦ What is the direction of the wound channel and what foreign objects are found in it?

♦ Did the injuries cause crippling injuries to the animal and how did this affect the period of loss of general economic use of the injured animal due to the injury?

♦ Were there any defects in the veterinary care provided to the animal that caused the injury, and if so, what was the severity of the injury, was there a loss of general economic and special use of the animal and, if so, what was the duration of the loss?

♦ Is there a causal link between the bodily injuries caused to the animal under the circumstances and the disorder of its health due to death?

♦ Could the animal itself have caused the injury?

♦ Did the injuries that were found on the injured animal cause physical pain and suffering? What are the signs of infliction of pain?

Considering the above, it is proposed to amend the Scientific and Methodological Recommendations on the Preparation and Appointment of Forensic Examinations (Order of the Ministry of Justice of Ukraine No. 53/5, 1998) with a list of questions in the authors' version that can be solved by a comprehensive forensic veterinary examination with the study of materials, substances, and products during the examination of live animals or their cadavers injured by glass fragments. To solve the expert tasks set out in the procedural document (decision of the investigator or prosecutor) or court decision, the authors of the present study proposed an algorithm for forensic examination of animals for injuries caused by stabbed objects and glass fragments removed from their bodies (Fig. 1).

To resolve identification and diagnostic issues related to foreign objects removed from the wounds of a live animal or its cadaver (in this study – glass fragments), the subject of the forensic examination may ask the following clarifying questions in addition to the main list of questions:

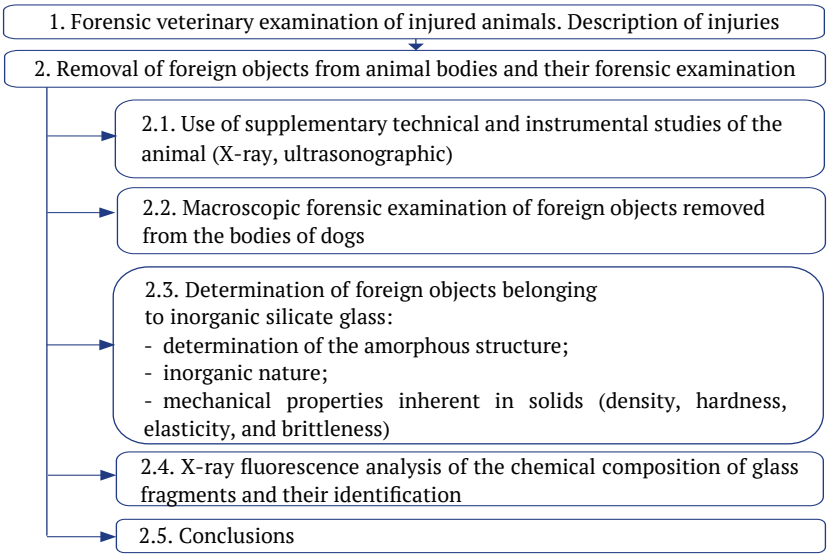


Figure 1. Algorithm for forensic examination of animals for injuries caused by stab wounds from objects removed from their bodies

***Sourse**: developed by the authors of this study*

♦ Are there any foreign objects in the animal's body? If so, what is their nature?

♦ What type of glass are the glass fragments removed from the soft tissues of the living animal (cadaver) and the fragments of the glass bottle (neck) removed during the examination of the crime scene?

♦ What is the chemical composition of the glass fragments recovered from the soft tissues of a living animal (cadaver) and fragments of a glass bottle (neck) found during the examination of the crime scene?

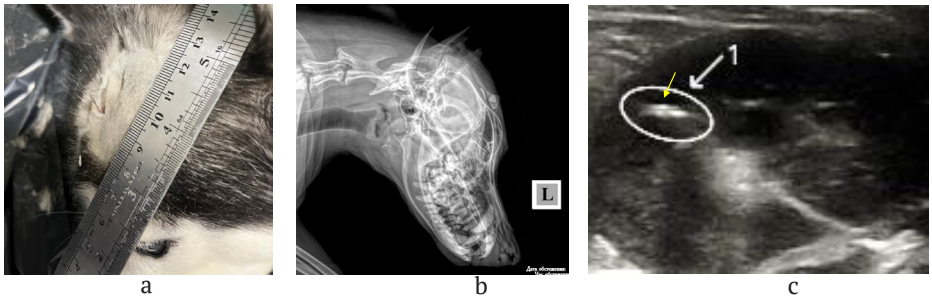
♦ Do the fragments of glass recovered from the soft tissues of the body of a living animal (cadaver) have a common genus (group) affiliation with the fragments of a glass bottle (neck) recovered during the inspection of the crime scene?

This list of questions is not exhaustive. The forensic veterinary expert may be asked other questions within the scope of the expert study.

Forensic veterinary examination of injured animals. Description of injuries

At the first stage of the forensic examination, it is necessary to conduct a clinical forensic veterinary examination of the injured animal or a pathological examination of the corpse. During a clinical forensic veterinary examination of a live animal or a pathological examination of a corpse, specific signs of damage caused by the action of piercing and cutting objects, including glass fragments, are revealed: the nature of the damage is a stab wound; its entrance opening is always linear in shape (Figs. 2a, 3a, 4a). The depth of the wound is much greater than the length, but it can be the same as the length of the glass fragment (if it is fixed in the skin and subcutaneous tissue and superficial layers of skeletal muscle) (Fig. 2a), or greater than its length (if it penetrates deep tissues or even into the body cavities of the animal with damage to internal organs) (Fig. 3a). The wound edges are smooth, even, intact, and slightly

cyanotic, with abrasions, drying, abrasion border, deposition border, and absence of tissue bridges in the wound channel (Figs. 3a, 4a). The wound ends are acute-angled, sometimes with notches and incisions; the wound channel has smooth or bevelled edges. The expert summary of the morphology of stab wounds inflicted on animals was confirmed by the findings of M. Ghanbari *et al.* (2024). The radiographs of a dog named Reks, taken in the vertebral and lateral projections, revealed one foreign body in the form of a light X-ray shadow, which was close to triangular in shape, located in the right frontal region (Fig. 2b). The ultrasonogram revealed signs of a dense hyperechoic elongated object without a pronounced acoustic shadow (Fig. 2c).

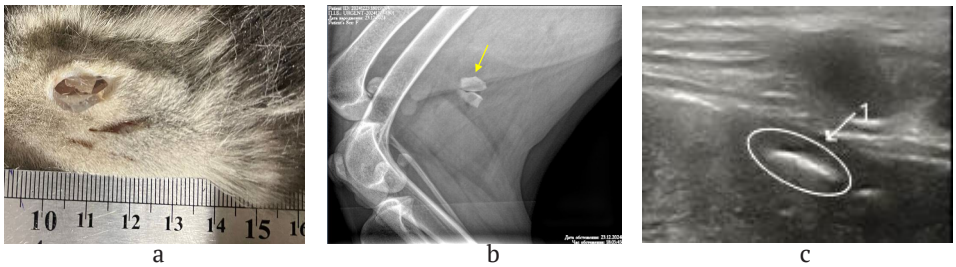


a b c

Figure 2. Glass fragment in the soft tissues of the right frontal area of a dog named Reks (Object No. 1)

***Note**: general view of the wound opening in the right frontal area of the dog and X-ray of the head and neck fragment with visualisation of a foreign body: a – external view of the puncture wound; b – view on the X-ray in the lateral projection (shown by an arrow); c – view on the ultrasonogram (1)*
***Sourse**: developed by the authors of this study*

A lateral view radiograph of a dog named Baks (Object No. 2) revealed two foreign elongated objects in the form of light X-ray shadows, close to rectangles in shape, located in the soft tissues of the lower third of the right thigh (Fig. 3b). The ultrasonogram in the same area revealed signs of soft tissue prolapse and a 9.9 mm hyperechoic, elongated, tuberculated object without an acoustic shadow (Fig. 3c).

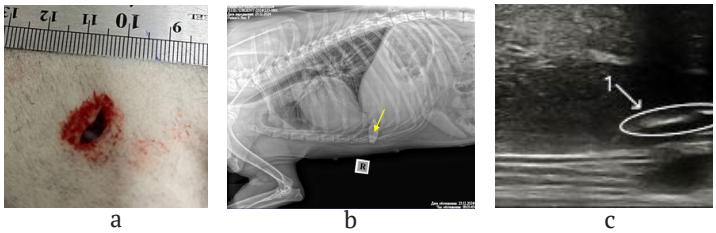


a b c

Figure 3. Glass fragment in the soft tissue of the left thigh of a dog named Baks (Object No. 2)

***Note**: general view of the wound opening in the left thigh of the dog and X-ray of a fragment of the animal's pelvic limbs with visualisation of a foreign body: a – external view of the puncture wound with a foreign body in the wound channel; b – view on the X-ray in the lateral projection (shown by an arrow); c – view on the ultrasonogram (1)*
***Sourse**: developed by the authors of this study*

The radiograph of a dog named Archi (Object No. 3), taken in the lateral and spinal projections, revealed one foreign elongated object in the form of a light radiopaque shadow, close to a pentagon in shape, located in the soft tissues of the lower third of the right thigh (Fig. 4b). In the same area, ultrasonographic signs of soft tissue prolapse up to 9.5 mm in size and two hyperechoic objects 9.2 mm and 3.9 mm in size, elongated, with a tuberous surface, without an acoustic shadow were visualised (Fig. 4c).



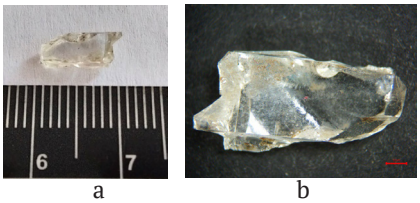
a b c

Figure 4. Glass fragment in the liver of a dog named Archi (Object No. 3)
***Note**: general view of the wound opening in the left rib wall and a lateral view of the animal's body with visualisation of a foreign body: a – external view of the stab wound in the right rib wall; b – view on the lateral projection X-ray (shown by an arrow); c – view on the ultrasonogram (1)*
***Sourse**: developed by the authors of this study*

The authors of the present study emphasise that the sensitivity for detecting vitreous foreign bodies by radiography and ultrasonography was 100% for all sensors. Foreign bodies detected in wounds using imaging methods were removed, documented, attached to the case files, and handed over to investigators in criminal proceedings for forensic examination. E. Bartelnik *et al*. (2022) pointed out the relevance of the identification, collection, and preservation of forensic veterinary evidence at the scene and during pathological examination. Ensuring high accuracy in forensic veterinary examinations of injured animals is an essential component of modern forensics, as such studies provide key information to establish the mechanism of injury, the time of their formation, and the possible tool used.

Forensic examination of foreign objects removed from the bodies of dogs

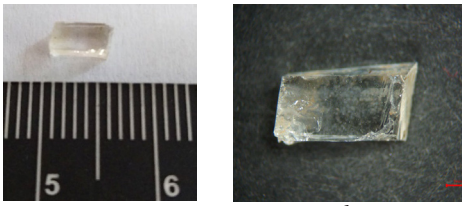
In the structure of a comprehensive forensic veterinary examination of materials, substances, and products, a separate determination of glass fragments removed from the bodies of injured dogs is performed by a forensic expert in the study of materials, substances, and products, including glass. Thus, during the examination of a transparent foreign object removed from the corpse of a dog named Reks (Object No. 1), it was found that it was a single transparent vitreous fragment with dimensions of 4 mm wide, 10 mm long, and 2.7 mm thick. It was polygonal in shape, with two flat-parallel surfaces of technological origin. On the surface of Object No. 1, there were layers of a brown substance that looked like blood (Fig. 5).



a b

Figure 5. A view of an object removed from a dog's wound (Object No. 1)
***Note**: a view of the object removed from the body of a dog named Reks: a – general view, b – detailed view*
***Sourse**: developed by the authors of this study*

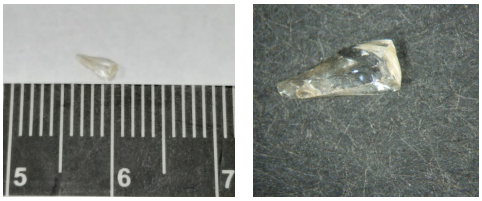
During the examination of the foreign object recovered from the cadaver of a dog named Baks (Object No. 2), it was found that it was a transparent object of a vitreous structure, with dimensions of 3 mm wide, 5.4 mm long, and 2.8 mm thick. The fragment was polygonal in shape with two flat-parallel surfaces of technological origin. On the surface of this object there were layers of a brown substance that looked like blood (Fig. 6).



a b

Figure 6. A view of an object removed from a dog's wound (Object No. 2)
***Note**: a view of the object removed from the body of the dog named Baks: a – general view, b – detailed view*
***Sourse**: developed by the authors of this study*

The examination of the foreign object recovered from the corpse of the dog named Archi (Object No. 3) revealed that it was a transparent glassy object with dimensions of 1.9 mm wide, 4.3 mm long, and 2.9 mm thick. The object had a complex pointed shape. The surface of this object had layers of a brown substance that looked like blood (Fig. 7).



a b

Figure 7. A view of an object removed from a dog's wound (Object No. 3)
***Note**: a view of the object removed from the body of the dog named Archi: a – general view, b – detailed view*
***Sourse**: developed by the authors of this study*

Examination of the object found at the scene (Object No. 4) revealed that it was a fragment of a glass bottle (neck) made of pure glass. The length of the neck fragment was 110 mm. Due to its design features the neck of the bottle had a variable outer diameter: the smallest (at the base of the neck) was 29 mm, the largest was 43 mm. The inner surface of the upper part of the neck contained a dispenser made of polymeric material. The upper part of the bottle neck was covered with a black polymer film tightly adjacent to the outer walls of the bottle with the printed inscription "JACK DANIEL'S", "Old No. 7 BRAND" in white. There were convex inscriptions on the surface of the neck, which were part of the glass neck. Due to the limited size of the neck fragment, the content of the inscription could not be determined. On the surface of the black polymer film, there were two fragments of an excise stamp and stickers marked "6" and "9". On the outer surface of the neck there was a sticker with the marking "5". On the inner surface of the neck there was a sticker with the markings "7" and "8" (Fig. 8).

Figure 8. General view of a fragment of a glass bottle (neck) recovered during the inspection of the scene
***Sourse**: developed by the authors of this study*

Thus, the analysis of the interim results of the study showed that during the forensic veterinary examination of the injured dogs, they were diagnosed with stab wounds, in the wound

channels of which foreign objects resembling glass were found. Therefore, at the next stage of the forensic examination, it was necessary to establish whether these foreign objects belonged to inorganic silicate glass.

Determination of the belonging of foreign objects removed from the bodies of dogs to inorganic silicate glass

According to the ideas about the structure and properties of glass, based on the concept of the glassy state, it is necessary and sufficient to identify three features to classify foreign objects removed from animal wounds as inorganic silicate glass: amorphous structure, inorganic nature, and mechanical properties of solids. Thus, the amorphous nature of the glassy state is manifested in the anisotropy of glasses due to the lack of directional orientation of structural formations. This orientation and, consequently, anisotropy are characteristic of crystals, in contrast to which glass microparticles do not exhibit the effect of pleochroism (double refraction) in polarised light. The results of the microscopic examination revealed that the fragments of objects Nos. 1-4 did not exhibit the pleochroism effect in polarised light. Accordingly, the absence of this effect indicated the amorphous structure of the substance.

The inorganic nature of the foreign objects removed from the dogs' wounds was established. The authors of the present study point out that all substances of organic origin, unlike inorganic ones, dissolve in organic solvents and/or interact with sulphuric acid and are subject to destruction at temperatures of 200-400°C. According to the results of the study of objects Nos. 1-4, during their treatment with solvents (dichloroethane, carbon tetrachloride) and sulphuric acid, including during heating, there were no noticeable changes in their shape and appearance, which confirmed the inorganic nature of the objects of study.

The mechanical properties of the foreign objects removed from the wounds of the dogs (Objects Nos. 1-3) and the object at the crime scene were also determined. According to G. Kaur *et al.* (2019), the mechanical properties of glass include density, hardness, elasticity, and brittleness. To determine the mechanical properties of objects recovered from dog wounds, specifically those inherent in glass, it is sufficient that the value of one of these properties is within the range of inorganic glasses. The appearance of the fragments is a qualitative characteristic of the fragility (brittle fracture) of the material. Therefore, microscopic examination of objects Nos. 1-4 recovered from animal bodies revealed signs characteristic of brittle fracture: chips, cracks, sharp shaped fragments with cutting edges, and a shell-shaped fracture surface (Figs. 5-8). Thus, the above studies of glass fragments recovered from the bodies of dogs proved that they belong to inorganic silicate glass in terms of their amorphous structure, inorganic nature, and mechanical properties.

X-ray fluorescence analysis of the chemical composition of glass fragments and their identification

According to V. Sharma *et al*. (2023), the identifying feature of glass fragments recovered from animal bodies to attribute them to a single object is their chemical composition. Notably, the elemental composition of glass is formed from the main components, additives specially introduced into the glass to give it the specified properties, and random impurities that enter the glass mass with raw materials or refractory of the furnace in which the glass is smelted. In this regard, XRF allows differentiating glass fragments recovered from dog wounds by their qualitative and quantitative elemental composition, as well as to determine the main and impurity elements in this composition. Thus, the elemental

chemical composition (in the range from atomic number 11, which belongs to sodium (Na), to atomic number 92, which belongs to uranium (U), of the objects of study is presented in Table 1.

Table 1. Chemical composition of objects Nos. 1-4, M ± m, n = 3

Chemical elements		Concentration of chemical elements (wt%)			
Atomic number	Name	Object No. 1	Object No. 2	Object No. 3	Object No. 4
14	Silicon (Si)	60.007 ± 0.302	60.205 ± 0.354	62.381 ± 0.671	60.036 ± 0.237
11	Sodium (Na)	33.958 ± 0.501	33.929 ± 0.655	31.580 ± 2.045	34.184 ± 0.365
20	Calcium (Ca)	2.585 ± 0.016	2.597 ± 0.018*	2.756 ± 0.036***	2.534 ± 0.012
12	Magnesium (Mg)	1.609 ± 0.058	1.586 ± 0.072	1.727 ± 0.186	1.623 ± 0.044
13	Aluminium (Al)	1.404 ± 0.043	1.444 ± 0.052	1.401 ± 0.113	1.409 ± 0.032
16	Sulphur (S)	0.099 ± 0.008	0.127 ± 0.010*	0.023 ± 0.034	0.085 ± 0.005
15	Phosphoros (P)	0.061 ± 0.024	0.022 ± 0.029	–	0.035 ± 0.002
26	Iron (Fe)	0.031 ± 0.001	0.032 ± 0.001***	0.035 ± 0.003**	0.025 ± 0.001
22	Titanium (Ti)	0.027 ± 0.005	0.029 ± 0.006	0.031 ± 0.012	0.022 ± 0.017
19	Potassium (K)	0.025 ± 0.004	–	–	0.022 ± 0.004
38	Strontium (Sr)	0.012 ± 0.000	0.011 ± 0.000	0.009 ± 0.001	0.011 ± 0.000
40	Zirconium (Zr)	0.007 ± 0.000	0.007 ± 0.000	0.006 ± 0.001	0.008 ± 0.000
23	Vanadium (V)	0.003 ± 0.002	0.002 ± 0.003	0.005 ± 0.006	0.003 ± 0.002
25	Manganese (Mn)	0.003 ± 0.001	0.004 ± 0.001	0.015 ± 0.003	0.002 ± 0.000
30	Zinc (Zn)	0.003 ± 0.000	0.003 ± 0.000	0.003 ± 0.004	0.001 ± 0.001
24	Chromium (Cr	–	0.002 ± 0.002	0.003 ± 0.001	–
46	Palladium (Pd)	–	–	0.023 ± 0.003	–

Note: **P < 0.05; **P < 0.01; ***P < 0.001 compared to the indicator of Object No. 4*
Source*: developed by the authors of this study*

According to the XRF results, it was established that the chemical elements listed in Table 1 are markers for establishing the generic characteristics of the investigated objects in the composition of objects Nos. 1-4, recovered from animal soft tissues. Objects Nos. 1-3, recovered from the bodies of dogs by nicknames: Reks, Baks, Archi, and a fragment of a glass bottle (neck) seized at the scene of the offence (Object No. 4) did not have the property of luminescence and fluorescence in ultraviolet rays with a wavelength of λ_{max} = 256 nm and with λ_{max} = 325 nm, as well as phosphorescence after the cessation of irradiation with ultraviolet rays. These coincident features are common to the material from which they are made, which indicated that they belonged to inorganic silicate glass.

The established chemical composition of Objects Nos. 1-4 suggested that the glass fragments recovered from the cadavers of dogs named: Reks (Object No. 1), Baks (Object No. 2), Archi (Object No. 3), and a fragment of a glass bottle (neck) seized during the inspection of the crime scene (Object No. 4) belonged to inorganic sodium aluminosilicate glass. Subsequently, the genus of the objects was established, i.e., their assignment to a specific set, following the classification accepted in science and technology. Therefore, based on the XRF results, the glass fragment (Object No. 1) recovered from the body of the dog named Reks and the fragment of the glass bottle (neck) (Object No. 4) recovered during the inspection of the crime scene were identical in terms of the qualitative composition of chemical elements and had no significant difference. Thus, the glass fragment (Object No. 1) and the bottle neck (Object No. 4) had a common origin.

Comparative studies of the chemical composition of Object No. 2, recovered from the body of a dog named Baks, with a fragment of a glass bottle (neck) (Object No. 4) revealed that Object No. 2 differed from the fragment of the glass bottle (neck) in the qualitative composition of chemical elements by the absence of potassium (K) and the presence of chromium (Cr). Differences in the quantitative composition of chemical elements were also revealed, specifically, the elemental composition of Object No. 2 significantly differed from the same Object No. 4 in the content of the following chemical elements: calcium ($P < 0.05$), sulphur ($P < 0.05$), and iron ($P < 0.001$), which indicated their different generic affiliation, i.e., the glass fragment (Object No. 2) and the bottle neck (Object No. 4) had different origins.

Comparative studies of the chemical composition of the glass shard (Object No. 3) and the fragment of the glass bottle (neck) (Object No. 4) revealed that Object No. 3 differed from the fragment of the glass bottle (neck) (Object No. 4) in the qualitative composition of chemical elements by the absence of potassium (K) and phosphorus (P) in Object No. 3 and the presence of chromium (Cr) and palladium (Pd). Furthermore, Object No. 3 did not differ significantly from Object No. 4 in terms of the quantitative composition of chemical elements, specifically, sodium, magnesium, aluminium, sulphur, titanium, strontium, zirconium, vanadium, manganese, and zinc. However, a quantitative significant difference in the content of chemical elements in Object No. 3 versus Object No. 4 was found, specifically: silicon ($P < 0.05$), calcium ($P < 0.01$), and iron ($P < 0.01$), which indicated that they were of different generic origin, with the glass fragment (Object No. 3) and the bottle neck (Object No. 4) were of different origin.

Notably, these qualitative and quantitative differences in the chemical composition of the elements are substantial features of forensic significance. This helped to state that objects Nos. 2 and 3, recovered from the bodies of dogs named Baks and Archi, respectively, did not have a common origin with the material of a fragment of a glass bottle (neck) (object No. 4) recovered during the examination of the scene. To summarise, the proposed algorithm for forensic detection and identification of glass fragments recovered from the wounds of injured animals will enable forensic experts to state the mechanism of formation of stab wounds under the circumstances and at the time specified in the criminal proceedings.

Conclusions

For the first time, an integrated approach to the detection and identification of glass fragments removed from animal bodies in the form of a comprehensive forensic veterinary examination with the study of materials, substances, and products was introduced into forensic science. During this examination of glass fragments removed from animal bodies, the expert commission can solve identification, diagnostic, and situational tasks. The algorithm of forensic examination of animals injured by glass fragments removed from their bodies comprises the following stages: visual forensic veterinary examination of injured animals and description of injuries; application of additional technical and instrumental studies of the animal (X-ray, ultrasonographic), removal of foreign objects from animal bodies and their forensic examination, which includes description of macroscopic parameters, determination of the foreign object's affiliation with inorganic silicate glass by determining its amorphous structure, inorganic nature, mechanical properties inherent in solids (density, hardness, elasticity, and fragility); X-ray fluorescence analysis of the chemical composition of glass fragments and their

identification; formulation of forensic expert opinions. X-ray and ultrasonographic methods of examining injured animals enabled an accurate visualisation of the presence of foreign objects in the body, establishing their number, shape, size, and location. However, they do not provide information on the type of foreign object.

The elemental composition of glass fragments removed from the bodies of injured animals and fragments found at the scene of the offence, determined by X-ray fluorescence analysis, is reliable, accurate, reliable for qualitative and quantitative analysis, and suitable for identifying glass fragments during a comprehensive forensic veterinary examination of materials, substances, and products. Glass fragments recovered from the bodies of dogs named Reks, Baks, Archi, respectively (Objects Nos. 1-3) and a fragment of a glass bottle (neck) (Object No. 4) recovered during the examination of the scene belonged to inorganic sodium aluminosilicate glass. The glass fragment (Object No. 1) recovered from the body of the dog named Reks and the fragment of the glass bottle (neck) (Object No. 4) recovered during the examination of the scene of the crime had a common generic affiliation by the material from which they were made. However, the glass fragments recovered from the bodies of dogs named Baks and Archi, respectively (Objects Nos. 2 and 3) did not have a common generic affiliation with the chemical composition of the glass bottle fragment (neck) (Object No. 4) recovered during the examination of the scene. The application of the algorithm for forensic examination of animals damaged by glass fragments removed from their bodies, developed and tested by the authors of the present study, will enable forensic experts to formulate categorical opinions. Further research will be aimed at resolving issues related to the detection and identification of substances of soil origin removed from animal wounds.

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Conflict of Interest

None.

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Судово-ветеринарна експертиза тіл тварин, травмованих осколками скла

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Анотація. Досудове розслідування правопорушень проти здоров'я і життя тварин неможливе без застосування правоохоронними органами або судом спеціальних знань, зокрема ветеринарних і технічних, довершеною формою яких є комплексна судова ветеринарна експертиза з дослідженням знарядь їх травмування: матеріалів, речовин та виробів. У таких випадках суб'єкт призначення судової експертизи ставить на вирішення судового експерта питання, які стосуються характеру, локалізації, ступеня тяжкості, виду знаряддя, яким могло бути заподіяне певне ушкодження тіла тварини. З огляду на зазначене, мета дослідження полягала в обґрунтуванні та розробці алгоритму судово-експертного виявлення та ідентифікації уламків скла, вилучених із тіл травмованих тварин. У роботі використовували комплекс сучасних методів дослідження, що включав рентгенографічний, ультрасонографічний, візуальний, мікроскопічний, фізико-хімічний, рентгенофлюоресцентний та статистичний аналіз, завдяки яким встановлено нові дані щодо інформативності рентгенографії та ультрасонографії як неінвазивних методів виявлення сторонніх предметів в тілах загиблих собак, а також можливості ідентифікації цілого знаряддя травми за окремими фрагментами (уламками скла). Завдяки отриманим результатам із застосуванням комплексного підходу для виявлення та ідентифікації сторонніх предметів, вилучених із тіл травмованих тварин, під час проведення судово-ветеринарних досліджень, вирішено низку важливих питань: підтверджено їх наявність у тілі тварин, приналежність до неорганічного силікатного скла з детальним описом його хімічного складу, а також ідентифіковано знаряддя травми за окремими його частинами. Напрацювання авторів статті позитивно вплинуть на ефективність проведення і результативність судової експертизи тварин, постраждалих внаслідок нанесення важких травм сторонніми предметами, вилученими з їхніх тіл. Водночас цей комплексний підхід

дає змогу підвищити ступінь обґрунтованості й об'єктивності висновку експерта як засобу доказування у судочинстві в категоричній формі та розширює доказові можливості органів досудового розслідування та суду

Ключові слова: комплексна судова експертиза; ідентифікація матеріалів; собаки; інструментальні та мікроскопічні дослідження; колото-різана рана; колюче-ріжуче знаряддя; жорстоке поводження з тваринами

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The effect of prolonged heat stress on haematological parameters of Holstein cows

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Abstract. Prolonged heat stress poses a significant challenge to the productivity and health of Holstein cows due to their heightened sensitivity to high temperatures and intensive metabolism. The purpose of this study was to investigate changes in haematological and immunobiological parameters in Holstein cows under prolonged heat stress conditions. The study included 18 cows in their second and third lactations, which were divided into two groups: one exposed to hyperthermia during the summer (August) (HYP, n = 8), and the other maintained under thermally comfortable conditions in the autumn (October), serving as the control group (CON, n = 10). Blood analysis was performed using methods commonly accepted in clinical veterinary practice. The results revealed significant reductions in red blood cell count (by 14.5%), haemoglobin level (by 11%), and haematocrit (by 8.4%) in HYP cows compared to CON ($P < 0.05$), indicating disruptions in erythropoiesis or reduced erythrocyte lifespan. Concurrently, an increase in mean corpuscular volume (by 8.9%) and platelet count (by 53%), alongside a decrease in mean platelet volume (by 20%), suggested adaptive metabolic and coagulation responses of the organism. A 48% decrease in serum lysozyme activity and a 36% increase in circulating immune complex levels indicated suppression of innate immunity and activation of compensatory immune defence mechanisms. The practical significance of the study lies in identifying potential biological markers for monitoring and managing heat stress, which could improve the health, productivity, and welfare of dairy cows

Keywords: dairy cattle; hyperthermia; blood parameters; erythropoiesis; innate resistance

Introduction

Heat stress (HS) represents a significant challenge to livestock production globally, particularly for high-yielding dairy cows, which are highly sensitive to elevated temperatures

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and humidity. HS disrupts physiological homeostasis, leading to reduced milk production, impaired immune responses, and compromised overall welfare. For instance, prolonged exposure to elevated temperature-humidity index (THI) levels has been shown to reduce milk production by 30-40%, particularly in regions where summer temperatures frequently exceed 40°C (Zeng *et al*., 2023). This decline is primarily attributed to metabolic alterations, including oxidative stress and systemic inflammation, which result from increased production of reactive oxygen species (ROS) and subsequent cellular damage (Sejian *et al*., 2022).

A.M. Ferreira *et al*. (2023) reported that chronic heat stress induces profound alterations in the innate immune response, leading to dysregulation of acute-phase proteins such as serum amyloid A, transferrin, and hepcidin. These changes reflect systemic inflammatory processes triggered by prolonged exposure to high ambient temperatures. The disruption of homeostasis under thermal stress conditions is closely associated with metabolic shifts, affecting protein synthesis and degradation pathways, ultimately influencing immune competence and overall physiological stability. This metabolic adaptation, while aimed at preserving cellular function, often compromises immune efficiency and antioxidant defence mechanisms, increasing susceptibility to inflammatory and oxidative damage (Mylostyva *et al*., 2022).

The physiological impact of HS extends to haematological parameters, reflecting the adaptive responses of dairy cows to thermal stress. Studies have highlighted significant reductions in red blood cell counts, haemoglobin levels, and haematocrit values, indicating disruptions in erythropoiesis and immune functionality (Burhans *et al*., 2022). According to S.H. Siddiqui *et al*. (2022), heat stress significantly affects immune responses by altering immunoglobulin levels and disrupting energy metabolism. Prolonged exposure to elevated temperatures has been shown to reduce IgM levels, indicating impairments in humoral immunity. Additionally, activation of the hypothalamic-pituitary-adrenal axis leads to increased glucocorticoid production, exerting immunosuppressive effects. This results in a reduction of T-lymphocyte counts and overall physiological deterioration during heat stress, increasing susceptibility to infectious diseases. Such disruptions compromise the ability of dairy cows to mount effective immune responses, making them more vulnerable to bacterial and viral infections, which can further exacerbate production losses and welfare concerns. Furthermore, the heightened metabolic demands during HS exacerbate oxidative stress, impairing immune responses and increasing susceptibility to diseases, including mastitis and metritis (Sachuk *et al*., 2018; Scerri *et al*., 2023).

Given the projected intensification of heat waves due to global warming, addressing HS in dairy cows has become a priority for sustainable livestock management. There has been a growing emphasis on non-invasive methods for assessing heat stress in animals. Non-invasive methodologies such as bioclimatic thermal indices and biomarker-based approaches, including cortisol analysis, offer valuable tools for quantifying the impact of HS and understanding adaptive mechanisms (Reolon *et al*., 2024). As noted by G. Hoffmann *et al*. (2021) and S. Farafonov *et al*. (2024), blood parameters continue to serve as reliable biomarkers for assessing the state of the body under hyperthermia, reflecting the adaptive responses of dairy cows to thermal stress. Studies by D.S. Park *et al*. (2021) reported a significant decrease in red blood cell count, haemoglobin levels, and haematocrit values, indicating impaired erythropoiesis and immune function. B. Yadav *et al*. (2024) highlighted that chronic heat exposure induces significant alterations

in both innate and adaptive immune responses, ultimately leading to systemic immunosuppression. Prolonged exposure to elevated temperatures results in a decline in white blood cell counts, reductions in neutrophil and lymphocyte populations, and disruptions in mean corpuscular volume and haemoglobin levels. These haematological changes indicate a diminished capacity to mount effective immune defences, thereby increasing susceptibility to infections and inflammatory conditions. Moreover, chronic heat stress has been shown to downregulate key toll-like receptors (TLR2 and TLR4), which are essential for pathogen recognition and immune activation. Concurrently, a predominance of regulatory T cells (Tregs) fosters an anti-inflammatory environment that may further impair immune surveillance and host defence mechanisms. Such shifts towards an immunosuppressive state under prolonged heat stress underscore the physiological vulnerabilities associated with sustained hyperthermia and highlight the need for adaptive strategies to maintain immune homeostasis and overall resilience. These strategies are particularly crucial for improving the resilience of Holstein cows, which, due to their high metabolic rates, are especially vulnerable to thermal stress.

The purpose of this study was to investigate the haematological and immunobiological parameters of Holstein cows under prolonged heat stress. This research sought to identify specific changes in blood and immune parameters that reflected the cows' physiological and metabolic responses, contributing to the development of effective strategies for improving their health, productivity, and resilience under thermal stress conditions.

Materials and Methods

Eighteen multiparous lactating Holstein cows in their second or third lactation were randomly assigned to one of two groups. The hyperthermia group (HYP, n = 8) was studied during the summer season in August, while the control group (CON, n = 10) was studied during the autumn season in October. Days in milk (DIM) for cows in both groups ranged from 117 to 152 days. The differences in DIM (LSM ± SE) between the HYP (130.2 ± 3.13) and CON (130.5 ± 2.81) groups were not statistically significant. The average daily milk yield (LSM ± SE) was 24.6 ± 0.45 kg in the CON group and 24.8 ± 0.48 kg in the HYP group, with no significant differences observed between groups. This study was conducted in accordance with the principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and approved by the Commission on Bioethics of the Dnipro State Agrarian and Economic University (protocol No. 5 dated 29 May 2018) and the requirements for humane treatment of animals.

The study was conducted on a commercial dairy farm located in the Dnipropetrovsk Oblast, Ukraine (48°28′44″ N, 35°36′46″ E). Dairy cows were housed in a naturally ventilated barn using a loose housing system. Sand was utilised as bedding in the cubicles, providing improved hygiene and comfort for the animals. All cows were fed a corn silage-based total mixed ration (TMR) throughout the year. The diets were balanced for essential nutrients following the National Research Council (2001) guidelines. The TMR composition included high-quality feedstuffs such as barley, oat, and corn grains, alfalfa silage, cereal hay, wheat straw, rapeseed, sunflower, and soybean meals, as well as dried beet pulp and mineral-vitamin supplements. The ingredients were thoroughly mixed in specialised mixers equipped with electronic scales to ensure homogeneity. Feeding frequency and rationing were monitored and controlled using a computerised system. The barn was equipped with a feeding alley and six water troughs, which were freely accessible to the cows,

ensuring constant availability of feed and water. This housing and feeding setup provided optimal conditions to maintain the physiological state and productivity of the animals.

The thermal environment within the barn was monitored using a thermohygrometer (Benetech GM 1360, Shenzhen Jumaoyuan Science and Technology Co., Ltd., Shenzhen, China). Key environmental parameters, such as air temperature and relative humidity, were recorded to calculate the temperature-humidity index (THI), which served as an indicator of heat stress (HS). The THI was determined using Kibler's (1964) formula (Mylostyvyi *et al.*, 2023). Blood sampling from HYP animals occurred on the 5th day of a recurring heatwave that lasted 10 days, with heat stress conditions persisting for a continuous period of 45 days prior to sampling. This period included multiple heatwaves, the most recent lasting 5 days, and a more distant heatwave lasting 9 days. At the time points of blood sampling from HYP cows, the minimum THI in the barn was 77.9, with values ranging from 77.9 to 78.6. Daily air temperatures during the HYP period reached a maximum of 34°C, accompanied by low relative humidity (26%). Conversely, in October, the control (CON) group was maintained under thermal comfort conditions, with a THI consistently below 68. Blood samples were collected 42 days after the last heat wave (lasting 8 days) and 21 days after the last day with heat stress conditions (THI ⩾ 72). During the control period, maximum daily air temperatures reached 19°C, with relative humidity ranging between 30% and 35%, corresponding to a THI of 63.1. These differences in climatic conditions between the experimental periods provided a basis for evaluating the impact of environmental stress on leukocyte indices and other physiological parameters.

Blood samples were collected via jugular vein puncture and directly transferred into 2-mL EDTA Vacutainer® tubes (Aichele Medico AG, Basel, Switzerland). For serum preparation, whole blood was drawn into Vacutainer® serum tubes without anticoagulants. Complete blood counts (CBC) were conducted using an automatic haematology analyser, Sysmex XS-1000i (Sysmex Corporation, Japan). The CBCs included the determination of total white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (HGB) concentration, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW-CV and RDW-SD), platelet (PLT) count, mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT).

The erythrocyte sedimentation rate (ESR) was measured using the Westergren method, which involves assessing the rate of erythrocyte sedimentation in a vertically positioned tube containing sodium citrate as an anticoagulant. Circulating immune complexes (CICs) were determined using the selective precipitation method with a polyethylene glycol-6000 solution (Hašková *et al.*, 1978). Serum immunoglobulin concentrations of classes A, G, and M (IgA, IgG, and IgM) were measured using an automatic biochemical analyser, Cobas Integra 400 Plus (Hoffmann-La Roche Ltd, Switzerland).

Serum lysozyme activity (SLA) and serum bactericidal activity (SBA) were evaluated using the photoelectric colorimetric method (Vlizlo *et al.*, 2012). SLA was determined based on the method of A.G. Dorofeychuk, with modifications to the temperature conditions of the reaction between cow serum and the *Micrococcus lysodeikticus* culture in phosphate buffer. SBA was assessed using the Michel Tefler method, as modified by O.V. Smirnova and T.A. Kuzmina, with a test culture of *E. coli*.

Initially, the data were presented as mean values (Mean) and the standard error of the mean (SE). Since the distribution of most indicators did not meet normality criteria, non-parametric

statistical methods were employed for further analysis. Statistical processing was carried out using Statistica 12 (StatSoft, Inc., Tulsa, OK, USA). Significant differences between samples were determined by the Mann-Whitney U test, and the probability level of $P < 0.05$ was considered significant.

Results and Discussion

Under prolonged HS (HYP group), significant changes were observed in haematological and immunobiological indicators compared to the CON group. WBC increased by 18% in the HYP group (Table 1), reflecting immune response activation under elevated THI conditions. HGB levels were 11% lower in HYP compared to CON ($P < 0.05$), indicating potential disruptions in erythropoiesis or reduced erythrocyte lifespan. RBC counts and HCT values declined by 14.5% and 8.4%, respectively, in the HYP group, suggesting a decrease in circulating erythrocyte volume.

Table 1. Haematological indicators (M ± SE)

Indicator	Experimental groups		p-value
	HYP	CON	
WBC, × 10^9/L	11.80 ± 4.01	10.00 ± 1.52	0.2144
HGB, g/L	87.90 ± 10.35	98.80 ± 16.99	0.0351
RBC, × 10^{12}/L	5.42 ± 0.67	6.34 ± 0.63	0.0051
HCT, %	24.24 ± 4.18	26.47 ± 2.80	0.1939
MCV, fL	44.57 ± 3.47	40.90 ± 3.01	0.0086
MCH, pg	16.17 ± 0.76	16.21 ± 0.75	0.9769
MCHC, g/L	366.80 ± 38.11	357.50 ± 51.28	0.9774
RDW-CV, %	15.12 ± 1.50	24.93 ± 1.60	0.0000
RDW-SD, fL	23.77 ± 4.16	33.10 ± 2.73	0.0008
PLT, × 10^9/L	394.10 ± 95.16	257.58 ± 82.82	0.0008
MPV, fL	6.03 ± 0.26	7.56 ± 0.44	0.0000
PDW, %	14.41 ± 0.20	8.67 ± 1.28	0.0000
PCT, %	0.23 ± 0.05	0.22 ± 0.02	0.5208
ESR, mm/h	2.17 ± 0.39	3.16 ± 0.13	0.0251

***Source**: developed by the author*

MCV increased by 8.9% ($P < 0.05$), indicative of macrocytosis likely caused by metabolic or hydration disturbances. Variability in erythrocyte volume (RDW-CV and RDW-SD) decreased by 39% and 28%, respectively ($P < 0.05$), suggesting adaptive mechanisms in haematopoiesis under stress. PLT rose by 53% ($P < 0.05$), while MPV decreased by 20% ($P < 0.05$), reflecting intensified production of smaller platelets. PDW increased 1.7-fold, suggesting enhanced coagulation mechanisms in response to vascular damage or inflammation. ESR in the HYP group was 31% lower than in CON, likely due to dehydration.

The immune response to prolonged HS involves complex adaptive mechanisms that regulate both humoral and cellular components. Changes in immune enzyme activity and antimicrobial defence reflect the body's attempt to maintain immune homeostasis under adverse environmental conditions. The impact of prolonged HS on immunobiological parameters was also significant (Table 2). SLA decreased by 48% ($P < 0.05$) in the HYP group, indicating reduced innate immune activity during HS.

Conversely, SBA increased by 24%, likely as a compensatory response to maintain systemic

defence. This shift in enzymatic activity suggests an adaptive mechanism aimed at counterbalancing the decline in lysozyme-mediated protection by enhancing serum bactericidal capacity. In parallel, changes in CIC and immunoglobulin levels reflect alterations in humoral immunity. CIC levels rose by 36%, indicating increased antigen release associated with cellular damage. Immunoglobulin levels (Ig A, Ig G, and Ig M) showed minimal differences between groups, with Ig A in HYP increasing by 43%, suggesting an adaptive immune response.

Table 2. Immunobiological indicators (M ± SE)

Indicator	Experimental groups		p-value
	HYP	CON	
Ig A, g/L	0.43 ± 0.26	0.31 ± 0.09	0.6904
Ig G, g/L	1.22 ± 0.09	1.17 ± 0.04	0.5476
Ig M, g/L	0.05 ± 0.01	0.05 ± 0.03	0.6904
CIC, AU	5.15 ± 1.70	3.78 ± 1.82	0.1811
SLA, %	22.99 ± 19.87	43.82 ± 16.36	0.0137
SBA, %	53.57 ± 20.17	43.16 ± 12.47	0.2029

***Source**: developed by the author*

Prolonged HS in Holstein cows induces significant alterations in haematological and immunobiological blood parameters, reflecting the systemic response to elevated THI conditions. The reduction in HGB levels and RBC counts suggests suppressed erythropoiesis, shortened erythrocyte lifespan, or haemodilution due to increased water intake for thermoregulation. Concurrently, an increase in MCV indicates metabolic adjustments or hydration changes aimed at maintaining oxygen transport. Enhanced platelet activity, marked by increased PLT counts and structural modifications, highlights coagulation system activation in response to stress.

Further supporting these observations, V. Sejian *et al*. (2022) highlighted that HS exacerbates oxidative stress, with elevated markers such as malondialdehyde (MDA) and reduced activity of superoxide dismutase (SOD). These alterations in oxidative stress markers are indicative of the systemic strain imposed by HS, and the resulting oxidative damage contributes to decreased erythropoiesis and compromised metabolic function. This aligns with the results of O.M. Azeez *et al*. (2022), who reported similar haematological changes in other livestock under heat stress, showing that oxidative stress plays a central role in HS-induced health issues.

PLT activity showed marked changes under HS conditions, with an increase in PLT count and a decrease in MPV, suggesting intensified production of smaller platelets. The elevation in PDW indicates enhanced coagulation mechanisms, likely triggered by vascular damage or inflammatory responses, as described by O.A. Saeed *et al*. (2021). These findings underscore the organism's effort to maintain haemostasis and prevent excessive blood loss under stress, a mechanism observed across several studies, including those by J.-H. Jo *et al*. (2021).

Immunobiological parameters revealed a distinct pattern of adaptation to HS. The significant reduction in SLA, indicative of innate immune suppression, was observed alongside an increase in SBA, reflecting compensatory mechanisms to preserve immune defence (Min *et al*., 2023). Elevated CIC levels also reflected immune activation associated with cellular damage due to HS, supporting the findings of V. Tejaswi *et al*. (2020), who noted similar immune

responses under thermal stress. These findings suggest that HS not only disrupts erythropoiesis but also triggers a complex immune response, which includes both suppression and activation of immune functions in an attempt to mitigate damage and preserve overall health.

Minimal variations in Ig A, Ig G, and Ig M levels, despite an observed increase in Ig A, suggest that adaptive immune responses stabilise over prolonged HS exposure. This stabilisation aligns with observations by S.L. Cartwright *et al*. (2021), who reported that high immune responders in Holstein cows demonstrated improved thermoregulation and cellular protection during HS, possibly due to enhanced expression of HSP70. This highlights the long-term nature of HS-induced immune modulation and suggests that dairy cattle may develop adaptive responses over time, depending on their individual immune capacity.

Findings by A. Rebl *et al*. (2020) and H. Kim *et al*. (2024) corroborated this, indicating that chronic HS leads to metabolic shifts and immune adaptations that help animals to cope with thermal challenges. For example, changes in blood metabolites, including increased HSP70 levels, were observed as protective responses to oxidative stress and cellular damage. However, these adaptations are not limitless, as prolonged exposure to high temperatures may ultimately compromise immune function and metabolic homeostasis, reducing productivity and increasing vulnerability to diseases (Steele, 2016). This is supported by findings from B. Stefanska *et al*. (2024), who reported similar effects of heat stress on immunometabolic indices during the close-up dry period in dairy cows.

Moreover, T.M. Scerri *et al*. (2023) highlighted the importance of environmental management in alleviating the negative effects of HS on dairy cattle. Strategies such as providing shade, cooling systems, and nutritional supplementation reduce the impact of HS, supporting both immune function and productivity. Similarly, R. Mylostyvyi *et al*. (2023) demonstrated that improving microclimate conditions within barns, particularly during high THI periods, mitigates the adverse effects of HS on milk yield and quality.

The increase in CICs, as reported by K. Pawlak-Osińska *et al*. (2019), further supports the idea that HS activates a compensatory immune response. This activation is associated with the release of antigenic components and subsequent CIC formation, playing a crucial role in systemic immune defence during HS. Additionally, alterations in gut microbiota composition and immune-related gene expression observed in sturgeons (Yang *et al*., 2022) and juvenile greater amberjacks (Hao *et al*., 2024) suggest that HS not only affects systemic immunity but also influences the microbiome, which is essential for maintaining homeostasis and overall health.

In conclusion, prolonged HS induces a multifaceted response in dairy cows, affecting both haematological and immune systems. The adaptive changes observed in RBC and WBC counts, along with variations in PLT and immune function, highlight the complex interplay between stress-induced metabolic shifts, oxidative damage, and immune modulation. The elevation in CICs and the reduction in innate immunity markers further emphasise the systemic adaptations to HS, providing valuable insights into the physiological challenges faced by dairy cows under elevated THI conditions. These results underscore the importance of integrated management strategies, including environmental cooling, nutritional support, and breed selection, to mitigate the negative impacts of HS and enhance the welfare and productivity of dairy cattle.

Conclusions

Prolonged heat stress significantly alters the haematological and immunobiological parameters of Holstein cows, reflecting the

physiological strain associated with elevated temperature-humidity index conditions. The study revealed a 14.5% reduction in red blood cell count, an 11% decrease in haemoglobin concentration, and an 8.4% decline in haematocrit in heat-stressed cows compared to the control group ($P < 0.05$). These findings suggest impaired erythropoiesis, reduced erythrocyte lifespan, or haemodilution as a compensatory mechanism for thermoregulation. Concurrently, an 8.9% increase in mean corpuscular volume indicates possible metabolic adjustments or alterations in hydration status, while a 53% elevation in platelet count and a 20% decrease in mean platelet volume suggest intensified coagulation processes, possibly in response to vascular damage.

Changes in immune function were also evident. Serum lysozyme activity, an important component of innate immunity, was 48% lower in the heat-stressed group, indicating immune suppression ($P < 0.05$). Moreover, circulating immune complex levels increased by 36%, reflecting antigen release and potential inflammatory responses associated with cellular damage. Immunoglobulin levels did not show significant differences between groups, although a notable increase in IgA suggests an adaptive immune response.

These findings highlight the physiological trade-offs Holstein cows experience under prolonged heat stress. The decline in erythropoiesis and immune activity may increase susceptibility to metabolic imbalances and inflammatory processes. To mitigate these effects, targeted nutritional interventions, such as antioxidant and amino acid supplementation, may support immune resilience and haematopoiesis. Additionally, improved environmental management strategies, including optimal ventilation, shading, and water cooling systems, can help to reduce the physiological burden of heat stress and maintain homeostasis.

Further research is required to investigate the long-term consequences of heat stress on immune resilience and haematopoiesis. Future studies should also focus on nutritional and environmental strategies to mitigate heat stress, including dietary interventions aimed at supporting erythropoiesis and immune function, and optimising barn microclimate and cooling systems. In addition, evaluating oxidative stress markers and their correlation with haematological changes may provide further insights into the mechanisms underlying heat stress adaptation.

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Conflict of Interest

None.

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Вплив тривалого теплового стресу на гематологічні показники голштинських корів

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Анотація. Тривалий тепловий стрес є серйозним викликом для продуктивності та здоров'я голштинських корів через їх підвищену чутливість до високих температур та інтенсивний метаболізм. Метою дослідження було вивчення змін гематологічних та імунобіологічних показників у голштинських корів за тривалого теплового стресу. У дослідженні використовували 18 корів другої-третьої лактації, з яких були сформовані дві групи. Одну групу в літній період (серпень) в умовах гіпертермії (HYP, n = 8), іншу – за комфортних умов восени (жовтень), яку вважали за контрольну (CON, n = 10). Аналіз показників крові проводили за допомогою загальноприйнятих у клінічній ветеринарній практиці методів. Результати продемонстрували значне зниження кількості еритроцитів (на 14,5 %), рівня гемоглобіну (на 11 %) та гематокриту (на 8,4 %) у корів HYP у порівнянні з групою CON ($P < 0.05$), що свідчить про порушення еритроцитопоезу або зниження тривалості функціонування еритроцитів. Водночас спостерігалося підвищення середнього обсягу еритроцитів (на 8,9 %) та кількості тромбоцитів (на 53 %) за зниження середнього обсягу тромбоцитів (на 20 %), що вказує на адаптаційні метаболічні та коагуляційні реакції організму. Зниження активності лізоциму сироватки крові (на 48 %) поряд із підвищенням рівня циркулюючих імунних комплексів (на 36 %) свідчить про пригнічення вродженого імунітету та активацію компенсаторних механізмів імунного захисту. Практична цінність дослідження полягає у виявленні потенційних біологічних маркерів для моніторингу та управління тепловим стресом, що сприятиме покращенню здоров'я та продуктивності молочних корів

Ключові слова: молочна худоба; гіпертермія; показники крові; еритропоез; природна резистентність

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Autonomic regulation of unsaturated fatty acid content in cow's milk

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Abstract. Investigating the role of the autonomic nervous system, as one of the key regulatory systems controlling metabolism in animals, is essential for assessing individual differences in the intensity and direction of metabolic processes. Therefore, studying the influence of the

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parasympathetic and sympathetic nervous systems on lipid metabolism, including fatty acids, is a relevant issue for ensuring dairy productivity in cows. This study aimed to provide a comparative assessment of the content of unsaturated fatty acids in cow's milk under varying types of autonomic regulation during the summer period. Experimental groups of Ukrainian Black-and-White dairy cows were formed based on sympathovagal balance, determined using a variation-pulsometry method. Gas chromatography was used to determine the fatty acid composition of the milk. The results revealed that the level of myristoleic acid in the milk of cows with vagotonia was 22% higher ($P < 0.001$) compared to that in cows with normotonia, and 7% higher ($P < 0.05$) compared to cows with sympathotonia. Palmitoleic acid levels in the milk of cows with vagotonia were 21% lower ($P < 0.001$) compared to those with normotonia, and 16% lower ($P < 0.001$) compared to cows with sympathotonia. In the milk of cows with sympathotonia, a 3.6% reduction ($P < 0.05$) in oleic acid content was recorded relative to cows with normotonia. The level of linoleic acid in cows with vagotonia was 19% lower ($P < 0.05$) than that in cows with normotonia, whereas in those with sympathotonia, it was 6% higher ($P < 0.05$). The content of α-linolenic acid in the milk of cows with vagotonia was 46% lower ($P < 0.05$) compared to normotonic cows, while in those with sympathotonia, it was 30% higher ($P < 0.05$). A relationship was established between autonomic nervous system activity and the content of unsaturated fatty acids in milk. The findings highlight the importance of exploring the regulatory mechanisms involved in lipid metabolism, particularly in dairy farming, where the individual productivity of cows is determined by the levels of fats consumed, absorbed, synthesised and metabolised within the body

Keywords: ruminants; gas chromatography; lipids; variation pulsometry; nervous system

Introduction

Examining and analysing the factors that influence fatty acid metabolism in cows' milk is a primary concern when evaluating the quality of dairy production. These factors include: the animal's genetics, environmental conditions (such as feeding regime, season, and housing), and physiological aspects (age, lactation stage, health status, and the functioning of systems responsible for metabolism). With a focus on the functioning systems within the animal's body, it is important to consider the nervous system, particularly the autonomic nervous system, which plays a significant role in regulating the body's metabolic processes. F. Di Gregorio *et al.* (2024) highlighted that each organism has individual differences in the way their central and peripheral nervous systems function. Consequently, the activity of different parts of the autonomic nervous system will vary, and this will be reflected in the metabolic processes occurring within the animal's body.

Milk synthesis is a rather energy-intensive process for high-producing livestock, as secreting a significant volume of milk requires a substantial amount of organic matter. To create these compounds, the animal's body undergoes biochemical processes of feed absorption and digestion, followed by the formation of complex molecules from the metabolic products. A. Zsombok *et al.* (2024) demonstrated that these biochemical processes within the body demand considerable energy expenditure and are maintained by the stability of homeostasis, which is regulated by the nervous system. It therefore follows that, due to the individual characteristics of cows' bodies, metabolic

processes occur differently from one animal to another, and this is particularly evident when the animal is under stress.

Z. Liu & S. Rochfort (2023) pointed out that for the synthesis of high-quality milk, the required lipid content falls within the range of 3% to 5%, which provides significant nutritional value for humans. Achieving this percentage necessitates a considerable number of fatty acids, which, according to these researchers, amounts to over 400 different types. These are present as triacylglycerols, phospholipids, and sphingolipids and constitute a significant portion of milk. K.E. Mitchell *et al.* (2023) demonstrated that the total number of lipid varieties exceeds 4,000, and this number continues to grow annually due to advancements in technologies for identifying these substances. Consequently, the cow's body expends a considerable amount of energy to produce the fat content necessary for milk synthesis, a process regulated by the nervous system to maintain homeostatic balance.

Scientists R. Gervais *et al.* (2023) found that investigating the structure and diversity of lipids in milk is a common aspect of assessing its quality, particularly concerning the composition of fatty acids, the levels of which are identified using mass spectrometry. G. Celano *et al.* (2022) reported on the use of methods for the quantitative or semi-quantitative determination of milk lipids. These methods rely on the application of multiple standards and their comparison with a reference sample.

In their research, L. Grille *et al.* (2023) found that saturated fatty acids are the most prevalent type of fatty acid in milk, with monounsaturated and polyunsaturated fatty acids present in smaller amounts. Milk fat content is the component that varies the most. One of the most significant factors affecting the amount of fat in milk is the cows' feed. Allowing animals to graze in pastures or including green forage in the diet of dairy cows kept indoors improves the fatty acid composition of their milk, which subsequently has a beneficial impact on human health. Under such conditions, when cows are on pasture, the levels of saturated fatty acids (C12:0, C16:0, C14:0) and the ratio of omega-6 to omega-3 fatty acids (n-6/n-3) are lower. Simultaneously, the levels of certain polyunsaturated fatty acids are significantly higher, such as C18:3 (n-3) and C18:2 (conjugated linoleic acid; cis9-trans11), which are important for human health. According to S.K. Gulati *et al.* (2022), allowing dairy cows to graze on pastures enhances the fatty acid profile of their milk by increasing the content of some health-promoting fatty acids, like n-3, and consequently reducing the n-6/n-3 ratio.

B. Walther *et al.* (2022) highlighted that the external environment is the primary and most significant factor influencing the milk production of cows, particularly in regions with seasonal climate changes. Consequently, the animal's body begins to adapt to these shifts by increasing the activity of its nervous system, especially the autonomic nervous system. The authors also noted that cow productivity tends to be more stable in areas with consistently temperate climates, as these animals can graze on pastures year-round, unlike those forced indoors during severe frost or intense heat. However, with changing climate patterns, more extreme weather conditions are emerging, which significantly impact the health and productivity of cows. Furthermore, considering the individual biological characteristics of each animal, their ability to adapt to such environmental changes varies, which is reflected in differences in the activity of their autonomic nervous systems. A straightforward example of this is the increased energy expenditure in cows during winter to maintain their body temperature, as well as the reduced intensity of their metabolic processes in summer to minimise heat production within their bodies. L. Grille *et al.* (2022)

demonstrated variations in the levels of fatty acids, specifically C18:2, C18:1, and C18:3 (n-3), in the milk of cows between the summer and winter seasons. Furthermore, the breakdown (catabolism) of fatty acids in cows experiencing heat stress led to an increase in the concentration of fatty acids in their blood plasma (particularly oleic and linoleic acids). This also resulted in a higher overall content of long-chain fatty acids and a decrease in the levels of short- and medium-chain fatty acids in their milk.

Research by O.V. Zhurenko *et al.* (2023) revealed that the presence of specific minerals in animal feed significantly influences cow productivity. Moreover, considering how the autonomic nervous system and its branches – the sympathetic and parasympathetic nervous systems – adjust metabolic processes within the animal, fundamentally alters the understanding of metabolic pathways and their impact on milk production in the mammary gland.

Scientists have extensively investigated the relationships between the levels of fatty acids in cows' milk and the influence of the autonomic nervous system. This study aimed to determine the impact of the autonomic nervous system tone as a factor regulating fatty acid metabolism, as reflected in the levels of unsaturated fatty acids in cows' milk.

Literature Review

The autonomic nervous system is a part of the peripheral nervous system. It has two main branches: the sympathetic and parasympathetic nervous systems. The sympathetic nervous system stimulates the cardiovascular system, increasing the force of the heartbeat. As T. LeBouef *et al*. (2023) identified that, by triggering the release of adrenaline, it activates the breakdown of nutrients, particularly carbohydrates and especially lipids. This generally promotes the mobilisation of nutrients and their active circulation throughout the body. This branch of the autonomic nervous system also inhibits digestion in the gastrointestinal tract, specifically the movement of the intestines. Conversely, the parasympathetic nervous system has the opposite effect, promoting the synthesis of nutrients and increasing the activity of the digestive system. It is important to note that these two systems constantly interact with each other. However, each living organism is unique and exhibits different levels of activity in the components of its autonomic nervous system. As a result, one might observe a greater influence of either the parasympathetic or the sympathetic nervous system. In other animals, these two branches function in a more balanced manner. Due to these individual characteristics, any changes in the cow's external or internal environment will affect its regulatory systems in different ways, and the resulting responses will also vary.

A.H. Rezakhani *et al*. (2024) established that the start of lactation in cows is significantly influenced by the activity of their regulatory systems and their individual ability to maintain the body's homeostasis, as well as the animal's overall health. According to these researchers, a normal lactation period relies on sufficient energy reserves built up during the dry period. The beginning of lactation is a substantial metabolic stress, controlled by the central nervous system through the action of the autonomic nervous system. A. Veshkini *et al.* (2024) emphasised the need to manage these energy shifts and develop strategies for controlling these processes. Currently, this is partly addressed by adjusting feed rations, but the effectiveness of this approach does not always yield the desired outcome.

The metabolism of lipids in cows and the development of balanced diets to improve their energy profile, thereby ensuring a positive lactation period, have been studied by W.B. Gallardo & I.A. Teixeira (2023) and C.D. Camell (2022). These studies highlighted the

importance of monitoring lipid concentrations in the animal's body, as lipids not only provide energy but also perform crucial bioactive physiological functions. Consequently, evaluating this aspect remains a highly relevant topic in scientific publications.

T.M. Knutsen *et al.* (2022) demonstrated that significant challenges exist in accurately measuring lipid concentrations in milk. Firstly, milk fat originates from two primary sources: *de novo* fatty acids, synthesised within the mammary gland from acetate and beta-hydroxybutyrate, and fatty acids derived from the bloodstream, which are obtained from the cow's diet and mobilised from the body's fat reserves. R.W. Shephard & S.K. Maloney (2023) highlighted the importance of proper energy metabolism and evaluating the sources of lipids entering the animal's system, as these factors will influence the fatty acid profile of the milk. Research into the essential components regulating lipid metabolism can help in predicting the optimal formulation of the animal's diet.

The importance of developing a strategic approach to feeding and managing cows during the transition period, which is crucial for maintaining the animals' health, productivity, and the farm's profitability, was emphasised in the study by S. Lashkari *et al.* (2024). Their findings indicate that the most critical challenge is meeting the cow's increasing energy demands for milk production at the start of lactation. To compensate for the high energy requirement in early lactation, the body mobilises fat reserves as an adaptive metabolic mechanism to prevent an energy deficit, leading to an increased concentration of non-esterified fatty acids in the blood plasma. The authors also noted the necessity of evaluating the systems that control lipid metabolism.

A.M. Bales *et al.* (2024) investigated the potential to adjust the fatty acid content of cows' milk by using feed supplements containing fatty acids such as palmitic and oleic acid in varying proportions. Three experimental groups of cows were established: the first was a control group, consuming a standard diet; the second group received a feed supplement composed of 80% palmitic acid and 20% oleic fatty acid; and the third group consumed a feed supplement containing 60% palmitic acid and 40% oleic fatty acid. The authors found that these feed supplements did not affect the cows' milk yield. However, they observed an increase in the fat content of the milk in the experimental groups where the modified feeding regime was implemented. Changes in the concentration of milk fatty acids were not significantly different when a low concentration of fatty acids was used in the animals' feed.

The factors that stimulate lipolysis were investigated by M.B. Oquendo *et al.* (2022). They found that some of the main factors in the release of lipids from storage are adrenergic agonists: isoprenaline and adrenaline. This variable also indirectly stimulates the synthesis of insulin by releasing lipids into the bloodstream. The primary driver for the release of adrenergic antagonists is the activity of the sympathetic nervous system, which is one of the first systems to respond to changes in the environment and helps the body adapt to unexpected alterations. M.A. North *et al.* (2023) noted significant changes in climatic conditions in the regions where cows are kept. These changes had a considerable negative impact on the animals' bodies, subsequently causing metabolic shifts under the influence of the sympathetic nervous system.

An analysis of the literature led to the conclusion that investigating the factors influencing the correction of the fatty acid composition of milk is an important issue. This is because the levels of fatty acids are crucial indicators when assessing milk quality and its nutritional benefits for humans. Adjusting the fatty acid

composition of milk, particularly the unsaturated fatty acids, is achieved through key factors such as a balanced nutrient content in the cows' feed rations, the state of their gut microbiota, and especially through neurohumoral regulation. Therefore, studying this topic is highly relevant in contemporary research.

Materials and Methods

The research was conducted in 2023 at the dairy farm of the LLC Obriy (Vyshneve Village, Orzhytskyi District, Poltava Region, Ukraine) using Ukrainian Black-and-White dairy cows. The analysis of the milk's fatty acid profile from the experimental cows was carried out at the interdepartmental educational and scientific laboratory for veterinary diagnostic research (Faculty of Veterinary Medicine, National University of Life and Environmental Sciences of Ukraine, Kyiv)

The animal-based experimental studies described in this research, specifically all procedures involving the cows, were conducted following the fundamental principles of bioethics. This adhered to Article 26 of the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986), Directive 2010/63/EU (2010), and the "General Ethical Principles of Experiments on Animals" adopted by the First National Congress on Bioethics (Law of Ukraine No. 249, 2012).

The animal groups were established using variation-pulseometric research (according to Bayevsky's method) and employing a Heart Mirror IKO electrocardiograph (Innomed, Hungary). Based on the electrocardiogram readings, a measure known as the heartbeat interval between the R-R wave segments on the recorded trace was determined, and this was expressed as the mode (*Mo*). The percentage value of the mode, which was most frequently observed over a sequence of 100 heartbeats, and represented as the amplitude of the mode (*AMo*), was then calculated. The difference between the maximum and minimum mode values within the selected readings was also calculated, which corresponded to the variation range (Δx) indicator. Following this, indices reflecting the activity of the autonomic nervous system were computed, including the autonomic balance index (ABI), the autonomic rhythm index (ARI), and the tension index (TI).

After conducting the variation-pulseometric study, three experimental groups of cows were formed: sympathotonics, vagotonics, and normotonics. Each experimental group consisted of five animals. Milk samples were collected from the animals in the experimental groups using individual milking machines during the morning milking session. The collected milk samples were transported using insulated containers.

Lipid extraction was carried out using the method developed by J. Folch *et al*. (1957). Milk was placed in a glass container with a ground glass stopper, and then a mixture of methanol and chloroform in a 2:1 ratio was added, with a volume ratio of 1:20 relative to the milk. The resulting mixture was thoroughly mixed and left to stand for 24 hours. The following day, the mixture was filtered, and a 0.74% potassium chloride solution was added to the filtrate in a 1:5 ratio. After settling, the solution was separated into two layers. The lower layer was collected, and the chloroform was evaporated off using a rotary evaporator. The remaining lipids were then used to analyse their fatty acid composition.

Chromatographic analysis was performed using a Trace GC Ultra gas chromatograph (USA) equipped with a flame ionisation detector. The chromatography of the samples was conducted under the following conditions: detector temperature +260°C, column temperature

+140-240°C. To identify the fatty acid components in the cow milk lipid samples obtained from the chromatography, a Supelco 37 Component FAME Mix standard (USA) was used.

Statistical analysis of the research findings was performed using the Microsoft Excel software package, with the probability of significant differences between the measured values assessed using Student's t-test ($P < 0.05$, $P < 0.01$, $P < 0.001$).

Results and Discussion

Based on the results of the chromatographic analysis of milk from cows predominantly showing normotonia, the levels of the following unsaturated fatty acids were determined: myristoleic acid, palmitoleic acid, oleic acid, linoleic acid, and α-linolenic acid. According to the statistical analysis, the results obtained from five cows were compiled based on key descriptive statistics and are presented in Table 1.

Table 1. Unsaturated fatty acid content (%) in the milk of the normotonic experimental group of cows during the summer period

Indicator	NVO	SE	M	SD	Asym.	Min.	Max.
Myristoleic acid (C14:1)	5	0.02	1.35	0.05	−0.11	1.29	1.42
Palmitoleic acid (C16:1)	5	0.05	1.89	0.11	−1.58	1.70	1.99
Oleic acid (C18:1n9c)	5	0.19	23.44	0.43	−1.54	22.71	23.86
Linoleic acid (C18:2n6c)	5	0.07	4.56	0.17	−0.08	4.39	4.73
α-Linolenic acid (C18:3n3)	5	0.04	0.61	0.08	−0.28	0.49	0.72

Note: *NVO – number of valid observations; SE – standard error; M – mean value; SD – standard deviation; Asym. – asymmetry; Min. – minimum value; Max. – maximum value*
Source*: authors' development*

Based on the statistical analysis (Table 1) of the fatty acid composition of milk lipids from the normotonic experimental group of cows, the myristoleic acid content in the cows' milk showed a low standard error, indicating minimal variability between the values. The standard deviation also indicated a low level of variability in the data. Furthermore, the asymmetry measure reflected a near-perfect symmetrical distribution of the data points, and the difference between the maximum and minimum values for this fatty acid was 0.13%, suggesting a very small range in the obtained results.

The palmitoleic acid content also exhibited a low standard error, again indicating little variability in the measured values. The standard deviation value pointed to a non-significant level of data variability, and the asymmetry measure demonstrated a reasonably symmetrical distribution of the data. The difference between the maximum and minimum values for this fatty acid was 0.29%, indicating a small range in the obtained results.

For the oleic acid content in the milk of the five cows, a low standard error with minimal variability in the values was observed. The standard deviation indicated a small degree of variability in the data. The asymmetry measure revealed a slight leftward skew, reflecting a small negative asymmetry. The difference between the maximum and minimum values for this fatty acid was 1.15%, reflecting a small range in the obtained data.

Regarding the linoleic acid content, a low standard error was found, suggesting little variability in the values, and the standard deviation indicated a negligible level of data variability. Conversely, the asymmetry measure showed a normal distribution of the obtained data,

indicating no significant asymmetry. The difference between the maximum and minimum values for this fatty acid was 0.34%, suggesting a small range in the obtained data.

For the α-linolenic acid content among the five animals, a standard error with minimal variability in the values was established. The standard deviation reflected slight variability in the data for this fatty acid. The asymmetry measure indicated a small negative skew, describing a relatively even distribution of the data. The difference between the maximum and minimum values was 0.23%, confirming a small range in the numerical data.

Based on the chromatographic analysis of milk lipids from cows predominantly showing sympathotonia, the levels of the following unsaturated fatty acids were determined: myristoleic acid, palmitoleic acid, oleic acid, linoleic acid, and α-linolenic acid. According to the statistical analysis conducted, the results obtained from the five cows involved in the study were processed based on key descriptive statistics and are presented in Table 2.

Table 2. Unsaturated fatty acid content (%) in the milk of the sympathotonic experimental group of cows during the summer period

Indicator	NVO	SE	M	SD	Asym.	Min.	Max.
Myristoleic acid (C14:1)	5	0.02	1.44	0.05	–0.13	1.38	1.49
Palmitoleic acid (C16:1)	5	0.02	1.58	0.04	–0.40	1.52	1.62
Oleic acid (C18:1n9c)	5	0.07	22.60	0.17	0.28	22.41	22.81
Linoleic acid (C18:2n6c)	5	0.05	4.85	0.11	0.08	4.71	4.98
α-Linolenic acid (C18:3n3)	5	0.05	0.79	0.12	0.60	0.64	0.97

Note: *NVO – number of valid observations; SE – standard error; M – mean value; SD – standard deviation; Asym. – asymmetry; Min. – minimum value; Max. – maximum value*
Source*: authors' development*

According to the statistical evaluation of the results from the chromatographic analysis of the cows' milk, the standard error for myristoleic acid was low, indicating negligible variability in the data. The standard deviation pointed to slight variability in the obtained values. The asymmetry measure was insignificant, indicating no data asymmetry, thus reflecting a normal distribution. The difference between the maximum and minimum values for this fatty acid was 0.11%, signifying a small range in its measured levels.

For palmitoleic acid, the standard error values were relatively small, indicating negligible variability in the measurements. The variability of the results was within acceptable limits, based on the standard deviation. The asymmetry measure indicated a slight negative skew, suggesting that the results approximated a normal distribution. The difference between the maximum and minimum values for this fatty acid was 0.10%, confirming a small range in its levels.

The oleic acid levels showed slight variation in the data and their variability, which is supported by the low standard deviation and standard error values. The asymmetry showed moderately small positive skew values, suggesting the distribution of the values was reliable. The difference between the maximum and minimum values for this fatty acid was 0.40%, further supporting the normal distribution of the obtained results.

The values for linoleic acid were characterised by low standard error and standard deviation, indicating minimal variability and fluctuations in the results. The asymmetry further confirmed a normal distribution for this fatty acid's levels, implying a virtual absence of

asymmetry. This was consistent with the difference between the maximum and minimum values, which was 0.27%.

For α-linolenic acid, the statistical analysis indicated slight variability and fluctuations in the data, which were supported by the standard error and standard deviation values. The asymmetry reflected a small positive skew, indicating a reasonable approximation to a normal distribution. The difference between the highest and lowest values for this fatty acid was 0.33%, corresponding to a small range in the raw data.

Based on the chromatographic analysis of milk lipids from cows predominantly showing vagotonia, the levels of the following unsaturated fatty acids were determined: myristoleic acid, palmitoleic acid, linoleic acid, oleic acid, and α-linolenic acid. According to the statistical analysis conducted, the results obtained from five cows were processed based on key descriptive statistics and are presented in Table 3.

Table 3. Unsaturated fatty acid content (%) in the milk of the vagotonic experimental group of cows during the summer period

Indicator	NVO	SE	M	SD	Asym.	Min.	Max.
Myristoleic acid (C14:1)	5	0.04	1.65	0.09	-0.50	1.53	1.75
Palmitoleic acid (C16:1)	5	0.04	1.49	0.09	0.41	1.37	1.62
Oleic acid (C18:1n9c)	5	0.04	23.57	0.10	0.24	23.45	23.70
Linoleic acid (C18:2n6c)	5	0.04	3.70	0.09	0.28	3.59	3.82
α-Linolenic acid (C18:3n3)	5	0.04	0.43	0.09	0.66	0.32	0.57

Note: *NVO – number of valid observations; SE – standard error; M – mean value; SD – standard deviation; Asym. – asymmetry; Min. – minimum value; Max. – maximum value*
Source*: authors' development*

According to the statistical analysis of the results from the chromatographic analysis of the cows' milk, the standard error for myristoleic acid was low, characterising a small variability in the data. The standard deviation indicated slight variability in the obtained values. The asymmetry showed a small negative skew, characterising a reliable symmetry in the data. The difference between the maximum and minimum values for this fatty acid was 0.22%, signifying negligible differences in their levels.

For palmitoleic acid, the standard error values were quite small, indicating negligible variability in the measurements. The variability of the results was within acceptable limits, based on the standard deviation values. The asymmetry value indicated a slight positive skew, suggesting that the results approximated a normal distribution. The difference between the maximum and minimum values for this fatty acid was 0.25%, confirming a small range in its levels.

The values for linoleic acid were characterised by low standard error and standard deviation, indicating minimal variability and fluctuations in the data. The asymmetry further confirmed a normal distribution for this fatty acid's levels, implying a virtual absence of asymmetry. This was consistent with the difference between the maximum and minimum values, which was 0.23%.

For α-linolenic acid, the statistical analysis indicated slight variability and fluctuations in the data, which were supported by the standard error and standard deviation values. The asymmetry reflected a small positive skew, indicating a reasonable approximation to a normal distribution. The difference between the maximum and minimum values for this fatty acid

was 0.25%, corresponding to a small range in the original data.

According to the obtained results regarding the content of unsaturated fatty acids in the cows' milk, it was found that the myristoleic acid content in the milk of the vagotonic experimental group increased by 22% ($P < 0.001$), and in the sympathotonic group by 7% ($P < 0.05$), when compared to the normotonic experimental group. It is worth noting that, among the three experimental groups of animals, the vagotonic group exhibited the highest levels of myristoleic acid. The lowest relative content of the fatty acid C14:1 was observed in the milk of the sympathotonic experimental group (Fig. 1).

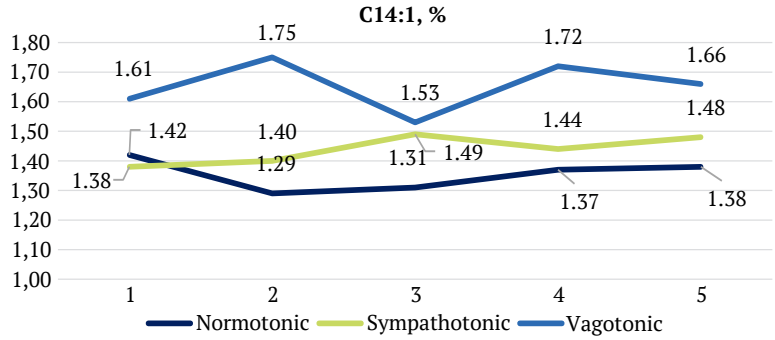

Figure 1. Myristoleic acid content in the milk of experimental groups of cows
***Note**: colours represent the experimental groups of animals*
***Sourse**: authors' development*

The palmitoleic acid content in the milk of cows in the vagotonic experimental group decreased by 21% ($P < 0.001$), and in the sympathotonic group by 16% ($P < 0.001$) compared to the normotonic group. Among the animals in the experimental groups, the highest relative content of this fatty acid (C16:1) was found in the normotonic cows. The animals in the sympathotonic and vagotonic experimental groups showed only slight differences in the levels of palmitoleic acid in their milk lipids (Fig. 2).

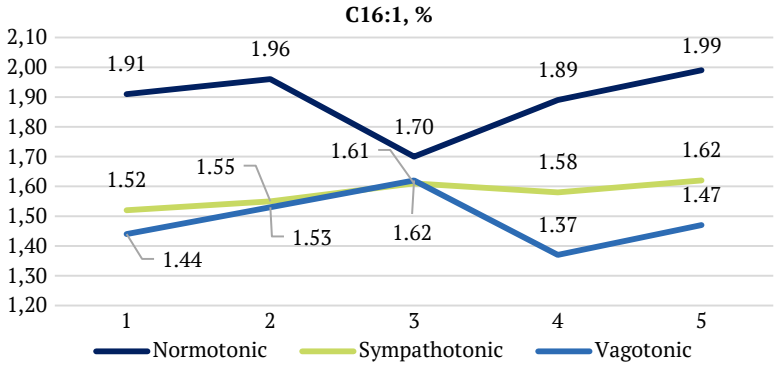

Figure 2. Palmitoleic acid content in the milk of experimental groups of cows
***Note**: colours represent the experimental groups of animals*
***Sourse**: authors' development*

The oleic acid content in the milk of cows in the sympathotonic experimental group decreased by 3.6% ($P < 0.05$) compared to the normotonic group. It is worth noting that, among the cows in the three experimental groups, the sympathotonic group had the lowest levels of oleic acid. The experimental groups of cows with a predominance of vagotonia and normotonia showed only slight differences in the levels of oleic acid in their milk lipids (Fig. 3).

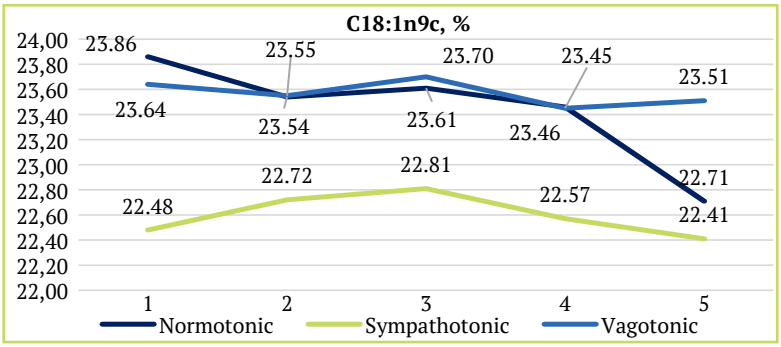

Figure 3. Oleic acid content in the milk of experimental groups of cows
***Note**: colours represent the experimental groups of animals*
***Sourse**: authors' development*

The linoleic acid content in the milk of cows in the vagotonic experimental group decreased by 19% ($P < 0.05$), while in the sympathotonic group, it increased by 6% ($P < 0.05$) compared to the normotonic group. Among the cows in the experimental groups, the lowest relative content of the fatty acid C18:2n6c was observed in the vagotonic animals. The cows in the sympathotonic and normotonic experimental groups showed only slight differences in the levels of linoleic acid in their milk lipids (Fig. 4).

The α-linolenic acid content in the milk of cows in the vagotonic experimental group decreased by 46% ($P < 0.05$), while in the sympathotonic group, it increased by 30% ($P < 0.05$) compared to the normotonic group. It is worth noting that, among the cows in the three experimental groups, the sympathotonic group had the highest levels of α-linolenic acid. The lowest relative content of the fatty acid C18:3n3 in the milk of cows was observed in the vagotonic experimental group (Fig. 5).

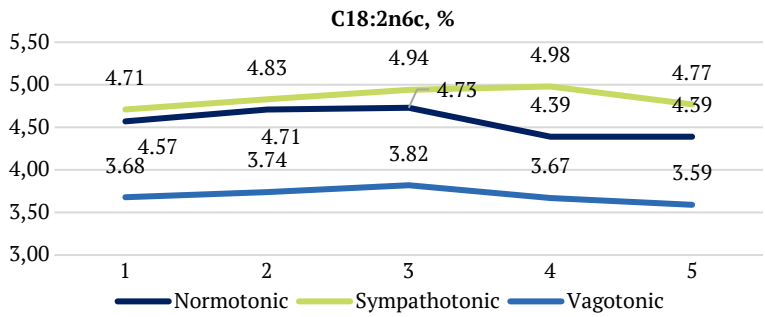

Figure 4. α-Linolenic acid content in the milk of experimental groups of cows
***Note**: colours represent the experimental groups of animals*
***Sourse**: authors' development*

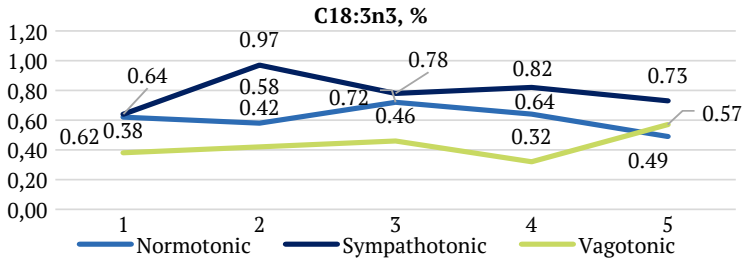

Figure 5. α-Linolenic acid content in the milk of experimental groups of cows
***Note**: colours represent the experimental groups of animals*
***Sourse**: authors' development*

A.M. Wanderley *et al.* (2022) demonstrated that the lipid content in milk constantly fluctuates within a defined range. These variations are due to several influencing factors, such as diet, housing conditions, the animal's genetic makeup, and physiological characteristics like neurohumoral regulation. The combination of these elements forms a list of important indicators that should be considered when raising cows. Indeed, without accounting for and adjusting these fundamental factors, the normal development of the animal will be compromised, leading to reduced milk yield and energy imbalances during lactation. Neglecting the control systems governing metabolic processes in cows can also undermine the effectiveness of any attempts to correct them.

Lipids are crucial for maintaining high productivity in dairy farming, as a cow's body consumes a significant amount of energy-rich compounds during milk synthesis. M.S. Gold *et al.* (2022) in their research highlighted the significant role of diet in maintaining the animals' energy metabolism. However, it is also necessary to investigate the influence of additional factors on lipid synthesis, with a focus on how this impact affects productivity. This study, therefore, highlights the importance of studying the role of autonomic regulation in fat metabolism to improve milk production in cows.

J. Shpirer *et al.* (2023) investigated the influence of diet on lipid metabolism in cows and the resulting changes in the milk's lipid profile. As previously mentioned, several factors regulate metabolism within an animal's body. The digestion, absorption, and utilisation of nutrients represent fundamental physiological processes. The authors demonstrated that a higher concentration of specific fatty acids in the diet leads to an improved lipid profile in the milk. For instance, increasing the levels of palmitic and oleic acids in the feed ration positively impacted the percentage of fat in the milk, and an increase in milk production was observed. This study emphasised the factors controlling the metabolic transformations of substances within the animals' bodies, which was evident in the differences in unsaturated fatty acid content in the milk, depending on the activity of the autonomic nervous system.

The specifics of fatty acid digestibility in the digestive tract and their subsequent production in the mammary gland under varying feed rations were investigated by A. Irawan *et al.* (2024). These researchers explored how the fatty acid composition of milk changes in response to physiological processes involved in the digestion and absorption of lipids within cows. They found that the inclusion of clover in the diet resulted in minor changes to milk composition, with increases observed in linoleic, α-linolenic, and stearic acid levels. When sainfoin silage was present in the diet, the levels of α-linolenic acid and linoleic acid improved. In the current

study, the impact on the fatty acid composition of milk was achieved through differing autonomic regulation, meaning variations in the activity of systems controlling metabolic intensity.

G. Satir *et al*. (2023) examined the role of palm oil in the diet of cows on fatty acid metabolism and their concentration in milk. Their analysis of milk showed an increase in palmitic acid content and a slight rise in the overall level of saturated fatty acids. The author highlighted the importance of studying lipid metabolism to enhance the fatty acid profile of milk, particularly in increasing the levels of essential omega-6 and omega-3 fatty acids. The present study established the influence of autonomic regulation on the content of omega-6 and omega-3 fatty acids, resulting from changes in their synthesis and breakdown processes.

E. Vargas-Bello-Pérez *et al*. (2022) investigated alterations in the lipid composition of milk when sunflower and rapeseed oil were included in the animals' diet. Evaluating the feed digestion processes, with the key aspects outlined, they found minor changes in the levels of butyric acid, α-linolenic acid, and linoleic acid. This indicated a dietary dependence on the feed consumed and its digestibility within the digestive system. The authors' own research focused on analysing changes in lipid concentration in cows' milk under the influence of the autonomic nervous system, which consequently showed significant alterations based on the chromatographic analysis data.

Having analysed the obtained results, including a comparative perspective with existing knowledge, conclusions can be drawn regarding the factors that influence the fatty acid composition of cows' milk. The overwhelming majority of research by other scientists studying lipid metabolism in the mammary gland focuses on the dietary dependence of consumed feed and its composition on the concentration of lipids in milk. However, their findings when assessing factors influencing lipid metabolism in the mammary gland often reveal only minor correlations. A possible explanation for this is the exclusion of systems responsible for metabolic processes within the animal's body, particularly neurohumoral regulation. By evaluating only a small part of this system, specifically the autonomic nervous regulation, significant differences in the lipid composition of milk in cows were revealed according to the study results. When analysing lipid metabolism in lactating cows, it is also important to consider individual differences within the animals' bodies. Categorising them according to their autonomic regulation will improve both the general understanding of metabolic processes and the development of methods for adjusting the intensity and direction of lipid metabolism in lactating cows.

Conclusions

It has been established that autonomic regulation influences the levels of unsaturated fatty acids in cows' milk. This is supported by the data from the chromatographic analysis of the lipid composition of milk from animals exhibiting normotonia, vagotonia, and sympathotonia. It was determined that a higher level of sympathotonia in cows is a contributing factor to increased levels of myristoleic, linoleic, and α-linolenic acids in their milk, compared to the group of animals with a balanced sympathovagal system. Specifically, the myristoleic fatty acid content, according to the chromatographic analysis, increased in the milk of sympathotonic cows by 7% ($P < 0.05$) and in vagotonic cows by 22% ($P < 0.001$) when compared to the normotonic experimental group. Conversely, the level of palmitoleic acid decreased in the milk of vagotonic cows by 21% ($P < 0.001$) and sympathotonic cows by 16% ($P < 0.001$), relative to its levels in the milk of the normotonic experimental group. The oleic acid content in the milk of sympathotonic cows decreased by 3.6% ($P < 0.05$)

compared to the normotonic experimental group. The level of linoleic fatty acid in the milk of sympathotonic cows increased by 6% ($P < 0.05$), while in vagotonic cows it decreased by 19% ($P < 0.05$) compared to the animals in the normotonic experimental group. The α-linolenic acid content in the milk lipids of sympathotonic cows increased by 30% ($P < 0.05$), while in vagotonic cows it decreased by 46% ($P < 0.05$) compared to the normotonic animals. Thus, the autonomic nervous system, depending on individual differences in the cows' physiology, such as varying activity of the sympathetic and parasympathetic nervous systems, has a distinct influence on fatty acid metabolism. This manifests as either an increase or decrease in lipolysis and lipogenesis, particularly during periods when external environmental factors exert their influence. This is evidenced by the differing levels of unsaturated fatty acids in the cows' milk, the percentage ratios of which depend on the intensity of lipid metabolism. This metabolism is controlled by the body's regulatory systems, which include the autonomic nervous system. Therefore, individual physiological characteristics of the animals, expressed as different tones of the autonomic nervous system – normotonia, sympathotonia, vagotonia – are an important indicator when analysing and adjusting lipid metabolism.

Further research will focus on the application of nanoaquachelate bioactive substances to high-yielding cows, with mandatory consideration of their individual characteristics, specifically their autonomic regulation. This will involve developing a methodology to enhance metabolic intensity within the body to improve overall cow productivity.

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Conflict of Interest

None.

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Вегетативна регуляція вмісту ненасичених жирних кислот у молоці корів

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Анотація. Вивчення ролі автономної нервової системи як однієї з провідних систем контролю за метаболізмом в організмі тварин важливе для оцінки індивідуальних відмінностей інтенсивності і напряму перебігу обміну речовин. Тому дослідження впливу парасимпатичної і симпатичної нервової системи на обмін ліпідів, у тому числі жирних кислот, є актуальним питанням для забезпечення молочної продуктивності корів. Метою цього дослідження було порівняльне оцінювання вмісту ненасичених жирних кислот у молоці корів за різної вегетативної регуляції у літній період. Формування дослідних груп корів породи українська чорно-ряба молочна здійснювалося відповідно до симпато-вагусного балансу шляхом проведення варіаційно-пульсометричного дослідження. Для ідентифікації жирнокислотного складу молока використовувався метод газової хроматографії. В результаті встановлено, що рівень мірістоолеїнової кислоти у молоці корів із ваготонією виявлявся вищим на 22 % ($P < 0,001$) порівняно з її вмістом у молоці корів із нормотонією, а із симпатотонією – на 7 % ($P < 0,05$). Пальмітолеїнова кислота у корів із ваготонією відмічався менше на 21 % ($P < 0,001$) порівняно з таким у молоці корів із нормотонією, а із

симпатотонією – на 16 % (P < 0,001). У молоці корів із симпатотонією зафіксовано знижений на 3,6 % (P < 0,05) вміст олеїнової кислоти порівняно з її концентрацією у корів із нормотонією. Вміст лінолевої кислоти у корів із ваготонією відзначався значеннями меншими на 19 % (P < 0,05) порівняно з її рівнем у молоці корів із нормотонією, а із симпатотонією – більшими на 6 % (P < 0,05). Вміст α-ліноленової кислоти у молоці корів із ваготонією виявлявся меншим на 46 % (P < 0,05) порівняно з таким у молоці корів із нормотонією, а у симпатотоніків – більшим на 30 % (P < 0,05). Виявлено залежність між активністю автономної нервової системи та вмістом ненасичених жирних кислот у молоці. Отримані результати підтверджують важливість вивчення питання ролі регуляторних механізмів обміну ліпідів, особливо у молочному скотарстві, де індивідуальна продуктивність корів визначається рівнем жирів, які споживаються, засвоюються, синтезуються та метаболізуються в організмі

Ключові слова: жуйні; газова хроматографія; ліпіди; варіаційна пульсометрія; нервова система

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Prevalence of hoof diseases in cows

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Abstract. An increasing incidence of orthopaedic disorders, including the development of purulent conditions, has been recorded among cows on dairy farms. These disorders are typically accompanied by localised inflammatory reactions, which adversely affect the overall physiological state of the animals, leading not only to reduced milk yield but also to premature culling. This study aimed to determine the prevalence of orthopaedic disorders in dairy cows, identify their forms and clinical characteristics, and develop approaches to local treatment. Monitoring studies were conducted at livestock farms in the Vinnytsia, Donetsk, Lviv, and Cherkasy regions. Data were obtained on the prevalence of orthopaedic pathology in dairy cows, including the identification of nosological forms, degrees of lameness, and the effectiveness of treatment applied. Lameness was assessed by observing cows in motion. The final diagnosis of the pathological condition was made following an examination of the distal limb segments and orthopaedic hoof trimming. As a result of the examination, aseptic pododermatitis was diagnosed in 7.5%-48.0% of cows within the observed herds; clinical signs of purulent infiltration at the base of the sole's dermis in the pedal segment of the hindlimbs were identified in 5.6%-18.0% of cows; and over 50.0% of cows were found to have papillomatous digital dermatitis over the past two years. By the sixteenth day following the course of treatment, two-thirds of the affected animals had recovered. Subsequent clinical evaluations of cows before and after local therapy revealed no signs of progression of

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purulent processes in the distal limb region. These findings indicate the positive effect of local treatment using Intra Hoof-fit Gel (Netherlands), the wound-healing Intra Repiderma Spray (Netherlands), and Intra Hoof-fit Tape (Netherlands) by day sixteen, resulting in treatment efficacy rates of 90.0%-93.3%. The results obtained are of practical value for both researchers and practising veterinary professionals and may contribute to the improvement of treatment protocols for orthopaedic disorders in dairy cows

Keywords: surgical pathology; animal welfare; orthopaedics; lameness; local therapy

Introduction

Hoof diseases and the resulting lameness in cows are a widespread problem of increasing importance in maintaining animal health. Preventative hoof trimming is a crucial procedure for promoting cow health. Specifically, it is considered optimal to perform orthopaedic hoof trimming at least twice a year to maintain hooves in a physiologically functional state. Regular hoof trimming prevents deformation of the horn capsule. Damage to the horn layer disrupts limb posture, leading to overstretching of tendons and the development of aseptic, and subsequently purulent, processes in the dermal base area.

R. Laven *et al.* (2024) expanded the understanding of the aetiology and pathogenesis of orthopaedic conditions in their research. They reported data showing that aseptic pododermatitis is accompanied by haemorrhages in the sole and white line. These haemorrhages are considered precursors to purulent pododermatitis and specific sole ulcers. The researchers concluded that understanding the development of haemorrhage in the sole horn is vital for determining the aetiology and pathogenesis of orthopaedic conditions and for assessing stress factors affecting the hoof. Their findings contribute to the development of evidence-based prevention and treatment plans for these animals.

Scientists D. Kucevic *et al.* (2022) studied 6,348 Holstein dairy cows from 5 farms to investigate the impact of housing systems (tie-stall vs. free-stall barns) on the prevalence of hoof diseases and culling rates. The researchers collected data on the causes of lameness, established diagnoses, and provided treatment. The animals underwent functional-corrective hoof trimming. Over the three-month study period, the average culling rate for cows in free-stall housing was 5.4%, compared to 4.9% in tie-stall housing. This difference was not statistically significant.

In their research, American scientists T.S. Marshall *et al.* (2024) in Missouri studied 34 cows with orthopaedic conditions for 4 weeks. They demonstrated that a hoof bath solution containing stannous fluoride (SnF2) was superior to a 5% copper sulphate solution for the treatment and prevention of digital dermatitis (DD) in dairy cattle. Their results showed that passing cows through the stannous fluoride bath once a week in a herd experiencing active infection transmission was more effective in preventing new lesions, but less effective in treating existing digital dermatitis lesions, compared to passing cows through a copper sulphate bath four times a week.

Purulent pododermatitis in cattle develops from aseptic pododermatitis when hooves become deformed with signs of horn sole breakdown. This was noted in an article by N.M. Khomyn *et al.* (2024). According to their data, the disease is partly caused by delayed

corrective trimming, hoof deformation, and poor housing conditions due to prolonged confinement (winter housing period). Their research showed that mechanical factors are the primary cause of local purulent processes in the dermal layer in the sole segment. When purulent inflammation develops, limb posture and weight distribution on the hoof sole are disrupted. In many cases, animals shift weight onto the healthy digit. Inflammatory exudate infiltrates the dermal tissues, initiating a local pathological process in the sole area, which may become purulent if microorganisms enter the affected site. Another cause of purulent pododermatitis is a puncture of the sole, followed by an infection of the injured area with pathogenic microorganisms, which mainly occurs during the pasture period.

According to research by L. Langova *et al.* (2020), lameness, which often results from hoof lesions, poses serious animal welfare problems on dairy farms. Hoof trimming is one method to prevent these lesions, but the overall effect and optimal trimming frequency are not fully understood. In a retrospective study, K. Grimm *et al.* (2024) analysed the relationship between hoof trimming frequency in first-lactation cows, their functional status, and culling during their second lactation. The authors concluded that reducing hoof lesions through trimming significantly improved animal welfare, productivity, and longevity.

As noted by Y. Saito *et al.* (2024), lameness is a symptom of distal limb lesions. The authors, based on their research, found that the disease, caused by hoof lesions, affected animal welfare and reduced dairy cow productivity. Furthermore, they reported that the prevalence of various types of hoof lesions varied depending on environmental conditions and the animal housing system on the dairy farm.

A. Dias *et al.* (2024) investigated the impact of hoof pain and lesion severity on animal mobility. The authors demonstrated that localised pain causes visible changes in cow movement. Animals spent longer periods lying down in stalls and were reluctant to stand. The authors found that sole ulcers increased movement activity, while digital dermatitis and sole haemorrhage, conversely, decreased animal mobility. In a static position, changes in hoof support and position were observed. Specifically, weight was shifted to the healthy limb, heel support was used, and weight distribution was uneven during stepping. The researchers suggested that changes in behavioural indicators related to support, weight distribution, and limb position help identify cows with hind-limb hoof lesions. Therefore, regular observation by farm staff could help identify lame cows requiring treatment.

M.I. Rodríguez *et al.* (2021) assessed the relationship between hoof pathologies and milk production in dairy farms in the Caaguazú Department of Paraguay. They examined 50 cows diagnosed with hoof pathologies during the declining phase of lactation. The researchers found that white line disease had the least impact on milk production.

Lameness is one of the most significant and economically damaging problems in cattle farming. M. Bayer *et al.* (2023) found that changes in movement activity are most often associated with limb lesions, particularly in the hind limbs. The authors noted that pain reactions in cows lead to depression and reduced milk production. Other factors contributing to limb dysfunction include feeding imbalances, poor hygiene, management errors, genetic predisposition, and breeding issues. A well-balanced diet, rich in macro- and micronutrients and amino acids involved in keratinisation, is essential for healthy hoof horn formation, preventing many orthopaedic lesions in cows. Proper keratinisation creates a resilient hoof horn that is impermeable and resistant to

external factors like injuries and the effects of bacterial and fungal keratinases, which break down disulphide bonds in the hoof horn.

P.T. Thomsen *et al.* (2020) found that cows undergoing hoof trimming late in pregnancy have a higher risk of abortion. The researchers determined that the probability of pregnancy termination is lower in Jersey cows and higher in cows pregnant with twins. The likelihood of pregnancy termination is also 2.4 times higher in cows that had their hooves trimmed within the last four weeks of gestation.

Y.-J. Chiu & J.-T. Hsu (2022) evaluated and demonstrated a link between behaviour and hoof lesions in lactating Jersey dairy cows. Specifically, they assessed body condition using an automated activity monitor (AfiTag II, AfiMilk, Afikim, Israel) attached to the right hind limb. The authors concluded that cows developing hoof lesions (80.0% with sole haemorrhages) had shorter lying durations (daily and in the early postpartum period). Therefore, they concluded that a reduced daily lying duration should be considered a risk factor for hoof diseases in lactating dairy cows.

H. Pirkkalainen *et al*. (2022a) found that hoof pathologies lead to lameness and animal welfare problems. This results in an increase in acute-phase proteins in the blood serum, which are markers of the inflammatory phase response. Specifically, the authors noted an elevation in serum amyloid A, haptoglobin, and interleukin-6 concentrations. The study evaluated the inflammatory response of cows with sole ulcers, pododermatitis, and digital dermatitis compared to healthy cows. It was demonstrated that serum amyloid A concentrations were significantly higher in cows with sole ulcers compared to the control group (cows without hoof lesions) over a two-week study period. These results indicate that sole ulcers initiate a prolonged systemic inflammatory response in dairy cows.

Therefore, this study aimed to determine the prevalence of orthopaedic conditions in cows on several dairy farms and to assess the effectiveness of local treatment using Intra Hoof-fit Gel, the wound-healing Intra Repiderma Spray, and Intra Hoof-fit Tape. Achieving this goal is important for improving the prevention and treatment of orthopaedic conditions in cows, which will reduce morbidity and enhance animal productivity. Furthermore, confirming the effectiveness of these topical treatments will optimise veterinary practices and improve the economic efficiency of dairy farming.

Materials and Methods

The research was conducted from December 2023 to November 2024 at dairy farms including: ALLC "Ridnyi Kray" (Yampil district, Vinnytsia region); LLC "Svitanok" (Pokrovsk district, Donetsk region); ALLC "Bilyi Stik" (Sokal district, Lviv region); and LLC "YuM-Vatutino" (Zvenyhorod district, Cherkasy region). Specific aspects of the research were carried out at the scientific laboratories of the Faculty of Veterinary Medicine, Poltava State Agrarian University (Poltava). All animals underwent clinical examination and clinical-instrumental assessment.

During the clinical examination of the cows, attention was paid to their body position: standing or lying. The position of the affected limb was noted, including whether the hoof was bearing weight (full weight bearing, lifting the limb off the ground, or only partial weight bearing on the heel or toe). The condition of the soft tissues surrounding the horn capsule was examined visually and by palpation. Palpation was also used to assess the presence or absence of increased pulse amplitude in the digital arteries. Hoof testers were used to examine the condition of the sole (pain upon pressure and flexibility of the sole horn).

Animal experiments were conducted following Order of the Ministry of Economic

Development, Trade and Agriculture of Ukraine No. 224 (2021) and in compliance with the international principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (European Convention on..., 1986), and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Treatment" (2006).

A total of 1,380 cows underwent an orthopaedic examination. Hoof-trimming procedures also included monitoring for limb diseases in cows. Digital colour photographs from literature sources (Espinasse, 1984) were used to identify each hoof lesion, according to the international classification system adopted at the 15th International Conference on Lameness in Ruminants held in Finland (International Veterinary Information Service, 2008).

The stages of hoof trimming involved mandatory restraint of the cow in a standing position within an orthopaedic crush. Hoof trimming included limb fixation in a comfortable position, removal of debris and loose old sole horn from the horn capsule surface using a hoof knife until elastic tissue was exposed, levelling of the hoof wall with nippers, and final smoothing with an angle grinder fitted with an orthopaedic milling cutter. Finally, a visual assessment of the hoof and limb position was performed on a level surface.

Hoof lesions were diagnosed during trimming based on visual examination, the presence of a specific odour in the pathological focus, and localised pain reactions in the cows, according to the international standardised diagnosis described by J. Espinasse (1984). Lesions were recorded as absent or present on the hind hooves. To determine the effectiveness of local treatment for digital dermatitis, 30 affected cows from each farm were selected using an analogue-based approach.

Based on the visual examination data, the following diagnoses were established and recorded: aseptic and purulent pododermatitis and digital dermatitis. After trimming and initial surgical debridement of the lesions, the affected cows were treated topically twice, with a five-day interval, using the antibiotic-free hoof gel Intra Hoof-fit gel (Intracare, Netherlands). The hoof was thoroughly cleaned before gel application. Subsequently, disposable wipes were used to remove purulent exudate and devitalised tissue thoroughly. After examination and confirmation of the absence of purulent exudate in the sole region and interdigital space, the surface was dried, and the topical medication was applied according to the instructions. Intra Hoof-fit Gel was applied in a thin layer to the affected surface using a brush. Per the study objectives, the topical medication was applied once every five days, and its therapeutic effect was subsequently evaluated. If necessary, the wound-healing Intra Repiderma Spray (Netherlands) was used. Before use, the canister was shaken. The spray was applied to the skin surface for 3 seconds from a distance of 15-20 cm to ensure even coverage. The product was allowed to dry for at least 30 seconds before applying an Intra Hoof-fit Tape (Netherlands). After the experiments were completed, the data was statistically analysed using Statistica 5.0 software (StatSoft Inc., USA), and the results were presented as mean (M) values in tables.

Results and Discussion

To understand the prevalence of orthopaedic conditions among dairy cows, a clinical examination of the animals present on the farms was conducted. The study involved visual inspection of the cows and, where necessary, local examination. The latter allowed the identification of cows with hoof pathologies, forming the experimental group (Table 1).

Analysing the results on the structure of

Table 1. Structure of digital region pathology in dairy cows

Clinical forms	Affected limbs																	
	Right forelimb			Left forelimb			Both forelimbs			Left hindlimb			Right hindlimb			Both hindlimb		
	Hooves																	
	L	M	B	L	M	B	L	M	B	L	M	B	L	M	B	L	M	B
AP	Ridnyi Kray ALLC (250 cows), Vinnytsia region																	
	-	-	-	-	-	-	-	-	17	-	-	-	-	-	-	-	-	84
	Svitanok LLC (350 cows), Donetsk region																	
	-	-	-	-	-	-	-	-	70	-	-	-	-	-	-	-	-	98
	Bilyi Stik ALLC (600 cows), Lviv region																	
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	45
PP	Ridnyi Kray ALLC (250 cows), Vinnytsia region																	
	-	-	-	-	-	-	-	-	-	9	4	5	1	4	8	-	-	14
	Svitanok LLC (350 cows), Donetsk region																	
	-	-	-	-	-	-	-	-	-	4	2	2	4	2	6	-	-	28
	Bilyi Stik ALLC (600 cows), Lviv region																	
	-	-	-	-	-	-	-	-	-	5	10	10	10	5	15	-	-	35
MD	Ridnyi Kray ALLC (250 cows), Vinnytsia region																	
	-	-	-	-	-	-	2	11	12	7	4	10	8	6	7	-	-	133
	Svitanok LLC (350 cows), Donetsk region																	
	-	-	-	-	-	-	-	-	65	-	-	13	-	-	24	-	-	263
	Bilyi Stik ALLC (600 cows), Lviv region																	
	-	-	-	-	-	-	-	-	45	-	-	-	-	-	-	-	-	235
	YuM-Vatutino LLC (180 cows), Cherkasy region																	
	-	-	-	-	-	-	-	-	78	-	-	-	-	-	-	-	-	88

Note: *AP – aseptic pododermatitis; PP – purulent pododermatitis; MD – Mortellaro's disease; L – lateral hoof; M – medial hoof; B – both*
Source*: authors' development*

orthopaedic pathology, it was found that during orthopaedic hoof trimming of 250 cows at ALLC "Ridnyi Kray", 40.4% showed aseptic pododermatitis (Fig. 1). In 84.0% of these cases, the disease was found on the hind limbs as oily yellow or bluish spots on the sole region. These animals spent long periods lying down and were reluctant to stand when attempts were made to move them. In a standing position, the affected animals shifted their body weight to the healthy limb. Palpation of these animals revealed an increased pulse amplitude in the digital arteries (a specific throbbing sensation upon palpation of the digital flexor tendons). When pressure was applied to the sole using hoof testers, localised pain and attempts by the animal to free the limb from restraint were noted.

The aforementioned is supported by re-

Figure 1. Aseptic digital pododermatitis of hind limbs
Note*: arrows indicate areas of sole horn infiltration with exudate*
Sourse*: authors' photo*

search from N.M. Khomyn *et al.* (2024). Specifically, the researchers found that cows with aseptic pododermatitis were reluctant to rise, often with groaning. At rest, they frequently shifted weight from limb to limb. Some animals exhibited a degree of postural tension during prolonged standing. Cows stood hunched, bringing their hind limbs under their body or pushing them back, carefully bearing weight on the hooves that showed deformation. This undoubtedly disrupted the even distribution of load on the sole area during weight bearing, leading to the formation of distorted hooves. The authors noted that deformation altered the sole region's topography, and weight-bearing occurred on only a small portion of the area, while the larger part of the sole did not touch the ground. This disrupted the even distribution of body weight on the sole area, resulting in the overloading of some parts and the unloading of others. Excessive overloading of certain sole parts over time led to profound functional changes and the development of chronic aseptic pododermatitis. The researchers believed that the locomotor characteristics of the hind limbs and the relatively small toe angle of their hooves predispose them to deformation and the development of aseptic pododermatitis.

In 14 cows (5.6%) from the same farm, purulent pododermatitis of the hind limbs was also diagnosed (Fig. 2). The latter was predominantly located on the lateral digits of the hind limbs in the heel region. These were primarily superficial purulent lesions.

Clinically, the manifestation of purulent

Figure 2. Purulent pododermatitis of hind limbs in cows
***Note**: a) areas of necrotic tissue on the dermal base in the sole segment; b) focus of purulent exudate; c) areas of damaged sole horn*
***Sourse**: authors' photo*

lesions on the dermal base in the sole segment was accompanied by the animal's desire to lie down for extended periods and reluctance to stand. The animals did not bear weight on the affected hoof, keeping them suspended. Affected cows periodically only touched the ground with the toe region, resulting in characteristic sole wear. Pain was observed upon the application of pressure to the affected area with hoof testers or even fingers, and polymorphonuclear purulent exudate was released when a funnel-shaped opening was cut into the sole region.

Approximately half of the animals examined over the two-year study period showed signs of Mortellaro's disease to varying degrees: redness of the skin in the coronary band and interdigital tissues and signs of tissue damage in the heel (Fig. 3). Palpation of the pathological areas on the affected limbs revealed significant pain. Minor accumulations of purulent exudate were observed on the surface of local lesions associated with this condition.

X. Ma *et al.* (2024) demonstrated that dig-

a b c

Figure 3. Animals affected by papillomatous digital dermatitis
***Note**: a) abnormal limb posture, heel weight bearing; b) horn separation in the sole region; c) areas of damaged sole horn*
***Sourse**: authors' photo*

ital dermatitis is a major cause of lameness in dairy cattle, particularly in animals housed in stalls for extended periods. The authors investigated the prevalence of digital dermatitis on three farms in Jiangsu, China. The disease was found on all three farms. All lesions were chronic. The prevalence of the disease in cows on the farms ranged from 7.3% to 10.8%.

Research on the prevalence of orthopaedic conditions in animals at LLC "Svitanok" (350 cows) revealed that the highest percentage of lesions in the existing herd was aseptic pododermatitis – 48.0%, which was more frequently diagnosed in the hind limbs (Table 1). This was primarily due to irregular hoof trimming in the animals and the formation of deformities. In 18.0% of the animals, signs of purulent pododermatitis were diagnosed on the hind hooves. This type of pathology developed as a result of microorganisms entering the dermal layer of the sole through damaged areas of the sole. In 75.0% of cases, cows were diagnosed with digital dermatitis over two years, with varying degrees of clinical symptom intensity. The research found significantly fewer cases of aseptic pododermatitis in cows at ALLC "Bilyi Stik", namely 7.5% of the existing herd. The proportion of purulent pododermatitis was 13.0%, and digital dermatitis was diagnosed in nearly half of the cows at various times during the study. At LLC "YuM-Vatutino" (180 cows), the majority of cows showed signs of digital dermatitis.

The data obtained largely aligns with the results of P. Patoliya *et al.* (2024). Specifically, the authors noted that the problem associated with orthopaedic pathology has become particularly acute due to the transition of animal husbandry to an industrial basis and the concentration of a large number of animals in limited areas. This problem has been especially exacerbated in the context of dairy farming specialisation, which is associated with a sharp change in animal feeding and housing. Hoof diseases in cows are common and cause significant losses to farms (Aliiev & Gavrilchenko, 2018). Farms suffered colossal losses in milk production due to reduced milk yield from cows with hoof lesions. When an animal was lame, production losses ranged from 20% to 50% or more. Simultaneously, lameness in cows negatively affected the reproduction process: a shortfall of up to 17 calves per 100 cows was observed, and the most productive cows had to be culled, leading to forced frequent herd rotation.

As noted by A.R. Mysak *et al.* (2020), 70%-80% of all animal diseases are non-contagious, with more than 50% of these being surgical, causing significant economic losses

to the livestock industry due to reduced productivity and premature culling of animals. Irrational, unbalanced feeding in terms of macro- and micronutrients, vitamins, and carbohydrates leads to metabolic disorders. This has resulted in researchers recording profound destructive degenerative changes in bones, joints, ligaments, and tendons, as well as impaired horn formation in hooves.

The data obtained is also confirmed by the results of other researchers. Specifically, R.C. Ebling *et al.* (2019) demonstrated that hoof lesions are a major cause of reduced productivity in the dairy industry and are caused by changes in cow movement activity and the frequency of hoof trimming. This is also supported by data from N.M. Khomyn *et al.* (2024). The most significant conclusion of this study was that to prevent the widespread occurrence of hoof diseases in cows, regular trimming of the horn capsule and the provision of a complete diet and regular exercise are necessary. Adherence to these principles will enable veterinarians to prevent the spread of hoof diseases in cows, including the development of inflammatory processes in the dermal base of the sole.

Following the identification of the main pathological forms, the efficacy of the topical application of Intra Hoof-fit Gel was determined. The rationale for choosing this particular treatment lies in the fact that Hoof-fit Gel contains copper, which is a component of the cytochrome oxidase enzyme, thereby supporting keratinisation processes, particularly the formation of disulphide bonds. Zinc, in conjunction with copper, exhibits antiseptic properties and is capable of neutralising a significant number of microorganisms present in the lesion. The chelated form of these elements enhances these properties. The soluble state of these elements ensures high, stable antiseptic properties. This is achieved through an increased number of active sites of copper and zinc. In their chelated form, the elements of the preparation act not only on the surface but also penetrate deep into the affected tissues, exerting a detrimental effect on microorganisms while simultaneously supporting keratinisation processes. Additionally, isopropyl alcohol, as an auxiliary component, enhances the antibacterial effect of the gel.

The chelated zinc in the gel promoted wound healing: the wound healed quickly. No antibiotic spray has such a wound-healing effect. The gel healed the hoof horn and surrounding tissue, thereby reducing the risk of new bacterial infections. Finally, the gel contains a powerful adhesive that prolongs the contact of the product with the infection on the hoof surface. The gel continues to work even when animals walk through slurry after treatment. Additionally, wound-healing Intra Repiderma Spray was used when necessary. This is a modern, antibiotic-free product with a more effective formula compared to purple iodine sprays and blue chlortetracycline sprays. Limb isolation and fixation of the medication were achieved using Intra Hoof-fit Tape. This is a green, elastic, polyester-based tape for external use on animals, with fabric and latex coating for better fixation when bandaging cattle hooves. The research results are presented in Table 2.

Analysis of the data revealed that for pu-

Table 2. Treatment efficacy for purulent pododermatitis and digital dermatitis in experimental farms

Livestock farm	Total animal, heads	Cows					
		Healed by day 12		Healed by day 16		Healed by day 24	
		heads	%	heads	%	heads	%
Purulent pododermatitis							
ALLC Ridnyi Kray	45	36	80.0	38	84.4	42	93.3
LLC Svitanok	48	33	68.7	41	93.3	48	100.0

Table 2. Continued

Livestock farm	Total animal, heads	Cows					
		Healed by day 12		Healed by day 16		Healed by day 24	
		heads	%	heads	%	heads	%
Purulent pododermatitis							
ALLC Bilyi Stik	90	76	84.4	83	92.2	88	97.7
Digital dermatitis							
ALLC Ridnyi Kray	30	25	83.3	28	93.3	28	93.3
LLC Svitanok	30	26	86.6	28	93.3	30	100.0
ALLC Bilyi Stik	30	24	80.0	27	90.0	30	100.0
LLC YuM-Vatutino	30	27	90.0	28	93.3	30	100.0

***Source**: authors' development*

rulent pododermatitis, by the 12th day of the study, over two-thirds of the total number of affected cows in all experimental farms had clinically recovered. The subsequent trend was also positive. The results demonstrated the high effectiveness of the chosen treatment method using the topical medication. Comparing the results from the 16th day of the study with those from the 12th day, it was found that at ALLC "Ridnyi Kray", an additional 2 animals (5.3%) recovered; at LLC "Svitanok", an additional 8 cows (19.6%) recovered; and at ALLC "Bilyi Stik", the number of affected animals decreased by 7 heads (8.5%).

During the examination on the 24th day of the experiment at ALLC "Ridnyi Kray", it was found that the proposed topical treatment resulted in the recovery of 9 out of 10 cows. Only three cows showed incomplete recovery with partial improvement. The high effectiveness of the proposed topical treatment was also observed in cows at ALLC "Bilyi Stik", where only two animals remained affected by the end of the study period. An even higher effectiveness was observed in cows at LLC "Svitanok". At the time of examination, no affected animals were found. The final number of recovered cows, compared to the 16th-day data, showed an increase: at ALLC "Ridnyi Kray", an increase of 4 heads (9.6%); at LLC "Svitanok", an increase of 7 heads (14.6%); and ALCC "Bilyi Stik", an increase of 5 heads (5.7%).

When treating digital dermatitis with the proposed method, the percentage of recovered animals by the 12th day of the study in the experimental farms ranged from 83.3% to 90.0% compared to the baseline data. The high efficacy of the drug components was evident in the local disappearance of signs of inflammation. The highest number of recovered cows was observed at LLC "YuM-Vatutino" (27 heads), and the lowest at ALLC "Bilyi Stik" (83.3%). Analysing the data obtained on the 16th day of observation, the percentage of recovered animals increased to 90.0-93.3%. Accordingly, the number of affected animals during this study period decreased by three (10.8%) at ALLC "Bilyi Stik", by two heads (7.2%) at LLC "Svitanok", and by one animal (3.6%) at LLC "YuM-Vatutino" compared to the 12th-day data.

The final observation results on the 24th day demonstrated the high efficacy of the topical treatment applied to animals suffering from digital dermatitis. In three out of four farms, no affected animals were found. Only at ALLC "Bilyi Stik" did the percentage of recovered cows remain at 93.3%. Compared to the previous 16th-day data, the number of affected cows decreased by two animals at ALLC "Bilyi Stik", LLC Svitanok, and LLC "YuM-Vatutino" (6.7%) and by three heads at ALLC "Bilyi Stik" (10.0%). Thus, it was established that hoof trimming twice a year

reduces the number of lame cows. Furthermore, Intra Hoof-fit Gel (Netherlands), wound-healing Intra Repiderma Spray (Netherlands), and Intra Hoof-fit Tape (Netherlands) showed high effectiveness in treating these animals. The proposed method resulted in the clinical recovery of the majority of animals by the 24th day.

The obtained results align with those of other researchers. For example, M. Ninkovic *et al.* (2021) found that pododermatitis and sole ulcers are widespread hoof diseases on small, non-commercial farms in Serbia. The authors noted that regular hoof trimming is crucial for the proper functioning of cow hooves. Their research demonstrated that regular hoof trimming (twice a year) and adequate housing conditions are essential for improving hoof health.

S. Praveen *et al.* (2024) pointed out that hoof lesions in dairy cows are typically treated with trimming. However, trimming itself can cause severe pain or exacerbate existing pain. The authors recommended using Tri-Solfen (Bayer Animal Health, Pymble, Australia) – a spray gel containing lidocaine, bupivacaine, and cetrimide – for topical treatment after trimming. Their research showed that the product effectively alleviated pain during and after hoof trimming. The present study yielded similar data, specifically, that timely hoof trimming (twice a year) prevents the occurrence of pathological conditions and that topical application of Intra Hoof-fit Gel, wound-healing Intra Repiderma Spray, and Intra Hoof-fit Tape resulted in the recovery of the majority of cows by the 16th day.

The obtained data is entirely consistent with research conducted by M. Waldbauer *et al.* (2024). The researchers found that hoof diseases, and consequent lameness in these animals, are quite common in cows. The authors noted that the economic losses due to hoof diseases in cows include increased costs for staff and animal treatment, and decreased productivity and fertility. As in the described studies, the researchers consider regular hoof trimming to be the main treatment strategy. The latter involves hoof trimming of the entire animal population at 6-month intervals.

M.B. Sadiq *et al.* (2021) described the prevalence of hoof lesions, their association with lameness, and related risk factors in dairy cows in Peninsular Malaysia. Data on hoof lesions were recorded after examining the distal limbs of 1,051 cows from 29 dairy farms. A total of 903 hoof lesions were recorded in 486 cows (46.2%). The highest percentage of lesions was recorded in the hind limbs (73.4%), and 51% of cows had more than one lesion. Non-infectious lesions predominated aetiologically (79.0%). In the present study, 40.4% of the examined animals were diagnosed with aseptic pododermatitis. Thus, the prevalence of pathology in the described studies was one-third lower.

According to H. Pirkkalainen *et al*. (2022b), digital dermatitis is the most common hoof disease in cattle in Europe. Copper sulphate and formalin are commonly used in hoof baths, but their use is problematic in many European countries due to health, environmental, and safety concerns. The authors noted that, based on their observations, the most effective method for treating animals is the use of hoof baths with acidified copper sulphate. This bath consisted of copper sulphate (2%) mixed with an organic acid for acidification and subsequent ionisation of the solution. Treatment of affected cows involved passing them through the acidified copper sulphate solution twice a day upon exiting the milking parlour. The proposed treatment method is one of the best and most widespread ways to combat digital dermatitis.

E. El-Shafaey *et al.* (2021) confirm the data obtained in this study. Specifically, the authors noted that the regular use of hoof care and cleaning products is an important element in preventing the development of hoof diseases, particularly digital dermatitis. Furthermore, the

effectiveness of topical application of a mixture of complex iron salts, zinc salts, and aluminium salts to affected animal hooves was established. The affected hoof areas underwent hygienic procedures and were sprayed with the preparation twice a week. Their research showed an almost six-fold higher treatment efficacy compared to the control group, which received topical copper sulphate. According to Serbian researchers M. Ninkovic *et al.* (2021), the health of cow hooves is directly related to regular trimming. Performing hoof trimming twice a year and providing adequate housing conditions are crucial for improving the health of the distal limbs. Aseptic and purulent pododermatitis, as well as sole ulcers, are among the most common hoof pathologies on small, noncommercial farms in Serbia. It is also well-known that a range of aseptic and purulent processes in the hoof region can be identified during preventative trimming. For instance, researchers examined 108 Simmental cows from 14 farms between March and October 2020 and diagnosed hoof diseases in 23.1% of the animals.

Therefore, scientific research aligns with the results of the current study regarding the importance of preventative hoof trimming to prevent the development of inflammatory diseases in the distal limbs of cows. Various methods proposed for treating cows with such diseases and used in modern veterinary medicine demonstrate their effectiveness. The use of the therapeutic agents proposed in the current study facilitated faster recovery in the majority of animals suffering from hoof pathologies.

Conclusions

This article presents the results of a study on the prevalence of various orthopaedic pathologies (aseptic and purulent pododermatitis, papillomatous digital dermatitis) in dairy cows. The study found that 7.5%-48.0% of the cows examined were diagnosed with aseptic pododermatitis, 5.6%-18.0% showed signs of purulent infiltration at the dermal base of the sole in the sole segment of the hind hooves, and more than half of the cows on the dairy farms had papillomatous digital dermatitis. Clinical assessment of the experimental animals revealed decreased motor activity, prolonged lying in stalls, moderate lameness during movement, and lifting of the affected limb off the ground when standing. Various forms of hoof pathology in the distal limbs showed localised pain in the affected area, signs of sole horn infiltration (in aseptic lesions at the dental base of the sole), swelling and detachment (in purulent infiltration at the dental base of the sole), and purulent lesions of soft tissues (in papillomatous digital dermatitis). Palpation of the affected animals' distal limbs revealed increased pulse amplitude in the digital arteries.

The topical application of Intra Hoof-fit Gel (Netherlands), wound-healing Intra Repiderma Spray (Netherlands), and Intra Hoof-fit Tape (Netherlands) demonstrated high treatment efficacy in affected animals by the 24th day. In three out of four farms, no cows with hoof pathologies were found. Only at Bilyi Stik ALLC (Lviv Region, Ukraine) did the percentage of recovered cows remain at 93.3%. Thus, a thorough analysis of cow treatment for various forms of distal hind limb pathologies, using topical medications fixed to the sole surface of the hoof after orthopaedic sole trimming, demonstrated the high effectiveness of the presented therapy method, which is supported by clinical research data.

Future research plans include testing other topical medications for the treatment of cows with orthopaedic conditions. The treatment protocol will involve similar orthopaedic hoof trimming and the application of the topical medication to the pathological defect. Specifically, the effectiveness of applying a powder containing copper sulphate, zinc oxide, iodoform, and potassium permanganate to the pathologi-

cal defect surface will be determined.

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Conflict of Interest

None.

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Поширення хвороб ратиць у корів

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Анотація. В умовах молочнотоварних ферм у корів реєструється зростання випадків ортопедичної патології, в тому числі з розвитком гнійних процесів. Такі процеси спостерігаються на тлі прояву локальної запальної реакції, яка негативно впливає на функціональний стан всього організму корів, призводячи не лише до зниження молочної продуктивності, а й до передчасного вибракування тварин. Мета роботи полягала у визначенні розповсюдження у молочних корів ортопедичної патології, встановленні її форм, особливостей клінічного перебігу та в опрацюванні методів локальної терапії. Проведено моніторингові дослідження на базі тваринницьких господарств Вінницької, Донецької, Львівської та Черкаської областей. Отримано дані щодо поширення ортопедичної патології у молочних корів, визначено її нозологічні форми, встановлено ступінь кульгавості та ефективність проведеного лікування хворих тварин. Для виявлення кульгавості оглядали корів під час руху. Заключне визначення форми патології здійснювали після огляду в корів дистального відділу кінцівок та за проведення ортопедичної розчистки ратиць. У результаті здійснення огляду в 7,5–48,0 % корів від наявного поголів'я діагностували асептичний пододерматит; у 5,6–18,0 % корів – клінічні ознаки гнійного просочення основи шкіри підошви в сегменті підошви на ратицях тазових кінцівок; у більше ніж 50,0 % корів –

папіломатозний пальцевий дерматит впродовж останніх двох років. Вже на 16 добу після проведеного курсу лікування дві третини хворих тварин одужували. Результати подальшого клінічного обстеження корів до і після проведеного курсу локальної терапії вказували на відсутність ознак прогресування розвитку гнійних процесів у дистальній ділянці кінцівки. Це свідчить про позитивний вплив локального застосування гелю для копит Intra Hoof-fit gel (Нідерланди), ранозагоювального спрею Intra Repiderma (Нідерланди) та бинту Intra Hoof-fit (Нідерланди) на 16 добу, що забезпечило ефективність лікування тварин на рівні 90,0–93,3 %. Отримані результати мають прикладне значення як для науковців, так і для практикуючих ветеринарних лікарів, що сприятиме удосконаленню протоколу лікування корів за ортопедичної патології

Ключові слова: хірургічна патологія; добробут тварин; ортопедія; кульгавість; локальна терапія

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Clinical and morphological indicators of halitosis in cats

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Abstract. The relevance of this study is due to the increasing number of complaints from cat owners presenting to veterinary clinics regarding the occurrence of unpleasant odours from the oral cavity (halitosis) in their pets. Accordingly, the article focuses on identifying diseases and their clinical manifestations most commonly associated with the development of halitosis in cats. To assess the presence and progression of halitosis, case histories were collected, and clinical examinations of the oral cavity were performed. The most frequent oral pathologies accompanied by unpleasant odours were identified. It was found that in 27% of the examined animals, halitosis developed against the background of chronic gingivostomatitis. In 14% of cases, it was associated with stomatitis and dental calculus, while in 13% it resulted from tooth resorption. Viral diseases of the oral cavity

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were diagnosed in 11% of the animals. Gingivitis (8%), periodontitis (7%), and oral neoplasms (7%) were among the least frequently diagnosed conditions in cats with halitosis. Morphological and biochemical blood studies in animals with chronic gingivostomatitis accompanied by halitosis revealed specific changes in haematological parameters, corresponding to underlying pathological processes. According to the results of clinical analysis of native blood from cats with chronic gingivostomatitis and clinical signs of halitosis, the white blood cell count increased by 88.6% ($P < 0.001$), eosinophils by 1.7 times ($P < 0.05$), while the number of platelets and red blood cells decreased by 1.5 times ($P < 0.001$) and 1.2 times ($P < 0.05$), respectively, in comparison with clinically healthy animals. These patterns indicate pronounced destructive changes in the cells of the oral mucosa and associated tissues and organs. This material is of practical relevance for veterinary practitioners and is important for use in the diagnosis and prognosis of diseases presenting with halitosis, as well as in selecting an optimal treatment strategy and monitoring its effectiveness

Keywords: oral cavity; gingivitis; stomatitis; chronic gingivostomatitis; tongue ulcer; squamous cell carcinoma

Introduction

Throughout 2024, a common concern raised by cat owners when visiting veterinary clinics was the presence of bad breath in their animals. This odour of exhaled air from the mouth, often described as offensive and pungent to humans, can either be a consequence of the animal's diet or may indicate the presence or development of specific underlying diseases. Therefore, in cases of persistent bad breath in a cat, it is recommended to consult a veterinary professional to determine the cause of the halitosis. Halitosis is defined as the unpleasant odour detected in an animal's breath upon exhalation by a person in close proximity.

According to J. Guilherme-Fernandes *et al.* (2024), halitosis in animals can be categorised as either physiological or pathological. Physiological halitosis typically occurs after the consumption of liquids or food and lasts for a few hours. In approximately 90% of these cases, the unpleasant smell originates solely from the oral cavity. J.M. Croft *et al.* (2022) have shown that this is primarily caused by the metabolic products of Gram-negative anaerobic bacteria (volatile sulphur compounds) present in the oral cavity, which break down proteinaceous substances found in saliva, shed epithelial cells, and food debris.

Pathological halitosis, as noted in the research by M. Soltero-Rivera *et al.* (2023), manifests as a clinical sign in a variety of diseases. The most common of these in cats are dental and gum disorders. According to the findings of these authors, between 50% and 90% of cats over four years of age experience some form of acute dental pathology. In their study, P. Kamlangchai *et al.* (2024) indicated that in chronic gingival and periodontal diseases in cats, specifically gingivostomatitis and periodontitis, the unpleasant odour from the animal's mouth is not just an initial symptom but also serves as an important indicator of the underlying issue.

The majority of bacteria within the oral microbiota, as established by P. Dai *et al.* (2024), exist in a symbiotic relationship, maintaining a dynamic balance. When this balance is disrupted, it can lead to disease. Indeed, disruption of the oral microbiota can play a crucial role in the development of oral tissue diseases and, consequently, be a cause of halitosis. The microbial

population of the oral cavity, according to the research of P.M. Oba *et al.* (2024), significantly influences periodontal disease. These authors suggest that investigating the oral microbiota can be used in conjunction with assessments of odour, dental plaque, and gingivitis to evaluate the impact of dental lesions on the animal's oral health. Microbial plaque is the aetiological agent of gingivitis, periodontitis, and halitosis, as noted in the study by E. Cunha *et al.* (2022), who demonstrated that controlling dental plaque plays a vital role in maintaining the physiological health of the oral cavity.

In their research, Y. Wei *et al.* (2024) reported that halitosis was observed in cases of gingivostomatitis resulting from various infectious or systemic diseases, including feline calicivirus, feline immunodeficiency virus, and feline leukaemia virus. Given the genetic diversity of the virus, these authors suggest that its wide spectrum of clinical presentations poses significant challenges in the diagnosis, treatment, and prevention of gum disease. A range of causes for halitosis not directly related to oral tissue damage was identified by D.H. Kim *et al.* (2023), including pathologies of the ear, nose, and throat, pulmonary conditions, gastrointestinal disorders, metabolic disturbances (such as renal failure and diabetes mellitus), and the administration of certain medications.

The findings of a study by U.I. Voloboieva & D.D. Bilyi (2024) have demonstrated that the prevalence of dental pathology in companion animals is on the rise, affecting over 70% of animals. Furthermore, the frequency with which oral cavity pathologies are detected is significantly influenced by the diagnostic methods employed: detection rates range from 20%-25% without general anaesthesia to 80%-100% with the use of anaesthesia.

Scientists have investigated specific pathological conditions within the oral cavity that are characterised by halitosis in animals. However, there remains a lack of comprehensive data regarding the findings of clinical examinations in affected animals and the corresponding changes in haematological parameters. These parameters reflect the progression of pathological processes within the animal's oral cavity and could play a crucial role in the diagnosis and prognosis of these conditions, as well as in guiding the selection of the most effective treatment strategies and monitoring their outcomes. Considering the widespread occurrence of oral pathology and the numerous potential causes of halitosis in animals, accurate differential diagnosis is essential. This study aimed to determine the informative value of clinical research methods in identifying the underlying causes of pathological halitosis in domestic cats.

Literature Review

Animal owners often perceive the offensive odour emanating from their pets' mouths as a mere inconvenience, failing to recognise that halitosis is frequently a symptom of underlying pathological conditions. According to J.G. Anderson & P. Hennet (2022) and U.I. Voloboieva *et al.* (2023), the majority of oral cavity diseases are identified through clinical examination, taking into account the animal's medical history. For more precise diagnostic differentiation, procedures such as gingival probing (in cases of periodontitis) or radiographic imaging of the head and jaw region are indicated. J.M. Croft *et al.* (2022) established that the primary cause of halitosis in animals is linked to the presence of bacteria within the oral cavity, which results from inflammatory processes leading to the development of gingivitis and periodontitis.

Building on the findings of C.M. Bollen & T. Beikler (2012), who noted that pathological halitosis is a symptom of disease, E. Cunha *et al.* (2022) discovered that gingival inflammation of varying severity is the primary aetiological

factor in the development of unpleasant breath in cats. Furthermore, research by A. Di Cerbo *et al.* (2015) indicates that physiological halitosis can occur in cats aged 5-7 months, associated with the inflammatory process in the gums during the transition from deciduous to permanent teeth. This information should be considered when taking a patient's history. According to research by D.O.L. Caravalho *et al.* (2025), the health of tissues within the oral cavity of animals is significantly influenced by dental diseases. These authors demonstrated that, depending on the severity of the underlying pathological process, dental conditions can clinically manifest as difficulty in eating, a reduced appetite, weight loss, and the presence of excessive salivation.

Regarding the presentation of pain, S. Taylor *et al.* (2024) noted that cats often conceal signs of discomfort, even when suffering from serious dental conditions such as periodontitis, chronic gingivostomatitis, apical periodontitis, and viral infections. However, in cases of purulent periodontitis, for example, which elicits a strong pain response in the animal, indicators of pain can be identified through changes in the cat's behaviour. The animal may become lethargic, lose its appetite, and avoid having its face touched. Chronic pain can also be caused by damaged teeth (due to dental caries or mechanical trauma), which can significantly impact the animal's quality of life and may clinically present as emaciation and dehydration.

M.X. Rodrigues *et al.* (2019) investigated periodontitis as a common and significant health concern in domestic cats. The composition of the subgingival microbiota in cats diagnosed with chronic periodontitis, aggressive periodontitis, and chronic gingivostomatitis has been insufficiently characterised. These researchers identified several key genera previously implicated in periodontal diseases (for example, *Treponema* and *Filifactor*) and also found in the oral microbiota (for example, *Moraxella* and *Capnocytophaga*) of healthy cats. Analysis of phylogenetic beta-diversity revealed that the microbiota of periodontally healthy cats differed from that of affected cats. While most of the bacterial genera known to be associated with periodontal disease were also identified in healthy cats, they were present in significantly lower relative abundance. Notably, alpha-diversity was found to be higher in the disease groups compared to their healthy counterparts. These findings suggest a pathological mechanism involving opportunistic behaviour by certain microorganisms.

M. Soltero-Rivera *et al.* (2024) drew attention to the specifics of feline chronic gingivostomatitis (FCGS). This condition is a debilitating disease for cats and presents a challenge for both veterinary surgeons and cat owners. It is an immune-mediated disorder associated with chronic viral infection in patients exhibiting higher alpha-diversity in their subgingival microbiome. These authors highlighted the multifactorial aetiology of FCGS, where clinical diagnosis relies on the examination of inflammatory lesions within the oral tissues and histological confirmation, rather than molecular diagnostic results. This limitation hinders the potential for early diagnosis. Research by D.O.L. Caravalho *et al.* (2025) has indicated that the aetiology of chronic gingivostomatitis remains to be fully elucidated. There is evidence potentially pointing towards a viral origin. Currently, dental extraction is the standard treatment approach, and cats that do not respond to this therapy may require lifelong medical management and, in some instances, euthanasia. Investigating the symptomatology of viral diseases, A.C. Fontes *et al.* (2023) noted that feline calicivirus is linked to a wide array of clinical presentations, particularly chronic gingivostomatitis, which is a common oral pathology in cats. R. Hofmann-Lehmann *et al.* (2022)

observed that calicivirus infection is a major predisposing factor for the development of pathology in the gingiva and oral mucosa of cats, although the precise pathogenesis of calicivirosis is not yet fully understood.

Diseases affecting the oral cavity, as demonstrated by N. Khomyn *et al.* (2020), such as fibrous periodontitis, can have a prolonged asymptomatic course, often only being detected during radiographic examination. In advanced cases, animals were observed to develop fistulae in the infraorbital region (the location of the fourth upper premolar tooth). Research findings from D.H. Kim *et al.* (2023) and M.K. Park & K.H. Song (2024) have shown that halitosis, salivation, reduced appetite, and an animal's reluctance to eat hard food can all indicate the presence of various oral pathologies in cats, including not only dental and oral mucosal lesions with ulceration, but also the formation of fistulae within the oral cavity, tissue necrosis, and the breakdown of neoplastic tissue.

Several scientific studies (Kamlangchai *et al.,* 2024; Soltero-Rivera *et al.,* 2024) have presented the results of developing and evaluating different treatment approaches for animals with oral pathologies of various origins. For instance, L.D.C. Araujo *et al.* (2022) and M. Zhang *et al.* (2024) reported that a composite probiotic used in their study modulated the oral microbiota in cats, supporting beneficial or commensal bacteria while inhibiting the growth of pathogenic ones, suggesting its potential for improving oral health in felines. A.J. Villatoro *et al.* (2022) proposed a novel treatment strategy for feline chronic gingivostomatitis, based on the use of mesenchymal stem cells and their regenerative and immunomodulatory properties. Research by C. Ohira *et al.* (2025) has established a strong link between halitosis and periodontal diseases, attributing it to volatile sulphide compounds, such as hydrogen sulphide and methyl mercaptan, produced by periodontal bacteria in the oral cavity. Furthermore, they suggest that oral administration of catechin may prevent periodontal diseases in both dogs and cats. According to D.O.L. Caravalho *et al.* (2025), surgical intervention, involving the extraction of molar and premolar teeth, is considered a preferable approach to prevent bacterial proliferation, halt the most intense inflammation, and improve the patient's quality of life. However, K. Oskarsson *et al.* (2021) pointed out that there is currently a lack of comprehensive information regarding the outcomes of communication between veterinary staff and pet owners concerning dental health and the prevention of dental pathologies in animals.

Therefore, pathological halitosis is a common symptom of numerous diseases, not only those localised within the oral cavity but also conditions affecting other organs and tissues. Establishing the underlying cause (aetiology) of halitosis necessitates a combination of various diagnostic approaches, including physical examination, laboratory tests, and instrumental investigations. The issue of early diagnosis remains a significant area of focus. Regarding the treatment of animals with pathologies associated with halitosis, several therapeutic modalities have been proposed, including antibiotic therapy, probiotics, stem cell therapy, and surgical intervention. Drawing upon the research findings presented in peer-reviewed publications, this article has outlined the most common clinical presentations of oral cavity diseases in cats, where the initial observation by owners is frequently the presence of an unpleasant odour from the mouth.

Materials and Methods

The research was conducted from August to December 2024 at the VetGeneration Holosiiv private veterinary clinic in Kyiv, Ukraine. To

identify pathologies localised within the oral cavity of cats and associated with the presence of halitosis, a thorough collection of medical histories and a comprehensive clinical examination were performed on 428 animals. The results of this process enabled the determination of the most prevalent pathologies among those examined. Ten animals diagnosed with chronic gingivostomatitis (the most frequently recorded condition) were selected for further investigation. During the study, blood samples were collected (from the lateral subcutaneous vein of the forearm) for the analysis of morphological and biochemical parameters to assess the overall health status of the patients.

For the complete blood count, biological samples were collected into tubes containing the anticoagulant ethylenediaminetetraacetic acid and analysed using a Heska Element HT veterinary haematology analyser (USA). This analyser was used to determine the following blood parameters: red blood cell count, white blood cell count, platelet count, haemoglobin concentration, and haematocrit level. For biochemical analyses, blood samples were stabilised with heparin and subsequently centrifuged for 10-15 minutes at a speed of 1,500-2,500 revolutions per minute. Following centrifugation, the supernatant – blood plasma – was collected, and the following biochemical parameters were measured: the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), as well as the concentration of glucose, creatinine, and urea nitrogen. These selected parameters were measured using a Fujifilm DRICHEM NX600 (Japan) automated biochemical analyser, specifically designed for veterinary clinics. Statistical analysis of the haematological data was performed using a personal computer with the BAF – Veterinary Medicine software and individual patient dental records. The Student's t-test was employed to determine statistical significance, with significance levels set at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

The scientific research involving animals adhered to the guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, approved in Strasbourg in 1986 (European convention..., 1986), and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006). All necessary procedures on the animals were conducted following the ARRIVE guidelines, ensuring compliance with the guiding principles of Directive of the European Parliament and of the Council No. 2010/63/EU (2010) on the protection of animals used for scientific purposes.

Results and Discussion

Given that pathological halitosis can be a symptom of various systemic disorders, a study was conducted on animals to determine the underlying causes of this unpleasant breath. The examination commenced with a visual assessment of the oral tissues, with particular attention paid to the teeth (presence of dental plaque or calculus, caries), gums (evidence of inflammation, ulcers, neoplasms), and the mucous membrane. The pathologies identified in the oral cavity of cats with halitosis following clinical examination are presented in Figure 1.

Chronic gingivostomatitis was the most frequently diagnosed condition associated with halitosis among the cats examined, accounting for 27% of cases. Stomatitis, dental calculus, and tooth resorption were identified as the primary causes of halitosis in 14%, 14%, and 13% of cases, respectively. The lowest prevalence of halitosis was observed in cats with gingivitis (8%), periodontitis (7%), and oral cavity neoplasms (6%). The proportion of feline viral diseases among all examined animals reached 11%.

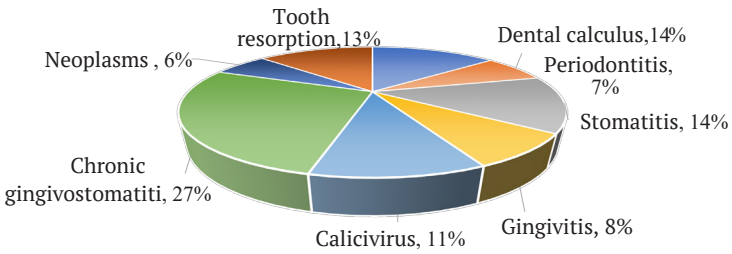


Figure 1. Prevalence of oral cavity diseases in cats with halitosis
***Note**: pathologies are presented as percentages and depicted using different colours*
***Sourse**: authors' development*

During the oral examination of cats exhibiting halitosis, attention was paid to the condition of the gingival mucosa, the dentition (tooth crowns), and the tongue. Notably, cases were observed during the study where halitosis was recorded in an animal in the absence of any apparent tissue lesions within the oral cavity (Fig. 2), as well as in cases of gingivitis (Fig. 3).

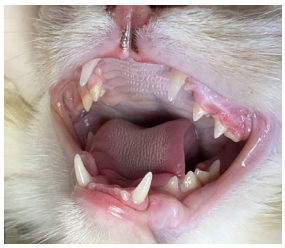


Figure 2. Oral cavity condition of a clinically healthy cat
***Note**: absence of pathological changes in the oral cavity of a cat with halitosis*
***Sourse**: authors' photo*

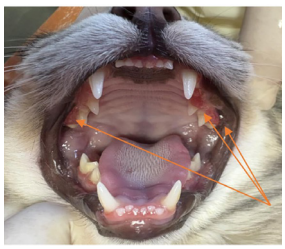


Figure 3. Gingivitis in a cat (moderate severity)
***Note**: arrows indicate areas of gingival recession*
***Sourse**: authors' photo*

Example 1. Semen, a 5-year-old male mixed-breed cat weighing 4 kg, is housed in an apartment. The clinical history revealed that the owners had noticed persistent bad breath for over a month prior to the consultation. Clinical examination of Semen (Fig. 2) revealed no pathological changes within the oral cavity tissues. The oral mucous membranes were pale pink, wellmoisturised, and free from swelling, rashes, or lesions. Discussions with the owners indicated frequent changes in the cat's diet to provide variety, as well as inconsistent portion sizes. Based on this information, further investigation was recommended, including a thorough clinical examination and both general and biochemical blood analyses, to determine the underlying cause of the pathological halitosis.

According to D.H. Kim *et al.* (2023), numerous causes of halitosis are not directly related to lesions of the oral tissues, including kidney disease, pancreatic disease, and diabetes mellitus. Research has established that renal disease, particularly in the form of chronic kidney disease, is associated with elevated blood urea nitrogen levels and a reduced rate of saliva production, which can predispose to halitosis. Furthermore, these authors identified several other metabolic conditions linked to enzymatic and transport abnormalities (such as trimethylaminuria) that result in the systemic production of volatile, unpleasant odours that are detectable in the breath.

Example 2. Baks, a 3.8-year-old male mixed-breed cat weighing 5.2 kg, is housed in an apartment. The clinical history revealed that the owners had noticed persistent bad breath for over two months before seeking veterinary attention. The cat became less interested in eating and preferred soft food, with frequent salivation observed. During the clinical examination of the animal (Fig. 3), areas of swelling and redness were noted in the mucous membrane of the oral cavity. Recession of the gums was also observed, where the gums began to separate from the crown of the tooth, creating an ideal space for the accumulation of food particles and bacteria. The findings, in this case, are consistent with the research of D.H. Kim *et al.* (2023), who noted that depending on the severity of gingivitis, cats may completely stop eating, turn their head unusually while eating, and exhibit bad breath.

According to M.K. Park & K.H. Song (2024), gingivitis is characterised by swelling and redness in the gum area, causing discomfort for the animals during feeding. In severe cases, bleeding from the gingival margins was also observed. Y. Wei *et al.* (2024) demonstrated that gingivitis resulting from systemic diseases can be accompanied by the spread of inflammation or ulceration from the gum tissues to the oral mucosa. These researchers noted that gingivitis, with subsequent inflammation of the remaining periodontal tissues, can lead to chronic oral infection, bacteraemia, pain, and ultimately, tooth loss. However, with adequate plaque control and thorough, consistent dental care at home, gingivitis is a reversible and manageable condition.

The oral cavity naturally contains bacteria that are typically non-pathogenic. However, if left undisturbed on the teeth, these bacteria multiply and form a soft, sticky film known as dental plaque. Subsequently, if this plaque is not removed, it can thicken and harden into dental calculus. This plaque develops on the teeth and gums, where bacteria utilise nutrients and produce acids and toxins that can damage tooth enamel and irritate the gums. During the clinical examination of cats with halitosis, patients were observed with changes characterised by the accumulation of dental plaque (Fig. 4) and the formation of dental calculus (Fig. 5).

Figure 4. Dental plaque in a cat
***Note**: arrows indicate areas of creamy-coloured dental plaque deposits*
***Sourse**: authors' photo*

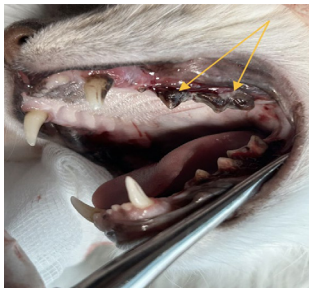


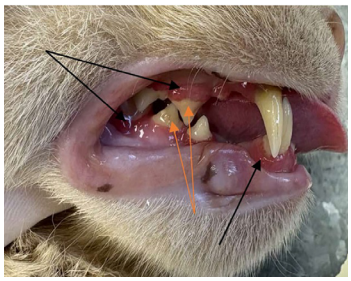
Figure 5. Dental calculus in a cat
***Note**: arrows indicate areas of black dental calculus deposits*
***Sourse**: authors' photo*

Example 3. Bublyk, a mixed-breed cat, 5 years old, weighing 5.8 kg, is housed in an apartment. According to the medical history, the owners noticed a persistent unpleasant odour from the cat's mouth over the course of the year. The cat consumed only soft food. During the clinical examination of the animal (Fig. 4), areas of creamy-coloured plaque accumulation were observed on the crowns of the

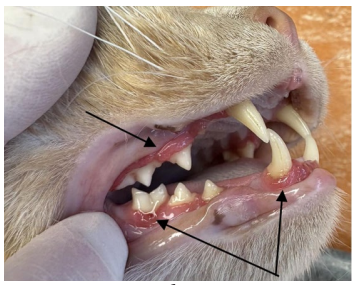
teeth, which consisted of a soft film of bacteria, saliva, and food that had formed on the tooth surfaces. J.M. Croft *et al.* (2022) established that while the bacteria in the feline oral cavity are generally considered commensal, inflammation only occurs when dental plaque, which harbours these bacteria, accumulates above the gum line, in the subgingival region, and at the base of the tooth. Furthermore, research by P. Dai *et al.* (2024) has demonstrated that pathologies within the oral cavity arise in a state of imbalance in its microflora.

Example 4. Begemot, a mixed-breed cat, 4.2 years old, weighing 4 kg, is housed in an apartment. According to the medical history, the owners noticed an unpleasant odour from the cat's mouth for a month. The cat consumed dry food but chewed primarily on the right side, with excessive salivation. During the clinical examination of the animal (Fig. 5), it was found that on the left side of the upper jaw, there was a hard, calcified black plaque on the teeth, known as dental calculus. This hard plaque was pressing on the gum tissues, causing pain and inflammation. C.M. Bollen & T. Beikler (2012) demonstrated that when dental plaque hardens through the absorption of minerals from both saliva and the gingiva itself, it transforms into dental calculus. Dental calculus has a rough surface to which pathogenic bacteria can readily attach. Therefore, it is not the calculus itself, but the bacteria colonising its surface, that triggers the inflammatory response. L.D.C. Araujo *et al.* (2022) noted that pathogenic bacteria accumulating on and below the gum line produce substances capable of damaging the cells forming the barrier between the gums and teeth. This allows bacteria access to the connective tissue beneath the teeth, leading to gingival inflammation and pain. M. Zhang *et al.* (2024) investigated that cats with a robust immune system may not exhibit an inflammatory response to the proliferation of pathogenic bacteria.

In the absence of routine oral examinations in animals, timely treatment of gingivitis, and the removal of dental plaque and calculus from the tooth crowns, oral diseases can progress to a chronic state. Indeed, among the cats examined in this study, the highest number of animals presented with clinical signs of chronic gingivostomatitis (Figs. 6a, 6b).



a



b

Figure 6. Condition of the gums and teeth in a cat with chronic gingivostomatitis

***Note**: a - before treatment, black arrows indicate inflamed and swollen tissues of the oral cavity; orange arrows indicate the presence of dental plaque on the crowns of the teeth; b -after treatment,arrows indicate a reduction in the inflammatory process in the gum area following treatment and the dentition after the removal of dental plaque*

***Sourse**: authors' photo*

Example 5. Vaska, a mixed-breed cat, 9 years old, weighing 3.2 kg, is housed in an apartment. According to the history, the owners had noticed an unpleasant smell in the cat's mouth for over two years. The cat eats very poorly, only soft food, and is losing weight. It

has become more aggressive. During the clinical examination of the animal (Figs. 6a, 6b), it was observed that the mucous membranes of the gums and oral cavity were inflamed, hyperaemic with bleeding areas, and swollen in the area of the gums and palate. Dental plaque was noted on the surface of the tooth crowns. During feeding, the cat exhibited salivation, pain, and enlargement of the mandibular lymph nodes. According to A.C. Fontes *et al.* (2023), feline gingivostomatitis is a common chronic inflammatory condition of the oral cavity with a not fully understood aetiopathogenesis, characterised by persistent, severe inflammation of the oral mucous membranes. The age, sex, and breed of the animal do not appear to influence the likelihood of developing chronic gingivostomatitis. M. Soltero-Rivera *et al.* (2024) established that the mucous membrane in cats suffering from gingivostomatitis is typically bright red, friable in texture, nodular, and prone to bleeding. P. Dai *et al.* (2024) found that the inflammation of the mucous membrane is most often symmetrical and bilateral, frequently accompanied by purulent discharge within the oral cavity, affecting the gums, the buccal mucosa, and the palate. The mandibular lymph nodes are usually enlarged. During yawning and eating, affected animals may vocalise in pain, stop abruptly, and twitch. The behaviour of these cats often changes. As P. Kamlangchai *et al.* (2024) reported, animals may become excessively aggressive or, conversely, consistently hide and avoid human interaction. In differentiating gingivostomatitis from periodontitis and gingivitis, L. Bashor *et al.* (2024) noted that gingivostomatitis invariably involves inflammation of the soft palate and pharynx, whereas, in periodontal diseases, inflammation is confined to the gums and tissues surrounding the teeth.

In the absence of treatment for the aforementioned conditions, the pathological process can extend to periodontitis, which may lead to the development of pathological tooth mobility and eventual tooth loss. Lesions associated with the progressive loss of tooth structure are most commonly observed at the neck of the tooth, where the crown meets the gum line. A cavity may form in the tooth, but often in the early stages, the problem may not be visually apparent and can only be diagnosed through radiography. In the initial phases of resorption, the animal may not exhibit any overt clinical signs. During the examination of cats with halitosis in this study, cases of tooth resorption were documented (Figs. 7, 8).

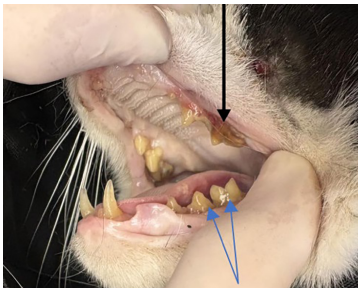


Figure 7. External tooth resorption in a cat (crown involvement)
***Note**: the black arrow indicates the location of the affected tooth, and the blue arrows point to dental plaque*
***Sourse**: authors' photo*

Figure 8. Internal tooth resorption in a cat (root involvement)
***Note**: the site of the pathological process within the oral cavity was identified during clinical examination*
***Sourse**: authors' photo*

Example 6. Smurfik, a mixed-breed cat, 6 years old, weighing 4.6 kg, is housed in an apartment. The medical history revealed that the owners had noticed an unpleasant smell in the cat's mouth for a month. The cat frequently refused food, and when eating, it chewed on the right side of its mouth with excessive salivation. It constantly tried to paw at its mouth. During the clinical examination (Fig. 7), a tooth lesion was detected, along with the presence of hard plaque on the tooth crown, inflammation, and bleeding of the gums around the affected tooth. Slight tooth mobility and a pink defect in the tooth at the junction with the gums were noted. J.G. Anderson *et al.* (2023) observed that by the time such a defect is detected, the tooth is often already significantly compromised. These types of resorptive lesions can vary in severity, ranging from relatively small defects at the gum line to extensive defects in the enamel of the tooth crown. Tooth resorption is not always associated with gingivitis. L. Bashor *et al.* (2024) established that tooth resorption is a process whereby the tooth structure is destroyed, starting from within and progressing to adjacent tissues. Tooth resorption is the most common cause of tooth loss in cats, with 30% to 70% of cats showing evidence of this destructive process. The underlying cause of tooth resorption remains unknown.

Example 7. Nafania, a mixed-breed cat, 6 years old, weighing 5.4 kg, is housed in an apartment. The medical history revealed that the owners had noticed an unpleasant smell in the cat's mouth for a week. The cat cried in pain during meals and refused solid food. During the clinical examination (Fig. 8), a defect was found on the inner surface of the tooth crown, and the area of the gums around the affected tooth was inflamed and painful. M.X. Rodrigues *et al.* (2019) demonstrated that tooth resorption is associated with significant pain, which leads to cats being reluctant to eat or refusing food altogether. Affected cats may also exhibit excessive salivation, turn their head to the side while eating, and become very irritable. Establishing a definitive diagnosis requires a thorough examination of the oral cavity and teeth, careful inspection for any lesions, and, if necessary, radiographic evaluation.

Among the causes of halitosis identified in the animals were infectious pathologies and neoplasms, both of which were associated with excessive salivation, a reduced appetite, and bleeding from the pathologically altered tissues. Oral tumours are often painful, particularly during feeding. A common initial sign of an oral tumour is a reluctance to eat, which is frequently accompanied by weight loss. Lesions of the oral cavity tissues were observed, specifically the appearance of ulcers on the tongue in cases of feline calicivirus infection (Fig. 9) and soft tissue neoplasms within the oral cavity (Fig. 10).

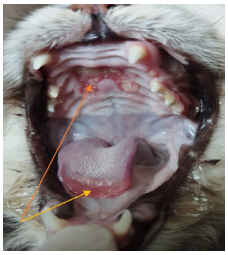


Figure 9. Lesions of the tongue and upper palate due to calicivirus in a cat
***Note**: arrows indicate the location of ulcers on the tongue and upper palate*
***Sourse**: authors' photo*

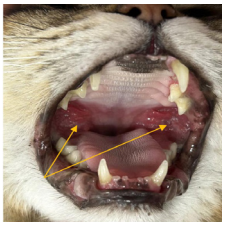


Figure 10. Soft tissue neoplasm in the oral cavity of a cat
***Note**: arrows indicate areas of tissue affected by the neoplasm*
***Sourse**: authors' photo*

Example 8. Simba, a mixed-breed cat, aged 5.5 years, weighing 5.8 kg, is housed in an apartment. According to the medical history, the owners noticed an unpleasant odour from the animal's mouth for a week. The cat completely refused food and exhibited excessive salivation. During the clinical examination (Fig. 9), numerous ulcers were found on the tongue and oral mucosa, along with inflammation in the gingival areas around the crowns of the teeth. Y. Wei *et al.* (2024) demonstrated that cats can be infected with FCV via the nasal, oral, or conjunctival routes. The primary site of viral replication is the oropharynx. Transient viraemia occurs three to four days post-infection, during which time the virus can be detected in many other tissues. FCV causes necrosis of epithelial cells, leading to the formation of vesicles on the tongue that subsequently develop into ulcers. In affected areas, the mucosa is infiltrated with neutrophils. Healing typically occurs within two to three weeks, although it can take considerably longer in some cases. A.C. Fontes *et al.* (2023) noted that the clinical signs can vary depending on the specific FCV strain, the age of the affected cat, and its living conditions. While some infections may be subclinical, a typical syndrome involving ulcers on the tongue and a relatively mild acute respiratory illness is commonly observed.

Example 9. Nora, a mixed-breed cat, aged 5 years, weighing 4.7 kg, is housed in an apartment. According to the medical history, the owners noticed an unpleasant odour from the animal's mouth for several days. The cat continually sat by its food bowl but did not eat, smacked its mouth, and there was a noticeable increase in saliva production. During the clinical examination (Fig. 10), tissue overgrowth was found in the oral cavity with swelling of the surrounding tissues, which was characterised by occasional bleeding. Cytological examination diagnosed an epithelial tumour (non-keratinising squamous cell carcinoma). According to J.G. Anderson *et al.* (2023) and D.H. Kim *et al.* (2023), this type of cancer is common in cats. It is particularly aggressive and tends to spread rapidly to adjacent tissues. This pathology is often associated with visible growths or ulcerated defects in the oral cavity, swelling of the oral tissues, excessive salivation, and loss of appetite and weight, all of which are consistent with the findings in this case.

Analysis of the patients' clinical histories revealed a considerably wide range of disease symptoms (clinical presentation). Indeed, chronic gingivostomatitis was the most frequently diagnosed condition among the examined animals presenting with halitosis. Table 1 presents the results of the clinical analysis of native blood from cats with halitosis attributed to chronic gingivostomatitis.

Table 1. Changes in morphological indicators of native blood in cats with halitosis due to chronic gingivostomatitis (m ± m, n = 10)

Blood parameters, units of measurement	Affected animals	Clinically healthy animals
White blood cells, 10^9/L	23.2 ± 1.8***	12.3 ± 1.0
Platelets, 10^9/L	286.0 ± 14.3***	420.0 ± 17.4
Red blood cells, 10^{12}/L	6.9 ± 1.4*	8.5 ± 0.6
Haematocrit, %	42.0 ± 3.5	48.0 ± 1.3
Haemoglobin, g/L	145.0 ± 12.2	162.0 ± 11.6
Eosinophils, %	3.1 ± 0.4*	1.8 ± 0.3

Note: **P < 0.05; ***P < 0.001, the difference is statistically significant compared to clinically healthy animals*
Source: *authors' development*

According to the data presented in Table 1, leukocytosis was observed in the native blood of animals with chronic gingivostomatitis. Specifically, affected cats showed an 88.6% increase in white blood cell count ($P < 0.001$) compared to clinically healthy animals. This leukocytosis in cats with halitosis suggests the presence of an infection within the body. Thrombocytopenia was also recorded in the native blood of the examined cats, with a 1.5-fold reduction in platelet count ($P < 0.001$) compared to clinically healthy animals. These animals also presented with erythrocytopenia, showing a 1.2-fold reduction in red blood cell count ($P < 0.05$), which may indicate the development of anaemia. However, the haematocrit and haemoglobin levels in the blood of these cats remained within the physiological range. The 1.7-fold increase in eosinophil count (eosinophilia) in the blood of cats with oral cavity pathology ($P < 0.05$) suggested the presence of an inflammatory process with an allergic component. Furthermore, as the condition progresses to a chronic form, the symptoms of inflammation change, significantly increasing the detriment to dental and overall health. Therefore, an investigation was conducted into the effect of chronic gingivostomatitis on the quantitative parameters of biochemical indicators in the blood plasma of cats with halitosis (Table 2).

Table 2. Changes in biochemical indicators in the blood plasma of cats with halitosis due to chronic gingivostomatitis ($M \pm m$, $n = 10$)

Parameter	Affected animals	Clinically healthy animals
Aspartate aminotransferase, U/L	56.3 ± 2.2***	40.0 ± 2.3
Alanine aminotransferase, U/L	90.0 ± 10.3	90.0 ± 6.0
Alkaline phosphatase, U/L	45.4 ± 2.3***	62.3 ± 4.2
Gamma-glutamyl transpeptidase, U/L	1.2 ± 0.4**	5.3 ± 1.2
Glucose, mmol/L	6.3 ± 1.1*	4.6 ± 1.3
Creatinine, μmol/L	186.6 ± 12.3***	70.0 ± 3.8
Urea nitrogen, mmol/L	10.2 ± 2.2***	5.3 ± 0.9

Note: **P < 0.05; **P < 0.01; ***P < 0.001 compared to clinically healthy animals*
Source: *authors' development*

Table 2 shows that the activity of aspartate aminotransferase in the blood plasma of cats with oral cavity pathologies was 1.4 times higher ($P < 0.001$) compared to clinically healthy animals. This increase in aminotransferase activity in the blood plasma of cats with chronic gingivostomatitis may indicate damage to the myocardium and muscle tissue. The activity of alanine aminotransferase in the blood of cats with oral cavity pathologies remained within the physiological range, suggesting no significant changes in liver function. The activity of alkaline phosphatase in the blood plasma of cats with oral cavity pathologies was 1.4 times lower ($P < 0.001$) compared to clinically healthy animals. This decrease in alkaline phosphatase activity in the blood of affected cats may indicate a disturbance in bone metabolism. The activity of gamma-glutamyl transferase in the blood plasma of cats with oral cavity pathologies was 4.4 times lower ($P < 0.01$) compared to clinically healthy animals, which could suggest alterations in the function of the hepato-biliary system. Additionally, the concentration of glucose in the blood plasma of cats with oral cavity pathologies was 1.4 times higher ($P < 0.05$)

compared to clinically healthy animals. The increased concentration of this carbohydrate in the blood of affected cats may be a consequence of stress, inflammatory processes, or metabolic disturbances. The creatinine concentration in the blood of these cats was 2.7 times higher ($P < 0.001$) compared to clinically healthy animals. An elevated creatinine concentration in the blood plasma primarily indicates impaired renal filtration capacity. Concurrently, the urea nitrogen content in the blood of cats with oral cavity pathology was 1.9 times higher ($P < 0.001$) compared to clinically healthy animals, further supporting the development of renal complications.

Therefore, based on the results of the haematological and biochemical investigations conducted, it can be concluded that oral cavity pathology manifesting as halitosis has a generalised impact on the health of affected cats. The development of this group of diseases may also occur in conjunction with functional impairments of the heart, kidneys, and hepato-biliary system. Halitosis is most frequently associated with chronic gingivostomatitis. Gingival inflammation with the formation of lesions or ulcers around the teeth and on the mucous membrane is a polyetiological condition with a pathogenesis linked to systemic pathological processes arising from dysfunctions in the body's most vital systems.

Conclusions

Clinical examination methods are informative in determining the causes of halitosis in cats. The clinical history revealed that, along with the presence of bad breath, owners frequently observed a decrease in their pet's appetite, difficulty eating, or even a complete refusal to eat. Increased salivation, smacking, and attempts to paw at the mouth were noted in almost all the cats examined. The animals had often experienced weight loss and had become more aggressive, likely due to the pain associated with their oral pathologies. The results of the clinical examinations of cats with halitosis showed that 8% of the animals had gingivitis, clinically characterised by hyperaemia and swelling of the gingival mucosa around the tooth crowns, along with moderate salivation. Periodontitis was diagnosed in 7% of the cats. This condition caused damage to the tissues surrounding the tooth, affecting the tooth itself. Dental calculus, formed by the hardening and mineralisation of dental plaque – a soft film of bacteria, saliva, and food debris on the tooth crown surface – was recorded in 14% of the animals, with the same prevalence as stomatitis. Tooth resorption was noted in 13% of the animals, which typically presented with poor food intake, frequent pawing at the mouth, and salivation. In these cases, the affected tooth was often mobile and required extraction as it acted as a source of infection within the oral cavity. In 11% of cats, halitosis was associated with viral infection, manifesting as ulcers on the tongue, oral mucosa, and palate. Neoplasms were diagnosed in 6% of the examined cats, with cytological examination of the oral tissues identifying squamous cell carcinoma. The most frequent diagnosis associated with halitosis in cats was chronic gingivostomatitis (27%), characterised by typical clinical signs such as lethargy and apathy, redness and swelling of the gums, intermittent gingival bleeding, and the formation of lesions and ulcers on the mucous membranes. However, halitosis in animals may not always be due to local oral pathologies. Underlying systemic diseases, such as heart, kidney, and hepato-biliary disorders, as well as anaemia, can also be significant causes of halitosis in cats, as supported by the results of the morphological and biochemical blood analyses. Leukocytosis ($23.2 \pm 1.85\ 10^9$/L, $P < 0.001$) and eosinophilia ($3.1 \pm 0.4\%$, $P < 0.05$) indicated the presence of

infection and an inflammatory reaction with an allergic component in these animals.

Future research will focus on evaluating the effectiveness of preventative and treatment strategies for cats with pathological processes in the oral cavity. Comprehensive studies are also planned to identify the early impact of oral lesions on other organ systems within the body.

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Conflict of Interest

None.

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Клініко-морфологічні ознаки в котів за галітозу

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Анотація. Актуальність дослідження зумовлена збільшенням скарг власників котів при зверненні до ветеринарних клінік щодо випадків появи в цих тварин неприємного запаху з ротової порожнини (галітозу). У зв'язку з цим, матеріал статті присвячено питанням встановлення захворювань та їх клінічних проявів, на тлі яких найчастіше виникає галітоз у котів. Для оцінки розвитку галітозу в цих тварин проводили збір анамнезу та клінічний огляд порожнини рота. Визначено найпоширеніші патології, що розвиваються в ротовій порожнині, які супроводжуються появою неприємного запаху. Встановлено, що у 27 % досліджених тварин галітоз виникав на тлі хронічного гінгівостоматиту, в 14 % котів він розвивався внаслідок прояву стоматиту та зубного каменю, у 13 % випадків його причиною була резорбція зуба, у 11 % особин діагностували вірусні захворювання ротової порожнини. Найрідше у тварин з галітозом діагностували гінгівіт (8 %), пародонтит (7 %) та новоутворення (7 %) в порожнині рота. Морфологічні та біохімічні дослідження крові у тварин із хронічним гінгівостоматитом, що супроводжувався галітозом, дозволили виокремити маркерні зміни гематологічних показників та їх відповідність патологічним процесам. Так, за результатами клінічного аналізу нативної крові в котів за хронічного гінгівостоматиту з клінічним проявом галітозу відмічали збільшення кількості лейкоцитів на 88,6 % ($P < 0,001$), еозинофілів в 1,7 раза ($P < 0,05$) та зменшення кількості тромбоцитів в 1,5 раза ($P < 0,001$), еритроцитів в 1,2 раза ($P < 0,05$) порівняно з клінічно здоровими тваринами. Встановлені закономірності свідчать про наявність виражених деструктивних змін у клітинах слизової оболонки ротової порожнини та взаємопов'язаних тканин і органів. Матеріал статті становить

практичну цінність для практикуючих ветеринарних лікарів та важливий для використання у діагностиці і прогнозуванні перебігу захворювань з клінічним проявом галітозу, виборі оптимальної стратегії лікування та моніторингу його ефективності

Ключові слова: ротова порожнина; гінгівіт; стоматит; хронічний гінгівостоматит; виразка язика; плоскоклітинний рак

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Morphological characteristics of the digestive tube organs in broiler chickens after feeding coarsely ground mussel shells

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Abstract. The relevance of the study is conditioned by the practical and scientific interest in feeding mineral feed additives from marine aquatic organisms and the effect of their particle

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size on the performance and condition of the digestive system in broiler chickens. In this regard, the purpose of the present study was to determine the effects of a feed additive made from sea mussel shells by coarse grinding on the growth rate and structure of the digestive organs of broiler chickens. To clarify this issue, histological studies of the tube-like digestive organs of chickens of the control and experimental groups were conducted, and their morphometric parameters were determined. Broiler chickens of the experimental group were fed with coarsely ground sea mussel shells in addition to the standard diet by free feeding. According to the findings of the study, the body weight of chickens in the experimental group stayed unchanged. In chickens of the experimental group, a decrease in the weight of the muscular part of the stomach and intestines and an increase in the length of the small intestine were found. An increase in the thickness of the epithelium and the area of the secretory portions of glands was observed in the crop and oesophagus, and an increase in the thickness of the cuticle and the mucosal lamina propria in the muscular part of the stomach. In the mucous membrane of the oesophagus and proventriculus, a larger number and area of lymphoid tissue accumulations in the form of small diffuse fields and lymph nodules were detected. In the duodenum of broiler chickens, the height of villi and the depth of crypts were lower, and in the caecum, the depth of crypts was lower. The morphological features of the crop, oesophagus, stomach, and small intestine, established upon the use of a mineral feed additive from mussel shells indicated the development of protective and adaptive reactions of the oesophagus and stomach to the traumatic effect of coarse particles of mussel shells, and the small intestine – to the increased calcium content in the chyme. The experimentally substantiated feeding of mineral feed additives from mussel shells to broiler chickens proved the need for their thorough grinding and dosed use in poultry feeding

Keywords: poultry; mineral feed additive; marine hydrobionts; oesophagus; stomach; intestine; morphometric parameters

Introduction

Chicken is a valuable poultry worldwide, providing people with meat and eggs. Presently, broiler chicken breeding is the most intensive livestock industry, characterised by a short growth period, high feed to animal protein conversion, and relatively low capital investment. Chicken meat is the most accessible product for multiple segments of the population, cultures, and religions around the world. According to Y. Wang *et al.* (2024), as the world's population and food consumption grows, poultry will be the most common source of meat. However, to be able to sustainably meet the growing demand, it is necessary to optimise all components of poultry production, considering the use of feed additives. N. Barzkar *et al.* (2024) pointed out that marine aquatic organisms can be one of the sources of unconventional feed additives in poultry feeding. L. Peshuk *et al.* (2023) emphasised that the hydrosphere occupies approximately 70% of the planetary surface, providing a wide range of diverse natural resources. Considering the global shortage of food resources necessary to maintain a healthy and active lifestyle, the study of alternative sources of human food and animal feed, including marine bioresources, is becoming increasingly relevant. According to J. Feng *et al.* (2025), in China, the use of marine biomass, primarily mussels, crabs, and scorpions, has

been identified as key species in the development of ecological "sea farming". According to O. Golubenko *et al.* (2023), the breeding of marine aquatic organisms plays a prominent role not only in maintaining the aquatic ecosystems of Ukraine but is also an essential component of such a profitable industry as aquaculture. A.A. Menchynska *et al.* (2022) proved that the development of technologies to produce various foods from marine aquatic organisms is promising for the food industry of Ukraine. The researchers experimentally confirmed the effectiveness of combining crustaceans with fish, animal, and plant raw materials. At the same time, hydrobionts are underutilised in the feeding of farm animals, despite their considerable potential.

Y. Zhang *et al.* (2022) found that for wild birds, aquatic organisms such as marine mussels are a regular food. S.A. Siddiqui *et al*. (2025) emphasised the significance of research aimed at the use of aquaculture facilities in animal diets in the context of the problem of shellfish shell utilisation. The latter are by-products of the primary processing of marine mussels, accumulating at processing plants, creating considerable environmental and economic challenges. H.K. Zanu *et al*. (2023) noted the practical and scientific significance of investigating the effects of mineral components and particle size distribution of mineral feed additives on the performance of poultry. Therewith, the researchers noted that the particle size of such additives affects the quantitative and qualitative indicators of productivity and depends on the duration of their presence in certain organs of the digestive tract of animals.

The use of marine mollusc shells in poultry feed is a long-standing practice in agriculture. The availability of significant extensive of marine aquatic organisms primary processing waste at the enterprises of Odesa region, as well as the potential economic effect, encouraged their use in livestock production as mineral feed additives. At the same time, due to the imperfect method of coarse grinding of sea mussel shells, the particles of this product have varying sizes and sharp edges, which can pose a potential danger to the health of poultry when fed. Considering the lack of information on the effects of the use of a mineral feed additive obtained by coarsely grinding sea mussel shells on the morphological and functional organisation of the poultry digestive system, it is prompt and relevant to explore this issue. The purpose of the present study was to determine the specific features of macro- and microscopic structure of the tube-like digestive organs in broiler chickens when using a mineral feed additive made from coarsely ground sea mussel shells in the diet.

Literature Review

The positive experience of using both aquatic organisms and their primary processing waste in poultry feeding is evidenced by a series of publications. According to E. Toyes-Vargas *et al.* (2018), the inclusion of 5% of crushed entrails of scallops, squid, shrimp, and blue mussel flour in the diet of laying hens increased the carotene content of egg yolks and was a better alternative to fish meal. W.D. Lee *et al.* (2021) found that the use of oyster shells in chicken feed contributed to higher bone mineral density in the lower leg. According to K.E. Buğdaycı *et al.* (2019), the positive effects of limestone and Mediterranean mussel shells on the productive and interior indicators of Japanese quail: body weight, eggs, feed consumption, feed conversion, yolk index, calcium and phosphorus content in the shell and serum was proved. R. Xing *et al.* (2019) found that a mineral feed additive made from scallop shells was a more effective source of active dicalcium phosphate than chicken eggshell powder. Its addition to the main diet increased the average

daily gain in live weight, muscle yield, and reduced the content of abdominal fat.

S.A. Lee *et al.* (2021) indicated that the particle size of a mineral feed additive affects the condition of the pectoral muscles of broiler chickens. The use of small limestone particles in the diet showed a tendency to improve the quality of pectoral muscles. B.S. Dongare *et al.* (2024) noted that the use of calcium from the diet by birds is influenced by its source, particle size, bioavailability, etc. Insufficient inorganic substances in bone tissue causes skeletal disorders in broilers with high body weight, which leads to significant economic losses. The use of specially coated limestone particles as a source of calcium contributed to an increase in body weight, better feed conversion, and greater bone strength in broiler chickens.

According to D. Joardar *et al.* (2020), a comparative study on the inclusion of limestone particles of varying sizes in the main diet of laying hens showed better mineral utilisation in the digestive tract when coarse particles were fed. At the same time, according to I.J. Bueno *et al.* (2016), the size of limestone particles in the feed did not affect the performance of poultry, both parent flocks and broiler chickens. The researchers did not observe any substantial difference in egg production, egg weight, shape index, and specific weight of eggs, eggshell weight, egg hatchability, body weight, and bone morphology of chicks.

According to W. Awad *et al.* (2008), high calcium content in the diet causes a decrease in the height of the villi of the small intestine in broiler chickens. However, according to Q.J. Wu *et al.* (2013), the intestinal villi height is lower when the mineral feed additive clinoptilolite is used in the diet of broiler chickens. According to R. Xing *et al.* (2019), the use of scallop shell powder in the feeding of broiler chickens contributed to a decrease in crypt depth and an increase in the ratio of villi height to crypt depth in the intestine.

Thus, as the literature analysis suggests, most researchers noted a positive impact on the state of poultry productivity of the use of marine aquatic organisms as a mineral feed additive. There is no consensus on the effects of the particle size of such additives on the state of the poultry organism. There is also no information on the effects of mineral feed additives made from marine aquatic organisms in the form of coarsely ground mollusc shells on the state of the digestive system, which was determined the purpose of the present study.

Materials and Methods

An experiment on feeding a mineral feed additive was conducted at the Budaki poultry farm in Odesa region on Ross 308 broiler chickens during 2024. Poultry were kept in a poultry house in group cages on the floor with soft bedding. The feed additive was produced by coarse mechanical grinding of Black Sea mussel (*Mytilus galloprovincialis*) shells to obtain particles up to 10 mm in size. The finished feed additive was used by free feeding along with the main diet.

Two groups of clinically healthy poultry of 14 days of age, 20 birds each, were formed according to the principle of pairwise analogues. After a preparatory period of 6 days, chickens of the experimental group from 20 to 42 days of age were additionally fed ground mussel shells as a mineral feed additive to the main diet. The feeder with the mineral feed additive was located next to the feeder containing the main feed. Broiler chickens of the control group received only the basic diet. The feed composition of the main diet included (by weight): maize – 32%, wheat – 25%, sunflower cake – 9%, extruded soybeans – 16%, soybean cake – 18%, and protein and vitamin complex *Biomix®Broiler* 1.5% at 15 g/kg of feed. During the experiment, the broiler chickens were allowed to walk and had

free access to clean and fresh drinking water. The chickens were kept and treated following the international principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (European Convention ..., 1986) and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006).

At 42 days of age, the live weight of chickens of the control and experimental groups was determined. After slaughtering the chickens, the weight of the glandular and muscular parts of the stomach, as well as the weight and linear indicators of individual intestines were determined. During the selection of internal organs, their topography, shape, size, and colour were determined. The boundary between the jejunum and ileum was determined by the tops of the caeca. The body weight of chickens and their digestive organs was measured using electronic scales A-250P (Poland), and the length of individual intestines was measured using a ruler. The relative weight of organs (%) was calculated as the ratio of their absolute weight to body weight, and the relative length of individual intestines (%) was calculated as the ratio of their absolute length to the length of the entire intestine.

Morphological studies were performed in the histological laboratory of the Department of Normal and Pathological Morphology of the State Biotechnological University (Kharkiv). For histological studies, pieces of the oesophagus, thoracic oesophagus, glandular and muscular parts of the stomach, duodenum, and jejunum were taken from their middle sections – 5 samples from each group of broiler chickens of different sexes. After fixation in a 10% solution of neutral formalin, the pieces of material were washed in running water, dehydrated and sealed in alcohols of increasing concentration, kept in intermediate media using chloroform and embedded in paraffin. Histological sections from paraffin blocks were made using a sled microtome MS-2 (Ukraine). Histological sections were stained with eosin and haematoxylin. Examination of histological specimens and micrographs were performed using a Jenamed 2 microscope (Carl Zeiss Jena, Germany). Microphotographs were taken using a Sigeta USMOS 5100 SMP camera (China). The morphometric parameters of the microstructures of the digestive organs were determined on histological specimens using an ocular square grid (N = 256) and Adobe Photoshop CS5 software on the obtained microphotographs.

The obtained digital data were processed statistically to determine the arithmetic mean (M) and statistical error (m). The reliability of the difference between the indicators was established according to the criterion of reliability (td) and Student's tables at three levels of probability $P < 0.05$, $P < 0.01$, and $P < 0.001$.

Results and Discussion

During the experiment, the feeding activity of broiler chickens was monitored. It was recorded that the birds of the experimental group showed a high attraction to the mineral feed additive, which was characterised by a specific "marine" scent reminiscent of fish. Additionally, an increase in the volume of drinking water consumed in this group was noted. At the initial stage of the study, the average body weight of 14-day-old chicks was 212.1 ± 13.3 g. At the end of the experimental period (42 days), the average body weight of broilers in the control group reached 1,284.3 ± 57.3 g, while in the experimental group this figure was 1,273.8 ± 60.3 g, which was 0.8% less than in the control group. The obtained findings were somewhat consistent with the data of N.R. Abdulla *et al.* (2016), who found a decrease in body weight of chickens with excessive calcium intake, which caused a decrease in the intensity of absorption of organic matter, crude protein, ether extract, and phosphorus.

According to V. De Gregorio *et al.* (2018), the intensity of animal growth is limited by the ability to absorb and digest feed, i.e., depends on the morphological and functional state of the digestive system. On the other hand, according to G. Lamprecht & P. Bodammer (2016), the composition of the feed itself, its physicochemical properties, determine the state of the digestive tract, which is essential in the prevention and treatment of its pathologies and is a vital element of adaptation. The results of determining the absolute and relative weight of the glandular and muscular parts of the stomach and intestines, as well as the absolute and relative length of individual intestines of broiler chickens when using a mineral feed additive made from coarsely ground sea mussel shells in the diet are presented in Table 1.

Table 1. Macroscopic parameters of digestive organs in experimental broiler chickens (M ± m, n = 5)

Indicator	Group	
	control	experimental
Weight of the glandular part of the stomach, g	5.9 ± 0.2	5.3 ± 0.2
Relative weight of the glandular part of the stomach, %	0.5 ± 0.02	0.4 ± 0.02
Weight of the muscular part of the stomach, g	23.1 ± 1.0	18.4 ± 1.0*
Relative weight of the muscular part of the stomach, %	1.8 ± 0.1	1.4 ± 0.1**
Intestinal weight, g	73.6 ± 4.5	55.5 ± 2.8*
Relative intestinal weight, %	5.7 ± 0.2	4.4 ± 0.2**
Absolute intestinal length, cm	150.3 ± 8.3	180.8 ± 10.4
Absolute length of the small intestine, cm	120.8 ± 6.0	152.0 ± 7.3*
Relative length of the small intestine, %	80.4 ± 2.2	84.1 ± 2.1
Absolute length of the large intestine, cm	29.5 ± 0.9	28.8 ± 1.3
Relative length of the large intestine, %	19.6 ± 1.3	15.9 ± 1.0
Absolute length of the duodenum, cm	27.1 ± 1.5	32.1 ± 2.0
Relative length of the duodenum, %	18.0 ± 0.2	17.8 ± 0.4
Absolute length of the jejunum, cm	82.3 ± 4.0	107.7 ± 6.8*
Relative length of the jejunum, %	54.8 ± 1.9	59.6 ± 1.7
Absolute length of the ileum, cm	11.4 ± 0.2	12.2 ± 0.5
Relative length of the ileum, %	7.6 ± 0.3	6.7 ± 0.4
Absolute length of the caecum, cm	23.3 ± 1.0	23.2 ± 0.5
Relative length of the caecum, %	15.5 ± 1.0	12.8 ± 0.7
Absolute length of the rectum, cm	6.2 ± 0.2	5.6 ± 0.2
Relative length of the rectum, %	4.1 ± 0.3	3.1 ± 0.4

***Note**: *P < 0.05; **P < 0.01, statistically significant in relation to the control*
***Sourse**: developed by the authors*

According to the data obtained, the use of the feed additive caused a decrease in the absolute and relative weights of both the stomach and intestines (see Table 1). Compared with the control group, the absolute weight of the glandular and muscular parts of the stomach of the experimental group chickens decreased by 10.2% and 20.3% ($P < 0.05$), respectively, while the intestinal weight – by 24.6% ($P < 0.05$). Their relative indices also decreased accordingly: the relative weight of the glandular part of the stomach – by 0.1%, the muscular part of the stomach – by 0.4% ($P < 0.01$), and the intestine – by 1.3% ($P < 0.01$).

In parallel with the decrease in weight characteristics, the broilers of the experimental group showed an increase in the linear length of the intestine by 20.6% compared to the control. Therewith, the increase in the total length of the digestive tract was mainly due to the small intestine, the absolute length of which increased by 25.8% ($P < 0.05$), while the large intestine showed a decrease of 2.4%. Such transformations led to an increase in the relative length of the small intestine by 3.7% and, accordingly, to a decrease in the relative length of the colon by a corresponding amount. Analysis of the morphometric parameters of individual intestinal sections revealed that in the experimental group, the absolute length of the duodenum increased by 18.5%, the jejunum – by 30.9% ($P < 0.05$), and the ileum – by 7.0%. The relative length of the duodenum underwent a slight decrease of 0.2%, the jejunum showed an increase of 4.8%, while the ileum – a decrease of 0.9%. Therewith, the absolute length of the large intestine, the relative length of the caecum and rectum did not change significantly. Thus, the use of a mineral feed additive caused the greatest macroscopic changes in the jejunum.

Thus, according to the findings of the study on the use of coarsely ground mussel shell particles in the diet of broiler chickens, there were significant changes in the morphometric parameters of the glandular and muscular parts of the stomach and jejunum. As reported by H. Zhang *et al.* (2019), the small intestine plays a key role in digestion and nutrient adsorption. According to N.A. Abd El-Azeem *et al.* (2023), the development of the gastrointestinal tract is crucial for maximising nutrient utilisation and productive growth of chickens. According to M.M. Kushch *et al.* (2019), the relative weight of the small intestine and its length were greater in heavy geese compared to light geese.

The experimental data obtained during the study demonstrate congruence with the observations of Q.J. Wu *et al.* (2013) on the lengthening of the small intestine and reduction of its weight when natural and synthetic clinoptilolite, a mineral with a high calcium content, is introduced into the broiler diet. An analogous trend can be observed in the report by R. Xing *et al*. (2019), who recorded an increase in the linear dimensions of the duodenum, small intestine, and ileum in broilers when using ground scallop shells as a source of active dicalcium phosphate.

Overall, the histological structure of the tube-like digestive organs of broiler chickens corresponded to the general patterns of their structure in other poultry species and was consistent with the information of R.R. Beheiry (2018) on their structure in domestic turkeys and D.S. Makhotina *et al.* (2020) on their structure in domestic ducks. Microscopically, the wall of both the oesophagus and thoracic oesophagus of broiler chickens consisted of three membranes: mucosa, muscularis, and adventitia. The submucosa was not found. The characteristic structure of the oesophageal wall was longitudinal folds. They were formed not only by the mucosa but also by the inner layer of the muscularis (Fig. 1).

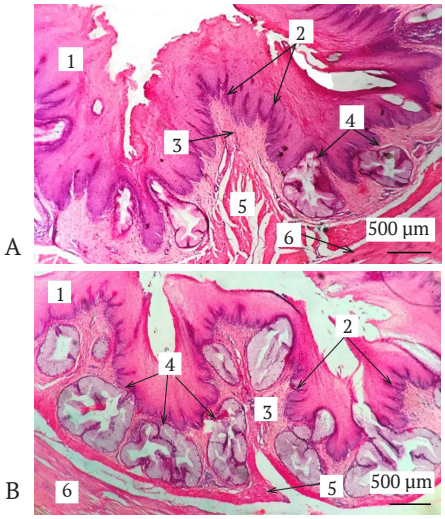

Figure 1. Secretory glands in the wall of the thoracic part of the oesophagus of chickens of control (A) and experimental (B) groups

***Note**: 1 – epithelial layer of the mucous membrane; 2 – basal membrane of the epithelium; 3 – lamina propria of the mucous membrane; 4 – secretory divisions of glands; 5 – inner layer of the muscular membrane: 6 – outer layer of the muscular membrane*

***Sourse**: developed by the authors*

The mucous membrane of the crop and oesophagus consists of two layers: the epithelial layer and the lamina propria. As can Figure 1 (A, B) shows, the epithelial layer (1) is formed by multilayered squamous non-squamous epithelium, while the lamina propria (3) is formed by loose fibrous connective tissue. The epithelial layer is separated from the lamina propria by a convoluted basement membrane (2). The lamina propria of the thoracic part of the oesophagus contains the secretory divisions of the alveolar tubular glands (4), which were round or oval in shape and formed by prismatic glandular epithelium. The glands were adjacent to the basal membrane of the epithelial layer with one edge, and to the inner layer of the muscularis membrane with the other (5). Due to the direct adhesion of the glands to the basal membrane, their secretory compartments opened into the epithelial layer, and no excretory ducts were found. The muscularis membrane consisted of two layers: the inner longitudinal and the outer circumferential (6), formed by bundles of smooth muscle tissue and separated by a thin layer of loose fibrous connective tissue.

The stomach of chickens consisted of two parts: glandular, or proventriculus, and muscular, or gizzard, which were connected by a short isthmus, which is consistent with the data of R. Pachauri *et al.* (2024) on its structure in other bird species. The surface of the mucous membrane of the stomach parts was uneven: in the proventriculus, it contained papillae, which opened the glandular ducts, and in the gizzard – low folds formed by the cuticle. In the wall of both the glandular (Fig. 2) and muscular parts of the stomach (Fig. 3) of broiler chickens, four membranes were distinguished: mucosa, submucosa, muscular, and serous.

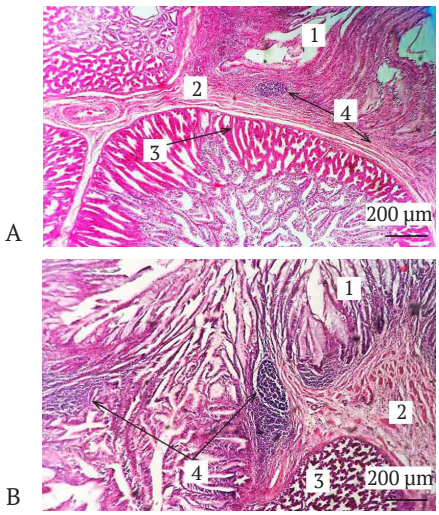

Figure 2. Mucous membrane of the proventriculus of chickens of control (A) and experimental (B) groupss

***Note**: histological preparation, stained with haematoxylin and eosin; 1 – epithelial layer of the mucous membrane; 2 – lamina propria of the mucous membrane; 3 – lobule tubes; 4 – lymphoid tissue*

***Sourse**: developed by the authors*

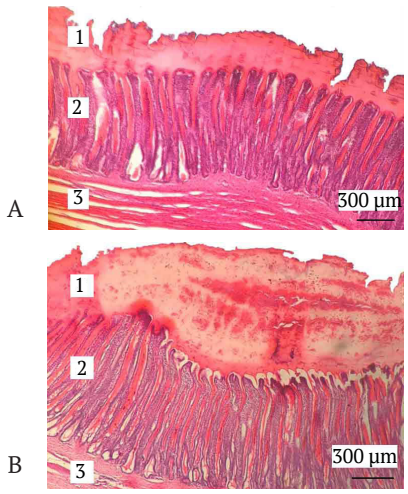

Figure 3. Cuticle and glands in the lamina propria of the gizzard of chickens of control (A) and experimental (B) groups

***Note**: histological preparation, stained with haematoxylin and eosin; 1 – cuticle; 2 – gastric glands; 3 – muscular membrane*

***Sourse**: developed by the authors*

The mucous membrane (Fig. 2) consisted of two layers: epithelial and lamina propria. The lamina muscularis mucosae was not detected. In the mucosa of the glandular part of the stomach, a glandular layer or superficial glands were noted, which were formed by protrusions of the lamina propria (2) covered with a single-layer prismatic epithelium (1), as well as deep glands located in the submucosa. Due to the significant content of such glands in the entire proventriculus wall, the submucosa was the thickest. The glands in the submucosa were densely arranged lobules of predominantly conical shape with a wider base and narrower apex, separated by thin layers of loose fibrous connective tissue. The lobules consisted of numerous tubes that radially converged to its central outflow duct (3).

In the muscular part of the stomach (Fig. 3), the epithelial layer of the mucosa was covered by a cuticle (1). The mucosa located under the cuticle formed the glandular layer (2), which consisted of densely arranged tube-shaped glands, representing the immersion of the epithelium in the lamina propria. The lower part of the glands was formed by secretory compartments, while the upper part – by excretory ducts that brought the secretion from which the cuticle was formed to the surface of the epithelial layer. The submucosa in the form of a wide strip separated the mucous membrane from the muscular membrane and was formed by a dense interweaving of connective tissue fibres. The muscular membrane (3) is formed by bundles of smooth muscle cells, mostly with a circular orientation.

In the small intestine of broiler chickens, the duodenum, jejunum, and ileum were identified. The duodenum formed a characteristic loop that contained the pancreas and was separated from the jejunum by the duodenal papilla. The jejunum formed 10-12 loops, the border with the ileum was set at the level of the tops of the caeca. The intestinal wall consisted of three membranes: mucosa, muscular membrane, and serous membrane (Fig. 4).

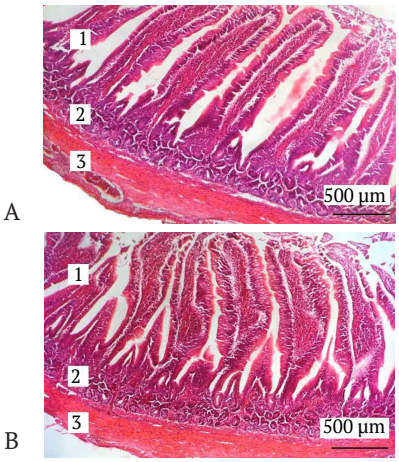

Figure 4. Duodenum of chickens of control (A) and experimental (B) groups

***Note**: Histological preparation, stained with haematoxylin and eosin, Sigeta; 1 – villi; 2 – crypts; 3 – muscular membrane*

***Sourse**: developed by the authors of this study*

The mucous membrane presented in Figure 4 consisted of three layers: epithelial, lamina propria, and muscularis mucosae. A feature of the mucous membrane relief was villi and crypts. Villi (1) were finger-shaped protrusions of the lamina propria covered with an epithelial layer. Crypts (2) were tube-shaped immersions of the epithelium into the lamina propria. The muscular lamina, in the form of a narrow chain of smooth muscle cells, was located directly under the bottom of the crypts and had a longitudinal direction of their location. The muscular membrane (3) of the intestinal wall consisted of two layers of smooth muscle tissue – a thicker inner circular layer and an outer longitudinal layer.

According to A.S. Davis *et al.* (2021), quantitative analysis of biological structures is a powerful tool that provides objective data that is challenging to obtain by other methods. It is morphometric studies that enable an unbiased morphological assessment of the state of the body's structures. The results of determining the morphometric parameters of the microstructures of the crop, thoracic part of the oesophagus, glandular and muscular parts of the stomach, duodenum, and jejunum of broiler chickens in the control group and with the use of a feed additive are presented in Table 2.

According to the findings obtained (Table 2), feeding broiler chickens with coarsely ground sea mussel shells caused changes in the microscopic parameters of the digestive tract organs. Thus, in the oesophagus and thoracic oesophagus, the thickening of the epithelial layer of the mucous membrane formed by multilayered non-squamous immature epithelium was noted by 39.0% ($P < 0.001$) and 11.8%, respectively. Furthermore, in broiler chickens of the experimental group, the area of the secretory glands in the thoracic part of the oesophagus (Fig. 1, note 4) increased by 38.5% ($P < 0.05$) compared to the control group. In contrast to the control group, in broiler chickens of the experimental group, some of the secretory compartments contained a much larger cavity filled with mucous secretion.

Table 2. Microscopic parameters of digestive organs in experimental broiler chickens (M ± m, n = 5)

Indicator	Group	
	control	experimental
Height of the epithelial layer of the crop mucosa, μm	457.5 ± 12.5	775.0 ± 15.0***
Area of the secretory glands of the thoracic oesophagus, μm^2	395.4 ± 35.5	547.5 ± 50.7*
Submucosal thickness of the glandular part of the stomach, μm	1,682.5 ± 17.5	1,478.3 ± 108.5
Area of lymphoid tissue accumulation in the submucosa of the glandular part of the stomach, μm^2	6,240.3 ± 341.5	17,026.3 ± 908.6***
Thickness of the cuticle of the mucous membrane of the muscular part of the stomach, μm	435.2 ± 27.5	621.3 ± 34.5**
Thickness of the glandular layer of the mucous membrane of the muscular part of the stomach, μm	465.3 ± 32.5	686.0 ± 38.3**
Thickness of the mucous membrane of the muscular part of the stomach, μm	902.4 ± 16.4	1,305.5 ± 79.8**
Height of duodenal villi, μm	1,534.9 ± 102.4	1,243.1 ± 56.0*
Depth of duodenal crypts, μm	344.7 ± 24.7	243.5 ± 64.7**
Ratio of villi height to duodenal crypt depth	4.5 ± 0.02	5.1 ± 0.2**
Height of the villi of the jejunum, μm	778.5 ± 27.5	735.4 ± 6.0
Depth of crypts of the jejunum, μm	274.8 ± 23.8	184.9 ± 19.1*
Ratio of villi height to depth of crypts of the jejunum	2.8 ± 0.1	4.0 ± 0.2*

***Note**: *P < 0.05; **P < 0.01; ***P < 0.001 statistically significant in relation to the control*
***Sourse**: developed by the authors*

A feature of the structure of the glandular part of the stomach in broiler chickens of the experimental group was a smaller thickness of the submucosa containing glandular lobules. Compared to the control group, its thickness was 12.1% smaller ($P < 0.05$). Lymphoid tissue in the form of primary lymph nodules and diffuse fields was found between the secretory glandular compartments, as well as in the mucosal lamina propria (Fig. 2, note 4). Compared to the control group, their area increased by 172.9% ($P < 0.001$).

The muscular part of the stomach of broiler chickens in the experimental group was characterised by a greater thickness of both the cuticle and the mucous membrane lamina propria, which contained the secretory portions of the tubular glands that synthesise its components (Fig. 3). Compared to chickens of the control group, the secretory compartments of such glands were wider and contained wide strands of secretion in the form of vertical columns that passed into the cuticle. The use of a mineral feed additive in the diet increased the thickness of the cuticle of the mucous membrane of the muscular part of the stomach of broiler chickens (Table 2) by 42.8% ($P < 0.01$), the thickness of the mucous membrane lamina propria by 47.4% ($P < 0.01$), and the thickness of the entire mucous membrane by 44.7% ($P < 0.01$) compared to the control group.

Considering the form of the mineral feed additive – coarsely ground fragments of sea mussel shells – it can be assumed that changes in the micro- and macrostructural state of the thoracic part of the oesophagus, as well as the glandular and muscular parts of the stomach of broiler chickens, occurred due to their mechanical effect. The fact that sea mussels are a regular food for wild birds can be explained by the use of defence and adaptive mechanisms that have evolved over a lengthy period of adaptation to this type of food specialisation (Khomych *et al*., 2021).

In broiler chickens of the experimental group, the study observed a decrease in the height of villi and the depth of duodenal crypts (Fig. 4) by 19.0% ($P < 0.05$) and 29.4% ($P < 0.01$), and in the jejunum – by 5.5% and 32.7% ($P < 0.05$), respectively. The findings of the study revealed that such a significant indicator of the morphological and functional state of the intestine of broiler chickens as the ratio of intestinal villi height to crypt length increased in the experimental group. Thus, in the duodenum, the ratio of villi height to crypt depth increased by 14.8%, in the jejunum – by 39.2%, which was, respectively, 5.1 ± 0.2 and 4.0 ± 0.2 against 4.5 ± 0.1 and 2.8 ± 0.2 in the control group.

T.D. Hinnant *et al.* (2025) reported that the absorption surface area of the small intestinal mucosa determines the ability of the entire intestine to absorb nutrients. Changes in villus height and crypt depth are prominent indicators of intestinal function. The intestinal crypts are the site of formation of enterocytes, the epithelial cells of the small intestine that ensure the performance of its functions. C.F. Marchini *et al.* (2011) found that a decrease in the depth of crypts and the height of duodenal villi of broiler chickens against the background of body weight loss is characteristic of such an unfavourable factor as heat stress. S. Gotoh *et al.* (2023) proved that the villi of the small intestine actively absorb calcium, and this process is more active at their tops.

The obtained findings of the study on the reduction of the height of the villi of broiler chickens when using mussel shells in the diet were consistent with the data of W. Awad *et al.* (2008) on the reduction of the height of the villi of the small intestine with a high calcium content in the diet of broiler chickens and contradicted the information of Q.J. Wu *et al.* (2013), according to which the use of clinoptilolite, which is a source of calcium, increased their height. The findings obtained in this study on the decrease in crypt depth and the increase

in the ratio of villi height to crypt depth of the intestine of broiler chickens using mussel shells were consistent with analogous data from R. Xing *et al.* (2019) on the use of scallop shell powder in broiler feed.

Thus, the presented studies of the morphological and functional state of the oesophagus and stomach of broiler chickens when fed mussel shells subjected to rough mechanical grinding indicated the need to use a mineral feed additive from mussel shells in the form of small particles or flour that would not have a traumatic effect on the mucous membrane of the digestive tract. Changes in the morphological parameters of the small intestine indicate a high calcium content in the diet, which requires the dosed use of a mineral feed additive, considering the needs of the poultry body and the total calcium content in the diet.

Conclusions

Free feeding of the mineral feed additive from mussel shells in addition to the main diet from 20 to 42 days of age did not affect the growth rate of broiler chickens, as indicated by the absence of a statistically significant difference in body weight of the control and experimental groups at the end of the experiment. At the same time, the findings of morphological studies revealed the effects of a mineral feed additive made from coarsely ground sea mussel shells on the anatomical and histological parameters of the structure of the tube-like digestive organs of broiler chickens: the oesophagus, glandular and muscular parts of the stomach, and intestines. At the end of the experiment, the absolute and relative weight of the muscular part of the stomach decreased by 20.3% and 0.4%, respectively, while the absolute and relative weight of the intestine decreased by 24.6% and 1.3%, respectively. Upon the use of a mineral feed additive, an increase in the length of the intestine of chickens by 20.6% was observed, which was caused by an increase in its small section by 25.8%. Moreover, such changes were associated with a 30.9% increase in the length of the jejunum. The length of both the large intestine and its individual intestines did not undergo statistically significant changes. In the crop of the chickens of the experimental group, an increase in the thickness of the protective epithelial layer of the mucous membrane by 39.0% was observed, in the thoracic part of the oesophagus – an increase in the area of the secretory glands by 38.5%, the mucous secretion of which facilitated the passage of feed and performed a protective function. Between the secretory portions of glands in the submucosa of the glandular part of the stomach, an increase in the area of such protective structures as lymphoid tissue accumulation by 172.9% was observed. In the muscular part of the stomach, the cuticle thickness increased by 42.8% and the thickness of the secretory layer of the mucosa – by 47.4%. Feeding a mineral feed additive contributed to a decrease in the height of villi and the depth of the duodenal crypts by 19.0% and 29.4%, respectively, and the depth of the crypts of the jejunum by 32.7%. Such changes in the parameters of the main microstructures of the intestine caused an increase in the ratio of villus height to crypt depth in the duodenum by 14.8% and in the jejunum by 39.2%.

The detected modifications of the morphological and functional organisation of the digestive tract suggested the formation of protective and adaptive reactions in response to mechanical stimulation by mussel shell particles. The recorded structural characteristics of the digestive organs correlated with the generally accepted principles of adaptation of biological systems as a dynamically labile structure to environmental factors. It is promising to investigate the degree of assimilation of nutrients in the diet by the body of broiler chickens using feed additives that differ in the size of mussel shell particles. Such an investigation will

enable the production of feed additives that would ensure their most efficient use.

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Conflict of Interest

None.

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Морфологічна характеристика органів травної трубки в курчат-бройлерів за згодовування грубо мелених стулок мідій

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Анотація. Актуальність дослідження зумовлена практичним і науковим інтересом щодо згодовування мінеральних кормових добавок із морських гідробіонтів і впливу розміру їх частинок на продуктивність і стан органів травлення в курчат-бройлерів. У зв'язку з цим, мета роботи полягала у визначенні впливу кормової добавки, виготовленої зі стулок морських мідій шляхом грубого подрібнення, на інтенсивність росту та будову органів травлення курчат-бройлерів. Для з'ясування цього питання проведені гістологічні дослідження трубкоподібних органів травлення у курчат контрольної і дослідної груп, визначено їх морфометричні показники. Курчатам-бройлерам дослідної групи додатково до стандартного раціону шляхом вільного згодовування використовували грубо подрібнені стулки морських мідій. За результатами дослідження маса тіла в курчат дослідної групи залишалася без змін. У курчат дослідної групи встановлено зменшення маси м'язової частини шлунка, кишечнику і збільшення довжини тонкого відділу кишечнику. У волі і стравоході відмічали збільшення товщини епітелію, площі секреторних відділів залоз, а у м'язовій частині шлунку – зростання товщини кутикули і власної пластинки слизової оболонки. У слизовій оболонці стравоходу і шлунку виявлено більшу кількість і площу

скупчень лімфоїдної тканини у вигляді дрібних дифузних полів і лімфатичних вузликів. У дванадцятипалій кишці курчат-бройлерів були меншими висота ворсинок і глибина крипт, а в порожній кишці – глибина крипт. Встановлені морфологічні особливості вола, стравоходу, шлунку і тонкого відділу кишечнику за використання мінеральної кормової добавки зі стулок мідій свідчать про розвиток захисно-пристосувальних реакцій стравоходу і шлунку до травмуючої дії грубих частинок стулок мідій, а тонкого відділу кишечнику – до збільшеного вмісту кальцію в химусі. За експериментально обґрунтованого згодовування мінеральних кормових добавок зі стулок мідій курчатам-бройлерам доведено необхідність ретельного їх подрібнення і дозованого використання в годівлі птиці

Ключові слова: сільськогосподарська птиця; мінеральна кормова добавка; морські гідробіонти; стравохід; шлунок; кишечник; морфометричні показники

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