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Milk phospholipids for correcting bile acid composition in rats with experimental fatty hepatosis

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Abstract. The unique functions of the liver require detailed study, since hepatopathology is a serious veterinary problem, which also negatively affects the productivity of farm animals. It is possible to clarify the pathogenetic mechanisms and its development using artificial modeling of hepatopathology and determine the therapeutic efficacy of hepatoprotective drugs, especially based on raw materials of animal origin. The aim of the study was to determine the effect of the use of milk phospholipids in the composition of the bioadditive "FLP-MD" on the secretion of bile acids by the liver in artificial modeling of fatty hepatosis in rats. To reproduce hepatopathology, laboratory rats were administered intragastrically with a 4% solution of tetracycline hydrochloride at a dose of 0.25 g/kg of body weight for seven days and the bioadditive was used at a dose of 13.5 mg/kg of body weight for nine days. Bile samples were collected from rats by conducting acute experiments. Six fractions of conjugated bile acids were determined in bile samples by thin-layer chromatography. It was found that in laboratory rats with experimental fatty hepatosis, the processes of biotransformation of primary and secondary cholates by conjugation with taurine were inhibited. In particular, a decrease in the concentration of taurocholic acid in the bile of sick animals by 20.5%-38.1% ($P < 0.01$), and of the complex of taurochenodeoxycholic and taurodeoxycholic acids by 21.8%-25.7% ($P < 0.05$) was recorded. In the case of using the bioadditive "FLP-MD" in rats with experimental fatty hepatosis, the concentration of taurocholic, taurochenodeoxycholic and taurodeoxycholic acids in bile significantly increased. The concentration of glycoconjugated bile acids and free cholates corresponded to their level in the control. The use of the bioadditive "FLP-MD" based on milk phospholipids in experimental fatty hepatosis eliminated the negative impact of the antibiotic in a toxic dose on the processes of biotransformation and the formation of cholates. This allows to recommend the bioadditive "FLP-MD" based on milk phospholipids as a hepatoprotective agent in the case of the use of antimicrobial drugs in animals

Keywords: bile acids; cholesterol; bioadditive "FLP-MD"; tetracycline hydrochloride; hepatocyte; hepatopathology

Introduction

One of the unique metabolic functions of hepatocytes is the conversion of cholesterol to the bile acids. Concentration changes of various cholates in the bile reflect the path of synthesis and biotransformation reactions and transport processes in the liver parenchyma. Bile acids are synthesised from cholesterol with the participation of enzymes specific exclusively for hepatocytes. Considering the importance of cholates in the liver as a unique site of their synthesis and biotransformation, it is important to investigate the effects of various factors, including pathological ones, on the functional state of the liver and on the transformation and transport of bile acids.

A. Allameh *et al.* (2023) and T. Nakamura *et al.* (2025) emphasised that the liver is damaged by many factors and, at the same time, numerous processes in hepatocytes are disrupted. I. Lupaina *et al.* (2021) and C. Silva *et al.* (2023) demonstrated that under the influence of various endogenous and exogenous substances, the cholate synthesising function of hepatocytes can be both stimulated and inhibited. In particular, the known property of antibiotics to cause serious disorders

of liver function can lead to changes in the synthesis, biotransformation and transport of bile acids. For example, doxycycline in excessive doses exhibits prooxidant properties and promotes the accumulation in tissues of toxic metabolites: aldehydes, ketones, hydroperoxides, resulting in increased lipid peroxidation, changes in the structure and permeability of cell membranes, impaired metabolic processes and developed cholestasis and inflammation of the liver (Varma *et al.*, 2021; Nikolajevic *et al.*, 2024). A.M. Liashevych *et al*. (2021) showed that during modeling of doxycycline-induced hypercholesterolemia and stress-induced hypercholesterolemia, the bile flow of conjugated bile acids is inhibited. C. Wei *et al*. (2022) indicated that the antibiotic tetracycline effects on the liver are hepatocellular injury, cytolysis, cholestatic injury, liver failure. D. Wupperfeld *et al*. (2022) showed that the antibiotic tetracycline inhibits succinate dehydrogenase, cytochrome oxidase, alkaline phosphatase, arginase, reduces the level of aerobic respiration and associated oxidative phosphorylation, disrupts the metabolism of proteins, fats, carbohydrates, pigments, and causes an increase in peroxide oxidation of lipids and causes destructive changes in hepatocyte membranes. As K. Taylor *et al.* (2025) in particular pointed out, the "targets" of the damaging effect of antibiotics on liver cells have been largely identified, and the use of antibiotics to model experimental liver damage has become widespread in research practice. Artificial pathology modeling is used to experimentally study the effectiveness of various drugs. V. Tiwari *et al.* (2025) noted that the study of the effects of tetracycline and its derivatives is due to their widespread use in medical practice and animal husbandry. Taking into account possibility of side effects, it is important to develop and study drugs with hepatoprotective properties. It is known that phospholipids have hepatoprotective effects across many different liver diseases. In particular, essential phospholipids are used for the supportive treatment of non-alcoholic fatty liver disease as biologically active substances that improve the fluidity of hepatocyte membranes and transmembrane transport (Wupperfeld *et al.*, 2024).

The liver's unique ability to synthesise bile acids from cholesterol, which consists of many energy-dependent enzymatic reactions in hepatocytes, can be significantly impaired under the influence of tetracycline. Disturbances of lipid metabolism and changes in the regulation of liver cell function by bile acids in conditions of simulated fatty hepatosis remain insufficiently studied. Given the need for long-term use of tetracycline in medical practice and possible side effects of the antibiotic on the liver, the search for effective hepatoprotectors is urgent. The aim of the study was to study the effect of the dietary supplement "FLP-MD", which contains milk phospholipids, unsaturated fatty acids and antioxidants, on the synthesis and biotransformation of cholates and their concentration in the bile of rats with fatty hepatosis, modeled using tetracycline hydrochloride.

Literature Review

The synthesis of bile acids from cholesterol and the transmembrane transport of cholates to the primary bile tubules are energy-consuming processes (Boyer & Soroka, 2021). As noted by V.M.P. de Bruijn *et al.* (2022) the body does not lose a significant amount of bile acids after its participating in digestion in the intestine. Bile acids biotransformed by the intestinal microbiota return to the liver with blood via the portal vein. This phenomenon is named bile acid enterohepatic circulation. Cholates, processed by the enzymes of the intestinal microbiome, are called secondary bile

acids. Enterohepatic circulation of bile acids occurs due to the coordinated functioning of the transport systems of the membranes of enterocytes and hepatocytes. In liver cells, secondary bile acids are biotransformed into primary ones, which most effectively perform the digestive function in the intestine.

Bile acids are essential components of bile necessary for digestion. They emulsify dietary fats, activate digestive enzymes, and affect the intestinal microbiota. It should be noted that bile acids are not only a necessary component of bile as a digestive secretion. They perform the functions of regulatory compounds, realising their effects through the corresponding intracellular nuclear receptors – farnesoid X receptor (FXR) (Qi *et al.*, 2024) and G-protein bound membrane receptors (Fleishman & Kumar, 2024; Jia *et al.*, 2024). The intestinal and hepatic transport systems regulated by farnesoid X receptors ensure the efficiency of enterohepatic circulation of cholates and lead to a negative feedback loop between the return of secondary bile acids to hepatocytes and the intensity of de novo bile acid synthesis. Activation of hepatocyte FXR by bile acids leads to the suppression of the expression of enzymes for primary cholate synthesis. F. Stellaard & D. Lütjohann (2021) found that when the flow of bile acids from the intestine decreases, the inhibition of synthesis is abolished and the processes of synthesis of primary bile acids from cholesterol are intensified in hepatocytes.

Changes in the content of bile acids in the internal environment of the body are primarily caused by changes in their synthesis, bile formation, and bile excretion. The synthesis of bile acids from cholesterol is a multi-step enzymatic process. Bile contains various bile acids that have different physicochemical properties, exert different detergent effects on biomembranes, and change the properties of bile as a digestive secret. S. Choudhuri & C.D. Klaassen (2022) noted that hydrophobic bile acids more effectively increase cholesterol absorption compared to more hydrophilic bile acids in the digestive tract. C.D. Fuchs *et al.* (2025) emphasised that trihydroxycholanic bile acids, such as cholate and its tauroconjugates and glycoconjugates, effectively regulate lipid metabolism in the body, as well as regulate the enterohepatic circulation of cholates. One of the important stages of the metabolic transformations of bile acids in hepatocytes is their conjugation with taurine or glycine. Y. Fu *et al.* (2025) found that conjugation of bile acids with taurine or glycine and changes in the ratio of different conjugated and free bile acids significantly affect the course of inflammatory processes in the gastrointestinal tract. At the same time, disorders of metabolic transformations of cholate cause cholestatic lesions of the liver and its fatty degeneration and significantly affect the metabolism in the body as a whole (Nimer *et al.*, 2021; Farooqui *et al.*, 2022).

The conversion of cholesterol to bile acids, which occurs in hepatocytes, requires the activity of many hepatospecific enzymes. The conversion of cholesterol into cholate is the most important mechanism for maintaining cholesterol homeostasis, and thus the prevention of many diseases associated with metabolic disorders and the accumulation of cholesterol in the body. J. Guo *et al.* (2024) indicated that bile acids, as well as intermediates of their biosynthesis, regulate the expression of genes whose products provide cholesterol homeostasis. In summary, bile acids play a central role in the digestion and absorption of dietary lipids in animals, while also participating in regulatory mechanisms that influence hepatic metabolism, intestinal immunity, and cholesterol balance. Alterations in bile acid synthesis or enterohepatic circulation may contribute to metabolic disturbances and hepatobiliary pathologies in veterinary species.

Materials and Methods

Research was carried out in scientific laboratories of National University of Life and Environmental Sciences of Ukraine and Taras Shevchenko National University of Kyiv. Experiments and biochemical analysis of the obtained biological fluids were carried out during 2023-2024. Studies were performed on white laboratory male rats with body weight 200 ± 50 g (n = 13), kept in the vivarium at room temperature 22-24°C with 14-hour light period and standard diet and free water access. All work with animals was carried out based on the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (1986), the Law of Ukraine No. 3447 (2006), which was confirmed by the conclusion of the Bioethics Commission of the National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine (Protocol No. 002/2023 of October 26, 2023).

The experiment included the following stages:

1) a control group of animals received water intragastrically for five days (control group);

2) the first experimental group of animals received intragastrically a 4% solution of tetracycline hydrochloride at a dose of 0.25 g/kg of body weight for seven days (self-rehabilitation group);

3) the second experimental group of animals received the milk phospholipid-containing bioadditive (Ukraine, author's development) daily intragastrically one hour before the introduction of tetracycline hydrochloride and for the next two days after the antibiotic administration (treatment group);

4) a day after the completion of intragastric administration of substances, an acute experiment was performed with cannulation of the bile duct to obtain bile;

5) biochemical analysis: in bile, the content of conjugated and free bile acids was determined by thin-layer chromatography;

6) a statistical analysis of the obtained results of measurements of the content of bile acids in animals of all experimental groups.

Liver damage in the form of fatty hepatosis was simulated by administering to rats a 4% solution of tetracycline hydrochloride intragastrically at a dose of 0.25 g/kg of body weight of the animal for seven days (Korolova *et al.,* 2023). The bioadditive made from milk phospholipids was administered daily to experimental animals one hour before tetracycline hydrochloride and for the next two days after the discontinuation of the antibiotic to observe cholate productive function of the liver. The daily oral dose of the bioadditive "FLP-MD" was 13.5 mg/kg of body weight. The bioadditive "FLP-MD" is an authors' development (Melnychuk *et al*., 2009). The composition of this bioadditive includes the following components: milk phospholipids with a set of fatty acids typical of cell membranes, a mixture of unsaturated fatty acids (oleic, linoleic, linolenic) extracted from linseed oil, and vitamins A and E as antioxidants.

The day before the start of the acute experiment with cannulation of the common bile duct, all animals were weighed and placed in a separate cage without access to food and with free consumption of water. The rats were then anesthetised with sodium thiopental (7 μg/100 g body weight, intraperitoneally). Immobilised animals underwent laparotomy and cannulation of the bile duct using a plastic cannula connected to a micropipette to bile collecting. Bile samples were collected every 30 min for 3 h in an acute experiment. Each bile sample was analysed for the content of bile acids of six different fractions.

Determination of bile cholates was performed by a modified method of thin layer chromatography (Melnychuk *et al*., 2009). Before extraction of cholate from the collected samples of liver secretion, to 1.9 cm^3 of a cold mixture of ethanol-acetone (1 : 3) was added

0.1 cm^3 of bile. Subsequently, the resulting mixture was kept at a temperature from -10°C to 0°C in a chamber for 30 min and then centrifuged for 10 min at 3,000-4,000 rpm. The obtained extracts were transferred into conical glass tubes and dried. The dry residue was dissolved in 50-100 µL of a mixture of ethanol water (6 : 4). Each sample in a volume of 5-10 µL was applied to chromatographic plates coated with Silicagel (Kavalier, Czech Republic). Free and conjugated bile acids were separated in a system containing amyl ester of acetic acid, toluene, butanol, acetic acid and water (3 : 1 : 1 : 3 : 1, respectively) in glass chromatographic chambers. Staining of the chromatograms was performed with a dye of the following composition: 15 cm^3 of glacial acetic acid, 1 g of phosphomolybdic acid, 1 cm^3 of sulfuric acid and 5 cm^3 of a 50% trichloroacetic acid solution. After staining, the chromatogram was dried at 60-70°C for 5 minutes. Quantitative determination of bile acid content was performed using a GP-920 densitometer (Shimadzu, Japan) at λ 620 nm. Modification of the chromatographic method contributed to the determination of six fractions of conjugated bile acids, in particular: taurocholic, taurochenodeoxycholic and taurodeoxycholic (mixture), glycocholic, glycochenodeoxycholic and glycodezoxycholic (mixed) and free bile acids: cholic, chenodeoxycholic and deoxycholic (mixture) acids (Veselskyi *et al.,* 2001).

The experimental data were processed by variation statistics methods using OriginPro 2018 (OriginLab Corporation, USA) and STATISTICA 7.0 (Stat Soft, USA) software packages. The obtained and digitised values of the research results indicators were checked for normality of the distribution of the general populations using the Shapiro-Wilk test. Significant differences between sample means were examined by parametric analysis of variance (ANOVA). Levels of variance were assessed using Levene's test. Post-test comparisons were performed using the Bonferroni test. The Kruskal-Wallis nonparametric ANOVA was used to determine reliable differences between the parameters of the distributions of the samplings, if non-equal variances were estimated with Levene's test. In all cases the results were considered reliable on condition of the probability value P under 5% ($P < 0.05$) (Deo & Ranganathan, 2024). The results were presented as the arithmetic mean ± standard deviation, n – number of experiments.

Results and Discussion

Effect of the milk phospholipid-containing bioadditive on the secretion of bile acid tauroconjugates under the conditions of experimental fatty hepatosis in rats

The influence of phospholipid-containing bioadditive components on the secretion of tauroconjugates of bile acids in the conditions of experimental fatty hepatosis in rats was investigated. It was found that, in the conditions of simulated tetracycline hydrochloride liver damage, the concentration of taurocholic, and taurochenodeoxycholic and taurodeoxycholic acids in the bile of rats is significantly reduced. The content of taurocholate in the hepatic secretion was lower by 20.5%-38.1% ($P < 0.01$) than the control values, and that of taurochenodeoxycholic and taurodeoxycholic acids was lower by 21.8%-25.7% ($P < 0.05$) (Table 1).

Thus, under conditions of tetracycline hydrochloride usage, the processes of biotransformation of bile acids (hydroxylation and conjugation of bile acids with taurine) are suppressed. When rats were administered the phospholipid-containing dietary supplement "FLP-MD" while taking tetracycline hydrochloride, the effect of the antibiotic on the processes of biotransformation and the formation of bile acids was significantly reduced. This is evidenced by the absence of statistically significant differences in the concentration of taurocholate in the first bile sample from the rats treated with

tetracycline hydrochloride and the bioadditive (treatment group) compared to the control, and a statistically significant increasinge of the taurocholate concentration in other samples of bile obtained from rats in the treatment group (tetracycline hydrochloride + bioadditive "FLP-MD"). The concentration of taurocholic acid in bile samples from the rats in the treatment group was even more significantly higher compared to the self-rehabilitation group. The use of the phospholipid-containing dietary supplement in animals with simulated fatty hepatosis led to an increase in the concentration of taurocholic acid in bile by 28.7%-86.3% compared to the indicators of animals in the self-rehabilitation group.

Table 1. Concentration (mg/100 mL) of bile acid tauroconjugates in the bile of rats during self-rehabilitation and administration of the phospholipid-containing bioadditive "FLP-MD" against the background of tetracycline-induced fatty hepatosis (n = 13), M ± SD

No. samples	Experiment time, min	Group of animals	Bile acid	
			taurocholic	taurochenodeoxycholic and taurodeoxycholic
1	30	Control	219.5 ± 15.9	125.4 ± 18.3
		Self-rehabilitation	174.1 ± 8.1**	90.7 ± 9.2*
		Treatment	224.78 ± 15.3	121.7 ± 6.1
2	60	Control	212.2 ± 1.3	113.4 ± 19.03
		Self-rehabilitation	168.75 ± 9.8*	88.7 ± 9.9
		Treatment	257.6 ± 4.8*	136.7 ± 6.6
3	90	Control	207.2 ± 23.2	108.4 ± 19.1
		Self-rehabilitation	160.2 ± 12.05*	83.1 ± 8.2
		Treatment	250.4 ± 12.0**	115.2 ± 14.3
4	120	Control	201.6 ± 23.0	103.5 ± 18.1
		Self-rehabilitation	152.8 ± 11.0**	78.1 ± 6.9*
		Treatment	258.5±12.7*	126.9 ± 10.6
5	150	Control	197.1 ± 24.3	98.1 ± 16.7
		Self-rehabilitation	138.4 ± 6.4**	74.1 ± 5.7*
		Treatment	238.5 ± 7.4**	112.1 ± 9.9
6	180	Control	189.0 ± 21.7	91.8 ± 13.2
		Self-rehabilitation	128.4 ± 6.8**	68.2 ± 3.7*
		Treatment	239.2 ± 3.4**	112.7 ± 4.2*

***Note**: * P < 0.05; ** P < 0.01, statistically significant differences compared to control*
***Source**: authors' development*

The concentration of taurochenodeoxycholate and taurodeoxycholate in the bile of the rats with fatty hepatosis treated with the phospholipid-containing bioadditive was similar to control concentration value in the first – fifth samples. The concentration of taurochenodeoxycholate and taurodeoxycholate in the sixth sample of the bile of the treated rats was higher by 22.8% (P < 0.05) than the control values. The concentration of taurochenodeoxycholate and taurodeoxycholate in bile samples from the rats in the treatment group was significantly higher compared to the self-rehabilitation group. The use of the phospholipid-containing dietary supplement in animals with simulated fatty hepatosis provided an increase in the concentration of taurochenodeoxycholate and taurodeoxycholate in bile samples by 37.5%-65.2% compared to the values in bile samples from the rats in the self-rehabilitation group (Table 1).

The described multidirectional changes in the content of bile acid tauroconjugates in the bile of rats during self-rehabilitation and administration of the phospholipid-containing bioadditive "FLP-MD" against the background of tetracycline-induced fatty hepatosis led to differences in their ratio in the hepatic secret obtained during the first thirty minutes of the acute experiment (Fig. 1). In the first 30-minute sample of bile obtained from animals of the control group, the proportion of taurocholic acid is 39%, taurochenodeoxycholic and taurodeoxycholic acids – 22%. During the tetracycline hydrochloride usage, the proportion of taurocholate decreased to 37%, and dihydroxycholane tauroconjugates decreased to 19% (Fig. 2).

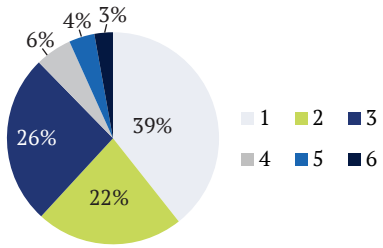

Figure 1. The ratio of 6 different fractions of bile acids in the first 30-minute sample of bile from the rats of the control group

***Note**: 1 – taurocholic, 2 – taurochenodeoxycholic and taurodeoxycholic (mixture), 3 – glycocholic, 4 – glycochenodeoxycholic and glycodezoxycholic (mixture), 5 – cholic, 6 – chenodeoxycholic and deoxycholic (mixture) acids*

***Source**: authors' development*

In the first 30-minute bile sample from the rats treated with the phospholipid-containing bioadditive "FLP-MD" in addition to tetracycline hydrochloride, the ratio of tauroconjugates of bile acids was changed significantly. Namely, the part of taurocholate tauroconjugates was 42%, while that of dihydroxycholane tauroconjugates was 23%. Note that in the control, the proportion of all bile acids conjugated with taurine was 61%. In the self-rehabilitation group of samples the percentage of bile acids conjugated with taurine was 56%. The use of the dietary supplement caused an increase in the proportion of taurocholates to 65% (Fig. 3).

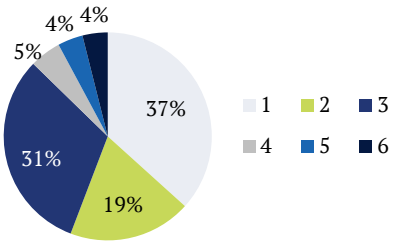

Figure 2. The ratio of 6 different fractions of bile acids in the first 30-minute sample of rat bile under the action of tetracycline hydrochloride

***Note**: 1 – taurocholic, 2 – taurochenodeoxycholic and taurodeoxycholic (mixture), 3 – glycocholic, 4 – glycochenodeoxycholic and glycodezoxycholic (mixture), 5 – cholic, 6 – chenodeoxycholic and deoxycholic (mixture) acids*

***Source**: authors' development*

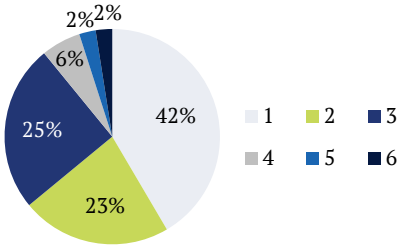

Figure 3. The ratio of 6 different fractions of bile acids in the first 30-minute sample of bile from the rats with the introduction of the phospholipid-containing bioadditive "FLP-MD" against the background of tetracycline-induced hepatosis

***Note**: 1 – taurocholic, 2 – taurochenodeoxycholic and taurodeoxycholic (mixture), 3 – glycocholic, 4 – glycochenodeoxycholic and glycodezoxycholic (mixture), 5 – cholic, 6 – chenodeoxycholic and deoxycholic (mixture) acids*

***Source**: authors' development*

Thus, the phospholipid-containing dietary supplement under conditions of experimental

tetracycline-induced fatty hepatosis contributed to the enhancement of the processes of conjugation of free bile acids with taurine. T. Miyazaki *et al.* (2023) in their studies on mice confirmed that both the synthesis of taurine and the pathways of amidation of free bile acids with taurine are regulated by the activation of intracellular nuclear receptors (FXR) sensitive to bile acids. In view of this, it could be assumed that factors that change the metabolism of bile acids in hepatocytes affect the further transformations of bile acids through their interaction with cholates. The described differences in the ratio of different fractions of tauroconjugated cholates persist throughout the experiment. Thus, in the control group in the last sample of bile, taurocholic acid was 42%, taurochenodeoxycholic and taurodeoxycholic acids were 21%. That is, in the sixth bile sample from rats in the control group, the total proportion of bile acids conjugated with taurine was 63%. Thus, in control, tauroconjugates were the predominant fraction of bile acids in rat bile (Fig. 4).

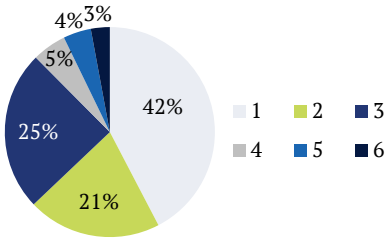

Figure 4. The ratio of 6 different fractions of bile acids in the last (180-minute) sample of bile from the rats of the control group

***Note**: 1 – taurocholic, 2 – taurochenodeoxycholic and taurodeoxycholic (mixture), 3 – glycocholic, 4 – glycochenodeoxycholic and glycodezoxycholic (mixture), 5 – cholic, 6 – chenodeoxycholic and deoxycholic (mixture) acids*

***Source**: authors' development*

In the last sixth sample of bile from the rats with tetracycline-induced hepatosis, the proportion of taurocholate was 34%, while that of taurochenodeoxycholate and taurodeoxycholate was 18%. As was observed, the proportion of taurocholic, and taurochenodeoxycholic and taurodeoxycholic acids in the last bile sample collected in the acute experiment from rats in the self-rehabilitation group was lower compared to the control. During the acute experiment, due to the interruption of the enterohepatic circulation of bile acids, the concentration of bile acids in bile was expected to decrease. In the conditions of the acute experiment with cannulation of the bile duct, hepatocytes needed to intensify the synthesis of bile acids to maintain their proper concentration in bile. It is noteworthy that in rats with tetracycline-induced hepatosis, the processes of synthesis of bile acid tauroconjugates were suppressed. The percentage of bile acid tauroconjugates in the sixth bile sample from the rats with tetracycline-induced hepatosis was 52% (Fig. 5).

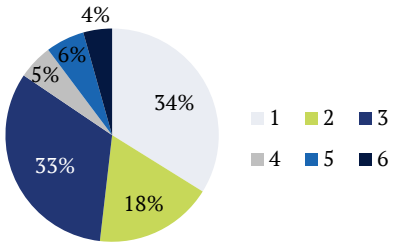

Figure 5. The ratio of 6 different fractions of bile acids in the last 180-minute sample of rat bile under the action of tetracycline hydrochloride

***Note**: 1 – taurocholic, 2 – taurochenodeoxycholic and taurodeoxycholic (mixture), 3 – glycocholic, 4 – glycochenodeoxycholic and glycodezoxycholic (mixture), 5 – cholic, 6 – chenodeoxycholic and deoxycholic (mixture) acids*

***Source**: authors' development*

Administration of the bioadditive based on milk phospholipids to animals with tetracycline-induced fatty hepatosis showed the effect of "drug protection" against the background of antibiotic usage, which was manifested in

maintaining the ratio of different fractions of free and conjugated bile acids. As shown in Figure 6, the proportion of taurocholate was 45%, while that of taurochenodeoxycholate and taurodeoxycholate was 21%. Thus, the percentage of taurocholate even exceeded the control figure, which was 42%. The total proportion of taurine-conjugated bile acids in the bile of the animals with fatty hepatosis that received the bioadditive was 66%.

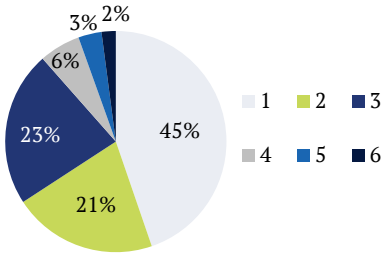

Figure 6. The ratio of 6 different fractions of bile acids in the last 180-minute sample of bile from the rats with the introduction of the phospholipid-containing bioadditive "FLP-MD" against the background of tetracycline-induced hepatosis

***Note**: 1 – taurocholic, 2 – taurochenodeoxycholic and taurodeoxycholic (mixture), 3 – glycocholic, 4 – glycochenodeoxycholic and glycodezoxycholic (mixture), 5 – cholic, 6 – chenodeoxycholic and deoxycholic (mixture) acids*

***Source**: authors' development*

As was found in the study, in conditions of simulated fatty hepatosis, the mechanisms of synthesis of bile acid tauroconjugates were suppressed. A particularly significant decrease in the fraction of tauroconjugates was detected in the last sixth sample of bile from animals in the self-rehabilitation group. The use of the dietary supplement led to an increase in the percentage of tauroconjugates in the bile of the rats in the treatment group. The percentage of taurocholic acid in the bile of the rats in the treatment group even exceeded the proportion of taurocholate in control samples. Thus, the effect of the phospholipid-containing bioadditive was to stimulate the secretion of tauroconjugates of bile acids into bile in animals with experimental fatty hepatosis.

Effect of the milk phospholipid-containing bioadditive on the secretion of bile acid glycoconjugates under the conditions of experimental fatty hepatosis in rats

Bioadditive influenced the secretion of glycoconjugates of chenodeoxycholic and deoxycholic bile acids in the conditions of experimental fatty hepatosis in rats. At the same time, the content of glycocholic acid in the bile of the animals with tetracycline-induced fatty hepatosis did not show significant changes compared to the control. Note that in the bile samples from the rats, which were administered the phospholipid-containing bioadditive "FLP-MD" and tetracycline hydrochloride, the concentration of glycocholates exceeded that in the control (Table 2).

Table 2. Concentration (mg/100 mL) of bile acid glycoconjugates in the bile of rats during self-rehabilitation and administration of the phospholipid-containing bioadditive against the background of tetracycline-induced fatty hepatosis (n = 13), M ± SD

No. samples	Experiment time, min	Group of animals	Bile acid	
			glycocholic	glycochenodeoxycholic and glycodeoxycholic
1	30	Control	144.2 ± 7.1	31.1 ± 3.4
		Self-rehabilitation	149.4 ± 13.1	23.1 ± 3.3*
		Treatment	135.7 ± 12.4	32.2 ± 3.7
2	60	Control	135.65 ± 11.3	27.4 ± 3.2
		Self-rehabilitation	146.7 ± 16.9	22.4 ± 3.1
		Treatment	140.8 ± 16.3	33.8 ± 2.8

Table 2. Continued

No. samples	Experiment time, min	Group of animals	Bile acid	
			glycocholic	glycochenodeoxycholic and glycodeoxycholic
3	90	Control	134.3 ± 3.9	27.4 ± 3.0
		Self-rehabilitation	142.6 ± 16.6	21.8 ± 0.7
		Treatment	140.6 ± 12.2	34.8 ± 5.2*
4	120	Control	128.0 ± 4.4	25.7 ± 3.04
		Self-rehabilitation	137.5 ± 16.4	21.1 ± 1.9
		Treatment	134.1 ± 13.2	30.4 ± 2.7
5	150	Control	121.3 ± 3.5	24.9 ± 2.4
		Self-rehabilitation	133.4 ± 15.1	20.3 ± 1.6
		Treatment	127.7 ± 13.9	34.0 ± 6.1*
6	180	Control	110.0 ± 5.3	23.4 ± 2.0
		Self-rehabilitation	123.7 ± 15.9	20.1 ± 1.9
		Treatment	121.3 ± 9.2	32.2 ± 6.6*

***Note**: * P < 0.05; ** P < 0.01, statistically significant differences compared to control*
***Source**: authors' developmen*

In the first 30-minute sample of bile obtained from animals of the control group, the proportion of glycocholic acid was 26% (Fig. 1) and during tetracycline hydrochloride usage the part of glycocholate increased to 31% (Fig. 2). In the first 30-minute bile sample from the rats treated with the phospholipid-containing bioadditive "FLP-MD" in addition to tetracycline hydrochloride, the ratio of glycocholic acids was close to that in the control. Namely, the proportion of glycocholate was 25% (Fig. 3).

A decrease in the concentration of glycochenodeoxycholic and glycodeoxycholic acids in the first sample of bile by 25.7% (P < 0.05) was observed in animals with self-rehabilitation under the conditions of simulated tetracycline hydrochloride liver damage (Table 2). In the bile of the rats, that were administered the phospholipid-containing bioadditive and tetracycline hydrochloride, the concentration of glycochenodeoxycholic and glycodeoxycholic acids was higher than control in the third, fifth and sixth samples (Table 2). Namely, the use of the phospholipid-containing bioadditive stimulated the processes that ensure the formation and secretion of glycoconjugates of dihydroxycholan bile acids – glycochenodeoxycholic and glycodeoxycholic bile acids. As a result, the content of this fraction of bile acids was 27.0%-37.6% higher than the control.

Effect of the milk phospholipid-containing bioadditive on the secretion of free bile acids under the conditions of experimental fatty hepatosis in rats

The milk phospholipid-containing bioadditive influenced the secretion of free bile acids under the conditions of experimental fatty hepatosis in rats. The synthesis of free bile acids under the influence of tetracycline hydrochloride in the dose used for modeling fatty hepatosis did not change statistically significantly. Therefore, in conditions of simulated fatty hepatosis, the mechanisms of synthesis of conjugated bile acids were primarily damaged, in particular the mechanisms of tauroconjugation. It is important to note that the concentration of free cholic acid, chenodeoxycholic acid and deoxycholic acid in the bile samples from the animals with tetracycline hydrochloride liver disease did not differ statistically significantly from the control (Table 3).

Table 3. The concentration (mg/100 mL) of free bile acids in the bile of rats during self-rehabilitation and the introduction of the phospholipid-containing bioadditive against the background of tetracycline-induced fatty hepatosis (n = 13), M ± SD

No. samples	Experiment time, min	Group of animals	Bile acid	
			cholic	chenodeoxycholic deoxycholic
1	30	Control	21.8 ± 3.6	15.8 ± 1.4
		Self-rehabilitation	18.6 ± 1.9	18.6 ± 1.9
		Treatment	13.4 ± 3.7	13.4 ± 3.7
2	60	Control	21.7 ± 3.3	15.4 ± 2.6
		Self-rehabilitation	25.3 ± 3.9	19.3 ± 1.6
		Treatment	20.0 ± 2.4	13.2 ± 4.6
3	90	Control	20.9 ± 2.9	14.9 ± 1.8
		Self-rehabilitation	24.2 ± 4.3	18.2 ± 1.8
		Treatment	20.8 ± 4.0	13.8 ± 5.4
4	120	Control	20.6 ± 2.5	14.1 ± 2.0
		Self-rehabilitation	24.5 ± 4.3	17.5 ± 1.8
		Treatment	18.0 ± 2.1#	11.8 ± 3.1#
5	150	Control	19.2 ± 2.5	13.3 ± 2.1
		Self-rehabilitation	23.0 ± 3.8	17.4 ± 2.0
		Treatment	18.6 ± 3.7	11.5 ± 3.5#
6	180	Control	19.1 ± 2.3	13.0 ± 2.8
		Self-rehabilitation	22.3 ± 3.6	16.5 ± 1.4
		Treatment	18.2 ± 3.4	11.1 ± 3.5

***Note**: # P < 0.05, statistically significant differences compared to self-rehabilitation group*
***Source**: authors' development*

It was discovered that the use of the phospholipid-containing bioadditive in rats with tetracycline-induced fatty hepatosis prevents the growth of the content of dihydroxycholan bile acids in the bile – chenodeoxycholic and deoxycholic acids – under the influence of an antibiotic at the dose used to model fatty hepatosis (Table 3). Note that the percentage of chenodeoxycholic and deoxycholic acids out of all bile acids in the bile of the first sample of the control group animals was 3% (Fig. 1). In the first and last samples of the bile of the self-rehabilitation group of rats, the percentage of chenodeoxycholic and deoxycholic acids out of all bile acids was 4% (Figs. 2 and 5). The smallest percentage (2%) of chenodeoxycholic and deoxycholic acids out of all bile acids was found in the first and last (sixth) samples of the bile of the treatment group (Figs. 3 and 6).

The category of primary bile acids includes cholic acid. This acid is synthesised by the so-called classical route, which begins with 7α-hydroxylation of cholesterol by CYP7A1 of liver. Because of the absence of notable variation in the concentration of cholic acid in the bile samples from the animals in experimental fatty hepatosis, it can be assumed that this link in the transformation of cholesterol during cholic acid synthesis does not undergo critical changes in the modelling pathology. It is known that dihydroxycholanic bile acids, in particular deoxycholic acid, have a more significant detergent effect on the biomembranes of liver parenchymal cells and bile duct cells. Thus, such damaging factors as antibiotics cause an increase in the content of dihydroxycholanic bile acids in the hepatobiliary system. In turn, dihydroxycholanic bile acids enhance the

damaging effect on liver cells. The phospholipid-containing bioadditive decreased the concentration of free dihydroxycholane bile acids in the bile of the rats with tetracycline-induced fatty hepatosis. The formation of more dihydroxycholane chenodeoxycholic acid in hepatocytes is the result of the enhanced activation of the so-called "acid" route of cholate synthesis, which starts with 27-hydroxylation by CYP27A1. It is important to note that the classical route of synthesis of bile acids from cholesterol usually takes place under physiological conditions. K. Nemeth *et al.* (2024) in particular noted that bile acid salts accumulate in the bile ducts and penetrate into cholangiocytes to a significant extent at low pH levels, at which the increased acidity of the bile ducts is a factor in accelerated apoptosis of bile duct cells. The cytoprotective mechanism of action of ursodeoxycholic acid explains its clinical use in the prevention of cholestasis and the treatment of cholangiopathies. It is known that endogenous conjugated tryhydroxycholanic bile acids in physiological concentrations do not exhibit a significant damaging effect on liver cells. The increase in the content of conjugated trihydroxycholanic bile acids under the influence of the dietary supplement, determined in the conducted studies, reveals one of the possible mechanisms of the hepatoprotective effect of the dietary supplement "FLP-MD", which was also described in the work of D.O. Melnychuk & V.A. Hryshchenko (2014).

Free bile acids are conjugated with taurine and glycine in hepatocytes. Conjugated bile acids are critical for lipid absorption in the gut. In liver cells, the enzyme bile acid-CoA amino acid N-acyltransferase ensures the formation of bile acid conjugates. Violation of this enzyme leads to significant losses of phospholipids and fat-soluble vitamins. A significant violation of the conjugation of compounds with taurine is observed in liver pathologies. According to D. Zhang *et al*. (2022), this may be related to the inhibition of taurine synthesis and transport in hepatocytes, and the inhibition of enzyme functioning. Disorders in the formation of bile acid conjugates with taurine were detected in the artificial modeling of fatty hepatosis in rats. The milk phospholipid-containing bioadditive eliminated the inhibition of the formation and secretion of tauroconjugates of bile acids in experimental fatty hepatosis. Decreased levels of bile acid tauroconjugates indicate significant changes in detoxification reactions during tetracycline hydrochloride usage. Namely, the conjugation of free cholates with taurine was inhibited. Instead, conjugation with glycine (an evolutionarily older metabolic pathway) in experimental fatty hepatosis was more stable, in particular the concentration of glycocholate did not change compared to control. The usage of the bioadditive based on milk phospholipids during tetracycline hydrochloride administration to the animals significantly eliminated the effects of antibiotics on the processes of biotransformation and formation of bile acids. This was confirmed by the absence of statistically significant differences with the control of the concentration of glycocholic acid, cholic acid and free dihydroxycholan bile acids in the bile of the rats that received the phospholipid-containing bioadditive for tetracycline-induced hepatosis. A special effect of the milk phospholipid-containing bioadditive was the increasinge of taurocholate, taurochenodeoxycholate and taurodeoxycholate secretion into the bile of the rats in the conditions of experimental fatty hepatosis. This can be explained by the enhanced formation of glycine and taurine conjugates with free bile acids under the influence of the dietary supplement containing milk phospholipids. Accordingly, the secretion of free dihydroxycholan bile acids under the influence of the bioadditive in rats with fatty hepatosis was reduced compared to the animals that did not receive the bioadditive but had fatty hepatosis.

The established patterns regarding the quantitative changes of six fractions of cholates in the bile of animals with fatty hepatosis indicated the manifestation of the effect of "medical protection" when using the milk phospholipid-containing bioadditive against the background of the introduction of antibiotics. Y. Ikeda (2020) noted that the specific interaction of bile acids with the transport protein ABCB4 in hepatocytes is necessary for the release of phospholipid molecules by ABCB4 into bile. The researcher suggested that understanding the mechanisms of bile acid-phospholipid interaction will help in the development of new therapeutic agents for cholestatic liver diseases.

As stated by X.Y. Yeo *et al.* (2023), it should be taken into account that changes in the composition of bile acids due to impaired metabolic transformations in the liver affect inflammatory reactions, permeability of the blood-brain barrier and synaptic functions of neurons. Thus, pathological changes in the synthesis, biotransformation, secretion into the primary bile tubules, detergent action of unconjugated dihydroxycholanic acids lead not only to damage to liver cells and progression of its damage. Changes in the physiological ratio of the different types of bile acids in bile, disorders in the biotransformation of cholates lead to violations of the mechanisms of nervous and humoral regulation of both liver functions and the organism as a whole. The determination of bile acid concentrations in biofluid samples is not yet standard practice in veterinary medicine. A special effect of the milk phosp The significant influence of bile acids on the functional state of the liver and the organism presented in this work and described by other researchers indicates the need for further thorough studies of the synthesis and biotransformation of cholates in normal and pathological conditions. The role of bile acids of various types in the pathogenesis of fatty hepatosis may be particularly significant.

In view of this, it is relevant to study the possible involvement of bile acids and enzymes that regulate their synthesis and biotransformation, as well as transport proteins that ensure the translocation of cholates through various domains of the plasma membrane of hepatocytes and enterocytes. Therefore, it is especially important to normalise the biotransformation of bile acids in hepatocytes. This allows to recommend the bioadditive "FLP-MD" as a hepatoprotective agent when administering potent drugs to animals that negatively affect liver cells and the processes of bile acid biotransformation.

Conclusions

The milk phospholipid-containing bioadditive eliminates the decrease in the concentration of bile acid tauroconjugates in the bile of the rats with experimental fatty hepatosis, which was detected during the study. This bioadditive stimulates the processes of conjugation of bile acids with taurine in hepatocytes. Under its influence, the concentration of bile acid glycoconjugates in the bile of the rats with fatty hepatosis approaches the control values. Therefore, the use of the milk phospholipid-containing bioadditive in animals stabilises the processes of conjugation of free bile acids with glycine in hepatocytes. The mechanisms of its effective action on the processes of conjugation of trihydroxycholanic and dihydroxycholanic bile acids are associated with the effect on the transmembrane transport of bile acids, taurine and glycine in hepatocytes, as well as with changes in the activity of the enzyme systems of cholate biotransformation. Under conditions of experimental fatty hepatosis, the milk phospholipid-containing bioadditive causes a decrease in the concentration of free bile acids in bile. The decrease in the secretion of cholic acid, chenodeoxycholic and deoxycholic acids with bile is due

to the stimulating effect of the components of the bioadditive on the conjugation of taurine with free bile acids in hepatocytes. An increase in the content of tauroconjugates of bile acids in bile significantly changes its physicochemical properties, efficiency in the processes of digestion, and the course of enterohepatic circulation of bile acids. The conjugation of substances with taurine and glycine is one of the effective detoxification processes in liver cells. The milk phospholipid-containing bioadditive acts on the mechanisms of conjugation in hepatocytes, stimulates the synthesis of tauroconjugates, which contributes to more effective detoxification of toxic compounds in the liver of animals with artificially modeled fatty hepatosis. Thus, the milk phospholipid-containing bioadditive reduces the content of toxic compounds in the internal environment of the body and, in particular, in the liver and promotes their effective removal from the body. Due to this, it is expected to slow down destructive changes in liver tissue in its fatty hepatosis.

The analysis of the ratio of conjugated and free bile acids in the bile of mammals with artificially modeled fatty hepatosis when using the milk phospholipid-containing bioadditive indicates prospects for studying the bioadditive as an effective means for improving the detoxification function of the liver. Further studies will be aimed at determining the features of changes in cholate secretion by the liver of rats during the development of experimental diclofenac-induced toxic hepatitis. It is planned to investigate the effectiveness of the influence of the components of the milk phospholipid-containing bioadditive "FLP-MD" on the molecular mechanisms of cholate synthesis and secretion in this hepatopathology and to conduct a comparative assessment with the patterns already described in this publication.

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Conflict of Interest

None.

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Фосфоліпіди молока в корекції жовчнокислотного складу жовчі в щурів за експериментального жирового гепатозу

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Анотація. Унікальні функції печінки вимагають детального вивчення, оскільки гепатопатологія є серйозною ветеринарною проблемою, яка також негативно впливає на продуктивність сільськогосподарських тварин. Використовуючи штучне моделювання гепатопатології, можливо з'ясовувати патогенетичні механізми її розвитку та визначати терапевтичну ефективність гепатопротекторних препаратів, особливо на основі сировини тваринного походження. Мета дослідження – визначити ефект від застосування фосфоліпідів молока у складі біодобавки «FLP-MD» на секрецію печінкою жовчних кислот за штучного моделювання в щурів жирового гепатозу. Для відтворення гепатопатології лабораторним щурам впродовж семи діб внутрішньошлунково вводили 4 % розчин тетрацикліну гідрохлориду в дозі 0,25 г/кг маси тіла та впродовж дев'яти діб використовували біодобавку в дозі 13,5 мг/кг маси тіла. Зразки жовчі в щурів відбирали шляхом проведення гострих експериментів. У зразках жовчі методом тонкошарової хроматографії було визначено шість фракцій кон'югованих жовчних кислот. Встановлено,

що в лабораторних щурів за експериментального жирового гепатозу гальмувалися процеси біотрансформації первинних і вторинних холатів шляхом кон'югації з таурином. Зокрема, фіксували зменшення у жовчі хворих тварин концентрації таурохолевої кислоти на 20,5–38,1 % ($P < 0,01$), а комплексу з таурохенодезоксихолевої та тауродезоксихолевої кислот – на 21,8–25,7 % ($P < 0,05$). У разі застосування біодобавки щурам за експериментального жирового гепатозу концентрація в жовчі таурохолевої, таурохенодезоксихолевої та тауродезоксихолевої кислот достовірно підвищувалася. Концентрація глікокон'югованих жовчних кислот і вільних холатів відповідала їх рівню в контролі. Біодобавка на основі фосфоліпідів молока за експериментального жирового гепатозу усувала негативний вплив антибіотика в токсичній дозі на процеси біотрансформації та утворення холатів. Це дозволяє рекомендувати біодобавку «FLP-MD» на основі фосфоліпідів молока в якості засобу гепатопротекторного профілю у разі застосування тваринам антимікробних препаратів

Ключові слова: жовчні кислоти; холестерол; біодобавка «FLP-MD»; тетрацикліну гідрохлорид; гепатоцит; гепатопатологія

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Determination of acute toxicity parameters of the extract of the basidial tree fungus *Inonotus obliquus* as a promising agent for veterinary medicine

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Abstract. The basidial tree fungus *Inonotus obliquus* (chaga) of the order *Hymenochaetales* is widely used in world medicine, as it is characterised by anti-inflammatory and antioxidant effects, and is effective in oncological, infectious and invasive diseases, as well as in diabetes, obesity, fatigue, liver and kidney pathologies, etc. However, the toxicological properties of chaga, which grows in Ukraine, require scientific justification. The aim of this study was to establish the parameters of acute toxicity of the aqueous extract of the basidial tree fungus *Inonotus obliquus*, which was obtained in natural conditions in Ukraine. The study was conducted on white Wistar rats according to generally accepted methods for conducting preclinical studies of new drugs. It was established that the median lethal dose of the test substance for rats after intragastric administration is 8,959 mg/kg according to the method of G. Kerber, 9,175.0 mg/kg according to the method of G. Pershin, and 9,175.0 mg/kg according to the method of B. Shtabsky – 8,890 (6,510.1 ÷ 11,269.9) mg/kg. According to the results obtained, it can be stated that the extract of the basidial wood fungus *Inonotus obliquus*, according to the classification of chemical substances by degree of danger (interstate standard 12.1.007-76), corresponds to class IV, and according to the classification of substances by toxicity – class V and the degree of toxicity "practically non-toxic substances". The established pathoanatomical changes, such as acute catarrhal gastritis, catarrhal-hemorrhagic or hemorrhagic enteritis, acute venous hyperemia of internal organs, dilatation of the wall of the right ventricle of the heart, edema of the subcutaneous tissue and skeletal muscles in the area of the right part of the scapula, manifested themselves identically in the body of the dead rats of the experimental groups and correlated with the dose of the studied extract. Determination of acute toxicity parameters is a prerequisite for studying the pharmaco-toxicological properties of medicinal substances, therefore the obtained results are promising for further preclinical and clinical studies with the aim of scientifically substantiating the use of medicinal raw materials from *Inonotus obliquus* obtained in Ukraine in veterinary medicine

Keywords: chaga; preclinical studies; laboratory animals; pathological changes; pharmacological effect

Introduction

The growing interest in the use of natural biologically active substances in veterinary medicine, in particular those obtained from medicinal mushrooms, has made this research relevant. The extract of the basidiomycete tree fungus Inonotus obliquus, which also grows in Ukraine, is considered a promising agent due to its wide range of pharmacological activity and potential for use in the prevention and treatment of animal diseases. However, the safety and acceptable doses of this extract for animals remain insufficiently studied, which necessitates the determination of its acute toxicity as a mandatory stage of preclinical studies.

The sterile (infertile) form of the basidiomycete tree fungus *Inonotus obliquus* of the order Hymenochaetales is called chaga or birch black fungus. Y. Lu *et al.* (2021) noted that the most favourable conditions for its growth are regions characterised by low temperatures. In Ukraine, chaga also occurs in the form of round (up to 30-40 cm in diameter) or elongated, dark-coloured growths formed by hardened hyphal networks of the fungus. J. Miina *et al.* (2021) indicated that the main raw material for the production of commercial goods from *I. obliquus* is its sterile conks, collected in natural conditions, mainly from birch trees.

Chaga was also obtained under experimental conditions. For example, according to data from P.T.Y. Ern *et al.* (2023), it was grown using artificial cultivation on potato dextrose agar or by inoculating birch trees.

It is known that chaga is characterised by a wide range of pharmacological effects. It has anti-inflammatory, antioxidant, antibacterial, antihelminthic and antiviral properties, and has a therapeutic effect in the complex treatment of people suffering from diabetes, obesity, cancer, liver and kidney pathologies, and fatigue. However, the above properties of chaga mushroom are not always scientifically proven and are based on observations of traditional folk medicine. It should be noted that the mechanism of pharmacological action has not been sufficiently studied. P.T.Y. Ern *et al.* (2023) pointed out the need to study the influence of various factors on the manifestation of the pharmacological effects of *I. obliquus*, as well as antagonism and synergism in the interaction between the constituent substances of chaga and other compounds or medicinal products.

S. Ghosh (2020) demonstrated that triterpenoids found in chaga are characterised by a wide range of biological activity. A study by R.W. Kou *et al.* (2021) established the anti-inflammatory effect of lanostane triterpenes and triterpenoids by inhibiting the activation of nitric oxide. Particular attention was paid to the study of biologically active substances of the fungus that have oncoprotective properties, such as betulinic acid. K.D. Kim *et al.* (2020) found that in PC3 prostate carcinoma and MDA-MB-231 breast carcinoma cell lines, when comparing the antitumour activity of ethanol, petroleum ether, ethyl acetate, butanol and water fractions extracted from *I. obliquus*, the petroleum ether extract had the highest cytotoxicity, which is explained by its high triterpenoid content. S.D. Chen *et al.* (2021) demonstrated a link between the antidiabetic properties of triterpenoids isolated from *I. obliquus* and their inhibitory activity against the brush border enzyme α-glucosidase. It was found that the use of an aqueous extract from chaga reduces the risk of liver damage under the influence of microcystin, as indicated by the absence of signs of oxidative stress, while glutathione and catalase levels remained unchanged. P.M. Ishfaq *et al.* (2022), investigating molecular docking, discovered a molecular mechanism involving the interaction of NF-κB-NIK with ergosterol peroxide. According to L.J. Dai *et al.* (2022), enzymatic analysis and inhibition kinetics analysis showed that polysaccharides isolated from *I. obliquus* exhibit significant inhibitory activity against the brush border enzyme α-glucosidase.

Despite the need for more extensive research into the pharmacokinetics and pharmacodynamics of the already known therapeutic properties of the basidiomycete tree fungus *I. obliquus,* it is important to consider the origin of this medicinal raw material. In Ukraine, the toxicological and pharmacological properties of *I. obliquus* have not been determined, and the use of its raw materials is not scientifically justified. In this regard, the aim of the scientific study was to determine the acute toxicity of the basidiomycete tree fungus *I. obliquus* collected in Ukraine as a prerequisite for further preclinical studies of samples of this medicinal raw material. The study is relevant for veterinary medicine, since establishing the toxicological profile of chaga opens up prospects for its use as an effective medicinal product for various diseases of infectious and non-infectious aetiology in animals and for their prevention. Therefore, along with studying the acute toxicity of chaga, an important task was to determine, based on an analysis of literary sources, the possible pharmacological effects it exhibits, with a view to developing new veterinary drugs.

Theoretical Overview

Analysis of scientific literature indicates considerable interest in the properties of the basidiomycete fungus *I. obliquus*, which determine its pharmacological action (Fig. 1). Accordingly, these properties correlate with the substances contained in it. Therefore, studies of the chemical composition of *I. obliquus* and the biological role of secondary metabolites (polysaccharides, polyphenols, etc.) in the body have become relevant.

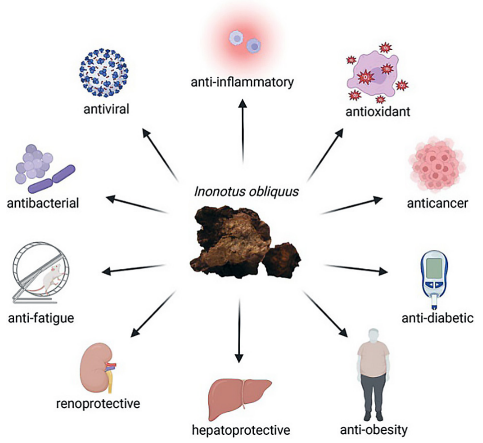

Figure 1. Pharmacological effects of exposure of *I. obliquus*

***Source**: based on materials from P.T.Y. Ern et al. (2023)*

Scientists have actively researched complex high-molecular compounds of polysaccharides from chaga. For example, S.D. Chen *et al.* (2021) studied the anti-inflammatory effect of polysaccharides from *I. obliquus*. The authors found a significant decrease in the expression of interleukin IL-17 and IFN-γ mRNA and a stimulating effect on the expression of IL-4 and IL-10. K. Yan *et al.* (2021) found a decrease in the expression of tumour necrosis factor-α (TNF-α), IFN-γ, IL-1β, IL-4 and IL-6 in macrophages infected with *Toxoplasma gondii.* Immunocytochemical analysis revealed that the inhibition of the inflammatory response is mediated by the prevention of NF-κB p65 translocation from the cytoplasmic space to the nucleus. Similar results were described by R. Sang *et al.* (2022), who found overexpression of inflammatory mediators through inhibition of excessive phosphorylation of the pro-inflammatory transcription factor NF-κB p65 and inhibitor IκBα in cells infected with *Toxoplasma gondii*.

Many scientists have studied the anti-cancer properties of chaga. Studies by B. Su *et al*. (2020) have shown a significant reduction in proliferation, migration and invasion, and increased apoptosis of MG-63 and U2OS osteosarcoma cells. The anti-diabetic effect of chaga is also explained by the presence of components such as polysaccharides. T. Xu *et al.* (2021) found that the use of preparations based on this mushroom increases insulin resistance, restores glycogen levels in the liver, and has an antihyperglycaemic effect. J. Yu *et al.* (2020) proved in experiments on mice that chaga polysaccharides reduce the degree of obesity in animals kept on a diet high in fat. The results of studies by Y.J. Chou *et al.* (2016) on the effect of *I. obliquus* polysaccharides on the body demonstrated their renoprotective effect and ability to reduce fatigue in animals.

Numerous studies have been devoted to investigating the components of *I. obliquus*, such as triterpenoids. Thus, L.S. Luo *et al.* (2021) found that in hyperuricaemia in mice, triterpenoids can bind more strongly to free enzymes than to enzyme-substrate complexes, in which this inhibitory interaction reduces inflammation. According to the work of K.D. Kim *et al.* (2019), betulinic acid, isolated from chaga, as a pentacyclic triterpenoid, has biological activity against obesity in a model of mice with HFD-induced obesity. Studies by A. Peng *et al.* (2022) have found that inotodiol, lanosterol and trametenolic acid, extracted from *I. obliquus*, have a protective effect against non-alcoholic fatty liver disease, in particular, effects against lipid

deposition, reduction of liver weight loss, reduction of triglyceride content in the liver, and restoration of unregulated alanine transaminase and aspartate aminotransferase levels were observed. J. Jin *et al.* (2022) demonstrated that the compound inonotuxoxide B, isolated from *I. obliquus,* has anti-fibrotic activity, as it inhibits the expression of α-smooth muscle actin and type I collagen. At the same time, it reduces the induced expression of α-SMA mRNA by platelet-derived growth factor-BB and activates phosphatidylinositol 3-kinase/protein kinase B and kinase signalling pathways regulated by extracellular signals, thereby inhibiting the viability and activation of PDGF-BB-stimulated hepatic stellate cells to protect against fibrosis. Q. Duan *et al.* (2022) observed the renoprotective effects of tramethenolic acid from *I. obliquus* in studies on white mice. In particular, blood urea nitrogen, creatinine, and urinary albumin levels increased, glomerular mesangial matrix expansion and collagen deposition decreased, contributing to the restoration of renal function. W.T. Basal *et al.* (2021) found that terpenoid compounds isolated from *I. obliquus* have antiviral properties.

Numerous studies have established that the antioxidant properties of the mushroom are due to the presence of flavonoid phenols in chaga (Abu-Reidah *et al.,* 2021). As shown in a review by K.A. Szychowski *et al.* (2021), phenolic compounds are capable of actively absorbing radicals and are characterised by their affinity for binding with the enzyme superoxide dismutase. R. Hao *et al.* (2023) investigated the effect of styrylpyranone polyphenols, namely feligridin E, on enhancing the activity of antioxidant enzymes. Y. Wang *et al.* (2021) demonstrated the iron-reducing activity of polyphenols isolated from *I. obliquus.* B.S. Hwang *et al.* (2016) described various methods for synthesising phenolic compounds from *I. obliquus*, while simultaneously enhancing their antioxidant activity.

Thus, the basidiomycete tree fungus *I. obliquus*, due to the presence of biologically active substances in its composition, can exhibit various types of pharmacological effects, which is a prerequisite for its use for therapeutic or prophylactic purposes in veterinary medicine. Such a medicinal product may have anti-inflammatory, antioxidant, antibacterial and antiviral properties, be used to treat cancer, diabetes, obesity, liver and kidney diseases, as well as to increase performance and combat fatigue. However, the toxicological and pharmacological characteristics are not fully scientifically substantiated, and potential antagonistic or synergistic interactions between *I. obliquus* and other compounds or medicinal products have not been established. Therefore, it is important to study the pharmacokinetics and pharmacodynamics of chaga samples obtained in Ukraine, as there is no such data available in the scientific literature.

The aim of this study was to analyse the main types of pharmacological action reported in the literature and to assess the results of acute toxicity tests of Inonotus obliquus extracts obtained from samples collected in Ukraine. The study will make it possible to investigate the toxicological and pharmacological characteristics of Ukrainian chaga samples, with a view to subsequently obtaining medicinal products with a broad spectrum of pharmacological action and creating new preparations for veterinary medicine.

Materials and Methods

The research was conducted in January 2025 at the scientific laboratory "Preclinical Studies of Medicines" of the Department of Vertebrate Physiology and Pharmacology of the National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine) in accordance with the tasks of the initiative topic "Research of the pharmacotherapeutic properties of new

veterinary chemotherapeutic, anti-inflammatory, antidote and metabolism-regulating agents" (state registration No. 0118U000544).

The object of the study was an aqueous extract of the basidiomycete tree fungus *I. obliquus* of the order *Hymenochaetales*. The raw material of the basidiomycete tree fungus *I. obliquus* of the order *Hymenochaetales* was collected in the Northern Polissya region (Sarny district, Rivne region). The aqueous extract was prepared according to the generally accepted method for preparing extracts from plant raw materials, which included the stages of grinding, dissolving in distilled water, and infusing for 10 days. The acute toxicity parameters of the aqueous extract of the basidiomycete wood fungus *I. obliquus* of the order Hymenochaetales were determined according to the methodological guidelines (Kotsyumbas *et al.,* 2006).

Acute toxicity was determined in white laboratory rats aged 3.5-4.0 months and weighing 180 g to 200 g. The animals were kept in plastic cages in accordance with the Regulations on the Maintenance and Use of Animals in Scientific Research and the Educational Process (2018) at a temperature of 18-20°C and relative humidity of 50%-55% under natural day-night light conditions. They were fed a complete feed mixture and had unlimited access to water and food. Before the experiment, the animals were kept in quarantine for 14 days, and feeding and watering were stopped three hours before the experiment. The test substance was administered intragastrically using a probe in the form of an aqueous extract at a rate of 5.0 mL per rat. The dose was calculated in mg of active substance per 1 kg of body weight.

To obtain preliminary information about the range of doses close to the median lethal dose, a preliminary study was first conducted, involving the administration of the test substance to three rats at doses differing by an order of magnitude. At the same time, distilled water was administered to animals in the control group (n = 3). This was followed by a detailed study in which 100% mortality was observed when the test substance was administered at the maximum dose, while no deaths were observed when the minimum dose was used.

The study was conducted on 36 white rats of the *Wistar* breed. They were divided into 6 groups (6 animals in each) according to the principle of analogues. The aqueous extract was administered to the animals in the experimental groups at doses of 2,500; 5,000; 7,500; 10,000; 12,500 and 15,000 mg/kg of body weight; the animals in the control group were administered distilled water in a volume of no more than 5.0 mL. The laboratory animals were observed for 14 days, noting changes in their clinical condition over time. The animals were under constant supervision during the first day after administration of the test extract, noting behavioural characteristics, food and water intake, and recording symptoms of intoxication and animal deaths.

The median lethal dose (LD_{50}) was calculated using the methods of G. Kerber and B. Shtafsky. The method of K. Miller and M. Teinter was used to calculate confidence limits, and based on the results obtained, the studied substance was classified according to its degree of danger and toxicity (Kotsyumbas *et al.,* 2006). According to G. Kerber's method, the average lethal dose was calculated using the formula:

$$LD_{50} = LD_{100} - \frac{\Sigma zd}{m}, \quad (1)$$

where LD_{100} is the dose of the substance under study that causes the death of all animals in the group; d is the interval between each two adjacent doses; z is the arithmetic mean of the number of animals that died under the influence of two adjacent doses; m is the number of animals in the group.

The mean lethal dose was also calculated using Pershin's method, using the following formula:

$$LD_{50} = \Sigma\,[(a+b)\,(m+n)]\,/\,200, \quad (2)$$

where *a, b* are the values of adjacent concentrations under study, mg/mL; *m, n* are the frequencies of fatalities at corresponding concentrations, %.

The dependence of the mortality rate *(Y)* on the dose *(X)* was described by a straight line equation with a slope coefficient *(a)* according to B. Shtafsky using the formula:

$$Y = aX + b, \quad (3)$$

where *b* is a free member.

The corresponding values of *a* and *b* were determined using the following formulas:

$$\alpha = (Y2 - Y1) : (X2 - X1); \quad (4)$$

$$b = (\Sigma Y - \alpha \Sigma X) : n, \quad (5)$$

where *X*1 and *X*2 are the values of the two extreme doses out of the three studied doses; *Y*1 and *Y*2 are the corresponding mortality rates; *n* is the number of specified (studied) doses, which is equal to 3.

Having obtained the values of *a* and *b,* we determined *X*:

$$X = (Y + b) : \alpha\,. \quad (6)$$

Thus, by substituting the values of *Y*, which were 50%, 84% and 16%, respectively, into the formula, *LD,* LD_{84} and LD_{16} were found.

Statistical processing of the obtained research results was carried out using a personal computer. According to the method of K. Miller and M. Teinter, confidence limits were calculated with the determination of σ and *m* (Kotsyumbas *et al.,* 2006). Accordingly, $2\sigma = LD_{84} - LD_{16}$, then the average error of the median lethal dose was calculated using the formula:

$$m = \frac{2\sigma}{\sqrt{N' x^2}}, \quad (7)$$

where *m* is the mean error of the mean value; σ is the standard deviation; N' is the total number of individuals in groups in which at least one animal died or survived.

After calculating the value of *m,* we found:

$$LD \pm mt, \quad (8)$$

where *t* is Student's criterion.

In the formulas above, the symbol m is used in different ways depending on the method. Specifically, according to G. Kerber's method, m means the number of animals in a group; in Pershin's formula, it means the frequency of fatalities (%); and in Miller-Tainter's method, it means the average error of the average value. To preserve the authenticity of the original approaches, the symbols are given in their original form.

Scientific research involving animals complied with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (1986) and Law of Ukraine No. 3447-IV (2006). All necessary interventions on animals were carried out in accordance with ARRIVE recommendations, without violating the guidelines of Directive of the European Parliament and of the Council No. 2010/63/EU (2010). The conclusion on the conduct of research was also approved by the Bioethics Committee of the National University of Life and Environmental Sciences of Ukraine, protocol No. 010/2024 dated March 28, 2024.

Results and Discussion

A preliminary study was conducted to compare toxicity at different mushroom infusion times, specifically examining extracts infused for 2 days and 10 days. The results showed greater toxicity when the extract was prepared for a shorter period of time. For the main study, the optimal dose range of the extract after ten days of infusion was determined in order to

establish the acute toxicity of *I. obliquus*. In the first minutes after intragastric administration of the extract of the basidiomycete tree fungus *I. obliquus,* all groups of rats showed a stress response to the intervention. Slight agitation followed by calming was observed. The results of determining the acute toxicity of *I. obliquus* in white mice are shown in Table 1.

Table 1. Results of acute experiments involving the administration of basidiomycete mushroom extract of *I. obliquus* to white rats

Dose, mg/kg b.w.	Number of animals in thegroup i	Number of animals killed per day				in total	%
		1	2	5	15		
2,500	6	-	-	-	-	0	0
5,000	6	-	-	-	1	1	17
7,500	6	-	-	-	2	2	33
10,000	6	-	-	-	3	3	50
12,500	6	-	-	5	-	5	83
15,000	6	-	1	5	-	6	100

***Note**: the results of an acute experiment involving the intraperitoneal administration of an aqueous extract of I. obliquus to white rats are shown*
***Source**: developed by the authors*

In rats of the sixth experimental group (at a dose of 15,000 mg/kg body weight), symptoms of depression appeared after 30 minutes. The laboratory animals hunched over and moved chaotically in the cage. After 2 hours, they became agitated, which gradually changed to depression, and an increase in heart rate and respiratory rate was observed. These signs persisted for some time, after which the rats assumed a lying position, performing swimming movements and slowly raising and lowering their tails. On the second day, one animal from the sixth experimental group died, and by the fifth day, 100% mortality was recorded in this group. The same results were observed in the fifth group (the test substance at a dose of 12,500 mg/kg body weight).

In other experimental groups, most animals gradually recovered to their physiological state, and by day 15, no mortality was observed in the first experimental group (test substance at a dose of 2,500 mg/kg body weight). At the same time, 17% mortality was observed in the second experimental group (test substance at a dose of 5,000 mg/kg body weight), 33% mortality in the third experimental group (test substance at a dose of 7,500 mg/kg body weight), 50% mortality in the fourth experimental group (test substance at a dose of 10,000 mg/kg body weight) and 83% mortality in the fifth experimental group (test substance at a dose of 12,500 mg/kg body weight). Thus, toxic effects after intraperitoneal administration to white rats are observed at doses of 5,000 mg/kg body weight and above. Under these conditions, the mortality rate in the experimental groups ranged from one to six rats (Table 2), based on which calculations were made to determine the LD_{50} (Table 3).

Table 2. Acute toxicity indicators of the extract of the basidiomycete tree fungus *I. obliquus* in white rats

Dose, mg/kg b.w.	Died/survived	Dose, mg/kg b.w.	Died/survived
2,500	0/6	10,000	3/3
5,000	1/5	12,500	5/1
7,500	2/4	15,000	6/0

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

Table 3. Toxicity indicators of the extract of the basidiomycete fungus *I. obliquus* determined using the method of G. Kerber

Dose, mg/kg b.w.	2,500	5,000	7,500	10,000	12,500	15,000
Survived	6	5	4	3	1	0
Died	0	1	2	3	5	6
z		0.5	1.5	2.5	4.5	5.5
d		2,500	2,500	2,500	2,500	2,500
zd		1,250	3,750	6,250	11,250	13,750

***Note**: z – arithmetic mean of the number of animals that died under the influence of two adjacent doses; d – interval between each two adjacent doses*
***Source**: developed by the authors*

Thus, LD_{50} was determined using formula (1), therefore:

$$m=6;\ LD_{100}=11{,}250\ mg/kg;$$
$$\Sigma zd=1{,}250+3{,}750+6{,}250+11{,}250+13{,}750=36{,}250;$$
$$LD_{50}=LD_{100}-\frac{\Sigma zd}{m}=15{,}000-\frac{36{,}250}{6}=$$
$$=15{,}000-6{,}041=8{,}959\ mg/kg.$$

Considering that it is recommended to use several methods to determine the acute toxicity parameters of new medicinal substances, these data were also calculated using G. Pershin's method (Table 4). Based on the data presented in Table 4, the results of the average lethal dose of the extract of the basidiomycete tree fungus *I. obliquus,* calculated using the method of G. Pershin, are as follows (formula 2):

Table 4. Toxicity indicators of the extract of the basidiomycete fungus *I. obliquus* determined using the method of G. Pershin

Dose, mg/kg b.w.	2,500	5,000	7,500	10,000	12,500	15,000
Observed results	0/6	1/5	2/4	3/3	5/1	6/0
Percentage of animals that died	0	17	33	50	83	100
$a+b$		7,500	12,500	17,500	22,500	27,500
$m-n$		17	16	17	33	17
$(a+b)(m-n)$		127,500	200,000	297,500	742,500	467,500

***Note**: a, b – values of adjacent concentrations studied, mg/mL; m, n – frequencies of fatalities at corresponding concentrations, %*
***Source**: developed by the authors*

$$\Sigma[(a+b)(m+n)]=127{,}500+200{,}000+$$
$$+297{,}500+112{,}5000=1{,}750{,}000;$$
$$LD_{50}=\frac{\Sigma\,[(a+b)(m+n)]}{200}=9{,}175.0\ \text{mg/kg}.$$

According to the data obtained from studies on rats, using formula (3), the LD_{50} was established using the method of B.M. Shtabsky (Table 5).

Table 5. Toxicity indicators of the extract of the basidiomycete tree fungus *I. obliquus* determined using the method of B.M. Shtabsky

Y	*X*
33,3	7,500
50	10,000
100	12,500
$\Sigma Y=183{,}3$	$\Sigma X=30{,}000$

***Note**: Y – percentage of mortality, X – dose*
***Source**: developed by the authors*

Thus, according to calculations using formulas (3)-(8), the parameters of the median lethal dose of the test substance for intragastric administration to rats, calculated using the method of B.M. Shtabsky, were LD_{50} = = 8,890 (6,510.1 ÷ 11,269.9) mg/kg.

The studies have established that the LD_{50} of the test substance for rats after intragastric administration is 8,959 mg/kg according to the method of G. Kerber, 9,175.0 mg/kg according to the method of G. Pershin, and 8,890 (6,510.1 ÷ 11,269.9) mg/kg according to the method of B. Shtafsky. These data allow us to conclude that the extract of the basidiomycete fungus *I. obliquus,* according to the classification of chemical substances by degree of danger, corresponds to hazard class IV, and according to the classification of substances by toxicity, it corresponds to class V and degree of toxicity "practically non-toxic substances" (Kotsyumbas *et al.,* 2006).

During the pathological autopsy of rats from experimental groups that died during the study of the acute toxicity of the extract of the basidiomycete tree fungus *I. obliquus*, it was established that the carcasses of animals had no external damage, natural openings were closed, there were no secretions, visible mucous membranes were pale pink in colour, dry with a bluish tint, and the hair coat had no visible changes. The pathological and anatomical changes in the experimental groups of animals (Fig. 2) were identical and differed only in the intensity of the changes, which correlated with the dose of the test substance.

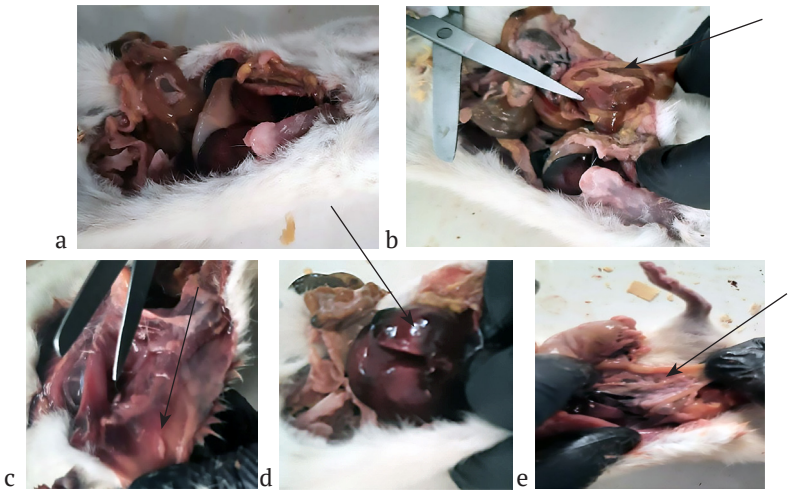

Figure 2. Pathological and anatomical changes in rats of experimental groups according to the determination of acute toxicity of the extract of the basidiomycete tree fungus *I. obliquus*
***Source**: authors' photos*

Acute catarrhal gastritis, catarrhal-haemorrhagic or haemorrhagic enteritis (Figs. 2a, 2b and 2e), acute venous hyperaemia of the liver (Fig. 2d), kidneys and spleen, protein dystrophy of the liver, dilatation of the right ventricular wall of the heart, oedema of the subcutaneous tissue and skeletal muscles in the right shoulder blade area (Fig. 2c), hyperaemia and oedema of the lungs. The changes detected in animals of all experimental groups indicate the presence of poisoning by a substance that entered the body through the alimentary tract.

Thus, the results obtained confirm the acute toxicity parameters of the extract of the basidiomycete tree fungus *I. obliquus* and the pathological changes in the body of laboratory animals under such exposure. The studies conducted are a prerequisite for the next stage of preclinical research and the creation of new highly effective herbal remedies for veterinary medicine. After all, a number of modern sources of medical literature confirm the wide range of pharmacological effects.

No results of studies on the acute toxicity of chaga have been found in the literature, either in Ukraine or abroad. A more relevant area of research worldwide is the study of the properties of the constituent substances of *I. obliquus* with a view to their use for therapeutic or prophylactic purposes, and the improvement of methods for obtaining extracts from natural and artificial chaga. S. Javed *et al.* (2019) demonstrated the anti-inflammatory activity of chaga using methanol as a solvent for its extraction and found a reduction in histamine-induced TNF-α in RAW 264.7 macrophages by more than 90%. In the next stage of research, evaluation of the methanol extract in a mouse microcirculation model revealed its ability to influence vasodilation, which is observed during inflammation. W. Alhallaf & L.B. Perkins (2022) found that *I. obliquus* extracts, especially those obtained by accelerated ethanol/water extraction and hot water soaking of powdered chaga, are capable of significantly inhibiting NO, TNF-α, IL-6, and IL-1β generation in RAW 264.7 macrophages, indicating the anti-inflammatory ability of the extract under study.

S.S. Tsai *et al.* (2017) found that extracts of *I. obliquus* have anti-cancer properties and extracts from chaga obtained as a result of fermentation in a liquid state exhibit antiproliferative effects on the HCT-116 cell line through activation of the mitochondrial apoptotic pathway by enhancing the expression of proapoptotic gene mRNAs and increasing the Bax/bcl-2 ratio. K. Ryu *et al.* (2017) found that after 14 days of oral administration of methanol extracts, there was a suppression of lung tumour formation and metastasis in C57BL/6 mice that had been injected with B16F10 melanoma cells. S. Arata *et al.* (2016) demonstrated significant inhibition of tumour development in a mouse lung carcinoma model under the influence of chaga extract by reducing tumour vascularisation. At the same time, the importance of body temperature in inhibiting tumour growth was indicated, as continuous measurement of the body temperature of mice showed that consumption of *I. obliquus* extract prevented a decrease in the body temperature of mice with LLC implants.

The antidiabetic properties of chaga extracts have been established. Thus, Z. Zhang *et al.* (2021) proved that administering extracts in doses of 250 mg/kg and 500 mg/kg to C57BL/6 mice with type 2 diabetes led to improved blood glucose levels and insulin resistance, increased glycogen levels in the liver and cholesterol, and decreased total cholesterol, triacylglycerols and cholesterol. S. Chen *et al.* (2022) describe that treatment of HFD+STZ-induced diabetic mice with *I. obliquus* at a dose of 150 mg/kg significantly improved the pathological condition of fatty liver infiltration and fatty degeneration of its cells, reducing the accumulation of lipid droplets in the liver. X. Ye *et al*. (2022) found that treatment with *I. obliquus* improved the functional status of other affected organs in diabetic mice, including the kidneys, pancreas, and colon.

The renoprotective effect of chaga has been established. K.H. Chiang *et al.* (2023) reported the renoprotective effect of ethanol-ethyl acetate extracts of *I. obliquus* on nephropathic mice, where the extracts were able to effectively reduce blood creatinine and urea nitrogen levels, improve glomerular atrophy and interstitial accumulation. J. Glamočlija *et al.* (2015) demonstrated the bacteriostatic

and bactericidal activity of alcohol and water extracts of *I. obliquus* against all studied Gram-positive and Gram-negative bacterial strains, with Staphylococcus aureus and Bacillus cereus being the most sensitive to the extracts. A. Milyuhina *et al*. (2021) found that although aqueous extracts of *I. obliquus* have antibacterial properties, the activity of the extracts is enhanced after microwave irradiation.

The results obtained are a prerequisite for preclinical and clinical studies of chaga samples obtained in Ukraine, with the aim of its scientifically based use in veterinary medicine for various pathologies in animals or for preventive measures. Such studies are extremely promising given the potential for creating veterinary herbal remedies based on chaga, the possibility of their use in the prevention of animal diseases; their introduction into feed as a nutraceutical; minimising the need for antibiotic therapy in veterinary medicine; creating new combinations of dietary supplements based on *I. obliquus* for animals of various species.

Conclusions

This article is devoted to the analysis of the results obtained from the study of the acute toxicity of the extract of samples of the basidiomycete tree fungus *I. obliquus* obtained in Ukraine (Rivne region). Preclinical studies, namely the establishment of the parameters of acute toxicity of the aqueous extract of chaga on laboratory animals, are a prerequisite for the experimental study of the toxicological and pharmacological properties of Ukrainian chaga samples with the subsequent production of medicinal products with a wide range of pharmacological effects. The creation of new highly effective veterinary herbal preparations for the treatment and prevention of diseases in various animal species will be of great importance in practical veterinary medicine and in ensuring the well-being of livestock.

The acute toxicity of the aqueous extract of *I. obliquus* was calculated using various generally accepted methods for preclinical studies of new substances. It was established that the LD_{50} of the studied substance for rats after intragastric administration is 8,959 mg/kg according to the method of G. Kerber, 9,175.0 mg/kg according to the method of G. Pershin, and 8,890 (6,510.1 ÷ 11,269.9) mg/kg according to the method of B. Shabsky. Therefore, the extract of the basidiomycete tree fungus *Inonotus obliquus*, according to the classification of chemicals by degree of danger, corresponds to hazard class IV, and according to the classification of substances by toxicity, class V and degree of toxicity "practically non-toxic substances". The pathological changes observed were identical in the bodies of dead rats from different experimental groups and depended on the amount of the test substance administered. Acute catarrhal gastritis, catarrhal-haemorrhagic or haemorrhagic enteritis, acute venous hyperaemia of the liver, kidneys, spleen, protein dystrophy of the liver, dilatation of the right ventricular wall of the heart, oedema of the subcutaneous tissue and skeletal muscles in the right shoulder blade area, hyperaemia and oedema of the lungs were observed.

The determination of acute toxicity of *Inonotus obliquus* extract allowed classifying the studied substance as low-hazard (hazard class IV) and practically non-toxic (toxicity class V). The obtained LD_{50} values indicate a high safety profile of the extract under conditions of intragastric administration. The toxicological testing conducted is a necessary prerequisite for further stages of preclinical development of a medicinal product for veterinary use and can be used for scientific justification of the safety of raw materials for pharmaceutical use in the livestock industry. Further research will be aimed at conducting

the next stages of preclinical and clinical studies to study the pharmacokinetics and pharmacodynamics of the active substances of *I. obliquus* in order to scientifically substantiate the use of medicinal raw materials from chaga obtained in Ukraine in veterinary medicine for the treatment or prevention of animal diseases of various aetiologies.

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Conflict of Interest

None.

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Визначення параметрів гострої токсичності екстракту базидіального деревного гриба *Inonotus obliquus* як перспективного засобу для ветеринарної медицини

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Анотація. Базидіальний деревний гриб *Inonotus obliquus* (чага) порядку *Hymenochaetales* широко використовується у світовій медицині, оскільки характеризується протизапальною і антиоксидантною діями, виявляє ефективність за онкологічних, інфекційних та інвазійних

хвороб, а також за цукрового діабету, ожиріння, втоми, патології печінки і нирок тощо. Проте, токсикологічні властивості чаги, яка росте на території України, потребують наукового обґрунтування. Метою цього дослідження було встановлення параметрів гострої токсичності водного екстракту базидіального деревного гриба *Inonotus obliquus*, який одержано в природних умовах України. Дослідження проведено на білих щурах лінії Wistar згідно загальноприйнятих методик проведення доклінічних досліджень нових лікарських засобів. Встановлено, що середня смертельна доза досліджуваної речовини для щурів за внутрішньошлункового введення становить за методом Г. Кербера – 8959 мг/кг, за методом Г. Першина – 9175,0 мг/кг, а за методом Б. Штабського – 8890 (6510,1 ÷ 11269,9) мг/кг. Згідно з отриманими результатами можна стверджувати, що екстракт базидіального деревного гриба *Inonotus obliquus* згідно з класифікацією хімічних речовин за ступенем небезпечності відповідає IV класу, за токсичністю – V класу і ступеню токсичності – «практично нетоксичні речовини». Встановлені патологоанатомічні зміни, такі як гострий катаральний гастрит, катарально-геморагічний або геморагічний ентерит, гостра венозна гіперемія внутрішніх органів, дилатація стінки правого шлуночка серця, набряк підшкірної клітковини та скелетних м'язів в ділянці правої частини лопатки, проявлялися ідентично в організмі загиблих щурів дослідних груп та корелювали з дозою досліджуваного екстракту. Визначення парамерів гострої токсичності є передумовою для вивчення фармако-токсикологічних властивостей лікарських речовин, тому отримані результати є перспективою для наступних доклінічних та клінічних досліджень із метою наукового обгрунтування застосування у ветеринарній медицині лікарської сировини з *Inonotus obliquus*, одержаної на території України

Ключові слова: чага; доклінічні дослідження; лабораторні тварини; патологоанатомічні зміни; фармакологічна дія

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

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

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

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

Introduction

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

According to T.S. Budnik & S.V Guralska (2022), the effectiveness of revaccination of poultry against viral and bacterial infections depends on the development of peripheral immune organs, which are based on lymphoid tissue. The latter contains numerous lymphocytes located in a mesh-like network of reticular tissue. In the immune organs, through the action of antigens (foreign substances), as noted by C. Ceccopieri & J.P. Madej (2024), lymphocytes differentiate into effector cells responsible for the development of specific (cellular and humoral) immunity. The process of morphofunctional differentiation and specialisation of immune organs occurs in a specific order: from the formation of a diffuse form as a result of the expulsion of lymphocytes from the lumen of blood vessels, to the emergence of prenodules without clearly defined boundaries and membrane, and the appearance of primary (without germinal centers) and secondary (with germinal centers) lymphoid nodules. The presence of secondary lymphoid nodules in the lymphoid tissue, as stated by N.J. Monisha *et al.* (2024a), signifies both its own morphofunctional maturity, as well as that of the structures that it forms, i.e. the ability to give a complete immune response in the form of a cellular reaction complex due to action of foreign substances with antigenic properties.

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

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

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

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

Literature Review

According to T. Budnik (2022), the effectiveness of preventive and therapeutic methods depends entirely on the condition of the immune system of birds. In turn, the functioning of immune system is possible only under the condition of a tight link of all specific immune responses and factors of innate immune reactivity. As noted by N.I. El-naseery *et al.* (2021) and E.C. Lavelle & R.W. Ward (2022), organs of the bird immune system are divided into central and peripheral. The emergence of blood cells, as well as the development and maturation of lymphocytes without significant influence of antigens, occur in the central organs. T-lymphocytes are formed in the thymus, while B-lymphocytes – in the cloacal sac, or the bursa of Fabricius. Along the blood flow, T- and B-lymphocytes are carried into the peripheral organs of the immune system (spleen, lymph nodes – in waterfowl, Harderian gland, immune formations of digestive and respiratory organs, and of skin), where their mature forms interact with antigens, which leads to the activation of the immune system and subsequent development of cellular and humoral immunity.

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

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

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

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

Materials and Methods

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

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

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

Table 1. Continued

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

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

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

Histological sections, 5-10 μm thick, were prepared on the sledge microtome MPS-2 (MedTech-Price, Ukraine), stained with Carazzi hematoxylin solution and eosin solution according to Van Gieson, Mallory and Weigert, and impregnated according to Kelemen. To calculate the area of lymphoid tissue in individual parts of the esophagus, the point counting method was employed using a binocular microscope with a measuring grid. The size of the lymphoid nodules was determined using the Olympus CX 43 microscope (Labdepo, Japan) and an ocular micrometer. Some histological specimens were photographed with a digital camera mounted in the Primo Star microscope (Carl Zeiss, Germany) and connected to a personal computer. The obtained results were recorded in protocols, and the statistical analysis of numerical values was performed using the software StatSoft Statistica 13.1 (2016). The following were calculated: the arithmetic mean and its error ($M \pm m$), statistical significance (P) according to Student and significance criteria – $P < 0.05$; $P < 0.01$; $P < 0.001$.

Results and Discussion

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

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

The inner layer of the mucous membrane of turkey esophagus is lined by a thick, stratified squamous epithelium with weak keratinisation, the process of which is best observed in the upper part of the organ (Fig. 1B). It is coated with a layer of mucus that, according to X. Zhang (2018) and T. Kataa & S.M. Ahmed (2022) protects the esophagus of birds from chemical and mechanical damage, as well as from pathogenic microorganisms and acts as a lubrication medium. The principal cell differentiation pathway of the superficial epithelium is represented by epithelial cells at successive stages of differentiation. In the epithelial cells of the basal layer, which are arranged in one row and have a cylindrical shape, the nuclei are oval, with a relatively loose chromatin structure and a well-defined nucleolus. Above them, there are polygonal-shaped cells of the spinous layer, followed by flat epithelial cells with partially destroyed nuclei. Under the epithelium, there is a layer of loose fibrous connective tissue, which forms the lamina propria of the mucous membrane. In the form of papillae, it digs into the lower layers of the epithelium. In this layer, elastic and collagen fibers, numerous small blood vessels and the glandular apparatus are found. The latter, according to L.P. Kharchenko (2014), functions by moistening the feed and providing mechanical protection. The author determined that the extent of development of the glandular apparatus depends on the type of nutrition of birds. Feeding on insects with a hard chitinous exoskeleton or on dry and hard plant-based fodder contributes to the formation of a well-developed glandular apparatus.

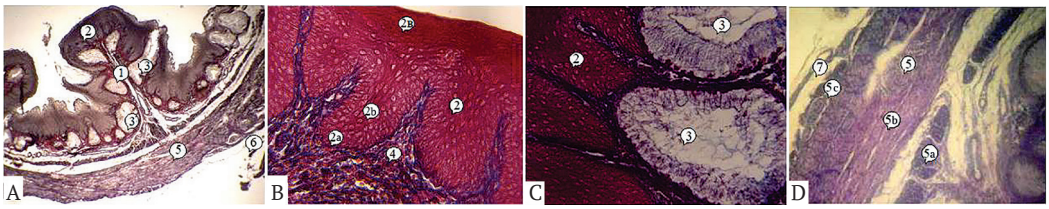

Figure 1. The cervical part of the esophagus of a control group turkey

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

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

According to research, the glandular apparatus of turkeys is represented by packages of alveolar-tubular esophageal glands with mucous secretions. The glands had an unequal shape, a substantial size and were separated from neighbouring glands by layers of loose fibrous connective tissue (Fig. 1C). Each gland was composed of terminal sections, formed by cylindrical epithelium and opening into the central cavity. External packages of glands are limited

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

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

F. Taki-El-Deen (2017) determined that in some bird species (domestic ducks and waders), the muscle tissue of the esophagus has a bilayer structure and is formed by an outer circular and an inner longitudinal layers. Externally, the esophagus is surrounded by the adventitious membrane in the cervical part and by the serous membrane in the thoraco-abdominal part. The adventitious and serous membranes are formed by loose fibrous connective tissue, which in the thoraco-abdominal part of the esophagus is covered by one layer of mesothelial cells (simple squamous epithelium). In those membranes, clusters of fat cells, small blood vessels, individual nerve nodes and nerve plexuses are found.

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

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

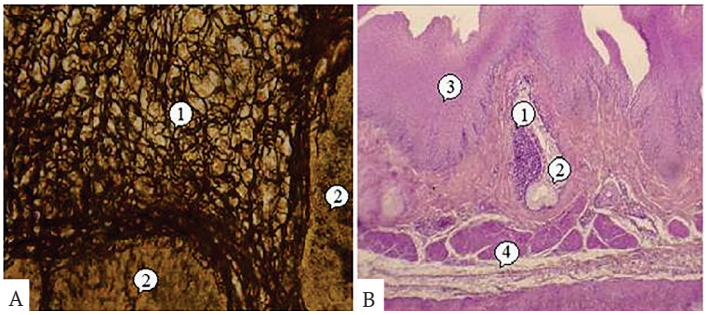

Figure 3. Diffuse lymphoid tissue of the cervical part of the esophagus of a 20-days old turkey from the experimental group

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

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

In the areas where immune formations are located, the latter came into contact with the epithelial layer, which is part of the lymphocyte microenvironment, or migrated into the lower layers of the epithelium. R. Sinha *et al.* (2023) found that among the lymphoid cells that have migrated into the epithelium, B-lymphocytes predominate, while the number of T-lymphocytes is much smaller, and plasma cells are found on the periphery of such clusters. Individual clusters of diffuse lymphoid tissue were also recorded in the wall of the secretory sections of glands between gland cells (Fig. 3B). As noted by N. Dyshliuk *et al.* (2024), local lymphoid tissue of the esophagus of birds along with its components is necessary for local immune responses. Diffuse lymphoid tissue was presented as a reticular tissue with a dense network of sinuous fibers, which were interconnected and formed uniform small and large network. The cytoplasm of reticulocytes formed short appendages. Their nuclei were irregularly shaped and contained a significant amount of heterochromatin. Between the elements of reticular tissue, lymphoid cells could be found. Some of them were connected to lymphoid chains. At the age of 30 days of turkeys from the experimental group, the development of diffuse lymphoid tissue continued, which appeared in some areas of the submucosal base on the border with the muscular layer. Inside and outside the tissue, singular lymphoid prenodules and nodules (primary and secondary) were recorded, the base of which, as is of diffuse lymphoid tissue, is formed by reticular tissue (Fig. 4A)

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

morphofunctional (anatomical) units of the immune system at the peak of their activity. They are one of the most important morphological components of the lymphoid tissue. M. Markowska *et al.* (2017) divided lymphoid nodules of the peripheral organs of the immune system into two types: perivascular, which are associated with the wall of hemo- and lymphomicrocirculatory channel, and lymphoepithelial, which are specialised nodules of mucous membranes that are directly involved in a specific response to a particular antigen.

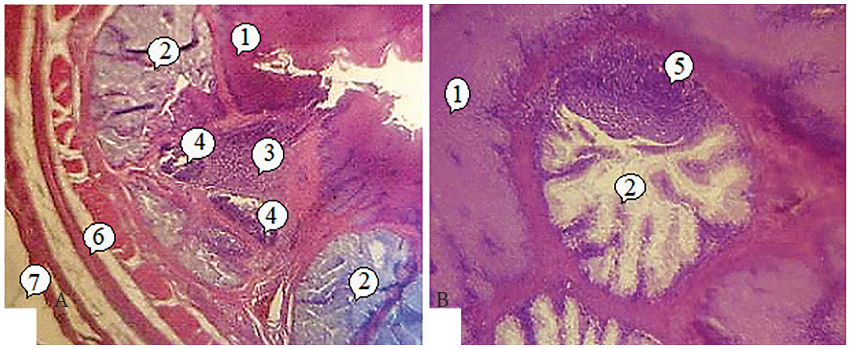

Figure 4. Immune formations in the esophagus of turkeys from the experimental group
***Note**: A – immune formations of the thoraco-abdominal section of the esophagus of a 30-days old turkey from the experimental group; B – primary lymphoid nodule in the wall of gland of the cervical section of the esophagus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

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

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

without germinal centers were observed. As found by L.P. Kharchenko (2014) and O. Lykova & L.P. Kharchenko (2021), lymphoid nodules of the esophagus are not present in all birds. They were not found by the authors in the European bee-eater and the chaffinch, and found in the rook, pheasant and quail. The observed nodules in birds of certain species had a close morphofunctional connection with the glands. According to the authors, presence or absence of the nodules in the esophageal wall correlates to their feeding specialisation. Immune formations in the mucosa of esophagus of turkeys from experimental and control groups occupy an unequal area and their content is greater in vaccinated birds (Table 2).

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

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

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

As noted above, in turkeys of the first group immune formations of the esophagus started appearing at 20 days of age, while in the second – at 30 days of age. In general, in poultry from the experimental group (from 20 to 50 days of age), their area increased by 268.14% (3.7 times), while in control (from 30 to 50 days of age) – by 98.44% (almost 2 times). The increase in the area of immune formations of the esophagus occurred unevenly. In turkeys from the experimental group, this value increased most intensively from 20 to 30 days of age (by 85.84%, P < 0.001), while in control – from 30 to 40 days of age (by 68.75%, P < 0.001). It was increasing less intensively in turkeys of 40 to 50 days of age (by 8.62% in experimental and by 17.59% in control groups). The lymphoid nodules of the esophagus of turkeys from both groups were primarily oval, round, and, some of them, triangular and pyriform with rounded tips. In the experimental group, they appeared at 30 days, while in the control group – at 40 days. Moreover, the sizes of secondary lymphoid nodules were relatively larger than that of primary (Table 3).

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

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

Table 3. Continued

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

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

In summary, in turkeys from the experimental group (from 30 to 50 days) the diameter of round primary nodules increased by 34.18%, the length of oval nodules – by 14.60%, their width – by 9.56%, while the same parameters in secondary nodules increased by 20.71%, 7.38 and 10.65% respectively. The diameter, length and width increased most intensively from 30 to 40 days, in primary lymphoid nodules – by 23.31 (P < 0.05), 11.2 and 6.91% (P < 0.05) respectively, and in secondary lymphoid nodules – by 18.98%, 4.9 and 8.81% respectively. In turkeys from the control group (from 40 to 50 days), the diameter of primary lymphoid nodules increased by 10.15%, length – by 3.77%, and width – by 0.64%, while in secondary lymphoid nodules the same parameters increased by 1.50%, 0.72 and 4.9% respectively. With the preservation of feed, the mucous membrane of the crop of turkeys from the experimental and control groups had a smooth surface with periodic low folds, while in the absence of feed, the folds were high and of considerable sizes. In the crop, the glandular part (from the esophagus) was clearly visible, as well as its opposite – non-glandular part (Fig. 5A).

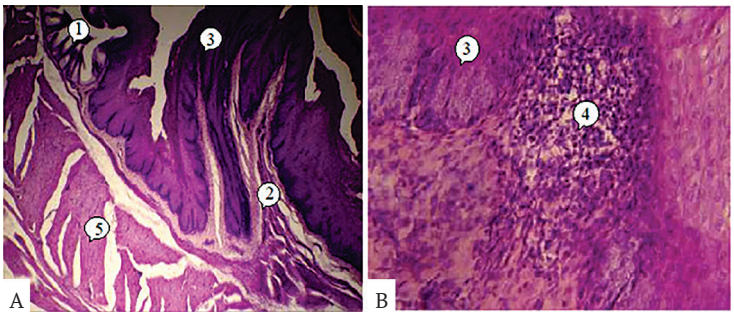

Figure 5. Structural and immune changes in the crop of turkeys from control and experimental groups

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

The general microstructure of the crop of turkeys is similar to the cervical section of the esophagus, that is, its wall is formed by three membranes: mucous, muscular and adventitious (Fig. 5A). In the mucous membrane, a thicker stratified squamous epithelium with many rows

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

In turkeys from the experimental group aged one and 10 days, immune formations in the mucous membrane of the crop were also not present. They were first recorded in its non-glandular and glandular parts in turkeys aged 20 days. Immune formations were present as diffuse form and individual diffuse clusters of lymphocytes that formed prenodules in some places. Such clusters were registered predominantly under the epithelium and near esophageal glands. Prenodules were located both in the diffuse lymphoid tissue and outside it. From the surface of immune formations, migration of lymphocytes into basal and spinous layers of the epithelium (Fig. 5B). Near or directly inside the clusters, blood vessels could be found. At the ages 30, 40 and 50 days of turkeys from the experimental group, an increase in amount of intraepithelial lymphocytes was observed, primary and secondary lymphoid nodules were detected. Diffuse form and prenodules were recorded in the wall of the glandular apparatus as well.

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

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

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

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

In summary, in turkeys from the experimental group (from 20 to 50 days), this value increased by 272.66% (by 3.7 times). Its most intensive increase was observed between 30 and 40 days of age (by 102.47%, P < 0.001), least intensive – in birds aged 40 to 50 days (by 26.65%) In turkeys from the control group (from 30 to 50 days), the area of immune

formations increased by 161.04% (by 2.6 times). Most intensively this value grew from 30 to 40 days, by 98.70% (P < 0.001), and least intensively – from 40 to 50 days, by 31.37% (P < 0.01). The shape of lymphoid nodules of the turkey crop was predominantly round and oval. In the experimental group, size of lymphoid nodules was somewhat larger than that of the primary ones (Table 5). In the period between 30 and 50 days of age of turkeys from the experimental group, the diameter of primary nodules increased by 33.67%, the length of oval ones – by 11.86% and their width – by 7.98%. Those values increased with a higher intensity in birds aged from 30 to 40 days, by 29.67% (P < 0.01), 7.69 and 6.21% respectively, and with a lower intensity from 40 to 50 days of age, by 5.34% (diameter of round nodules), 3.87% with confidence P < 0.05 (length of oval nodules) and 1.66% (width of oval nodules).

Table 5. Sizes of lymphoid nodules of the crop of turkeys, μm, M ± m, n = 6

Bird groups	Lymphoid nodules					
	Primary			Secondary		
	round	oval		round	oval	
		length	width		length	width
			30 days			
Experimental	133.67 ± 6.35	260.0 ± 16.81	150.33 ± 11.77	163.17 ± 3.30	282.17 ± 2.37	160.33 ± 8.59
Control	-	-	-	-	-	-
			40 days			
Experimental	173.33 ± 10.08**	280.0 ± 5.60	159.67 ± 12.95	178.67 ± 3.61**	286.0 ± 1.49	165.17 ± 2.55
Control	161.17 ± 7.75	273.67 ± 7.91	148.17 ± 2.18	162.83 ± 11.70	274.67 ± 9.22	159.17 ± 3.46
			50 days			
Experimental	178.67 ± 4.05	290.83 ± 4.20*	162.33 ± 5.42	188.67 ± 2.98	293.83 ± 7.97	177.67 ± 2.55**
Control	167.5 ± 5.14*	280.0 ± 14.94	154.0 ± 8.97*	178.67 ± 0.81	282.83 ± 2.86	167.67 ± 2.05*

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

From 30 to 50 days, the diameter of secondary lymphoid nodules increased by 15.63%, length of oval ones by 4.13% and their width by 10.82%. Most intensively the diameter of such nodules increased from 30 to 40 days of age (by 9.50%, P < 0.01), least intensively – from 40 to 50 days of age, by 5.59%, 2.73 and 7.57% respectively. Length and width of oval lymphoid nodules increased with a higher intensity from 40 to 50 days (by 2.73 and 7.57%, P < 0.01), and with a lower intensity from 30 to 40 days of age, by 1.36 and 3.02% respectively. From ages 40 to 50 of turkeys from the control group, values of diameter, length and width of oval primary lymphoid nodules increased respectively by 3.93%, 2.31 and 3.93%, while in secondary lymphoid nodules – by 9.73%, 2.97 and 5.34%. Thus, the paper describes the development of lymphoid tissue that forms the basis of immune formations during vaccine prophylaxis of turkeys of the Big-6 breed on the early stages of postnatal period of ontogenesis and establishes the terms of its morphofunctional maturity, which must be taken into account by specialists when carrying out vaccination and revaccination of birds in poultry farms.

Conclusions

The development and formation of morphofunctional maturity of immune formations of

the esophagus and the protrusion of its wall – the crop – in turkeys of the Big-6 breed with respect to age, which is an important criterion during the preventive immunisation of birds, was studied. It has been shown that in vaccinated and unvaccinated turkeys, the development of lymphoid tissue, which forms the basis of immune formations, occurred in the same sequence (formation of diffuse form, prenodules, primary and secondary nodules), however this process proceeded more intensively in vaccinated poultry. Immune formations were recorded in the plane of the mucous membrane and had a close morphofunctional connection with the epithelial layer, forming a "lymphoepithelium", the esophageal glands, and in deep areas of the submucosa at the border with the muscular layer. Reticular fibers, intertwined, formed a fine- and large-cell network in the diffuse lymphoid tissue, surrounded the lymphoid nodules, forming their membrane, and were found in the center of primary lymphoid nodules, or in secondary lymphoid nodules as separate fragments. Collagen and elastic fibers in the form of thin bundles were recorded only in the membrane of lymphoid nodules, but not in their central part. In vaccinated turkeys, diffuse lymphoid tissue in the walls of the esophagus and the crop was first recorded at the age of 20 days, and lymphoid nodules appeared at the age of 30 days, while in unvaccinated birds diffusely arranged lymphocytes appeared on the 30th day, and lymphoid nodules on the 40th. The area of immune formations of turkeys from both groups increased with age, and was somewhat larger in vaccinated birds. Lymphoid nodules had a predominantly round and oval shape. Their sizes changed with increasing age of the birds and reached maximum values in birds aged 50 days, moreover, the diameter of the round and the length and width of the oval secondary lymphoid nodules exceeded those of primary nodules. Prospects for further studies could be aimed at studies of the features of the structure and functions of immune formations of the esophagus of turkeys in later stages of the postnatal period of ontogenesis, and at conducting a comparative assessment with other species of domestic poultry.

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Conflict of Interest

None.

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Вплив вакцинації на розвиток імунних утворень у стравоході індиків

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

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

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Identification of methicillin-resistant *Staphylococcus aureus* from nasal carriage of goat and their antimicrobial resistance profiles

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Abstract. The methicillin-resistant *Staphylococcus aureus* (MRSA) is a global challenge in veterinary medicine due to its responsibility for nasal infection in goats. The present study was carried out to estimate the prevalence of this bacterium in goats and to detect their antimicrobial resistance profiles. A total of 153 nasal swab samples were collected from goats, and a questionnaire was used to collect necessary data related to this study. A standard laboratory analysis was done to recognise MRSA and to determine its antimicrobial sensitivity. Among 153 samples, 20.9% were

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found positive for *Staphylococcus aureus* based on results of cultural and biochemical properties. Overall, 4.58% of the samples were identified to be *mecA* gene positive. The prevalence of MRSA was higher in young goats aged below 1 year at 7.14%. In the case of female goats, the prevalence was 5.05%, which was greater than males at 3.70%. In Black Bengal goats, the prevalence was highest at 11.11%, and in the semi-intensive rearing system, the prevalence was 7.89%. Methicillin-resistant *Staphylococcus aureus* showed 100% resistance against Cefoxitin and Oxacillin. The organism also showed 42.86% resistance to Ampicillin, Erythromycin, and Oxytetracycline, followed by 14.29% to Ceftriaxone and Gentamicin, and 28.57% to Ciprofloxacin, respectively. The findings of 4.58% MRSA and multidrug-resistant MRSA in goats indicate a significant public and animal health concern. By identifying goats as potential carriers of MRSA, the findings emphasise the need for improved surveillance, biosecurity, and antibiotic stewardship in livestock farming. These results can guide veterinarians, farmers, and policymakers in developing effective strategies to control the spread of resistant bacteria from animals to humans, particularly in regions where close human-animal interactions are common

Keywords: prevalence; *mecA* gene; Antimicrobial Sensitivity Testing (AST); staphylococcal infection; risk factors

Introduction

The livestock sector has been playing a crucial role in the socio-economic development of Bangladesh. Bangladesh had a livestock population of more than 4,535.95 lakh, with 271.17 lakh goats and this livestock sector contribute about 1.80% in the GDP of Bangladesh (Bangladesh Bureau of Statistics, 2024). In livestock sector, the goat is called a poor man's cow. In developing countries like Bangladesh, small-income residents can easily rear goats with less investment in a minimum area. About 40.6% of the total population in Bangladesh may be directly or indirectly engaged with agriculture and its sub-sector as a source of income generation (Bangladesh National Portal, 2022). Goat farming plays an important and potential role in poverty reduction, income generation, contribution to food and nutrition security and employment (Barua *et al*., 2021). Goat farming provides employment opportunity which are associated with feed production, veterinary services, and marketing and play a great role for direct income generation of farmer through selling of meat, milk, skin, and live goat (FAO, 2018). As goat farming require less feed cost due to they mainly depend on tree leaves and green grass so net benefit is high 2,142.00 BDT per household (Haque *et al*., 2023), which is profitable. But this sector is challenging due to outbreak of many infectious, non-infectious, and production-related diseases in goat during rearing time.

For infectious diseases, many viruses and bacteria are responsible. Among different microbial pathogens, S. Piva *et al*. (2021) confirmed that *S. aureus* can cause many diseases such as abscesses, pneumonia, abortions, stillbirths and meningitis in goats and mammals. Y. Titouche *et al*. (2024) demonstrated that *S. aureus* is the main causative agent of mastitis in small ruminants such as goats. According to them, 15-25% of the goat population harbours this bacterium in the nasal cavity. A. Sakr *et al*. (2018) emphasised in their study that as a commensal organism, approximately 30% of the human population carries *S. aureus*

in their nasal passages. As A.F. Mechesso *et al*. (2021) argued, nasal carriage of *S. aureus* in livestock is a reservoir for human infection. *Staphylococcus aureus* poses a potential public health threat and the potential for zoonotic transmission of staphylococci among livestock and pets. According to E.O. Omoshaba *et al*. (2020), goats and sheep play an important role in the transmission of zoonotic staphylococcal infections and infections caused by other pathogens due to the close contact between the animals and their owners. Several risk factors contribute to *S. aureus* infections in goats, including those related to the animal, the environment, and farm management practices. *S. aureus* is one of the major carriers of new and re-emerging antibiotic resistance determinants represent a health risk for humans and animals (Abdullahi *et al*., 2023). As identified in their study by M. Pal *et al*. (2020), they are a major public health concern, especially given the rise in multidrug-resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA strains linked to livestock, which are classified as livestock-associated MRSA (LA-MRSA). There are few studies which were conducted to know the MRSA in goat. According to a study by M. Altaf (2019), methicillin-resistant *S. aureus* (MRSA) develops resistance by acquiring the *mecA* gene, which encodes the PBP2a protein, which aids in bacterial cell wall synthesis. Indiscriminate use of antibiotics in livestock management is a key factor in the rapid development and spread of AMR which has devastating effects in low and middle income countries. O. Herawati *et al*. (2023) wrote about this in their work. According to studies by K.M. Muwonge *et al*. (2025), *S. aureus* isolates from nasal swabs of goats were found to be resistant to several antibiotics.

As antimicrobial resistance is a burning issue, more attention is needed to control the infection and develop an effective treatment protocol against this infection. There are few studies which was conducted to know the MRSA in goat. So, the current research study aimed to estimate the prevalence of nasal carriage of *S. aureus* in goats, to detect the *mecA* gene containing methicillin-resistant *S. aureus* from goats and to know their resistance percentage against several antibiotics.

Materials and Methods

A cross-sectional study was conducted at the S.A. Quaderi Teaching Veterinary Hospital (SAQTVH), Chattogram Veterinary and Animal Sciences University (CVASU), from February to June 2023. The study population consisted of goats admitted to the clinic for treatment or routine examination. A total 153 swab sample were collected from the nostrils of 153 goats having respiratory problems. Samples were immediately inoculated into Mueller-Hinton broth supplemented with 6.5% NaCl (Oxoid, UK). Microbiological analyses were performed at the Poultry Research and Training Centre (PRTC) laboratory, CVASU. Data regarding the animals' age, sex, breed, and rearing system were systematically recorded using a structured questionnaire.

Isolation and identification of *Staphylococcus aureus*

The isolation of *S. aureus* organism involved placing swab samples into Mueller-Hinton broth supplemented with 6.5% NaCl, followed by incubation at 37°C overnight. Subsequently, the samples were streaked onto blood agar plates and incubated at 37°C for 24 hours to promote bacterial colony development. Colonies exhibiting typical features – such as hemolysis, round shape, smooth texture, and shiny surface – were subjected to Gram staining. Those identified as Gram-positive cocci underwent catalase testing according to standard protocols. Colonies that were both catalase-positive and Gram-positive

cocci were preliminarily classified as staphylococci. These suspected Staphylococcus colonies were further cultured on Mannitol Salt Agar and incubated at 37°C for an additional 24 hours to facilitate more precise identification and differentiation (Fig. 1).

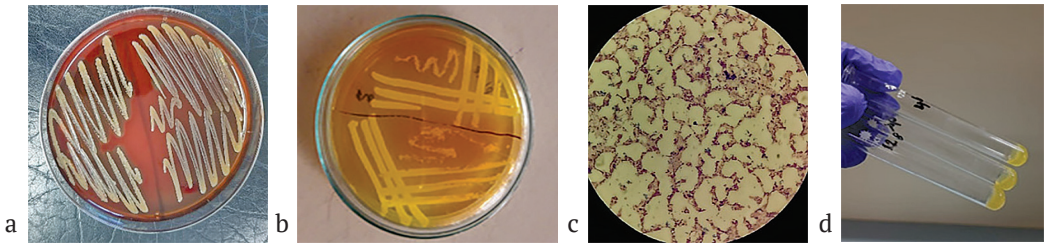

Figure 1. Isolation and identification of bacteria
***Note**: a – whitish and yellow colour colony on blood agar; b – golden yellow colour colony on mannitol salt agar; c – Gram's staining; d – coagulation in tube coagulase test*
***Source**: authors' original images from this experiment*

Colonies yielding a yellowish colour were suspected as *Staphylococcus aureus* species (Islam *et al.*, 2019). The presumptive *S. aureus* colonies on mannitol salt agar were then transferred onto blood agar for additional testing to confirm their identity by incubating at 37°C for 24 hours (Rahimi *et al.*, 2015). The positive samples were tested for coagulase using horse plasma.

Molecular identification of the *mecA* gene using polymerase chain reaction (PCR)

Staphylococcus aureus organisms that are resistant to specific antimicrobial drugs like oxacillin and cefoxitin were classified as MRSA and were tested in the laboratory (Broekema *et al.*, 2009). Initially identified MRSA were further examined by molecular diagnosis through PCR to detect the presence of the *mecA* gene as described by A.R. Larsen *et al.* (2008). Primers used were *mecA* P4 (Forward) 5'-TCCAGATTACAACTTCACCAGG-3' and *mecA* P7 (Reverse) 5'-CCACTTCATATCTTGTAACG-3'. The PCR amplification program was done by following the protocol of A.R. Larsen *et al.* (2008). The MRSA ATCC 33591 strain served as the positive control, while nuclease-free water was used as the negative control to ensure the accuracy and reliability of the PCR results (Fig. 2). The PCR products were visualised using gel electrophoresis.

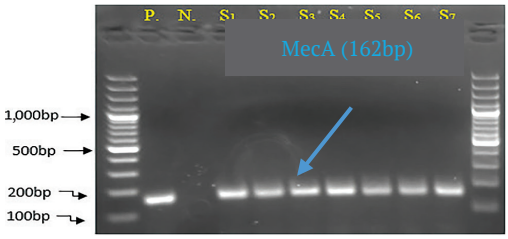

Figure 2. PCR products amplified using *mecA* gene-specific primers of MRSA
***Note:** P – positive control; N – negative control; S1-S7 – sample ID*
***Source**: authors' original images from this experiment*

The antimicrobial resistance test was performed by the disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) (2020) guidelines. The antibiotic resistance test results, based on the sizes of the zones of inhibition, are interpreted as resistant (R), intermediately resistant (I), or sensitive (S) to the antibiotics tested against the isolates shown in Table 1. Methicillin resistance was assessed by measuring the zone of inhibition around oxacillin and cefoxitin drugs. *S. aureus* isolates that demonstrated resistance to a minimum of three distinct classes of antimicrobial agents were considered to be multidrug-resistant (MDR) (Li *et al.*, 2014).

Table 2. The antimicrobials used, their concentrations, and Zone diameter interpretative standards for *Staphylococcus aureus*

Antimicrobial agent with disc code and Concentration (µg)	Diffusion zone breakpoint (mm)		
	S	I	R
Ampicillin (AMP_{10})	⩾29	27-28	⩽26
Oxacillin (OX_1)	⩾13	11-12	⩽10
Ceftriaxone (CRO_{30})	⩾23	20-22	⩽19
Cefoxitin (FOX_{30})	⩾22	–	⩽21
Gentamicin (CN_{10})	⩾15	13-14	⩽12
Ciprofloxacin (CIP_5)	⩾21	16-20	⩽15
Oxytetracyclines (TE_{30})	⩾15	12-14	⩽11
Erythromycin (E_5)	⩾23	14-22	⩽13
Sulfamethoxazole-trimethoprim (SXT_{25})	⩾16	11-15	⩽10

***Note**: S – sensitive; I – intermediate; R – resistance*
***Source**: created by the authors based on CLSI (2020)*

The collected data from goat-rearing farmers and the laboratory were then entered and tabulated into MS Excel 2010 spreadsheets. Data were analysed using the Statistical Package for Social Sciences 20 computer software package. This study was conducted by the ethical guidelines for animal research. The animals were kept and all manipulations were carried out by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986).

Results and Discussion

Prevalence of *Staphylococcus aureus* and *mecA* gene containing MRSA in the nasal carriage of goat

Among the total of 153 nasal swab samples collected from goats, 32 samples (20.9%) were identified as positive for *S. aureus* based on standard cultural characteristics and biochemical tests (coagulase test). These preliminary findings confirmed the presence of *S. aureus* colonisation in a notable proportion of the sampled goat population. To determine the presence of methicillin-resistant strains, all 32 *S. aureus* isolates were subjected to antimicrobial susceptibility testing specifically targeting resistance to Oxacillin and Cefoxitin, which are commonly used indicator antibiotics for the detection of MRSA. The antibiotic susceptibility testing was performed using the disc diffusion method following CLSI guidelines.

Out of these 32 isolates, 7 showed phenotypic resistance to both Oxacillin and Cefoxitin. These resistant isolates were then further confirmed through molecular analysis by polymerase chain reaction (PCR) targeting the *mecA* gene, a well-established genetic marker for methicillin resistance. All 7 isolates that exhibited phenotypic resistance were found to be mecA-positive, confirming them as MRSA. The findings (Table 2) indicate that MRSA was present in 7 out of 153 total goat nasal swab samples tested. Furthermore, when considering only the *S. aureus*-positive cases, the prevalence of MRSA was found to be 21.88% (7 out of 32).

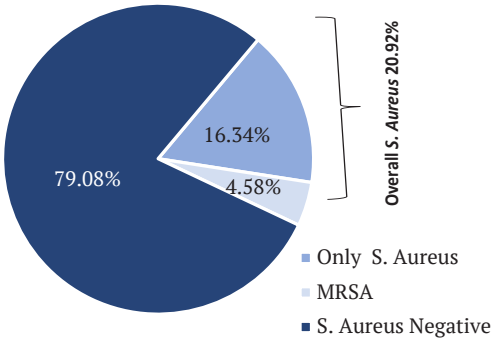

Figure 3. Prevalence of S. Aureus and MRSA in the Study location
***Source**: authors' original images from this experiment*

In this study, various potential risk factors including age, sex, breed, and rearing system – were analysed to determine their association with Methicillin-resistant *S. aureus* positivity in the animal population (Table 2). Although differences in MRSA prevalence were observed across categories, statistical analysis showed that none of these factors were significantly associated with MRSA infection ($P > 0.05$). Age-wise distribution showed that animals under one year of age had the highest MRSA prevalence (7.14%), followed by those older than three years (4.05%) and those between one and two years (3.92%). The chi-square test ($\chi^2 = 0.519$, $P = 0.772$) indicated this variation was not statistically significant despite the slightly higher rate in younger animals. Similarly, the analysis of sex revealed that female animals had a higher prevalence (5.05%) compared to males (3.70%), but this difference was also not statistically meaningful ($\chi^2 = 0.145$, $P = 0.703$), suggesting that sex does not play a major role in determining susceptibility to MRSA.

Table 2. The risk factors for the presence of MRSA in goat

Risk factor		Number of MRSA-positive cases	Prevalence,%	χ^2 value	P value
Age	< 1 year	2 (28)	7.14	0.519	0.772
	1-2 year	2 (51)	3.92		
	> 3 year	3 (74)	4.05		
Sex	Male	2 (54)	3.70	0.145	0.703
	Female	5 (99)	5.05		
Breed	BB	1 (9)	11.11	1.883	0.597
	JP	1 (14)	7.14		
	JP Cross	3 (99)	3.03		
	Other cross	2 (31)	6.45		
Rearing system	Intensive	4 (115)	3.48	1.276	0.259
	Semi-intensive	3 (38)	7.89		

***Note**: BB – Black Bengal; JP – Jamunapari; P < 0.05 – significant at 5% level; P > 0.05 – non-significant; χ^2 – chi-square value*
***Source**: created by the authors*

Breed-wise analysis showed varying MRSA prevalence, with the highest in BB (Bangladeshi indigenous breed) animals (11.11%), followed by JP (Japanese breed) at 7.14%, other crossbreeds at 6.45%, and JP crossbreeds at 3.03%. However, despite these apparent differences, the chi-square value ($\chi^2 = 1.883$, $P = 0.597$) again indicated no significant association. Notably, the highest prevalence in BB animals might be influenced by the small sample size ($n = 9$), which limits the statistical power to detect a true effect. Regarding the rearing system, all animals in this study were reared under an intensive system, with an MRSA prevalence of 3.48%. The chi-square test ($\chi^2 = 1.276$, $P = 0.259$) showed no significant relationship between rearing system and MRSA status, which might also reflect limited variability in the rearing condition data.

Taken together, the findings indicate that none of the studied demographic or management factors had a significant influence on MRSA prevalence among the animals. These results suggest that MRSA infections may be more strongly associated with other factors not covered in this analysis, such as antibiotic

usage patterns, environmental contamination, farm-level biosecurity measures, or contact with human carriers. Further research with a larger sample size and more comprehensive data, including antimicrobial usage and hygiene practices, is needed to better understand the epidemiology and risk factors of MRSA in animal populations.

Antimicrobial resistance pattern of methicillin-resistant *Staphylococcus aureus*

The antimicrobial susceptibility testing conducted on the 7 MRSA isolates revealed important insights into the resistance patterns of these bacteria in goats (Fig. 4). Among the antibiotics tested, Sulphamethoxazole-trimethoprim showed the highest level of effectiveness, with 85.71% of the isolates being susceptible. This indicates that this combination of antibiotics remains a strong candidate for treating MRSA infections in goats. Its high susceptibility rate suggests that it could still reliably inhibit bacterial growth and thus be useful in clinical management. Following Sulphamethoxazole-trimethoprim, Ceftriaxone demonstrated considerable antimicrobial activity, with 71.43% of the MRSA isolates showing susceptibility. Ceftriaxone is a broad-spectrum third-generation cephalosporin commonly used in both human and veterinary medicine, and its relatively high effectiveness against MRSA in this study highlights its potential as an alternative therapeutic agent.

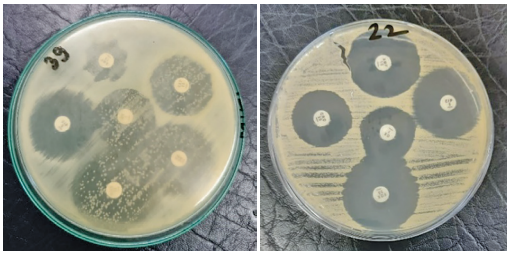

Figure 4. The sensitivity and resistance profile of MRSA against different antimicrobial drugs
***Source**: created by the authors*

In stark contrast, the isolates showed very low sensitivity to several other commonly used antibiotics. Ampicillin, Erythromycin, and Ciprofloxacin each exhibited only 14.29% susceptibility, indicating that these drugs are largely ineffective against the MRSA strains tested. This poor response reflects the widespread resistance mechanisms present in MRSA, making treatment with these antibiotics unreliable and likely to fail in clearing infections. Gentamicin and Oxytetracycline showed moderate susceptibility, each with 28.57% effectiveness against the isolates. While these drugs may still play a role in some treatment regimens, their lower susceptibility rates suggest that they should be used cautiously, preferably only after susceptibility testing confirms their efficacy for a specific case (Fig. 5).

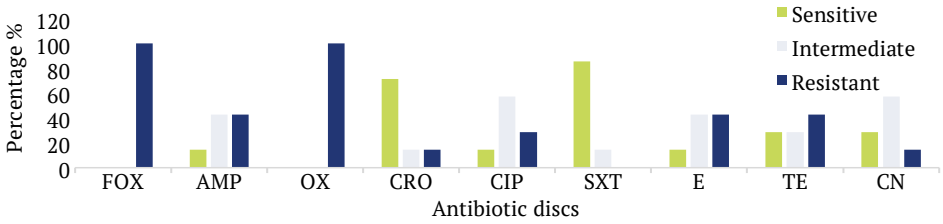

Figure 5. Antimicrobial susceptibility and resistance profile of MRSA in goat
***Note:** Cefoxitin (FOX); Ampicillin (AMP); Oxacillin (OX); Ceftriaxone (CRO); Ciprofloxacin (CIP); Sulfamethoxazole-trimethoprim (SXT); Erythromycin (E); Oxytetracycline (TE); Gentamicin (CN)*
***Source**: created by the authors*

The highest level of resistance was observed against Cefoxitin and Oxacillin, with 100% of the isolates resistant, confirming their methicillin-resistant status. Among the other antibiotics tested, the isolates showed 42.86% resistance to Ampicillin, 42.86% to Erythromycin, 42.86% to Oxytetracycline, 28.57% to Ciprofloxacin, and 14.29% to both Ceftriaxone and Gentamicin. In terms of intermediate resistance, the isolates displayed 57.14% intermediate resistance to both Ciprofloxacin and Gentamicin, 42.86% to Ampicillin and Erythromycin, 28.57% to Oxytetracycline, 14.29% to Sulphamethoxazole-trimethoprim, and Ceftriaxone (Fig. 5).

The observed resistance patterns raise significant concerns regarding the management of MRSA infections in goats. The limited effectiveness of several frontline antibiotics could lead to treatment failures, prolonged infections, and increased risk of transmission both within animal populations and potentially to humans. These findings emphasise the critical need for prudent use of antibiotics, guided by susceptibility testing, to slow down the emergence and spread of resistant strains. Overall, the data suggest that Sulphamethoxazole-trimethoprim and Ceftriaxone currently remain the most effective options for managing MRSA infections in goats in this study setting.

In the present study, 4.58% of nasal swab samples collected from goats tested positive for the presence of the *mecA* gene, confirming MRSA colonisation. This prevalence, although slightly lower than the 7.3% reported by A. Moroz *et al*. (2020), still indicates a noteworthy presence of MRSA in the sampled goat population. Conversely, a study from Tunisia by H. Gharsa *et al*. (2012) reported a lower MRSA prevalence, highlighting considerable variability in MRSA carriage rates globally. Such variations can be attributed to multiple factors, including geographic location, differences in animal husbandry practices, variation in the sampled animal populations, and differences in laboratory methodologies or sampling techniques. This variation underscores the importance of localised surveillance to better understand the epidemiology of MRSA in different regions and animal species.

To explore whether host factors or management systems influenced MRSA prevalence in this study, Chi-square (χ^2) tests were conducted on categorical variables such as age, sex, breed, and rearing system. The results showed no statistically significant associations ($P > 0.05$) between these variables and MRSA colonisation. This lack of significant correlation suggests that factors beyond the examined demographic and management variables might contribute more prominently to MRSA carriage in goats. These could include environmental contamination, farm hygiene practices, antibiotic usage patterns, or contact with humans and other animals, which were not assessed in the present study. Future research should consider incorporating these additional variables to better understand the dynamics of MRSA transmission and persistence in livestock.

Regarding antimicrobial susceptibility, the MRSA isolates demonstrated multidrug resistance but with varying degrees of sensitivity to different antibiotics. Most notably, Sulphamethoxazole-trimethoprim (SXT) was highly effective, with 85.71% of isolates showing susceptibility. This finding is in close agreement with the 87.23% susceptibility reported by C.A. Nsofor & O.O. Patience (2019), suggesting that SXT remains a promising option for treating MRSA infections in goats. The high susceptibility to SXT may be attributed to its mechanism of action and potentially limited misuse in veterinary settings compared to other antibiotics. Resistance patterns to other antibiotics revealed more concerning trends. Ampicillin resistance was observed in 42.86% of isolates,

closely matching the 40.43% resistance rate reported this authors. This highlights the persistent challenge of beta-lactam resistance among *S. aureus* strains. Similarly, Oxytetracycline resistance was found in 42.86% of isolates, consistent with the 44% tetracycline resistance rate documented by B. Egyir *et al*. (2020). These resistance levels indicate that both beta-lactam and tetracycline classes may have limited efficacy in treating MRSA infections in goats, possibly due to overuse or misuse in livestock.

Resistance to Ciprofloxacin was recorded at 28.57%, which is somewhat higher than rates reported by E.O. Omoshaba *et al*. (2020), but still signals emerging resistance to fluoroquinolones. Additionally, resistance to Ceftriaxone and Gentamicin was relatively low at 14.29% each, closely mirroring findings from M. Vitale *et al*. (2019). These moderate resistance levels suggest these antibiotics may still hold some therapeutic value but warrant cautious use and continued monitoring. The multidrug resistance observed among MRSA isolates in this study is a cause for concern. It reflects the growing global challenge posed by antimicrobial-resistant pathogens in veterinary medicine, which not only threaten animal health but also pose zoonotic risks. Resistant MRSA strains may be transmitted to humans through direct contact or via the food chain, complicating treatment of infections and increasing healthcare burdens.

These findings highlight the critical need for regular antimicrobial resistance surveillance, judicious antibiotic use, and enhanced biosecurity measures within livestock production systems. Furthermore, the consistency of resistance patterns across different geographic regions emphasises that antimicrobial resistance in MRSA is a global issue, influenced by local antibiotic usage practices and microbial ecology. Implementing One Health approaches that integrate human, animal, and environmental health perspectives is essential to controlling the spread of MRSA and safeguarding antibiotic efficacy for both veterinary and human medicine.

Conclusions

The occurrence and antimicrobial resistance profile of methicillin-resistant *Staphylococcus aureus* in goats, with a prevalence of 4.58% confirmed through PCR targeting the *mecA* gene. Although this prevalence is lower than some previous reports, the presence of MRSA in apparently healthy goats is significant. It indicates that goats may serve as asymptomatic carriers of MRSA, posing a potential zoonotic risk to individuals in close contact with livestock, such as farmers, veterinarians, and slaughterhouse workers. The detection of MRSA in livestock raises concerns regarding the potential transmission of resistant bacteria to humans and the environment. Antimicrobial susceptibility testing of the MRSA isolates revealed a worrying pattern of multidrug resistance. All seven isolates demonstrated 100% resistance to both Oxacillin and Cefoxitin, confirming their methicillin-resistant status. In addition, resistance was observed against commonly used antibiotics such as Ampicillin, Erythromycin, Oxytetracycline, and Ciprofloxacin, highlighting the risk of therapeutic failure in clinical cases. However, the isolates showed relatively high susceptibility to Sulphamethoxazole-trimethoprim and Ceftriaxone, indicating these antibiotics may still be effective treatment options against MRSA infections in goats. The study also investigated associations between MRSA occurrence and various host-related and management factors, including age, sex, breed, and rearing system. Chi-square analysis revealed no statistically significant association between MRSA prevalence and any of these variables ($P > 0.05$), suggesting that MRSA colonisation may be influenced by other unmeasured factors

such as antibiotic use history, farm hygiene, or environmental exposure.

Despite the valuable findings, the study was limited by a relatively small sample size (153 goats) and was conducted in a specific geographic area. As a result, the data may not fully represent the broader goat population in Bangladesh. Further studies with larger and more diverse samples are needed to better understand the epidemiology of MRSA in livestock. In conclusion, the detection of MRSA and its multidrug resistance in goats emphasises the need for routine surveillance, responsible antimicrobial use, and improved farm biosecurity. A One Health approach, integrating human, animal, and environmental health strategies, is essential to control the spread of antimicrobial-resistant pathogens and protect both public and animal health. Future research should also explore the molecular characterisation of resistance genes, whole-genome sequencing of MRSA isolates, and longitudinal studies to assess transmission dynamics between animals, humans, and the environment.

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Conflict of Interest

None.

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Виявлення носового носійства метицилін-резистентного *Staphylococcus aureus* у кіз та профілю стійкості збудника до антимікробних препаратів

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Анотація. Метицилін-резистентний *Staphylococcus aureus* (MRSA) є глобальною проблемою у ветеринарній медицині через його здатність викликати носові інфекції у кіз. Дослідження було проведено з метою оцінки поширеності цієї бактерії у кіз та визначення її профілю стійкості до антимікробних препаратів. Загалом було зібрано 153 зразки мазків з носа кіз, а також використано анкетування для збору необхідної супутньої інформації. Було проведено стандартний лабораторний аналіз для виявлення MRSA та визначення його чутливості до антибіотиків. Серед 153 зразків у 20,9 % було виявлено *Staphylococcus aureus* на основі культурних та біохімічних властивостей. Загалом, у 4,58 % зразків було виявлено наявність гена *mecA*. Поширеність MRSA була вищою серед молодих кіз віком до 1 року й становила 7,14 %. У самок поширеність становила 5,05 %, що більше, ніж у самців – 3,70 %. У кіз породи Black Bengal поширеність була найвищою – 11,11 %, а в умовах напівінтенсивного утримання – 7,89 %. Метицилін-резистентний *Staphylococcus aureus* виявив 100% стійкість до Цефокситину та Оксациліну. Також спостерігалася стійкість 42,86 % до ампіциліну, еритроміцину та окситетрацикліну, 14,29 % – до цефтріаксону та гентаміцину, та 28,57 % – до ципрофлоксацину. Виявлення MRSA у 4,58 % зразків та мультирезистентного MRSA у кіз свідчить про серйозну загрозу для громадського та ветеринарного здоров'я. Ідентифікація кіз як потенційних носіїв MRSA підкреслює необхідність посиленого епіднагляду, біобезпеки та відповідального використання антибіотиків у тваринництві. Ці результати можуть стати основою для ветеринарів, фермерів і політиків при розробці ефективних стратегій стримування поширення резистентних бактерій від тварин до людей, особливо в регіонах з тісними контактами між людиною і тваринами

Ключові слова: поширеність; ген *mecA*; тестування чутливості до антимікробних препаратів (AST); стафілококова інфекція; фактори ризику

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The influence of hermanium and iron nanocomposites on saturated fatty acid levels in pig blood, depending on vegetative regulation

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Abstract. The study's relevance is related to developing new methods for increasing pig productivity by balancing metabolic processes in the body. The main objective was to determine changes in saturated fatty acid levels in pigs when using a feed supplement of nanoaquachelates, taking into account individual characteristics of the body. The experimental groups of animals were formed using a pulsometric study, which resulted in three groups of 10 pigs, which were then divided into two groups, control and experimental, with five animals each. The experimental group was given a mixture of nano-compounds of iron in a dose of 3 mg/day and germanium in a dose of 0.01 mg/day. The content of saturated fatty acids was assessed using gas chromatography. The results of the study showed that the blood of pigs in the control group with normotonia had increased levels of saturated fatty acids: myristic ($P < 0.01$), heptadecanoic ($P < 0.05$), and behenic ($P < 0.01$). In the blood of pigs in the experimental group with sympathicotonia, the content of saturated fatty acids decreased: myristic ($P < 0.01$), pentadecanoic ($P < 0.01$), heptadecanoic ($P < 0.01$), and behenic ($P < 0.01$). In the blood of pigs in the control group with vagotonia, the content of saturated fatty acids increased compared to the experimental group. It was found that fatty acids with the highest proportion in experimental normotonic pigs, such as palmitic acid, were 12.1% lower, and stearic acid was 10.7% lower ($P < 0.01$). In control pigs with sympathotonics, the palmitic acid content was 15.4% higher ($P < 0.01$) and stearic acid was 17.8% higher ($P < 0.001$). In experimental pigs with vagotonia, the palmitic acid content was lower by 14.8% ($P < 0.01$) and stearic acid by 11.8% ($P < 0.001$). The results of the study showed a positive effect of the mixture of iron and germanium nanocomposites on the indicators of saturated fatty acid metabolism and the formation of pigs following the individual characteristics of the organism, which improved the accuracy of the data obtained

Keywords: metabolism; nanopreparation; productive animals; autonomic nervous system; lipids

Introduction

Modern pig breeding and fattening farms have a well-established system for producing animal raw materials based on cyclicality and stable livestock productivity. Proper regulation of this system requires a balanced diet that is adjusted at all stages of pig rearing. However, this technology has a significant drawback: when attempting to reduce the time needed for an animal to reach the required body weight, problems arise with metabolic processes in its body. This leads to a significant decline in productivity and further economic losses for the farm. Attempts to solve this problem involve adding feed additives to the pigs' diet to improve the stability of homeostasis in the animal and compensate for the adverse effects of intensive metabolism.

S. Rao *et al*. (2023) identified several methods for identifying factors related to the animal and how much and what feed additives it needs to consume to improve body weight gain. This chain omits one point: it does not consider the individual characteristics of pigs, which reduces the effectiveness of the feed additives. Z.X. Rao *et al. (*2023) proved that feed additives in pig farming are in high demand. Many different feed additives exist, including various acidifiers, probiotics, minerals, etc. The composition of these feed additives varies widely and theoretically affects the growth rate of animals and improves homeostasis. Despite such positive prospects for using these additives in pig feed, their cost must be economically viable for the farm. In addition, they are also designed to

help pigs adapt to the effects of negative environmental factors, which is especially important during weaning, regrouping and relocation to new places depending on the rearing cycle.

Several factors contribute to increased pig productivity: diet, genetics, housing conditions, metabolic rate and individual characteristics. Q. Tang *et al.* (2023) found that maintaining stable pig productivity largely depends on lipid metabolism, especially fatty acids. It has been proven that animals need a significant amount of amino acids for active muscle growth. Still, with insufficient energy metabolism, fatty acids are used as fuel, reducing the herd's productivity. To prevent this, it is necessary to adjust lipid metabolism. The main goal of increasing pig productivity is stabilising lipolysis and lipogenesis, with particular attention paid to fatty acids.

Y. Feng *et al.* (2023) studied the effectiveness of feed additives in lipid metabolism in pigs. It was established that the primary goal was to improve lipid metabolism and use non-traditional feed, which would be economically beneficial for the farm. Grape seed extract was used for pigs during the fattening period. The positive results included improved productivity ($P < 0.05$), increased high-density lipoprotein content, and reduced triacylglycerol and total cholesterol levels in the animals ($P < 0.05$). At the same time, the antioxidant protection status improved. Y. Liu *et al. (*2024) studied the usage of various feed additives to improve pig productivity and ensure a high level of antioxidant protection. According to these authors, using more natural feed additives rather than artificially created ones is better. Therefore, it is proposed that flavonoids from mulberry leaves be used as an alternative feed additive. The use of flavonoids from mulberry leaves contributed to increased pig productivity, which was reflected in body weight gain, better feed conversion, and improved lipid metabolism.

M. Han *et al.* (2024) used the feed supplement *Eucommia ulmoides* to improve metabolic processes in pigs. It was found that the extract from the leaves of this plant is highly effective in stimulating animal growth and stabilising lipid metabolism and antioxidant protection. Using *Eucommia ulmoides* in pigs for 60 days helped regulate lipid metabolism, resulting in normalised liver enzyme activity and reduced lipogenesis, including increased antioxidant protection.

V.O. Ivanov *et al.* (2024) investigated the effect of the feed supplement Nanoverm on pig performance. The results showed an increase in the body weight of pigs that additionally consumed the feed supplement and an increase in total protein content on the 28th day of use due to the albumin fraction. The authors noted the economic feasibility of using Nanoverm and its positive effect on the pig organism.

The study of ways to increase pig productivity is of great interest to the scientific community. The development of methods to improve lipid metabolism in pig farming is more relevant than ever, and the study of using nanoaquachelate preparations, taking into account the individual characteristics of the animal organism, opens up new horizons for researchers. The study aimed to determine the effect of nanoaquachelate preparations on pigs regarding the tone of the autonomic nervous system and the levels of saturated fatty acids in blood plasma lipids.

Literature Review

The balanced lipid breakdown processes make animal weight gain stable and productive. With sufficient energy, the body uses amino acids more efficiently to build muscle mass. Many factors influence maintaining a stable lipid metabolism, including ambient temperature, diet, breed, age, sex, activity of regulatory systems, and others. Considering the extensive list of variables that directly or indirectly play a role in balancing homeostasis in pigs, it can be seen

that modern methods of improving lipid metabolism take the above factors into account.

N. Khan *et al.* (2025) found that lipid metabolism is an essential link in the development of pigs. C.G. Yao *et al.* (2021) proved that any direction in the study or improvement of lipid metabolism begins with the alimentary pathway, i.e., the diet. Further, in most cases, the issues concern the microflora of the animal's intestines and the subsequent distribution of digested components to their locations in the body. At the same time, the state of the nervous system, particularly the autonomic nervous system, is often not considered. This peripheral nervous system component plays a leading role in regulating homeostasis, which also applies to lipid metabolism.

J. Imai & H. Katagiri (2022) argued that the autonomic nervous system, which has afferent and efferent nerve fibres, communicates between peripheral organs and the central nervous system. This feature helps analyse metabolic processes in pigs and compensate for energy deficiencies on time, which is essential for stimulating metabolism. An example of this is regulating the hepatoportal system by the vagus nerve, which helps regulate glucose levels, namely its synthesis and breakdown. In addition, the efferent nerve endings of this nervous system are involved in the control of energy metabolism by promoting lipolysis in white adipose tissue and enhancing thermogenesis in brown adipose tissue. M. Hardoňová *et al.* (2021) established a significant relationship between the content of triacylglycerols in the body and the sympathetic nervous system. According to the data obtained, different types of neurons that express melanocortin and the hormones leptin and insulin are modulated by the peripheral nervous system, namely the autonomic nervous system, thereby controlling the metabolism of triacylglycerols. Lipolysis processes depend on the activity of the sympathetic nervous system, reflecting the catabolic role of this type of autonomic nervous system. Therefore, studying autonomic regulation in lipid metabolism is fundamental in analysing the body's homeostasis.

T. Teng *et al.* (2023) studied the energy balance in pigs under the influence of heat. First, it was found that during the colder months, the metabolism of high-molecular compounds increases to produce energy. This causes the release of significant amounts of both glucose and lipids, which are then broken down. At the same time, the gut microbiota is crucial as it helps digest feed and form short-chain fatty acids, which are then used to synthesise triacylglycerols. H. Ohorodnichuk & V. Zagamula (2022) conducted comprehensive research on improving pigs' metabolism to obtain high-quality products and ensure their productivity growth. The scientists used a complex feed additive called "MikoStop", which positively affected pigs' body weight gain. Z. Xu *et al.* (2021) studied the effect of low temperatures on the body of pigs. The researchers found that exposure to low temperatures reduces the concentration of triacylglycerols in animals by maintaining thermogenesis. The opposite transport of cholesterol occurs with the help of high-density lipoproteins and their subsequent conversion to bile acids. Thus, with short-term modulation of the effect of low temperatures on the pig organism, changes in the fatty acid composition were found, namely in the total content of saturated and unsaturated fatty acids.

Z. Tian *et al.* (2021) studied the inclusion of probiotics as feed additives in pigs' diet to improve amino acid and fatty acid metabolism. According to the results, using a probiotic based on Lactobacillus reuteri one positively affected the content of glutamic and inosinic acids. The authors claimed that protein synthesis in pigs was likely to increase and the fatty acid profile to change due to improved feed digestion,

which positively affected metabolic processes. E.R. Grela *et al.* (2021) demonstrated the need to improve microbial digestion of feed to normalise fatty acid content in pigs during the fattening period. The study aimed to determine the effect of the prebiotic inulin on improving metabolic processes in animals, preventing the occurrence of antioxidant stress and improving the fatty acid profile. At the same time, the primary task was to avoid the significant use of antibiotics in the pig diet and replace them with prebiotics, with a subsequent positive effect on metabolism. However, using this feed additive had a negligible impact on the intensity of metabolism.

H. Zhou *et al.* (2021) investigated the effect of short-chain fatty acids on lipid metabolism in pigs and their impact on the intestinal microflora. The study was based on determining the effect of exogenous sources of fatty acids, rather than those synthesised during digestion by microorganisms. After 21 days of using the modified diet, an increase in protein synthesis ($P < 0.05$) and changes in metabolism associated with the biosynthesis of unsaturated fatty acids were observed.

Materials and Methods

The study was conducted throughout 2023 at the pig farm of Koshet LLC in the village of Chapivtsi, Mukachevo district, Zakarpattia region, on pigs of the Large White breed. The experimental groups of pigs were formed by studying the individual characteristics of autonomous regulation using the pulsometric method, followed by statistical evaluation of the obtained indicators, which allowed forming three groups of 10 animals each: normotonic (pigs with a predominance of normotonic processes in the nervous system), sympathicotonic (pigs with a predominance of sympathicotonic processes in the nervous system) and vagotonic (pigs with a predominance of vagotonic processes in the nervous system). The experimental studies involving pigs described in this paper, namely all manipulations with pigs, were carried out following the basic principles of bioethics, by Law of Ukraine No. 3447-IV (2006), the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986), Directive 2010/63/EU of the European Parliament and the Council (2010) and the "General Ethical Principles for Animal Experiments" adopted by the First National Congress on Bioethics (Law of Ukraine No. 249, 2012).

The pre-formed pigs were divided into two subgroups – control and experimental, five heads each. The experimental group of pigs was fed a feed supplement containing nano-compounds of iron (3 mg/day) and germanium (0.01 mg/day) per head for 40 days. Pig blood plasma was used to study the content of saturated fatty acids. Blood was collected from pigs through the cranial hollow vein at the jugular groove on the left or right side, 1-2 cm to the side of the trachea and 1-2 cm above the sternum, using a sterile syringe with a 1% heparin solution in a volume of three drops per 10 cm^3 blood. The collected blood was placed in a thermocontainer at a temperature of 4°C. Blood plasma was obtained by centrifugation for 10 minutes at 2,000 rpm.

To investigate the content of saturated fatty acids in pig blood plasma, lipids were extracted using the method of J. Folch *et al.* (1957), which was based on the use of blood plasma as a lipid-containing medium, from which lipids soluble in chloroform were extracted using a mixture of chloroform and methanol in a 1:2 ratio and subsequent addition of 0.74% potassium chloride, and the fat was concentrated using a reverse refrigerator. Chromatographic analysis was performed using a Trace GC Ultra chromatograph (USA) with a flame ionisation detector. A standard sample of Supelco 37 Component FAME Mix was used to identify the results of the chromatographic study and determine saturated fatty acids in pig blood lipids. The study was

performed in three parallel groups. Statistical processing of the obtained indicators of saturated fatty acids in pig blood was performed using Microsoft Excel with an assessment of the probability of differences in indicators of the blood and evaluation using Student's t-test with significance levels: $P < 0.05$, $P < 0.01$, $P < 0.001$.

Results and Discussion

After analysing the blood lipid chromatography indicators of pigs in the control group of normotonic animals using descriptive statistics, a corresponding data table was formed based on the percentage content of the fatty acids studied (Table 1).

Table 1. Descriptive statistics of saturated fatty acid indicators in the blood plasma of pigs in the control group of normotonic animals (n = 5)

Indicator	C14:0	C15:0	C16:0	C17:0	C18:0	C22:0
Average, %	0	0	2	0	27.55	1.12
Standard error, %	0	0	0	0	0	0
Standard deviation, %	0	0	0	0	0	0
Asymmetry	0	0	1.09	0	1	0
Minimum, %	0	0	23.92	0	27.01	0
Maximum, %	0	0	25.81	0	28.45	1.26

***Note**: C14:0 – myristic acid; C15:0 – pentadecanoic acid; C16:0 – palmitic acid; C17:0 – heptadecanoic acid; C18:0 – stearic acid; C22:0 – behenic acid*
***Source**: authors' development*

Descriptive statistics of saturated fatty acid levels in the blood lipids of normotonic pigs are statistically significant. In particular, it was found that the standard error and standard deviation for palmitic acid were higher, ranging from 6.25 to 8.5 times, and for stearic acid – in the range from 4.8 to 6.5 times, compared to other fatty acids. However, considering that the average values of these saturated fatty acids are significantly higher than those of others, these figures were within relatively favourable limits. When assessing asymmetry, it was found that myristic and behenic acids had low negative asymmetry, while pentadecanoic acid had medium asymmetry. Heptadecanoic acid showed positive low asymmetry, while palmitic and stearic acids had medium asymmetry. It should also be noted that the differences between the minimum and maximum values of the studied saturated fatty acids are insignificant, ranging from 0.08 to 1.89% depending on the type of fatty acid. The following statistical data were obtained after examining the fatty acid levels in the pigs' blood in the normotonic experimental group, which were given a mixture of iron and germanium microelements (Table 2).

Table 2. Descriptive statistics of chromatography indicators in blood lipids of pigs in the experimental group of normotonics (n = 5)

Indicator	C14:0	C15:0	C16:0	C17:0	C18:0	C22:0
Average, %	0	0	21.64	0	24.60	1
Standard error, %	0.02	0.03	0	0	0.44	0
Standard deviation, %	0	0	1.41	0	0	0
Asymmetry	0	0	0	-0.56	-1.38	0

Table 2. Continued

Indicator	C14:0	C15:0	C16:0	C17:0	C18:0	C22:0
Minimum, %	0	0	20.50	0	23	1
Maximum, %	0	0	2	0	25.51	1

***Note**: C14:0 – myristic acid; C15:0 – pentadecanoic acid; C16:0 – palmitic acid; C17:0 – heptadecanoic acid; C18:0 – stearic acid; C22:0 – behenic acid*
***Source**: authors' development*

According to descriptive statistics of blood lipids in pigs, it has been proven that the average values formed had insignificant differences. At the same time, the standard error and standard deviation values for palmitic acid were 15-20 times higher, and for stearic acid – 14.0 to 20.0 times higher than for other fatty acids. When analysing the asymmetry of fatty acid indicators, a slight positive symmetrical asymmetry was found in myristic and pentadecanoic acids. Palmitic and behenic acids were characterised by moderate positive asymmetry. Heptanoic acid was characterised by negative average asymmetry, and stearic acid by more pronounced negative asymmetry. The value of this indicator was not critical, which can be explained by the content of these fatty acids. When evaluating the minimum and maximum values, a slight discrepancy within the range of 0.16% to 2.75% of the initial data is worth noting. After analysing the data on saturated fatty acids in the blood plasma lipids of pigs in the control group of sympathicotonic animals using descriptive statistics, a corresponding data table was formed depending on the percentage content of the fatty acids studied (Table 3).

Table 3. Descriptive statistics of blood lipid parameters in pigs of the control group of sympathicotonic animals (n = 5)

Indicator	C14:0	C15:0	C16:0	C17:0	C18:0	C22:0
Average, %	0	0	2	0	24.44	0
Standard error, %	0	0	0	0	0	0
Standard deviation, %	0	0.03	1	0	0	0
Asymmetry	-	0	0	0	1	0
Minimum, %	0	0	22.5	0	23.5	0
Maximum, %	0	0	24.96	0	25.92	0

***Note**: C14:0 – myristic acid; C15:0 – pentadecanoic acid; C16:0 – palmitic acid; C17:0 – heptadecanoic acid; C18:0 – stearic acid; C22:0 – behenic acid*
***Source**: authors' development*

According to statistical analysis, the chromatographic analysis of lipids in the blood of pigs in the control group with sympathicotonia showed that the average values for fatty acid content were highly reliable and showed insignificant differences in the values of the corresponding indicators. This was confirmed by the standard error and standard deviation values obtained, which were relatively low, especially for myristic, pentadecanoic, heptadecanoic and behenic acids. At the same time, the standard error and standard deviation were significantly higher for palmitic acid, corresponding to a range of 15.5 to 16.0 times, and for stearic acid, within a range of 13.4 to 14 times, relative to the data for other saturated fatty acids. Considering the values of these acids, the standard error and standard deviation were relatively low.

Regarding asymmetry, myristic, pentadecanoic and behenic acids differed in their low negative asymmetry, while palmitic and heptadecanoic acids showed moderate asymmetry. Despite its high asymmetry, the content of stearic acid corresponded to the normal numerical distribution. The differences between the minimum and maximum values of the six saturated fatty acids were within the acceptable range of 0.07% to 2.43%. After examining the descriptive statistics of lipid content in pigs' blood in the experimental sympathotonics group, the corresponding statistical data were compiled (Table 4).

Table 4. Statistical analysis of lipid indicators in the blood of pigs in the experimental group of sympathotonics (n = 5)

Indicator	C14:0	C15:0	C16:0	C17:0	C18:0	C22:0
Average, %	0	0	2	0	20.74	0
Standard error, %	0	0	0	0	0	0
Standard deviation, %	0	0	0	0	0	0
Asymmetry	1.0	1.22	0	0	0	0
Minimum, %	0	0	20.1	0	20.33	0
Maximum, %	0	0	21.38	0	21.18	0

***Note**: C14:0 – myristic acid; C15:0 – pentadecanoic acid; C16:0 – palmitic acid; C17:0 – heptadecanoic acid; C18:0 – stearic acid; C22:0 – behenic acid*
***Source**: authors' development*

When evaluating the data from the chromatographic analysis of blood lipids in pigs of the experimental group with sympathicotonia, characteristic values of the spectrum of saturated fatty acids were determined. It should be noted that in pigs of this group, the standard error and standard deviation values were predominantly relatively low. However, the standard error and standard deviation values for palmitic acid were higher, ranging from 7 to 7.8 times, and for stearic acid, 5.0 and 5.5 times, unlike other fatty acids. Considering the numerical data on asymmetry, the highest values were found in myristic and pentadecanoic acids, indicating significant asymmetry in the sequence of numbers. Other saturated fatty acids, such as palmitic, heptadecanoic and behenic acids, were characterised by moderate asymmetry, while palmitic acid had low asymmetry. The distribution of data within the range of 0.07% to 1.25% between the maximum and minimum values of saturated fatty acids in the experimental group of sympathotonics indicated a slight discrepancy in the results of their chromatographic study in pig blood plasma. The results of descriptive statistics on the content of lipids in the blood of pigs in the control group of vagotonics are presented in Table 5.

Table 5. Statistical analysis of lipid indicators in the blood of pigs in the control group of vagotonics (n = 5)

Indicator	C14:0	C15:0	C16:0	C17:0	C18:0	C22:0
Average, %	0	0	2	0	24.44	0
Standard deviation, %	0	0	0.49	0.02	0	0
Standard deviation, %	0	0	1	0	0	0
Asymmetry	-	0	0	0	1	0

Table 5. Continued

Indicator	C14:0	C15:0	C16:0	C17:0	C18:0	C22:0
Minimum, %	0	0	22.5	0	23.5	0
Maximum, %	0	0	24.96	0	25.92	0

***Note**: C14:0 – myristic acid; C15:0 – pentadecanoic acid; C16:0 – palmitic acid; C17:0 – heptadecanoic acid; C18:0 – stearic acid; C22:0 – behenic acid*
***Source**: authors' development*

According to the statistical assessment of the lipid content in pigs' blood in the control group of vagotonic animals, it was established that the data obtained for six fatty acids were within the permissible limits. The average values had relatively small standard errors and standard deviations, characterising them as numerical data with insignificant discrepancies. This emphasises the reliability of the results obtained in the chromatographic study. The asymmetry index for myristic, pentadecanoic, palmitic and behenic acids was characterised by a negative average asymmetry, while heptadecanoic acid showed a positive average asymmetry. Stearic acid was characterised by high positive asymmetry, which is not critical for the calculated indicator. When evaluating the minimum and maximum values of all saturated fatty acids in the control group of vagotonic animals, it was determined that they ranged from 0.07% to 2.43%, which indicated a slight discrepancy in the initial data values. Descriptive statistics were used to analyse the blood lipid spectrum of pigs in the experimental group of vagotonics that were given a mixture of iron and germanium nanocomposites (Table 6).

Table 6. Statistical analysis of blood lipid levels in pigs from the experimental group of vagotonic animals (n = 5)

Indicator	C14:0	C15:0	C16:0	C17:0	C18:0	C22:0
Average, %	0	0	21	0	2	1.06
Standard error, %	0	0	0	0	0	0
Standard deviation, %	0	0	0	0	0	0
Asymmetry	0	0	1.60	0	0	0
Minimum, %	0	0	20.71	0	20.82	0
Maximum, %	0	0	22	0	21.99	1

***Note**: C14:0 – myristic acid; C15:0 – pentadecanoic acid; C16:0 – palmitic acid; C17:0 – heptadecanoic acid; C18:0 – stearic acid; C22:0 – behenic acid*
***Source**: authors' development*

According to the descriptive statistics of the blood lipid chromatograms of pigs in the experimental group, it was determined that the average values are highly reliable and show insignificant differences. This is confirmed by the obtained standard error and standard deviation values, which were relatively low, especially for myristic, pentadecanoic, heptadecanoic and behenic acids. It should be noted that the standard error and standard deviation were significantly higher for palmitic acid, ranging from 7.8 to 18.0 times, and for stearic acid, ranging from 5 to 11.3 times. This indicated that these indicators were relatively low. Regarding the asymmetry index, myristic, pentadecanoic, stearic, and behenic acids showed moderate positive asymmetry, while heptadecanoic acid showed negative asymmetry. Despite its high asymmetry, palmitic acid corresponded to a statistically correct distribution of data.

The differences between the minimum and maximum values of the six fatty acids ranged from 0.05% to 1.79%, corresponding to the normal range. Comparing the indicators of saturated fatty acids, the blood lipids of pigs in the normotonic groups showed a higher content of myristic acid by 1.7 times ($P < 0.01$) in animals that did not receive a mixture of nanoaquachelates in their diet, unlike the pigs in the experimental group. The relative content of pentadecanoic acid did not differ between groups of pigs with a predominance of normotony (Fig. 1).

Figure 1. Content of myristic (C14:0) and pentadecanoic (C15:0) fatty acids in blood plasma lipids of normotonic pigs
Source: *authors' development*

The content of heptadecanoic acid increased by 22.81% ($P < 0.05$) in the blood plasma lipids of pigs with a standard diet compared to normotonic pigs that additionally received feed additives of nanoaquachelates. Conversely, behenic fatty acid increased by 2.95% ($P < 0.01$) in pigs with a standard diet of normotonic pigs, unlike normotonic pigs in the experimental group (Fig. 2). When studying the level of saturated fatty acids, the percentage of which was found to be the highest, it was found that the content of palmitic acid in pigs of the experimental group increased by 13.81% ($P < 0.01$) compared to normotonic pigs that had a regular diet. The content of stearic fatty acid in the blood of normotonic pigs increased by 11.97% ($P < 0.01$), compared to those that were not given nanoaquachelate feed additives, unlike the experimental group of animals (Fig. 3).

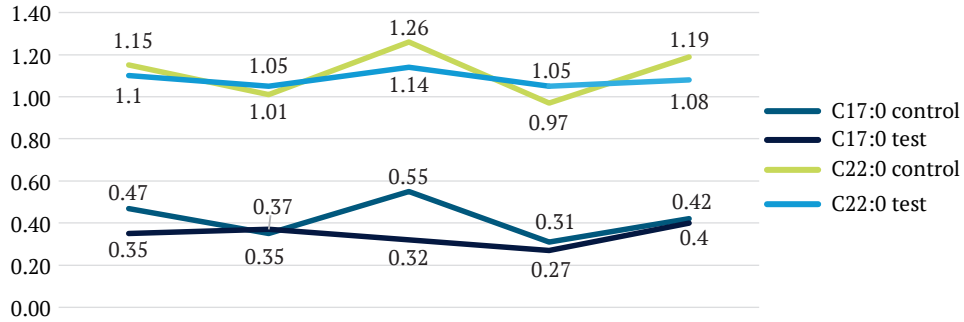

Figure 2. Indicators of heptadecanoic (C17:0) and behenic (C22:0) acids in the blood lipids of pigs in the experimental and control groups of normotonics
Source: *authors' development*

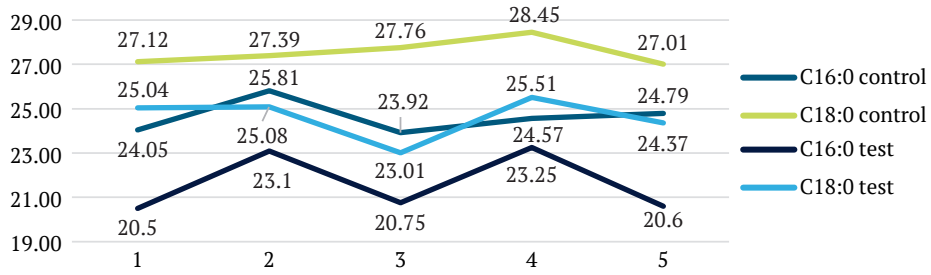

Figure 3. Indicators of palmitic (C16:0) and stearic (C18:0) acids in normotonic pigs
***Source**: authors' development*

Comparing the levels of saturated fatty acids in the blood plasma lipids of pigs in the control and experimental groups of sympathotonics, a decrease in myristic acid by 34.16% ($P < 0.01$) was observed in those that received an additional feed supplement compared to those that had a standard diet. Pentadecanoic acid decreased by 17.74% ($P < 0.01$) in sympathotonics pigs compared to the control (Fig. 4).

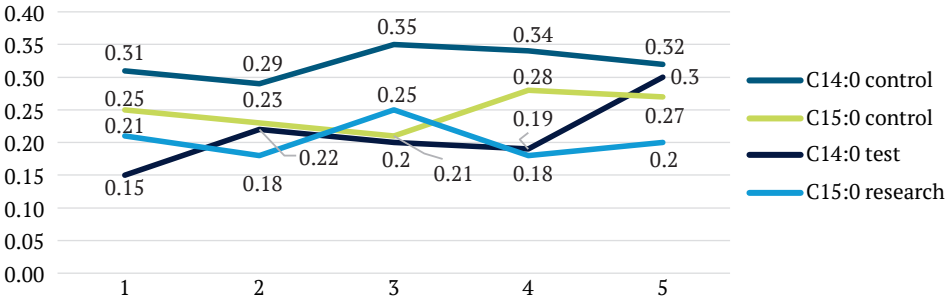

Figure 4. Levels of myristic (C14:0) and pentadecanoic (C15:0) acids in blood plasma lipids of pigs in the experimental and control groups of sympathotonics
***Source**: authors' development*

The content of heptadecanoic acid decreased by 19.39% ($P < 0.01$) in the blood lipids of pigs with sympathotonics that were given a feed supplement compared to animals in the control group that did not consume nanoaquachelate feed supplements. The content of behenic fatty acid decreased by 35.59% ($P < 0.01$) in pigs with sympathotonics that were given a feed supplement compared to control sympathotonics (Fig. 5). When studying the content of saturated fatty acids with the highest percentage, it was determined that palmitic acid in the blood lipids of pigs in the experimental group of sympathotonics decreased by 13.32% ($P < 0.01$), and stearic acid decreased by 15.17% ($P < 0.001$) compared to the control group of animals (Fig. 6). Comparing the levels of saturated fatty acids in the blood plasma lipids of pigs in the control and experimental groups of vagotonic animals, the content of myristic acid in the blood plasma lipids of pigs that did not receive a mixture of nanoaquachelates in their diet increased by 45.83% ($P < 0.01$). However, the content of pentadecanoic acid increased by 91% ($P < 0.05$) relative to the experimental group (Fig. 7).

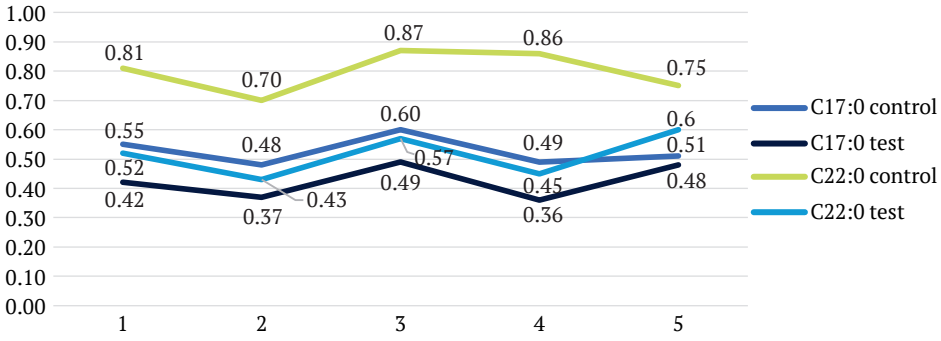

Figure 5. Levels of heptadecanoic (C17:0) and behenic (C22:0) acids in blood plasma lipids of sympathotonics pigs

***Source**: authors' development*

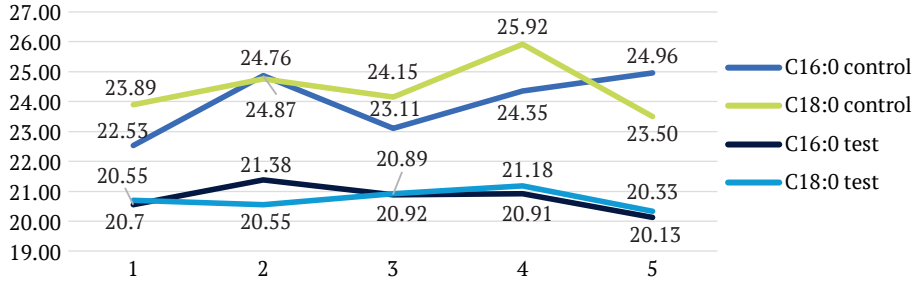

Figure 6. Indicators of palmitic (C16:0) and stearic (C18:0) acids in the blood plasma lipids of sympathicotonic pigs

***Source**: authors' development*

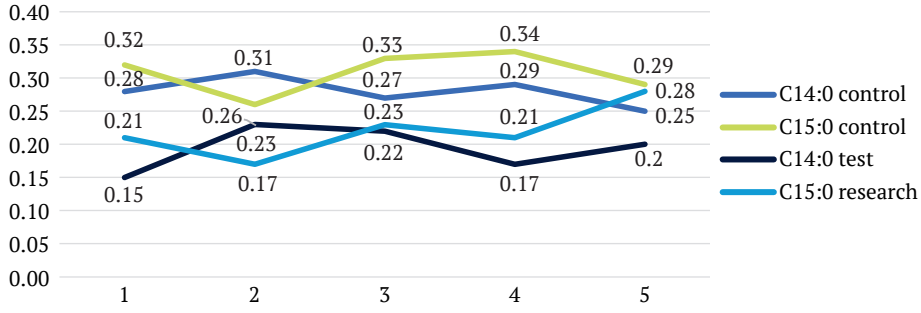

Figure 7. Indicators of myristic (C14:0) and pentadecanoic (C15:0) acids in the blood plasma lipids of vagotonic pigs

***Source**: authors' development*

The content of heptadecanoic acid increased by 27.64% ($P < 0.05$) in pigs of the control group with vagotonia compared to pigs that consumed feed additives of nanoaquachelates. The level of behenic fatty acid also increased by 21.24% ($P < 0.01$) in the blood lipids of pigs in the control group of vagotonic pigs, unlike vagotonic pigs in the experimental group (Fig. 8).

The content of palmitic acid in the blood lipids of pigs in the control group of vagotonic animals, which were not given nanoaquachelate feed additives, increased by 17.35% (P<0.01), and stearic acid content increased by 13.45% (P < 0.001) compared to the experimental group (Fig. 9).

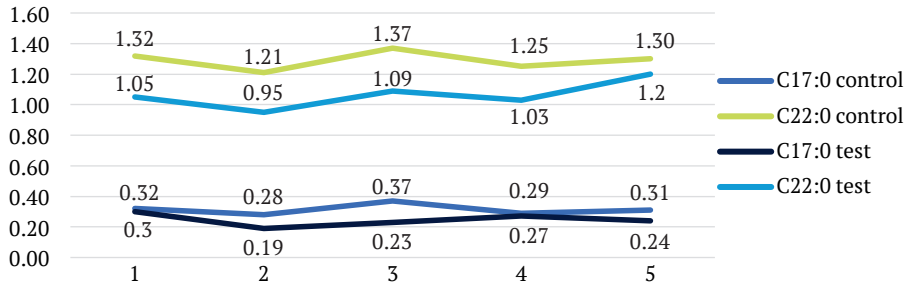

Figure 8. Content of heptadecanoic (C17:0) and behenic (C22:0) acids in blood lipids of vagotonic pigs
***Source**: authors' development*

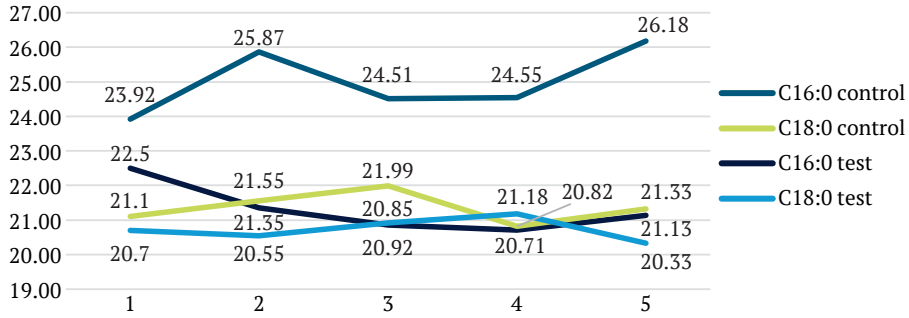

Figure 9. Levels of palmitic (C16:0) and stearic (C18:0) acids in the blood lipids of vagotonic pigs
***Source**: authors' development*

D.O. Melnychuk & V.A. Hryshchenko (2014) established that lipid metabolism in mammals is influenced by both exogenous (ecopathogenic) and endogenous factors, and also found a corrective effect of adding milk phospholipids to the diet of animals at a dose of 13.5 mg/kg body weight. H. Zhang *et al.* (2022) investigated the fact that diet significantly affects lipid metabolism, especially regarding the composition of the diet and the feeding period. It has been proven that excessive feed intake inhibits lipid metabolism, accumulating saturated fatty acids in the animal's body without their further involvement in synthetic processes. According to the current study's results, additional feed additives improved lipid metabolism, as evidenced by a decrease in fatty acid levels in the blood lipids of pigs.

M. Scerra *et al.* (2024) investigated the effect of bergamot pulp and olive oil on lipid metabolism, especially fatty acids. The use of nanoaquachelates caused an increase in the content of myristic, pentadecanoic, palmitic, heptadecanoic and stearic acids in the range from 17.4% to 47.2% (P < 0.05). Compared with the results presented in this study, the nanopreparation led to a decrease in fatty acid levels in pig blood plasma from 13.45% to 45.83%

($P < 0.001$), which may indicate an increase in lipid metabolism in the body. It is also important to note that more accurate results of chromatographic analysis were obtained due to the formation of pigs following the individual characteristics of their vegetative regulation.

M. Xu *et al.* (2022) investigated the effect of a proanthocyanidin feed supplement from grape seed extract on lipid metabolism in pigs. According to the results obtained, the scientists noted a decrease in the content of myristic, pentadecanoic, palmitic, heptadecanoic and stearic acids in the range from 10% to 6% ($P < 0.05$) at a maximum concentration of the dietary supplement at a dose of 200 mg/kg. Compared with the study's results, there was a decrease in the content of saturated fatty acids, which may indicate better efficiency of the nanopreparation. At the same time, the standard error and standard deviation decreased, possibly due to the distribution of animals into groups based on vegetative regulation.

L. Zhang *et al.* (2021) investigated the effect of a feed supplement in the form of branched-chain amino acids. According to the results obtained, the authors demonstrated a gradual decrease in the concentration of total lipids in the bloodstream and an increase in triacylglycerols ($P < 0.05$), the main transport compounds of fatty acids. These researchers noted the positive effect of the feed supplement on lipid metabolism. Compared to the results of this study, the concentration of saturated fatty acids decreased significantly, and the formation of pigs following vegetative regulation had a positive effect on the results obtained. Z. Guo *et al.* (2021) investigated the effectiveness of the feed supplement dihydromyricetin. According to the results obtained, the reduction in saturated fatty acid content ranged from 2.5% to 3.6%, and it is also worth noting the high P-value, which indicated a significant discrepancy in the data. According to the results obtained, the significance coefficient P was significantly lower due to the distribution of animals according to individual characteristics.

O. Kovalchuk *et al.* (2024) used a feed supplement with the same composition as in this study. According to the results of their research, there were changes in lipid metabolism in the triglyceride indicators between the control and experimental groups of pigs, which were 24.3% higher in the experimental group ($P < 0.05$) and non-esterified fatty acids, which increased in the control group by 8.0% ($P < 0.05$). Comparing our results regarding lipid metabolism and the use of nanoaquachelate feed additives, it should be noted that taking into account the individual characteristics of pigs' organisms gives more accurate results when using a modified diet. Therefore, the formation of animals according to the activity of autonomous regulation, based on our study, provides more detailed data.

After analysing the results of other researchers and comparing them with those described in this paper, it should be noted that the effectiveness of each feed additive varies considerably. It should also be noted that the region, country and breed of pigs are significant factors, as climatic conditions and access to the same feed additives vary from farm to farm within a country. Therefore, it is understandable why scientific works differ significantly in their approach to the issue of additional components to the feed ration that improve lipid metabolism in pigs. However, despite these differences, the processes of lipolysis and lipogenesis occurring in pigs are unchanged. Therefore, according to the results obtained, it is clear that the physiological differentiation of pigs according to the individual characteristics of their vegetative regulation makes a significant contribution to the study of the feed additives used and improves the accuracy of the results obtained. It should also be noted that the feed additive used in the

experiment, a nanopreparation of a mixture of germanium and iron, has significant results in lipid metabolism in pigs.

Conclusions

Quantitative changes in lipid metabolism indicators, namely saturated fatty acids, were determined when pigs with different autonomic nervous system tone were fed a feed mixture of iron and germanium nanocomposites. It was found that the blood plasma lipids of pigs in the experimental group with different autonomic nervous system tone showed a decrease in the content of saturated fatty acids. Thus, the content of myristic fatty acid in the blood lipids of pigs with a modified diet decreased from 34.16% to 45.83% ($P < 0.01$), pentadecanoic fatty acid – from 17.74% to 27.64% ($P < 0.05$; $P < 0.01$), palmitic acid – from 13.32% to 17.35% ($P < 0.01$), heptadecanoic acid – from 19.39% to 27.64% ($P < 0.05$; $P < 0.01$), stearic fatty acid – from 11.97% to 15.17% ($P < 0.01$; $P < 0.001$), behenic acid – from 2.95% to 35.59% ($P < 0.01$). According to the studies, using a nano-mixture of iron and germanium positively affected saturated fatty acids, as evidenced by a decrease in their relative content in the plasma lipids of pigs. These results reflect the stability of homeostasis in the body of the experimental pigs, which was ensured by using nanoaquachelates in their diet. According to the statistical analysis of the results of the chromatographic study, there was a significantly lower discrepancy between the numerical values obtained from the five animals in the experimental and control groups with different vegetative regulation. The low values of the standard error and standard deviation confirm this. Using the pulsometric study indicator for more accurate formation of experimental groups of pigs contributed to a better analysis of the effect of nanoaquachelates on fatty acid indicators in the body of these animals. The prospect of further research lies in adjusting the doses of feed additives of iron and germanium microelements according to pigs with different vegetative regulation, since it is already known that animals in each of the normotonic, sympathicotonic, and vagotonic groups had specific characteristics regarding the content of saturated fatty acids when using iron and germanium nanocomplexes. Taking this into account, selecting an individual dose of feed additive for pigs according to the characteristics of the activity of the vegetative nervous system will help to effectively correct lipid metabolism and ensure the stable productivity of these animals.

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Conflict of Interest

None.

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Вплив наносполук германію і заліза на показники насичених жирних кислот у крові свиней залежно від вегетативної регуляції

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Анотація. Актуальність виконаного досліду пов'язана із розробкою нових методів підвищення продуктивності свиней завдяки збалансуванню метаболічних процесів в організмі. Головною метою було визначити зміни показників насичених жирних кислот в організмі свиней при використанні кормової добавки наноаквахелатів з урахуванням індивідуальних особливостей організму. Формування дослідних груп тварин виконували за пульсометричним дослідженням, відповідно до якого було отримано три групи по 10 свиней, кожна з яких була поділена на дві підгрупи – контрольну і дослідну по 5 тварин. Дослідній групі за допомогою випоювання задавали суміш наносполуки мікроелементів заліза в дозі 3 мг/добу та германію – 0,01 мг/добу. Вміст насичених жирних кислот оцінювався

за допомогою методу газової хроматографії. За результатами виконаного дослідження встановлено, що у крові свиней контрольної групи із нормотонією збільшувався вміст насичених жирних кислот: міристинової ($P < 0,01$), гептадеканової ($P < 0,05$), бегенової ($P < 0,01$). У крові свиней дослідної групи з симпатотонією зменшувався вміст насичених жирних кислот: міристинової ($P < 0,01$), пентадеканової ($P < 0,01$), гептадеканової ($P < 0,01$), бегенової ($P < 0,01$). У крові свиней контрольної групи із ваготонією вміст насичених жирних кислот збільшувався порівняно з дослідною групою. Встановлено, що у дослідних свиней нормотоніків жирні кислоти з найбільшою часткою, такі як пальмітинова – менша на 12,1 %, а стеаринова кислота – на 10,7 % ($P < 0,01$). У свиней симпатотоніків контрольної групи спостерігався більший вміст пальмітинової кислоти на 15,4 % ($P < 0,01$) та стеаринової – на 17,8 % ($P < 0,001$). У дослідних свиней ваготоніків – менший вміст пальмітинової на 14,8 % ($P < 0,01$) та стеаринової кислоти на 11,8 % ($P < 0,001$). За результатами дослідження відмічався позитивний вплив суміші наносполук заліза і германію на показники обміну насичених жирних кислот, а розподілення свиней відповідно до індивідуальних особливостей організму, покращило точність отриманих даних

Ключові слова: обмін речовин; нанопрепарат; продуктивні тварини; автономна нервова система; ліпіди

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Prevalence and diagnostic features of external otitis in dogs and cats in veterinary clinics in Poltava

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Abstract. The relevance of the conducted research was due to the spread of otitis of various aetiologies among dogs and cats. The aim of this work was to study the prevalence of external otitis in small animals, to determine the clinical symptoms of otitis depending on different forms of progression in order to improve the diagnosis of the disease. To achieve this goal, haematological and microscopic types of studies were carried out. Cytological examination of imprint smears established the presence of epiteliocytes, lymphocytes, cerumen and dirt, and in some cases

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otodectic infestation was detected. The diagnosis of infectious diseases was carried out using immunochromatographic methods, where otitis was an accompanying disease. Leucocytosis, neutrophilia and lymphopenia were established, which developed during the acute phase of inflammation, while the chronic process was accompanied by less pronounced changes in the leucogram. Monitoring research of the intensity of otitis spread among dogs and cats was conducted in the city of Poltava in the period from March 2024 to February 2025. The obtained data indicated a significant prevalence of this pathology among dogs in the spring period. In the veterinary clinic "Aibolit" 793 cases of otitis were established, of which 705 in dogs, which amounted to 88.9%, and 88 in cats, which corresponded to 11.1%. In the veterinary clinic "MAXVET" 680 cases of otitis were recorded, among which 609 in dogs, which amounted to 89.6%, and 71 in cats, which made up 10.4%. Breed predisposition to the development of otitis in brachycephalic breeds was revealed. The most common aetiological factors of otitis were established: mechanical injuries, parasitic infestations, and complications as a result of infectious diseases. Clinical symptoms manifested as local redness, swelling of the external ear, itching and pain signs, which were accompanied by the animals' restlessness and constant scratching of the ears and the formation of exudate. The practical value of the obtained results lay in the identification of patterns of spread and aetiological factors of otitis, which expanded the possibilities of the diagnosis

Keywords: aetiology; ear diseases; cytology; haematology; otodectosis

Introduction

The full functioning of the auditory organ was an important condition for maintaining the vital activity of small domestic animals. Any disturbances in the functioning of the auditory system could significantly affect the emotional state of the animal, causing depression, anxiety, or sudden outbursts of aggression. Otitis was a common inflammatory syndrome of infectious, allergic, parasitic, or traumatic aetiology, characterised by lesions of the external, middle, or inner auditory canal, accompanied by local and systemic clinical manifestations and could lead to functional disorders of the auditory system.

D.L. Datsiuk *et al*. (2024) noted that inflammation of the ears in animals significantly affected the quality of life, led to exhaustion, decreased immunity, long-term and costly treatment. In addition, the inflammatory process was usually often complicated by secondary infection. These data were reflected in most of the studies by T. Nuttall (2023), E. Ludewig *et al*. (2024), M. Chupryna (2024). Otitis was no longer considered an isolated disease, but more and more often a complex syndrome, which was often a consequence of an underlying disease, caused by the natural predisposition of the animal's body. The most common primary causes of otitis included foreign bodies (insects, trauma, plant awns), hypersensitivity (in particular, atopy and food allergy), keratinisation disorders (most often primary idiopathic seborrhoea and hypothyroidism) and ear mites, especially in cats. Therefore, systematic diagnosis was necessary to identify the causes and contributing factors, which included: collection of anamnesis data, clinical examination, otoscopic and cytological examination, determination of cultural features and sensitivity, as well as assessment of otitis media and biopsy (in severe and recurrent cases). Depending on the underlying cause, additional tests might be required. Treatment of otitis consisted of identification

and elimination of the main factors and predisposing factors, mechanical removal of foreign elements and washing of the auditory canal, local therapy, systemic therapy if necessary, client education, further monitoring, and if necessary – preventive and supportive therapy.

J. Juhola *et al*. (2024) indicated breed predisposition to the development of otitis in dogs. Otitis was recorded in different breeds, but mixed-breed dogs, pugs, Pekingese, French bulldogs, labradors, clumber spaniels, Jack Russell terriers, chihuahuas, spitz, German shepherds and Cane Corso were more often affected. In general, otitis was one of the most common diseases in dogs. This was associated with bacteria and yeasts, which were considered secondary causes in the occurrence of otitis. M. Gracz *et al*. (2024) proved the presence of altered microRNAs in the serum of dogs affected by external otitis. MicroRNAs could modulate the host's immune response and provide biomarkers for the diagnosis of several inflammatory and infectious disorders. With the application of bioinformatics, it was found that serum microRNAs could participate in the modulation of the host's immune response. The authors demonstrated for the first time that with the help of microRNAs it was possible to effectively determine the number of serum components and its profile, which changed between healthy dogs and those affected by otitis, which could serve as potential biomarkers of the disease.

Thus, otitis in small animals was a common problem of various aetiologies, and therefore there was a wide choice of diagnostic methods. Timely and correct diagnosis, determination of the main causes of the disease, made it possible to apply the correct treatment approach. Therefore, the purpose of the work was to carry out an analysis of the main causes, patterns, and frequency of otitis in dogs and cats, depending on the season and the breed.

Literature Review

Otitis externa was one of the most common pathologies in dogs and cats, requiring long-term and complex treatment. According to J. Bajwa (2019), treatment of otitis included local and complex therapy. A frequent cause of otitis externa, especially with prolonged or improper treatment, was the excessive growth of yeast-like fungi *Malassezia* spp. In the study of S. Hobi *et al*. (2024), it was noted that *Malassezia* dermatitis was a common problem in both dogs and cats, often associated with other dermatological disorders, as well as antibiotic-induced yeast overgrowth. These data were consistent with the study of M. Tuyakhov (2024), in which *M. pachydermatis* was detected in 30% of cases of otitis in dogs, and rare cases of Candida albicans (3.3%) were also recorded, often in association with *Staphylococcus aureus*, *S. pseudintermedius* and *Proteus* spp.

Diagnosis of otitis required a thorough clinical examination and laboratory evaluation. As noted by L.S. Jacobson (2002), it was important to take into account not only infectious but also non-infectious factors that could cause otitis, in particular allergies or anatomical features. In more complicated cases or chronic forms, especially with inadequate housing conditions, otitis media or interna could develop. From an epidemiological point of view, otitis had many preconditions. D.G. O'Neill *et al*. (2021) studied the frequency and risk factors of otitis externa in dogs in the United Kingdom. It was found that certain breeds had a higher risk of the disease, and that care and hygiene conditions were also of great importance. A. Arkhypenko & V. Ushkalov (2021) found that the bacterial community of the serum of healthy dogs was characterised by high variability, with the most common type represented by *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes* and *Fusobacteria*. Analysis of both alpha- and beta-diversity between pairs of samples from the left

and right ears of the same dog in the affected group showed greater differences than between paired samples of healthy dogs. In addition, there was a decrease in the number of bacteria in the affected group compared with the control and an increase in the variability of the population structure among animals affected by otitis, which was often associated with the predominance of one bacterial taxon over others. At the same time, *Staphylococcus* and *Pseudomonas* proved to be bacterial genera responsible for most of the differences between the two groups, due to differences in the structure of the bacterial community. The microbiota of the serum in healthy dogs turned out to be a complex bacterial population that underwent significant changes in animals affected by otitis.

Recurrent otitis externa was a common problem in dogs and cats. Thus, according to T. Nuttall (2023), local treatment of each outbreak was successful in the short term, but repeated cycles of inflammation and infection led to chronic inflammatory changes, pain, and antimicrobial resistance. This made outbreaks more frequent and more difficult to control. Over time, changes became irreversible and required total ablation of the auditory canal (lateral osteotomy). Most operations could be avoided if recurrent otitis was treated at an early stage. This required a different way of thinking and approach to diagnosis and treatment. All recurrent ear infections in dogs were secondary infectious processes. To achieve a good long-term outcome in the treatment of the disease, it was important to take all factors into account during diagnosis. It was especially necessary to consider possible complications that developed, in particular the formation of otohaematomas, which prolonged the treatment and rehabilitation process of animals. Traditionally, treatment was carried out in two phases: induction, to induce remission, and then long-term maintenance therapy – to prevent relapses. Treatment had to be appropriate for each dog, but usually involved ear cleaning, local antimicrobial therapy, and administration of topical or systemic glucocorticoids. New methods of treating infection and inflammation would lead to the use of additional options in the future. Understanding the triggers of recurrent otitis in dogs would help clinicians to plan effective treatment regimens that significantly improved the quality of life of the patients and the owners. D.L. Datsiuk *et al*. (2024) pointed out the significant prevalence of combined preparations, which on the one hand were effective in the treatment of otitis, and on the other hand were safe for animals. Such preparations usually had to demonstrate anti-inflammatory, antiparasitic, analgesic and immunomodulatory effects simultaneously. Therefore, the search for and development of new preparations in the system of complex preventive and therapeutic measures, especially for the external ear, was a relevant and promising direction.

A. Lorek *et al*. (2020) confirmed that short focused MRI scanning made it possible to detect inflammation of the mucous membranes, as well as to distinguish avascular material and vascularised soft tissue in the tympanic cavity. During otoscopy of dogs' ears, the tympanic membrane was found intact in 6 of 41 cases (15%), ruptured in 16 of 41 (39%), and invisible in 14 of 41 (34%) cases. At the same time, analysis of cytological samples revealed rod-shaped microorganisms associated with inflammation. M. Gracz *et al*. (2024) indicated the wide use of computed tomography (CT) for dogs and cats for the purpose of rapid and accurate diagnosis, especially in prolonged inflammatory otitis. CT analysis proved to be more sensitive for demonstrating damage to the tympanic bone wall and more effective for detecting abnormal content inside the tympanic bulla. Otitis externa was a common multifactorial disease. J. Korbelik *et al*. (2018) proved that its prevalence among

dogs reached 10%-20%. A frequent aetiological factor was fungi of ten different types. In the microbiota of all affected animals, *Malassezia* genera predominated, accounting for 55.7%-98.4%. In the affected ears there was a decrease in the number and diversity of fungi, with a significantly higher relative number of *Malassezia*. The mycobiota of fungi in dogs' ears was much more complex than revealed in cultural studies.

The study of otitis externa in dogs and cats was a relevant task of veterinary medicine, as it was a common multifactorial disease accompanied by complex inflammatory processes. Timely diagnosis, a deep understanding of pathogenesis, the use of modern imaging methods and the development of effective and safe treatment regimens made it possible to prevent the development of chronic disease, avoid surgical intervention and improve the quality of life of animals. This emphasised the need for a comprehensive approach to the prevention and treatment of otitis in veterinary practice.

Materials and Methods

The research was carried out in the period from 2024 to 2025 in private veterinary clinics of Poltava ("Aibolit", "MAXVET"). The epizootic study was conducted by the method of statistical calculation of clinical cases recorded in the studied veterinary clinics in the autumn, winter, spring and summer periods. In total, 1473 clinical cases were studied, of which 793 in the veterinary clinic "Aibolit" and 680 in the veterinary clinic "MAXVET". The diagnosis of otitis was carried out comprehensively, on the basis of anamnesis data, clinical signs and laboratory studies.

To determine the breed predisposition of dogs to the occurrence of otitis depending on the morphological type of breed (brachycephalic or non-brachycephalic), an analysis of clinical cases recorded in the veterinary clinics was carried out. The data included the breed, age, sex of the animal, as well as the presence of diagnosed external otitis. Breeds were classified as brachycephalic (for example, pug, French bulldog, Pekingese) and non-brachycephalic in accordance with the typical anatomical characteristics of the skull. To brachycephalic cat breeds were attributed, in particular, Persian, Exotic, Himalayan cats, which had a shortened skull structure and narrowed auditory canals. To assess the prevalence of otitis among each group, the percentage ratio of otitis cases to the total number of dogs of the respective group was calculated.

The research and analysis of aetiological factors of otitis in dogs and cats were carried out by means of differential diagnosis of parasitic invasion, bacterial infection and a complex of clinical changes that characterised otitis. In case of suspicion of otodectosis, microscopy of the taken material was carried out for the purpose of detecting *Otodectes* mites. Material from both ears was studied, even in cases of unilateral otitis. Material from the left and right ear was studied separately. The material was taken by immersing a cotton swab into the auditory canal and applying it to a glass slide with a drop of glycerine. All microscopic studies were carried out using a Micromed microscope (XS-5520 LED, China) at magnifications x64, x160. Cytological study of the obtained material was carried out for the purpose of assessing the cytogram (leukocytes, as well as epithelial cells) and microorganisms (bacteria, fungi). For cytological examination, smears of the taken material were prepared. The content of the proximal part of the horizontal canal was mainly studied. For this, an otoscope was carefully introduced into the ear, into the lumen of which, without touching the wall of the vertical canal, a thin cotton swab was introduced. By rotating the cotton swab, a sample of the content (earwax and/or exudate) appeared on the tip of the swab. By this approach, the formation of conglomerates in the auditory canal was avoided, without causing compaction of the content.

Before staining the obtained material, it was fixed by heating the glass slide over a flame. Haematological stains were used for staining preparations (Diff-Quik®, Quick-Diff, Wright-Giemsa, May-Grünwald and others). This process took place in four stages: in the first – haematological eosin-methylene blue stain (according to May-Grünwald) was applied for 2 min; in the second – an aqueous solution of azure-eosin stain (10 drops of Romanowsky-Giemsa stain per 10 mL of buffer), in the volume of 2 mL per slide, exposure 3 min; the third – consisted of washing with water; the fourth – envisaged drying. After staining the preparations, microscopy and evaluation of the preparation were carried out, starting from the smallest magnification (x64). Then the prepared samples were studied under higher magnification (x160). In this case, leukocytes, erythrocytes, epithelial cells, as well as neoplastic cells, yeasts, and bacteria were identified. For a more detailed examination, a magnification of x640 was used, which allowed examination of the cytoplasm of neutrophils and macrophages to detect phagocytosed bacteria as indisputable evidence of infection. By this criterion, pathogenic microorganisms were differentiated from commensals.

Haematological studies were carried out on the basis of the veterinary clinics "Aibolit" and "MAXVET" using the haematology analyser Mindray BC-20S (China). The following indicators were determined: haemoglobin content (g/L), number of erythrocytes (10^{12}/L), MCH (pg), leukocytes (10^9/L), basophils (%), eosinophils (%), neutrophils (%), lymphocytes (%) and monocytes (%). For comparison, three groups of dogs (n = 15) were formed. The first group – control, included clinically healthy animals (n = 5); the second group – included dogs with symptoms of chronic otitis (n = 5), in which itching, redness of the skin in the area of the auricle were noted; the third group – was formed of dogs that had manifestations of acute otitis (n = 5), such as the presence of swelling of the auricle and surrounding skin, the appearance of ear discharge, the presence of deposits in the form of scales and crusts inside the auricle and an unpleasant odour. Except for the control group of animals, the reference analysis, for the following indicators: haemoglobin, g/L; erythrocytes, 10^{12}/L; mean haemoglobin content in erythrocyte (MCH), pg; leukocytes, 10^9/L; basophils %, eosinophils %, neutrophils %, lymphocytes %, and monocytes %, was carried out on the basis of the data of J.W. Harvey (2001).

Differential diagnosis of infectious diseases was carried out comprehensively, taking into account anamnesis data, clinical symptoms and the results of express tests. The research with animals was carried out according to the requirements of international principles European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (1986), Law of Ukraine No. 3447-IV (2006). Statistical processing of the research results included determination of Student's t-test ($P < 0.05$, $P < 0.01$, $P < 0.001$, the results were considered statistically significant with varying degrees of reliability in relation to the values of the corresponding indicator in the control). For statistical processing of the data, the computer programme STATISTICA 7.0 (Stat Soft, USA) was used.

Results and Discussion

The study of the prevalence of otitis registered in the veterinary clinic "Aibolit" established that the largest number of cases was registered in the spring period – 293. Otitis was most often diagnosed in dogs – 250 cases (85.3%). At the same time, the number of otitis cases by aetiological factor amounted to: 174 – allergic, 51 – infectious bacterial, 12 – traumatic, 11 – infectious fungal, 2 – parasitic. In 43 cats, otitis was registered, which amounted to 14.7%.

At the same time, the number of otitis cases by aetiological factor amounted to: 21 – traumatic, 13 – allergic and 9 – parasitic. In the summer period, 222 cases of otitis were recorded. Otitis was most often registered in dogs – in 199 cases (89.6%). At the same time, the number of otitis cases by aetiological factor amounted to: 112 – allergic, 68 – infectious bacterial, 11 – parasitic, 4 – infectious fungal and 4 – traumatic. In cats the number of cases amounted to 23 (by aetiological factor: 7 – parasitic, 15 – traumatic and 2 – allergic). The results of the number of registered otitis cases in dogs and cats in the veterinary clinic "Aibolit" are presented in Figure 1.

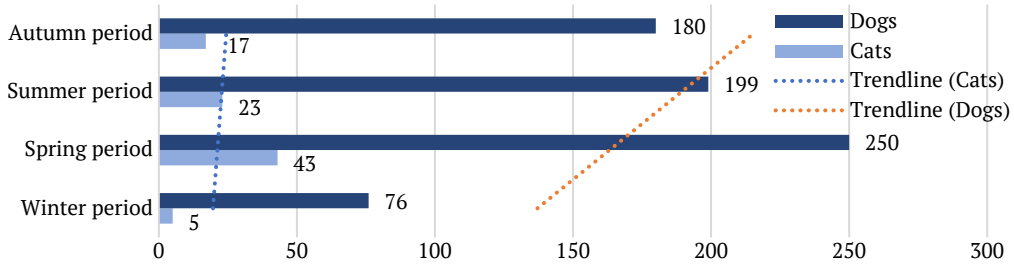

Figure 1. The number of registered cases of otitis in dogs and cats (individuals) in the veterinary clinic "Aibolit" (Poltava)

***Source**: authors' development*

In the autumn period, 197 cases were established, of which in dogs – 180 (91.4%). At the same time, the number of otitis cases by aetiological factor amounted to: 41 – allergic, 101 – infectious bacterial, 26 – infectious fungal, 11 – traumatic and 1 – parasitic. In cats 17 cases of otitis were registered, of which: 5 – parasitic, 7 – traumatic and 5 – allergic. The smallest number of cases was registered in the winter period – 81, of which in dogs – 76 cases (93.8%), of which: 58 – allergic, 28 – infectious bacterial and 4 – infectious fungal. In 5 (6.2%) cats, otitis was diagnosed, of which: in 1 – allergic and 4 – traumatic. The obtained data indicated a significant prevalence of otitis in dogs and most often the disease was registered in brachycephals (pugs, French and English bulldogs, Pekingese) – 368 cases (Fig. 2).

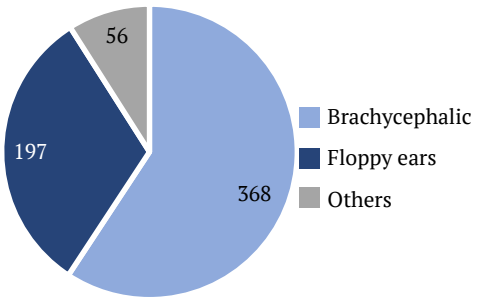

Figure 2. Quantitative ratio and breed predisposition of dogs (individuals) to the occurrence of otitis

***Source**: authors' development*

Similar results were also recorded in the veterinary clinic "MAXVET". In particular, during the study period 680 cases of otitis were registered, of which: in dogs – 609, which amounted

to 89.6%; in cats – 71, which corresponded to 10.4%. The research results of the number of otitis cases in dogs and cats depending on the season are presented in Figure 3.

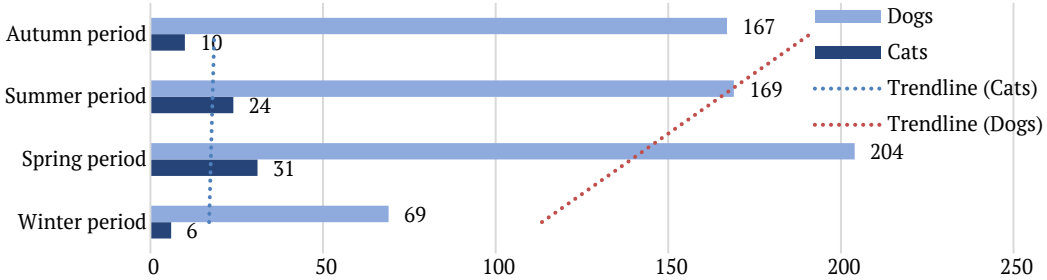

Figure 3. The number of registered cases of otitis in dogs and cats (individuals) in the veterinary clinic "MAXVET" (Poltava)

***Source**: authors' development*

The largest number of otitis cases was registered in the spring period – 235. Otitis was most often registered in dogs – 204 cases (86.8%), of which: 113 – allergic, 46 – infectious bacterial, 10 – infectious fungal, 25 – traumatic and 10 – parasitic. In cats, otitis was registered in 31 cases (13.2%), of which: 7 – parasitic, 16 – traumatic and 8 – allergic. In the winter period otitis was diagnosed in 75 cases. In dogs – 69 cases (92%), of which: 43 – allergic, 1 – infectious viral (complication after carnivorous plague), 15 – infectious bacterial, of which 8 were caused by staphylococci, 7 by streptococci; 2 – infectious fungal and 8 – traumatic. In cats 6 cases of otitis were registered (8%), of which: 4 – parasitic (otodectosis), 1 – allergic and 1 – traumatic.

In the summer period 193 cases of otitis were registered. In dogs – 169 (87.6%), of which: 101 – allergic, 33 – infectious bacterial, 5 – infectious fungal, 19 – traumatic and 11 – parasitic. In cats 24 cases of otitis were registered (12.4%), of which: 7 – parasitic, 15 – traumatic and 2 – allergic. In the autumn period 177 cases of otitis were diagnosed. In dogs – 167 (94.4%), of which: 45 – allergic, 69 – infectious bacterial, 46 – infectious fungal, 5 – traumatic and 2 – parasitic. In cats 10 cases of otitis were registered (5.6%), of which: 1 – parasitic, 5 – traumatic and 4 – allergic. The obtained data indicated a significant prevalence of otitis in dogs, most often it was registered in brachycephals – 404 cases (Fig. 4).

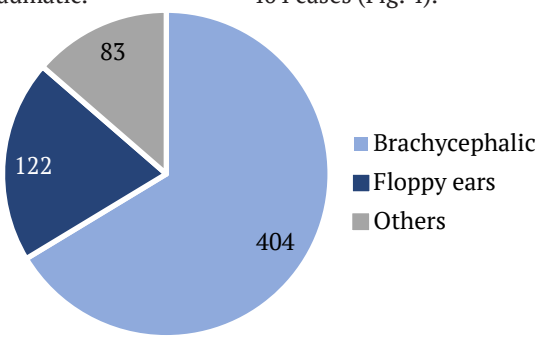

Figure 4. Quantitative ratio and breed predisposition of dogs (individuals) to the occurrence of otitis

***Source**: authors' development*

R. Schuenemann *et al.* (2022) indicated that the only breeds that suffered from chronic otitis were French bulldogs with a prevalence of 35/66 (53%) and pugs with a prevalence of 20/79 (25%). Similar results were described by D. Krainer & G. Dupré (2021), who found that otitis media was more common in French bulldogs than in pugs and was not related to the size of the nasopharynx. Clinical symptoms in dogs with otitis were characterised by a set of non-specific signs, discharges were rarely registered, which were recorded during a prolonged inflammatory process with the subsequent layering of secondary microflora. Cytological examination of ear deposits established the presence of lymphocytes and conglomerates of epithelial cells that had dystrophic features. Unlike bacteria and yeasts present in small numbers in healthy ears, leukocytes appeared in the external ear space exclusively during inflammation by exocytosis through damaged epithelium.

During the study, the diagnosis of otitis in dogs and cats in both clinics was carried out according to a set of non-specific symptoms, among which the most common were: redness and swelling of the external ear (Fig. 5A), constant itching and pain, which manifested as animal restlessness and constant scratching of the ears; formation of exudate, which was released, sometimes with an unpleasant odour. In more complex cases, inflammation of the middle and inner ear could develop, accompanied by the spread of infection, loss of balance, changes in animal behaviour. Diagnosis of these conditions required additional research methods (X-ray, magnetic resonance imaging or computed tomography) in order to assess the depth of the lesion. Carrying out cytological research was effective in all cases of otitis in dogs and cats. Cytological examination could detect and assess the presence of microorganisms, leukocyte cells (Fig. 5B) and mycelium in otomicoses. To detect microorganisms and assess the inflammatory response in the external ear, cytological research was used. Cytological examination of ear deposits in otitis established the presence of epitheliocytes, lymphocytes, sulphur, and dirt. Epitheliocytes (epidermal cells) were present in small numbers.

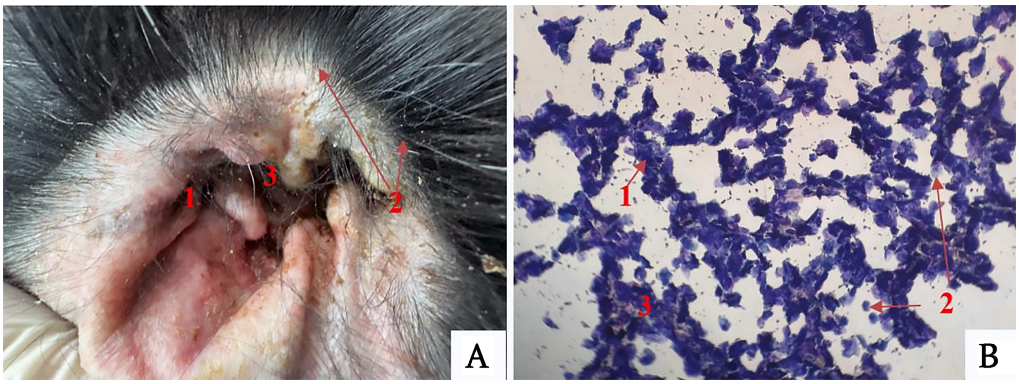

Figure 5. Examples of diagnostic examination results for the detection of otitis

***Note**: A – clinical case of otitis in a dog: 1 – hyperaemia of the skin of the external ear, 2 – discharge of serous exudate, 3 – accumulation of serous exudate and dirt in the lumen of the ear canal. B – cytology of serous exudate from the lumen of the ear canal: 1 – epitheliocytes, 2 – lymphocyte cells, 3 – dirt*

***Source**: photo by the authors*

Otodectosis invasion in cats was an extremely widespread acariasis invasion. *O. cynotis* was a common commensal of the skin microbiota of the external auditory canal of cats, and its impact on the body of cats was possible only with a decrease in the general resistance of the body and dysfunction of organs and systems. In the studied material (crusts and exudate taken from the ears of the studied animals), both mites at different stages of development and the eggs were detected (Figs. 6, 7).

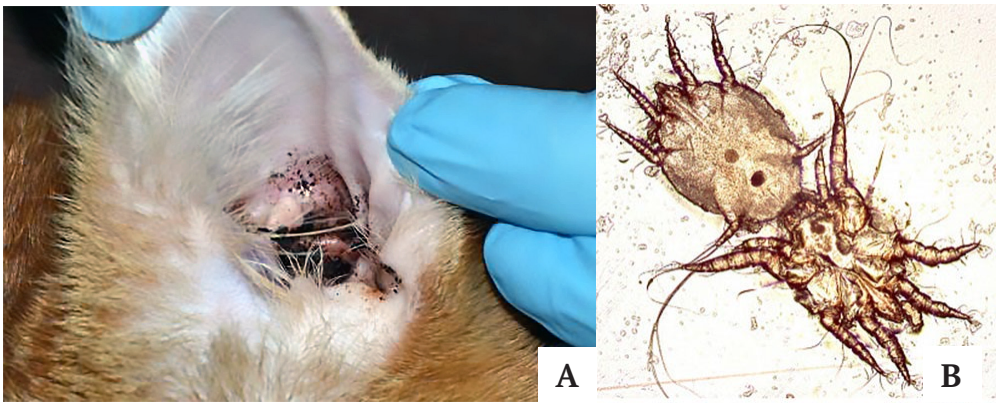

Figure 6. Clinical case of otodectosis in a cat and mite invasion of the genus Otodectes spp
***Note**: A – hyperaemia of the skin of the external ear and layering in the form of serous exudate and dirt in the lumen of the ear canal of the cat are shown; B – mite invasion Otodectes spp., which was detected using microscopy*
***Source**: photo by the authors*

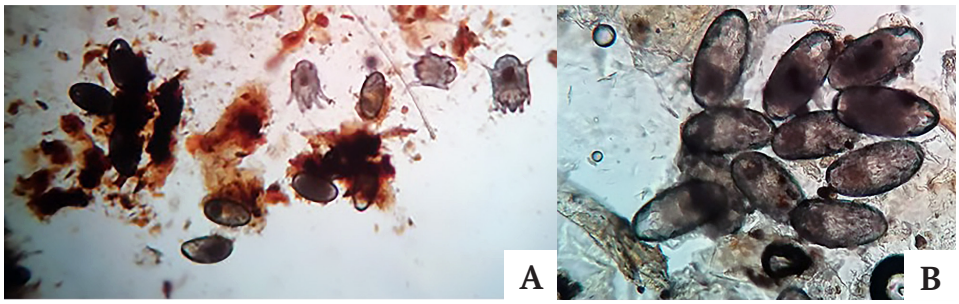

Figure 7. Otodectes cynotis mites at different stages of development
***Note**: A – Otodectes cynotis mites at different stages of development and mite eggs in a sample taken from a cat's ear, ×40; B – Otodectes cynotis mite eggs in a sample taken from a cat's ear, ×100*
***Source**: photo by the authors*

From Figures 6 and 7, it was seen that the pathogen had a flat oval-shaped body, its size varied from 0.6 to 0.7 mm. In the material taken from the sick animal, mites and eggs were found both as single specimens and in large numbers. Considering the localisation

site of the detected mites, as well as the features of the morphological structure of the body, the final diagnosis was established – feline otodectosis. In urban areas, the most common habitat of cats was flats or private houses, as well as streets and yards. Analysing the level of infestation of animals depending on the keeping conditions, it was found that animals kept in private houses or flats without access to the yard were affected the least.

During the study period in veterinary clinics of Poltava, the highest prevalence of otitis among dogs was noted. In the veterinary clinic "Aybolit" 793 cases of otitis were established, of which in dogs – 705, which accounted for 88.9%, in cats – 88, which corresponded to 11.1%. In the veterinary clinic "MAXVET" 680 cases of otitis were registered, among which in dogs 609 cases were recorded, which accounted for 89.6%, in cats – 71 cases, which accounted for 10.4%. A natural predisposition was found in brachycephalic breeds – pugs, bulldogs, and Pekingese. It was established that cats of different breeds turned out to be prone to otodectosis invasion. It was worth noting that the level of infestation in different cat breeds was unequal. In particular, acarological studies proved that crossbreeds were the most susceptible to *O. cynotis* mite infestation. In addition, it was established that such breeds as Scottish Fold, Siamese and Persian cats were quite susceptible to otodectes infestation. The least affected were Burmese, Maine Coon and Bengal cats.

According to the studies carried out on haemoglobin content, the number of erythrocytes and the mean haemoglobin content in the erythrocyte (MCH), it was possible to determine the general state of the organism and the course of pathological changes that occurred in different forms of otitis, as well as to detect latent changes in the organism, the degree of damage, severity of the disease course and existing complications. The research results were presented in Table 1.

Table 1. Indicators of erythropoiesis in the blood of dogs with otitis of different forms

Indicator	Reference data according to J.W. Harvey (2001	Control group, n = 5	Sick dogs (acute otitis), n = 5	Sick dogs (chronic form of otitis), n = 5
Hemoglobin, g/L	124.0-169.0	148.34 ± 3.67	95.63 ± 1.68**	97.61 ± 5.85**
Erythrocytes, 1012/L	5.2-8.8	6.98 ± 0.27	3.73 ± 0.13***	4.21 ± 0.23***
Mean haemoglobin content in erythrocyte (MCH), pg	19.0-25.6	21.82 ± 0.68	24.80 ± 0.44**	23.45 ± 0.82*

Note**: the difference between groups of clinically healthy and sick dogs was reliable at P < 0.05*, P < 0.01**, P < 0.001, compared with the values of the control group*
***Source**: authors' development*

The comparative assessment of erythropoiesis indicators in clinically healthy dogs and in dogs with otitis showed that in dogs with the acute form of otitis, haemoglobin levels decreased by 35.5% (P < 0.01), and with chronic course – by 34.2% (P < 0.01) compared with the control group. This indicated the development of anaemic syndrome against the background of the inflammatory process, which was especially pronounced in the acute stage of the disease. The number of erythrocytes decreased by 46.6% (P < 0.001) in acute otitis and by 39.7% (P < 0.001) in chronic otitis compared with the control group. Such a pronounced erythrocytopenia might have been a consequence of both suppression of erythropoiesis and increased destruction of erythrocytes or blood loss. In the acute course of otitis, the mean haemoglobin

content in the erythrocyte (MCH) on the contrary increased by 13.7% (P < 0.01), and in chronic – by 7.5% (P < 0.01) compared with the control group. This might have indicated compensatory activation of the megaloblastic type of erythropoiesis or a macrocytic nature of anaemia. The obtained results indicated a pronounced anaemic reaction of the dogs' organism to the development of otitis, which was manifested by a decrease in haemoglobin content and erythrocyte count. The increase in MCH could be regarded as a compensatory reaction aimed at maintaining oxygen transport against the background of a reduced number of erythrocytes. The research results indicated that in dogs with otitis, especially with acute course, significant disturbances of the erythropoiesis system were observed. The established decrease in haemoglobin content and erythrocyte count with simultaneous increase in MCH indicated the development of anaemia with signs of compensation. The obtained results could be used as additional criteria for assessing the severity of the inflammatory process and the effectiveness of treatment of sick animals. The clinical condition analysis of dogs with otitis also envisaged the study of the leukocyte profile of the blood. The obtained results are presented in Table 2.

Table 2. Indicators of leukocyte profile in the blood of dogs with otitis of different courses

Indicator		Reference data according to J.W. Harvey (2001)	Control group of animals, n = 15	Sick dogs (acute otitis), n = 5	Sick dogs (chronic form of otitis) (n = 5)
Leukocytes, 109/L		5.9-13.4	8.24 ± 0.55	13.39 ± 0.25 ***	13.04 ± 0.49***
Basophils, %		0.0-1.0	0.4 4 ± 0.13	0.82 ± 0.06	0.35 ± 0.04
Eosinophils, %		3.0-4.0	3.4 1 ± 0.13	4.26 ± 0.20***	2.58 ± 0.43***
Neutrophils, %	Juvenile	0.0-1.0	0.2 6 ± 0.09	0.44 ± 0.17	0.17 ± 0.12
	Band	2.0-5.0	3. 29 ± 0.24	0.54 ± 0.17	3.18 ± 0.16
	Segmented	46.0-57.0	47.25 ± 1.05	56.14 ± 0.46***	45.43 ± 0.16***
Lymphocytes, %		32.0-45.0	34.43 ± 1.13	22.48 ± 1.14**	34.21 ± 1.98
Monocytes, %		2.0-3.0	2.6 8 ± 0.13	1.93 ± 0.22	1.04 ± 0.15

Note**: the difference between groups of clinically healthy and sick dogs was reliable at P < 0.05*, P < 0.01**, P < 0.001, compared with the control group*
***Source**: authors' development*

As a result, it was noted that the number of leukocytes in the blood of dogs with acute otitis increased by 62.5% (P < 0.001), and with chronic course – by 58.3% (P < 0.001) compared with the control group. These data indicated an active inflammatory reaction of the organism, typical for both forms of the disease. In the case of acute otitis, the number of basophils in the blood increased by 1.9 times, whereas with chronic course – it decreased by 1.3 times, but remained within the physiological range. This indicated a minimal contribution of basophil cells to the pathogenesis of otitis. The number of eosinophils in acute otitis increased 1.3 times (P < 0.001) compared with the control group, which might have indicated increased sensitivity or the presence of an allergic component in the course of the inflammatory process. In contrast, with chronic otitis, there was a decrease of 1.3 times, which was probably associated with a decrease in the intensity of the immune response. In the quantitative changes of segmented neutrophils, a divergent dynamic was noted: in acute otitis the content increased 1.2 times (P < 0.001) compared with the control group of dogs, which indicated

an active phagocytic response. In the case of chronic process, on the contrary, there was a slight decrease ($P < 0.001$), which might have been a sign of adaptation or immune exhaustion. In the case of lymphocytes in acute otitis in dogs, a decrease in the number by 1.5 times ($P < 0.01$) compared with the control group was recorded, which might have indicated redistribution of the cell pool in favour of neutrophils in the acute phase of inflammation. In the case of chronic otitis, the number of lymphocytes in the blood of sick dogs remained practically unchanged. The number of monocytes also decreased, but no statistically significant difference was observed. This might have indicated reduced activity of the monocyte-macrophage system in long-term or recurrent inflammation.

Thus, the obtained data indicated that in dogs with otitis, especially in acute form, pronounced changes in the leukogram were observed, which reflected functional tension of the immune system in response to inflammation. The main markers of the acute phase of otitis were leukocytosis, neutrophilia, eosinophilia and lymphopenia. In chronic process, less pronounced changes were observed, but a characteristic gradual decrease in the number of monocytes and eosinophils in the blood was revealed, which indicated transformation of the inflammatory process into a less active phase. The described results could be used as diagnostic criteria for assessing the stage and dynamics of the disease.

The established regularities indicated the presence of an inflammatory type of leukocyte reaction in dogs with otitis, which manifested as pronounced leukocytosis, neutrophilia, lymphopenia and changes in the number of eosinophils and monocytes. In particular, in acute otitis the total number of leukocytes increased, which corresponded to the classical picture of the acute phase of inflammation. These changes, as noted by R. Schuenemann *et al.* (2022), might have been the result of activation of the immune system, in particular stimulation of myelopoiesis under the influence of pro-inflammatory cytokines (IL-1β, TNF-α), which increased the release of neutrophils from the bone marrow. Dominant neutrophilia, which was observed in acute otitis, was a typical manifestation of the infectious–inflammatory process in dogs. It ensured a phagocytic response and was an early marker of bacterial infection. Lymphopenia, registered in animals with acute otitis, was often a component of stress-leukogram, caused by endogenous secretion of glucocorticoids in response to pain or systemic inflammation. Whereas in chronic otitis, the number of lymphocytes remained stable, which indicated a decrease in the activity of the immune response or the development of immunological tolerance.

As noted by T. Nuttall (2023), an increase in the number of eosinophils in the blood during otitis in dogs might have indicated the involvement of hypersensitive or allergic reactions in the pathogenesis of the disease, particularly against the background of parasitic infection or mycotic invasion. A decrease in the number of eosinophils in the case of chronic course of the disease was probably associated with exhaustion of the immune response or suppression of the eosinophil pool. A significant decrease in the number of monocytes in chronic otitis reflected prolonged load on the monocyte–macrophage system, depletion of reserve or transition of the inflammatory process into a less active phase. Thus, the obtained haematological indicators corresponded to the modern understanding of the pathophysiology of otitis in dogs. Leukocytosis with neutrophilia and lymphopenia were informative criteria of the acute phase of inflammation, whereas chronic process was accompanied by less pronounced but stable changes in the leukogram. Special attention should have been paid to the

assessment of eosinophils, since an increase in the number might have been a sign of additional allergic or parasitic component, which had important therapeutic significance.

The results of the conducted study confirmed the high prevalence of otitis in dogs (88.9%-89.6%) compared with cats (11%-14%). Most often otitis was recorded in spring, which was associated with seasonal exacerbation of allergies, increased parasite activity, as well as changes in climatic conditions, which might have contributed to the accumulation of pathogenic microorganisms in the lumen of the ear canal. In dogs, allergic and infectious-bacterial forms of otitis predominated. In cats, traumatic and parasitic forms were most often diagnosed, in particular otodectosis (*Otodectes cynotis*), which was consistent with the data of B. Mosallanej *et al.* (2011). A natural predisposition to otitis was established in brachycephalic breeds (pugs, bulldogs, Pekingese), which was associated with the anatomical features of the auditory canals, and the obtained data coincided with the studies of L.N. Gotthelf (2004).

Cytological examination of the ear content proved its informativeness in establishing the aetiology of otitis. Microscopy itself, as emphasised by R.S. Rosales *et al*. (2024), was an important primary diagnostic stage, which made it possible to identify inflammatory cells, fungal structures, parasites or bacterial microflora. An important complicating factor in treatment was antibiotic resistance of pathogens, as revealed by G. Borriello *et al*. (2020). Cases caused by *Pseudomonas aeruginosa* required special attention, as this bacterium was able to form biofilms, which significantly reduced the effectiveness of antibiotic therapy (Tanveer *et al*., 2024).

Compared with the clinical data obtained in this study, which confirmed a high level of leukocytosis, neutrophilia, eosinophilia in acute forms of otitis and the development of anaemic syndrome (reduction of Hb, RBC), the results of C. Lecchi *et al.* (2021) indicated an increased number of pro-inflammatory cytokines (IL-1β, TNF-α). It was also important to take into account the data of S. Hobi *et al.* (2024), which indicated a disturbance of the normal bacterial composition of microorganisms as a potential therapeutic feature, especially in recurrent forms of otitis. J.W. Harvey (2001) described in detail the diagnostic and therapeutic strategies, which fully coincided with the approaches implemented in veterinary clinics of Poltava: cytological diagnostics, antibiotic sensitivity testing, local and systemic therapy depending on the form and aetiology of the disease. In addition, the results of the present study were consistent with the data of L.S. Jacobson (2002), who pointed out the importance of considering morphological changes in the chronic course of the disease, as well as with E. Milne *et al.* (2020), who described the use of antibiotic therapy effective against resistant strains of microorganisms. Prevention of chronic otitis was possible only under conditions of comprehensive veterinary supervision, as indicated by M.B. Mascarenhas (2022).

Thus, otitis was a common disease among dogs and cats, especially in the spring period, with a predominance of cases among mixed-breed dogs, pugs, French bulldogs and labradors. The most frequent causes were mechanical injuries, parasitic infestations and complications of infections, and the symptoms were itching, pain, exudate and redness of the auricle. Acute otitis was accompanied by anaemia and pronounced leucocytosis, whereas in the chronic course a moderate decrease in haemoglobin and a persistent increase in the level of leucocytes were noted.

Conclusions

The obtained data indicated a significant prevalence of otitis among dogs, especially in the spring period. In the veterinary clinic “Aibolit”

793 cases of otitis were identified, of which 705 were dogs, which amounted to 88.9%, and 88 were cats, which amounted to 11.1%. In the veterinary clinic "MAXVET" 680 cases of otitis were identified, of which 609 were dogs, which corresponded to 89.6%, and 71 were cats, which amounted to 10.4%. Otitis was registered in different breeds, but it was most often observed in mixed breeds, pugs, French bulldogs, Pekingese, Labradors, Clumber spaniels, Jack Russell terriers, Chihuahuas, Spitz, German shepherds and Cane Corso. It was established that cats of different breeds were prone to otodectosis invasion. It was worth noting that the level of invasion in different cat breeds was not the same; it was proved that the most susceptible were cats: crossbreeds, Scottish Fold, Siamese and Persian. The least vulnerable breeds were Burmese, Maine Coon and Bengal cats. The most frequent aetiological factors of otitis media were: mechanical injuries, parasitic invasions and complications of infectious diseases. Diagnosis of otitis media in dogs and cats was carried out on the basis of a set of non-specific symptoms, among which the most common were: redness and swelling of the external ear, constant itching and pain, manifested by animal restlessness, constant scratching of the ear, exudate formation, sometimes with an unpleasant odour. Cytological examination of ear deposits in otitis media showed the presence of some epithelial cells, lymphocytes, sulphur, and dirt. Inclusions of melanin granules were found in the cytoplasm of keratinocytes. Haematological indicators in the acute course of otitis were characterised by a decrease in haemoglobin level by 35.5% and the development of anaemia. In addition, an increase in the blood of the number of leukocytes by 65.2%, segmented neutrophils by 1.2 times, basophils by 1.9 times and eosinophils by 1.3 times was found, which was caused by the acute inflammatory process against the background of the underlying disease. The chronic course of otitis was characterised by a decrease in blood haemoglobin content by 34.2% and an increase in the number of leukocytes by 58.3%, which was caused by the prolonged course of the inflammatory process.

In-depth study of the aetiological factors of otitis in dogs and cats, taking into account breed, age and seasonal features, remained a relevant and promising area of veterinary medicine. Further research had to be aimed at improving methods of early diagnosis, in particular non-invasive laboratory and visualisation approaches, as well as at a comprehensive assessment of immunological, haematological and cytological changes in various forms of otitis. This made it possible to improve the personalised approach to the treatment and prevention of the disease, taking into account the individual characteristics of animals.

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Conflict of Interest

None.

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Поширеність та діагностичні особливості зовнішніх отитів у собак та котів в умовах ветеринарних клінік м. Полтава

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Анотація. Актуальність проведених досліджень зумовлена поширенням отитів різної етіології серед собак та котів. Метою цієї роботи було дослідити поширеність зовнішніх отитів у дрібних тварин, визначити клінічні симптоми отитів в залежності від різних форм перебігу для покращення діагностики захворювання. Для досягнення поставленої мети проводились гематологічні та мікроскопічні види досліджень. Цитологічним дослідженням мазків відбитків встановлено наявність епітеліоцитів, лімфоцитів, сірки та бруду, подекуди виявлено отодектозну інвазію. Діагностику інфекційних хвороб здійснювали за допомогою імунохроматографічних методів, де отит був супутнім захворюванням. Встановлено лейкоцитоз, нейтрофілію та лімфопенією, що розвивались під час гострої фази запалення, тоді як хронічний процес супроводжувався менш вираженими змінами в лейкограмі. Моніторингове дослідження інтенсивності поширення отитів серед собак та котів проводилось в місті Полтава у період з березня 2024 року до лютого 2025 року. Отримані дані свідчать про значну поширеність цієї патології серед собак у весняний період. У ветеринарній клініці «Айболить» встановлено 793 випадки отиту, з них у собак – 705, що становило 88,9 %, а у котів – 88, що відповідало 11,1 %. У ветеринарній клініці «MAXVET» зафіксовано 680 випадків отиту, серед яких у собак зареєстровано 609, що становило 89,6 %, а у котів – 71, що складало 10,4 %. Виявлено породну схильність до розвитку отиту в брахіцефальних порід. Встановлено найпоширеніші етіологічні чинники отитів: механічні пошкодження, паразитарні інвазії та ускладнення внаслідок інфекційних захворювань. Клінічні симптоми проявлялися локальним почервонінням, набряком зовнішнього вуха, свербіжем та больовими ознаками, що супроводжувалось занепокоєнням тварин і постійним розчухуванням вух та утворенням ексудату. Практична цінність отриманих результатів полягає у виявленні закономірностей поширення та етіологічних чинників отитів, що розширює можливості їх діагностування

Ключові слова: етіологія; хвороби вух; цитологія; гематологія; отодектоз

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Nutritional effects of calcium and phosphorus on chronic kidney disease in cats: A literature review

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Abstract. The relevance of this study is conditioned by the significant prevalence of chronic kidney disease in cats and the prominent role of calcium-phosphorus metabolism disorders in its progression and the development of complications, which requires further scientific attention to improve approaches to animal treatment. The purpose of this review was to summarise modern scientific data on the influence of calcium and phosphorus on the course of chronic kidney disease in cats and to formulate recommendations for nutritional support. A systematic approach was employed to search and analyse scientific literature for 2020-2025. The review analysed the results of modern studies on the diagnostics of chronic nephropathy in small carnivores, clinical recommendations, and expert opinions in the field of veterinary medicine. The study found that the excess phosphorus content in the diet of animals directly correlates with the acceleration of disease progression, the development of secondary hyperparathyroidism, soft tissue calcification, and deterioration of the functional state of the kidneys. At the same time, excessive decrease in phosphorus concentration can cause hypercalcemia, loss of appetite, vomiting, and other metabolic disorders. The optimal diet for animals with chronic kidney disease is a calcium to phosphorus ratio within the range of 1.1:1-1.5:1. The study considered role of modern biomarkers, such as symmetrical dimethylarginine

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and fibroblast growth factor-23, in the early diagnosis and monitoring of chronic kidney disease. The study also analysed the efficacy of dietary therapy, particularly the use of phosphate binders, active forms of vitamin D, probiotics, antioxidants, and agents for the correction of acid-base homeostasis. A review of the literature confirmed that efficacious nutritional management is a key factor in the control of chronic kidney disease. The findings obtained emphasised the significance of developing optimal dietary strategies that account for both the phosphorus level and the calcium to phosphorus ratio in the blood serum. This will inhibit the progression of the disease and improve the quality of life of cats with chronic kidney disease

Keywords: mineral homeostasis; veterinary dietetics; electrolyte metabolism; metabolic disorders; hypercalcemia; hyperphosphatemia; phosphate binders

Introduction

Chronic kidney disease is one of the most widespread pathologies in cats, especially among older animals. It substantially reduces the quality of life, as it is accompanied by a gradual and irreversible loss of kidney function. According to R.C.C. Costa *et al*. (2024), the disease can occur due to a variety of causes, including genetic predisposition, past infections, or toxicity. The kidneys play a key role in maintaining homeostasis: eliminating metabolic products, regulating electrolyte balance, and fluid volume. If these functions are insufficient, toxic substances accumulate in the blood, which adversely affects all body systems.

One of the most effective tools for managing chronic kidney disease (CKD) is dietary therapy, which is aimed at reducing the metabolic load on the kidneys. According to V.J. Parker (2021), controlling phosphorus, protein, and calcium levels in the diet is key to slowing disease progression, reducing the risk of mineral disorders, and improving the overall condition of the animal. Diets moderately reduced in phosphorus and protein, supplemented with essential nutrients, can reduce uraemia, stabilise parathyroid hormone levels, and improve quality of life. Although protein restriction is a classic component of dietary therapy for chronic kidney disease, recent literature reviews in humans suggest that too severe protein reduction (<0.6 g/kg/day) may not have an additional nephroprotective effect and increases the risk of protein-energy deficiency, especially in frail patients. According to J.W. Kim & S.J. Yang (2025), excessive protein restriction also contributes to the development of sarcopenia – loss of muscle mass and functional activity, which is especially dangerous for elderly patients. This emphasises the need for a balanced and individualised approach to protein correction in the diet, which is likely to be relevant for cats with CKD, considering the similarity of pathophysiological processes and sensitivity to muscle loss in older animals.

One of the most prominent aspects of the pathogenesis of chronic kidney disease is the disturbance of calcium and phosphorus metabolism. Under physiological conditions, these minerals ensure the strength and integrity of bone tissue. However, cats with this pathology often develop calcium and phosphorus imbalances, which, according to S.-H. Tsai *et al*. (2024), contributes to the development of secondary hyperparathyroidism, bone resorption, soft tissue mineralisation, and even the development of cardiovascular complications. The level of phosphorus in the diet plays a

crucial role in maintaining kidney health in cats. An excess of this mineral is associated with an increased risk of structural damage to renal tissue and accelerated disease progression. Apart from the phosphorus content, a significant factor is its ratio with calcium.

Olad J. Stockman (2024) highlighted modern aspects of nutritional therapy for chronic kidney disease in cats, particularly the effects of phosphorus and the calcium to phosphorus ratio on disease progression. The researcher noted that an imbalance between these elements may contribute to the development of mineral disorders, although their exact impact on clinical outcomes is still understudied. Stockman also emphasised the variability of phosphorus bioavailability in diets, the potential risks of excessive restriction, and the lack of clear recommendations for target intakes. Previous studies showed that specialised diets with controlled phosphorus content can be an effective component of therapy, helping to reduce clinical manifestations of the disease and maintain patients' stable condition. The researcher emphasised the significance of a comprehensive nutritional approach that includes not only phosphorus regulation, but also a balanced ratio of protein and other macronutrients to prevent secondary disorders such as hypoproteinaemia or hypophosphataemia.

According to W. Zhang *et al*. (2024), a high dietary calcium to phosphorus ratio is associated with a greater prevalence of nephroliths, which can cause ureteral obstruction and create risks for urinary infections, which worsens the development of kidney disease. Although these values may vary between cats, focusing on calcium/phosphorus ratios rather than absolute concentrations appears reasonable for the prevention of mineral disorders, particularly in patients with chronic kidney disease. This emphasises the expediency of dietary adjustment regarding the calcium to phosphorus ratio as one of the key nutritional parameters in the treatment of CKD.

R.F. Geddes *et al*. (2021) also noted that excessive phosphorus restriction can lead to hypercalcaemia, especially in the early stages of renal dysfunction. The researchers found that mitigating phosphorus restriction in cats with stage 2 kidney disease helped to normalise ionised calcium levels, suggesting the need for a cautious approach to dietary correction.

Considering the significance of improving approaches to the treatment of chronic kidney disease in cats, the purpose of the current study was to analyse current research on the role of calcium and phosphorus in the progression of CKD to develop recommendations for optimal dietary nutrition of animals. For this, the objectives were to scientifically substantiate the role of calcium and phosphorus in the pathogenesis of chronic kidney disease in cats, to analyse the efficacy of dietary therapy and means of correcting mineral imbalances, and to identify diagnostic approaches to monitor the state of mineral metabolism to optimise therapeutic tactics and improve the quality of life of animals.

To fulfil this purpose, a systematic literature review was applied, the search for which was conducted through the scientific databases PubMed, Scopus, and Web of Science. The initial search yielded over 100 publications, of which 41 scientific sources were selected for analysis after filtering by inclusion criteria. The inclusion criteria included publications in peer-reviewed journals, studies of the effect of calcium and phosphorus on the functional state of the kidneys in cats, availability of empirical data, or a detailed review of the results of clinical application of dietary approaches. The results of clinical and experimental studies, official veterinary recommendations, and expert opinions on metabolic changes in chronic nephropathy in cats were analysed. Particular

attention was paid to biomarkers of early diagnosis, mechanisms of metabolic disorders, and the efficacy of nutraceutical and pharmacological approaches to correcting phosphorus-calcium imbalance.

Mineral metabolism disorders in chronic kidney disease

In cats, the course of chronic kidney disease is accompanied by a disturbance in mineral metabolism, particularly calcium and phosphorus levels, which affects its progression. New studies confirmed that these changes can substantially affect the further development of the disease and the quality of life of animals. D.J. Chew (2022) noted that in cats with chronic kidney disease, the mechanism of phosphorus excretion through the kidneys is impaired, which causes its accumulation in the blood. This can lead to the development of hyperphosphatemia, which is a trigger for secondary hyperparathyroidism. Dietary therapy is the key method of inhibiting the progression of chronic kidney disease in cats. V.J. Parker (2021) indicated that key aspects of dietary restrictions include controlling phosphorus levels, balancing protein, regulating sodium intake, maintaining acid-base homeostasis, and correcting essential nutrient deficiencies.

C.J. Vorland *et al.* (2020) investigated the effects of chronic kidney disease progression on intestinal phosphorus absorption using rats, which have become a model for chronic kidney disease and mineral metabolism disorders. The researchers found that, despite the deterioration of kidney function, disease progression does not reduce the efficiency of phosphorus absorption in the intestine. This study provided new insights into the role of the gut in regulating phosphorus levels when suffering from CKD, as it was previously thought that renal dysfunction directly reduced the body's ability to absorb phosphorus.

D.E. Jewell *et al.* (2022) studied metabolic changes in cats with chronic kidney disease and calcium-oxalate uroliths, finding marked changes in metabolic processes that are essential for the development of these diseases. Their findings highlighted that the impaired calcium and phosphorus metabolism that often accompanies CKD may be associated with metabolic changes that contribute to the development of uroliths. The study also pointed to the significance of metabolic analysis as a tool for understanding the pathophysiology of kidney disease in cats. Specifically, it enables only a more accurate diagnosis of mineral metabolism disorders, but also the creation of personalised treatment and prevention strategies that include the correction of metabolic changes in these patients.

J. Stockman (2024) indicated that elevated blood phosphorus levels are associated with faster progression of chronic kidney disease in cats. This is conditioned by the fact that hyperphosphatemia stimulates the production of parathyroid hormone, which causes bone resorption, increasing calcium mobilisation. Long-term exposure to this mechanism can lead to calcification of kidney and cardiovascular tissues. J. Lin *et al.* (2021) noted that early monitoring of calcium and phosphorus levels can predict the risk of complications in cats with chronic kidney disease. The researchers found that cats with a high phosphorus to calcium ratio had a significantly shorter life expectancy compared to those with balanced levels in the early stages of the disease. Assessment of calcium and phosphorus levels is a crucial element of the diagnosis and monitoring of chronic kidney disease in cats, as their imbalance affects the progression of the disease and the effectiveness of dietary correction. P.-K. Tang *et al.* (2021) noted that monitoring these parameters helps to assess the risk of renal calcification or secondary hyperparathyroidism.

Diagnostics of chronic kidney disease in cats

Serum biochemical parameters such as creatinine, urea, and symmetrical dimethylarginine are used to determine the stage of chronic kidney disease and assess the functional state of the kidneys. According to J.A. Hokamp & M.B. Nabity (2016), SDMA is a more sensitive biomarker than creatinine, particularly in animals with low muscle mass, where creatinine levels may stay within normal limits. The combined use of SDMA and creatinine enables a more precise assessment of glomerular filtration rate, especially in the early stages of CKD. This approach provides greater diagnostic accuracy by compensating for the limitations of each marker when used in isolation.

Another element of the diagnostics is urine analysis. V. Pedrinelli *et al.* (2020) emphasised the significance of assessing urine specific gravity and proteinuria. A decrease in the concentration capacity of urine indicates tubular damage, while the presence of protein in the urine indicates glomerular damage. Proteinuria is an independent predictor of the progression of chronic kidney disease and is associated with a shorter life expectancy. R.C.C. Costa *et al.* (2024) noted that basic diagnosis includes determination of urine specific gravity, serum creatinine and SDMA levels. Creatinine continues to be a standard marker of renal function, but it does not always enable early detection of pathology due to its dependence on muscle mass. In this context, SDMA is a more sensitive indicator, because its increase may precede the increase in creatinine. This was confirmed by L. Hahn & C. Callaband (2022), who demonstrated that SDMA can diagnose chronic kidney disease in the preclinical phase and has a high prognostic value. Specifically, the level of ionised calcium in the blood serum is a more accurate indicator of the body's supply of this mineral than the total calcium level. R.F. Geddes *et al.* (2021) emphasised the significance of the ionised fraction in assessing mineral metabolism disorders, especially in the setting of reduced albumin levels, which often accompany renal pathology. In their study, the researchers emphasised that monitoring the level of active calcium enables a more accurate interpretation of the clinical situation and the selection of suitable correction tactics.

Laboratory analysis of urine, particularly the determination of specific gravity and protein-creatinine ratio, helps to identify early signs of proteinuria and reduce the concentration capacity of the kidneys. Furthermore, L. Hahn & C. Callaband (2022) emphasised that a comprehensive urine assessment together with renal ultrasound allows not only detecting early functional disorders but also visualising structural changes in tissues. Specifically, urine analysis, including the determination of specific gravity, protein-creatinine ratio and sediment, allows assessing the concentration capacity of the kidneys and the degree of proteinuria, which are vital indicators of glomerular damage. Ultrasound diagnostics of the kidneys allows detecting morphological changes such as increased parenchymal echogenicity, reduced organ size, mineralisation, or cystic formations. Particularly significant is the detection of calcification, which may indicate a long-term disorder of mineral metabolism and the progression of a chronic process.

Another promising method for assessing renal function is renoscintigraphy, a radioisotope test that provides information on perfusion, filtration capacity, and symmetry of the kidneys. This method is based on the injection of a radiopharmaceutical followed by recording its uptake, transport, and excretion by the kidneys using a gamma camera. Renoscintigraphy enables a separate assessment of the functional activity of each kidney, which is particularly valuable in cases of asymmetric lesions.

It also helps to differentiate between a decrease in glomerular filtration rate and obstructive changes or vascular disorders. Despite its limited availability in routine veterinary practice, it is considered to be extremely informative and promising, especially in specialised centres or research institutions. Thus, the combination of general clinical, visual, and functional examination methods, such as urinalysis, ultrasound, and renoscintigraphy, provides a multidimensional assessment of the functional state of the kidneys and enables the prompt detection of both structural and functional changes, which is critical for choosing the optimal tactics in the treatment of animals.

Role of FGF-23 in the diagnosis of chronic kidney disease and mineral metabolism disorders in cats

Apart from conventional biomarkers, recent studies have pointed to the significance of fibroblast growth factor-23 in the pathogenesis of chronic kidney disease. This hormone, which is produced by osteocytes and osteoblasts, ins instrumental in the regulation of phosphate homeostasis and vitamin D metabolism. Its level is significantly increased in chronic kidney disease, which can have both compensatory and pathological effects on the animal's body. S. Seiler *et al.* (2009) found that in cats with chronic kidney disease, FGF-23 levels increase markedly, especially in the later stages of the disease. This is because the kidneys lose their ability to effectively excrete phosphorus, and the body tries to compensate by increasing the secretion of FGF-23. However, this compensation mechanism can have negative consequences:

♦ inhibition of calcitriol: FGF-23 blocks the synthesis of active vitamin D, which reduces intestinal calcium absorption;

♦ exacerbation of secondary hyperparathyroidism: calcitriol deficiency stimulates the production of parathyroid hormone, which can lead to osteodystrophy and soft tissue calcification;

♦ accelerating the progression of chronic kidney disease: elevated levels of FGF-23 correlate with a decrease in glomerular filtration rate, which means a rapid deterioration in kidney function.

J. Lin *et al.* (2021) showed that FGF-23 levels can be used as a predictor of survival in cats with chronic kidney disease. Cats with elevated levels of this hormone have a significantly worse prognosis, as it indicates a critical disruption of phosphate balance and a systemic inflammatory response. Monitoring of FGF-23 levels in patients with chronic kidney disease can be useful for: early detection of phosphorus imbalance before clinical signs of hyperphosphatemia appear; evaluation of the effectiveness of dietary therapy (a decrease in FGF-23 levels after the introduction of a low-phosphorus diet or phosphate binders may indicate improvement in kidney health); and prediction of complications (high FGF-23 levels may signal the need for more aggressive phosphorus control). This study showed that the use of low-phosphorus diets and phosphatebinders can help reduce FGF-23 levels in cats with chronic kidney disease. This confirms the hypothesis that FGF-23 is a sensitive indicator of phosphorus load.

L. Rodríguez-Osorio *et al.* (2015) found that the administration of phosphate binders such as sevelamer significantly reduced FGF-23 levels, which helps to reduce the manifestations of secondary hyperparathyroidism. This highlights the value of phosphorus control as a key factor in the management of chronic kidney disease. The researchers emphasised the role of phosphorus control at all stages of CKD as a key element of treatment. However, FGF-23 is not a routine biochemical marker available for widespread use in clinical practice. Its determination requires specialised laboratory methods, which may limit its use in veterinary medicine.

Nevertheless, further research in the field of nephrology may help to expand the possibilities of using FGF-23 as part of a comprehensive approach to the diagnostics and treatment of chronic kidney disease in cats.

Ca:P balance – clinical significance of the ratio and dietary impact

Nutrition plays a key role in the management of CKD in dogs and cats. A properly formulated diet can not only stabilise mineral metabolism but also reduce the burden on the kidneys, which helps to inhibit the progression of the disease. Specifically, controlling the content of phosphorus and calcium in the diet helps to reduce the risk of developing hyperphosphatemia and hypercalcaemia, factors that can worsen kidney function and the general condition of the animal. According to L.A. Nagode *et al.* (1996), increased phosphorus concentrations promote the development of calcium-phosphate complexes that are deposited in tissues, particularly in the kidneys, which accelerates their damage. Controlling phosphorus levels is an essential part of the treatment of chronic kidney disease in cats. However, dietary phosphorus restriction requires careful monitoring of blood calcium levels to prevent hypercalcaemia. According to scientists, a significant reduction in phosphorus levels reduces the stimulation of parathyroid hormone secretion, which can lead to increased intestinal calcium absorption under the influence of activated vitamin D (calcitriol). As a result, blood calcium levels may increase, which potentially contributes to calcification of tissues, including renal parenchyma.

P.-K. Tang *et al.* (2021) noted that phosphorus plays a key role in the pathogenesis of CKD, affecting both renal haemodynamics and systemic processes. Elevated blood phosphorus levels are associated with a decrease in glomerular filtration rate, activation of parenchymal fibrosis, and chronic inflammation, which contributes to the progression of nephron damage. The researchers also noted that impaired phosphorus and calcium metabolism in CKD affects the musculoskeletal system. Specifically, a decrease in calcitriol levels and the development of hypocalcaemia stimulate the secretion of parathyroid hormone, which leads to bone resorption and decreased bone mineralisation. Although clinical manifestations in the form of fractures are rare, these changes indicate a systemic nature of the disease. Therewith, an excess of calcium and phosphorus contributes to vascular calcification and a subsequent decrease in their elasticity, which can have dire consequences for both the renal and cardiovascular systems. A combination of dietary restrictions and the use of phosphate binders is effective in reducing blood phosphorus levels. L. Danks (2015) pointed out the risk of hypophosphatemia in case of excessive restriction of this element, which can cause muscle weakness and impaired energy metabolism. A balanced ratio of protein and phosphorus in the diet is significant, as insufficient protein intake can lead to muscle loss, which is a negative factor for the animal's health.

Calcium has vital physiological functions, including nerve conduction, muscle contraction, and blood coagulation. R.F. Geddes *et al.* (2021) noted that cats with chronic kidney disease often have disturbances in calcium homeostasis due to phosphorus imbalance. When phosphorus levels in the blood increase, the amount of free (ionised) calcium decreases, which is a stimulus for increased secretion of parathyroid hormone. As pointed out by S.A. Brown *et al*. (2008), the decrease in ionised calcium levels is caused by its binding to excess phosphorus in the blood, which leads to the development of insoluble complexes. Elevated levels of parathyroid hormone cause calcium to be mobilised from bones, which can lead to osteodystrophy and bone resorption.

This process is widespread in cats with chronic kidney disease and requires careful monitoring. Dietary phosphorus restriction requires careful monitoring of blood calcium levels to prevent hypercalcaemia. Therefore, A.J. Felsenfeld *et al.* (2015) recommended the use of calcium-free phosphate binders, such as lanthanum carbonate or sevelamer, in cats with chronic kidney disease, which enables effective control of phosphorus levels without increasing calcium levels. These scientists stated that hypercalcaemia can also occur in chronic kidney disease, especially with prolonged use of active forms of vitamin D or impaired calcium excretion mechanisms due to reduced renal function.

Hypercalcaemia is an essential factor that can contribute to the development and progression of chronic kidney disease in cats. One of the key mechanisms of renal damage in hypercalcaemia is the deposition of calcium-phosphate complexes in the renal parenchyma. L.A. Nagode *et al.* (1996) pointed to an increased risk of calcification in the renal tubules and interstitium with elevated plasma calcium levels, which impairs renal function and contributes to the progression of chronic nephropathy. R.F. Geddes *et al.* (2021) noted that elevated calcium levels lead to renal vasoconstriction, which can reduce renal blood flow and impair renal perfusion. This contributes to the development of ischaemic nephron damage and the progression of chronic kidney disease. Furthermore, hypercalcaemia can cause nephrogenic diabetic polyuria, which is accompanied by impaired renal tubular concentration. According to S.A. Brown *et al*. (2008), excessive plasma calcium concentration reduces the sensitivity of the renal tubules to the antidiuretic hormone (vasopressin), leading to polyuria, water loss, and secondary dehydration. R. Pusoonthornthum *et al.* (2011) recommended that calcium levels in cats with chronic kidney disease should be carefully monitored, as even a moderate increase in calcium concentration can negatively affect renal function. This is especially true for animals receiving calcium-based phosphate binders or activated forms of vitamin D.M. Coady *et al.* (2019) noted that the severity of ionised hypercalcaemia and hypocalcaemia is associated with the aetiology of this condition in dogs and cats.

The optimal ratio of calcium and phosphorus in the diet is crucial for maintaining mineral homeostasis in cats with chronic kidney disease. R.M. Korman & J.D. White (2013) noted that the balance between these elements in the diet affects their plasma levels, which is critical for preventing mineral metabolic disorders. A study conducted by S.A. Brown *et al*. (2008) showed that even with controlled phosphorus intake, excessive levels of calcium in the feed can lead to an increase in its concentration in the blood serum. This phenomenon is especially evident in cats with chronic kidney disease, as impaired renal function affects the calcium excretion mechanisms. Increased calcium intake in conditions of reduced phosphorus levels in the feed can increase hypercalcaemia, which negatively affects renal function. L.A. Nagode *et al.* (1996) emphasised that in animals with chronic kidney disease, the Ca:P ratio in the feed should be stable, as fluctuations in this ratio can lead to instability of calcium and phosphorus levels in the blood. Specifically, diets with a ratio below 1:1 can contribute to the development of hypocalcaemia, while excess calcium in the presence of phosphorus deficiency can increase the risk of calcium salt deposition in tissues. J. Stockman (2024) recommended maintaining a calcium to phosphorus ratio of 1.1:1-1.5:1, as this helps to maintain a stable level of these elements in the blood. The researcher also noted that it is not only the absolute amount of these minerals that is vital, but also their balance, as even with optimal levels of phosphorus and calcium, their incorrect ratio can cause undesirable changes in the kidneys.

Furthermore, according to R.F. Geddes *et al.* (2021), controlling the calcium-phosphorus ratio in the diet is essential to prevent the development of secondary complications. Scientists addressed the need to balance these minerals depending on the stage of chronic kidney disease, as a change in one of them can lead to compensatory mechanisms that further complicate the pathological process. Recent research by H. Kramer (2019) indicated the potential use of probiotics to reduce phosphorus absorption in chronic kidney disease. This approach has not yet been validated for veterinary practice but may be the subject of further research. This area may be especially useful for animals that do not tolerate phosphate binders or have concomitant digestive system diseases that complicate the uptake of medications.

Role of phosphate binders, ketosteroids, and probiotics

Phosphate binders are key pharmacological agents for controlling phosphorus levels in cats with chronic kidney disease. Their use helps to reduce the risk of secondary hyperparathyroidism and soft tissue calcification, which are frequent complications of mineral imbalance in renal failure. These medications work by binding phosphorus in the gastrointestinal tract, preventing its absorption into the bloodstream. According to V.J. Parker (2021), controlling blood phosphorus concentrations in cats with chronic kidney disease involves primarily reducing its intake from the diet, and in case of insufficient effectiveness, adding oral phosphate binders administered with food, such a restriction of phosphorus intake from the diet helps to reduce its systemic level and improve the metabolic state of the animal.

There are several key types of phosphate binders, which have different mechanisms of action, efficacy, and safety profiles:

♦ Calcium binders. They effectively reduce the concentration of phosphorus, however, as noted by G.J lder & J. Center (2017), their excessive use can lead to hypercalcaemia and increased soft tissue calcification, which can complicate the course of the disease.

♦ Aluminium hydroxide-based binders. These compounds have a high affinity for phosphorus, but their prolonged use is accompanied by a risk of aluminium intoxication. K.G. Beita *et al*. (2024) highlighted the potential toxicity to the nervous system, which limits the long-term use of this group of medicines.

♦ Non-complex binders. For example, sevelamer, which does not contain calcium, shows a lower risk of hypercalcaemia. At the same time, according to L. Rodríguez-Osorio *et al*. (2015), its effectiveness in reducing phosphorus may be somewhat lower compared to calcium medicines, especially in patients with high hyperphosphatemia.

Overall, the efficacy of phosphate binders depends on the type of medicine, dosage regimen, dietary background, and the stage of renal dysfunction. They can be a vital element of complex therapy aimed at stabilising phosphorus levels and reducing the risk of calcifications. At the same time, according to E. Biasibetti *et al*. (2018), their use requires an individual approach, consideration of the animal's clinical condition, and regular monitoring of possible side effects.

This approach may be complemented by the use of ketosteroids, nitrogen-containing substitutes for essential amino acids that do not contain nitrogen but are converted into amino acids in the body. H.-Y. Chen *et al*. (2021) showed that combining ketosteroids with phosphate binders in people with renal impairment can reduce the burden on nitrogen metabolism, lower phosphorus levels, and contribute to an overall improvement in metabolic status. The researchers noted that this combination has a

synergistic effect, slowing down the progression of the disease by reducing the production of uremic toxins, improving protein balance, and controlling phosphate metabolism. This approach can also be considered as a promising one in veterinary medicine for animals with signs of hypoproteinaemia, which are not recommended to have a high protein intake but need amino acid support.

F. Perondi *et al*. (2025) investigated the effect of a probiotic feed supplement on stool and clinical parameters in dogs with chronic kidney disease and gastrointestinal disorders. They used a combination of probiotics, including *Lactobacillus* spp., *Bifidobacterium* spp., and *Enterococcus faecium*, which are widely known for their gut health support properties. According to the study, the use of the probiotic supplement helped to improve stool consistency and reduce the frequency of diarrhoea episodes. Thus, the researchers concluded that probiotic support is a promising additional component of therapy in dogs with kidney disease, especially in the presence of gastrointestinal complications.

G. Meineri *et al*. (2021) confirmed the potential role of nutraceutical support in the comprehensive treatment of chronic kidney disease in dogs. The use of combined supplements containing prebiotics, probiotics, and antioxidants was associated with a statistically significant reduction in urea, creatinine, phosphorus, and SDMA levels. Furthermore, patients with concomitant gastrointestinal disorders showed improvements in faecal score, appetite, and overall clinical condition. Although the results are encouraging, the researchers emphasised the need for further investigation to examine the mechanisms of action and confirm the long-term effectiveness of this approach. E. Ephraim & D.E. Jewell (2020) investigated the effects of dietary supplements such as betaine and soluble fibre on metabolic changes and faecal microbiome in dogs with early chronic kidney disease. The findings revealed a positive effect of these supplements on metabolic parameters and gut microbiota. However, the researchers noted that these findings required further study to better understand the effectiveness of such dietary adjustments in the long-term management of chronic kidney disease.

Role of vitamins, nutrients, and electrolytes in maintaining mineral homeostasis

Vitamins, trace elements, and other nutrients play a significant role in the regulation of mineral metabolism and metabolic state of the body. In cats with chronic kidney disease, abnormalities in calcium, phosphorus, magnesium, potassium, and acid-base homeostasis are widespread changes that substantially affect the prognosis. Their prompt detection and correction can reduce the risk of complications and stabilise the course of the disease. R.V.A. Zafalon *et al.* (2020) studied vitamin D metabolism in dogs and cats, emphasising its significance in regulating calcium and phosphorus metabolism. The researchers noted that disorders in vitamin D metabolism can contribute to the development of various pathologies, including chronic kidney disease, due to mineral imbalances and tissue calcification.

Vitamin D is a key regulator of phosphorus and calcium metabolism. It stimulates the absorption of calcium and phosphorus in the intestines and contributes to normal bone mineralisation. According to the researchers, in cats with renal failure, the level of active vitamin D decreases, which leads to the development of secondary hyperparathyroidism and tissue calcification. This study showed that optimising vitamin D levels may be significant for controlling the progression of CKD and maintaining normal calcium and phosphorus levels in

the animal's body. Disorders of vitamin D-dependent calcium-phosphorus metabolism in CKD have not only biochemical but also structural consequences. A. Shipov *et al.* (2014) demonstrated that cats with this pathology show a marked deterioration in bone microstructure, i.e., a decrease in the density and strength of both trabecular and cortical bone. Such changes may indicate the development of renal osteodystrophy and an increased risk of pathological fractures.

Vitamin K also plays a significant role in the regulation of calcium metabolism, specifically, through the activation of osteocalcin and other Vitamin K-dependent proteins. M. Cozzolino *et al.* (2019) pointed out that deficiency of this vitamin in people with renal impairment is associated with an increased risk of vascular calcification and a deterioration in the coagulation profile. Studies showed that Vitamin K deficiency reduces the activity of matrix Gla protein, one of the key inhibitors of vascular calcification. Furthermore, an inverse relationship between Vitamin K levels and the severity of cortical artery calcification was found in patients with renal insufficiency.

Another aspect that was studied is the effect of potassium levels in cats with chronic kidney disease. D.J. Polzin (2013) noted that hypokalaemia is a frequent complication in cats with stage 2-3 chronic kidney disease, with a prevalence of up to 20%-30%. It can be accompanied by polyuria, polydipsia, muscle weakness, lethargy, and in severe cases, the development of hypokalemic myopathy. V.J. Parker (2021) recommends the addition of potassium supplements, such as potassium gluconate or citrate, to the diet to correct this imbalance. Furthermore, an inverse relationship between blood potassium concentration and the risk of developing systemic hypertension is indicated, which emphasises the significance of monitoring potassium status as part of the comprehensive treatment of chronic kidney disease. The study found that maintaining optimal potassium levels in the diet can reduce the manifestations of hypertension in cats with chronic kidney disease, which is a crucial factor in preventing further deterioration of kidney function. The researcher also described the role of antioxidant therapy in maintaining kidney function in chronic kidney disease. Parker found that the addition of vitamin E and omega-3 fatty acids to the diet of cats with chronic kidney disease can help reduce inflammation and improve renal glomerular function. Antioxidants are essential for reducing oxidative stress, which is one of the key mechanisms of nephron damage in chronic kidney disease.

In a study on mice with induced chronic kidney disease caused by a high-fat diet, H. Lu *et al.* (2025) demonstrated that supplementation with magnesium hydride nanoparticles reduced renal fibrosis and improved renal function. These findings suggest the potential of innovative forms of magnesium as a nutraceutical to influence the progression of CKD under conditions of metabolic stress but require further evaluation in clinical settings.

The approach to the treatment of cats with chronic kidney disease also includes monitoring of acid-base homeostasis. R. Pusoonthornthum *et al.* (2011) showed that metabolic acidosis is a widespread problem in chronic kidney disease in cats and needs to be corrected by dietary intervention or the addition of alkaline agents such as sodium bicarbonate. Metabolic acidosis adversely affects calcium and phosphorus metabolism, contributing to bone demineralisation. The researchers revealed that controlling blood pH may be critical to preventing osteodystrophy in cats with chronic kidney disease. Thus, chronic metabolic acidosis can exacerbate bone health and cause catabolic processes in the body, which is critical to consider when planning treatment.

Comparison of veterinary and medical approaches

The treatment of chronic kidney disease in cats is largely based on the principles developed in human medicine. At the same time, there are substantial differences between veterinary and humane protocols conditioned by both physiological features and the availability of medical technologies and drugs. In human medicine, chronic renal failure treatment protocols include a wide range of pharmaceuticals, such as phosphate-binders, angiotensin-converting enzyme inhibitors (ACE-inhibitors), diuretics, electrolyte balance agents, and immunosuppressants for the treatment of secondary hyperparathyroidism. According to S.-H. Tsai *et al.* (2024), the use of immunosuppressive therapy can reduce parathyroid hormone activity and prevent osteodystrophy. Furthermore, L. Hahn & C. Callaband (2022) noted that medical protocols often include a kidney transplant or dialysis programme, which is the standard of care in end-stage disease. In veterinary practice, the treatment of renal dysfunction in cats is based on somewhat simplified but effective approaches. Transplantation or dialysis are rarely used due to the prohibitive cost, complexity, and lack of adequate infrastructure. The key focus is on dietary therapy with a limited phosphorus and protein content, the use of phosphate binders, both calcium and non-complex, and hydration support. Both approaches have a series of shared features. Specifically, both medicine and veterinary medicine actively use biomarkers to assess the functional state of the kidneys, monitor blood electrolyte composition and acid–base homeostasis, and prescribe vitamin D and K supplements to regulate mineral metabolism. However, according to R.F. Geddes *et al.* (2021), veterinary protocols still have less pharmacological variability and less often include invasive interventions. A promising area for both fields is the introduction of the latest technologies, such as cell therapy. According to S. Clark (2024), the use of stem cells opens new opportunities for the regeneration of renal tissue and the potential restoration of its function. Such approaches, as well as the expansion of the panel of biomarkers for early diagnosis, can bring veterinary nephrology closer to the standards of evidence-based human medicine and substantially improve the quality of life of patients.

Promising areas of research and future interventions

Chronic kidney disease in cats continues to be one of the most pressing problems in veterinary medicine. Despite the advances made in its diagnostics and treatment, effective control over the course of the disease is often complicated by the limitations of modern methods. In this regard, promising areas of research are being developed to improve early detection, introduce new interventions, and improve the quality of life of patients. One of the key factors in successful treatment is prompt diagnostics. Current markers, such as creatinine and symmetrical dimethylarginine concentrations, have limited sensitivity in the early stages. Therefore, special attention is paid to the search for new biomarkers. S. Seiler *et al.* (2009) pointed out the potential of fibroblast growth factor-23, whose level increases before the onset of clinical symptoms. This opens opportunities for early intervention and better prognosis.

The use of stem cells is also of considerable interest. N. Tavakolidakhrabadi *et al.* (2024) demonstrated that the introduction of mesenchymal cells can promote renal tissue regeneration, reduce inflammation, and stabilise functional parameters. This approach gives hope not only for maintenance but also for partial restoration of the affected structures. Another development vector is molecular technologies, specifically gene therapy. J.L. Peek & M.H. Wilson (2023) described the possibilities of gene

modification aimed at normalising metabolic processes in the nephron. Genome editing and targeted molecular interventions are considered as a future alternative or complement to conventional approaches. Additionally, new pharmacological agents are being developed to optimise mineral metabolism. According to J. Stockman (2024), phosphatase inhibitors and stimulators of active vitamin D synthesis can reduce the risk of secondary hyperparathyroidism, improve calcium-phosphorus balance, and slow the course of the disease.

Most approaches to the treatment of chronic kidney disease in cats focus on supportive care and slowing the progression of the disease. However, recent studies are increasingly drawing attention to the possibility of actively influencing the pathogenesis of the disease through immunological mechanisms. Among the innovative strategies, the development of vaccines that can not only modulate the immune response but also potentially improve kidney function by reducing inflammation and fibrosis is of particular interest.

R. Sugisawa *et al.* (2016) found that the structure of the AIM (Apoptosis Inhibitor of Macrophage) protein in cats may be a key factor in their susceptibility to developing chronic kidney disease. Specifically, it was showed that in cats, AIM binds extremely strongly to IgM, which prevents its release in response to renal insult or toxin-induced damage. Under normal conditions, free AIM promotes the removal of necrotic cells and prevents the development of tubular obstructions, but this mechanism is impaired in cats. The use of exogenous AIM in model animals improved the clearance of cellular residues and reduced the severity of renal damage, demonstrating the potential of this molecule as a therapeutic agent for the prevention or treatment of CKD in cats. If successfully completed, this approach could be a prominent breakthrough in the prevention and treatment of animals with chronic kidney disease, especially in case of its early detection. Thus, the future treatment of renal failure in cats is associated with an integrated approach: early diagnostics using sensitive biomarkers, cell and gene therapy, as well as pharmacological modulation of metabolism, which forms a new paradigm of veterinary nephrology.

The results of the literature analysis showed that the ratio of calcium and phosphorus plays a key role in the development and progression of chronic kidney disease in cats. An optimal balance of these elements is necessary to maintain a stable mineral metabolism, reduce the risk of secondary hyperparathyroidism, avoid hypercalcaemia, and prevent renal tissue calcification. Studies revealed that elevated blood phosphorus levels correlate with the progression of chronic kidney disease and impaired renal function. According to V.J. Parker (2021), phosphorus restriction is one of the key measures in the treatment of chronic kidney disease. Reducing phosphorus levels through dietary therapy or the use of phosphate binders can effectively regulate the level of this element and reduce the risk of complications. However, according to L.A. Nagode *et al.* (1996), excessive phosphorus reduction can cause hypercalcaemia, especially when using calcium-containing phosphate binders.

Evaluation of the impact of calcium showed that its elevated level contributes to the development of nephrocalcinosis, which increases structural damage to the nephron. According to A.J. Felsenfeld *et al.* (2015), hypercalcaemia in cats with chronic kidney disease can aggravate renal failure due to impaired glomerular filtration capacity. On the other hand, M. Coady *et al.* (2019) noted that reduced calcium levels caused by hyperphosphatemia stimulate the activity of parathyroid hormone, which leads to bone loss and impaired overall metabolism.

The use of modern diagnostic methods, such as determining the concentration of creatinine, urea, SDMA, calcium, and phosphorus, allows for effective monitoring of kidney function in cats with chronic kidney disease. This enables not only the accurate assessment of the intensity of disease progression, but also prompt adjustment of therapy, including diet and the use of phosphate binders. Complete (clinical) urinalysis and ultrasound are significant additional tools for detecting structural changes in the kidneys, which contribute to more accurate prognosis and treatment of CKD. Considering these factors in combination is a prerequisite for achieving optimal results in controlling the course of the disease and improving the quality of life of animals.

Conclusions

In the context of the purpose of this study, the current literature data on the role of the serum calcium-phosphorus ratio in chronic kidney disease in cats were analysed and the optimal approaches to the effective correction of this indicator were determined. As a result of the literature analysis, the data on the influence of mineral metabolism on the course of chronic renal failure in animals were systematised and the main mechanisms of complications were outlined. The study examined the physiological role of calcium and phosphorus, analysed the mechanisms of their imbalance in kidney damage, and determined the consequences of hyperphosphatemia and hypercalcaemia, as well as the effectiveness of their correction with dietary therapy and phosphate binders. Particular attention was paid to the evaluation of diagnostic approaches, including laboratory and visual monitoring of changes in mineral metabolism and renal function. The presented results confirmed the significance of maintaining an optimal calcium to phosphorus ratio to prevent the development of secondary hyperparathyroidism, tissue calcification, and glomerular filtration disorders in cats with renal pathology. It was found that the interval of 1.1:1-1.5:1 is the safest and most suitable in the long-term dietary therapy of sick animals. The study systematised the methods for evaluating the effectiveness of therapy, including monitoring of biochemical parameters of blood and urine, as well as changes in the renal parenchyma by ultrasound examination.

The synthesis of available data allows forming a modern idea of the pathophysiological role of calcium-phosphorus metabolism in chronic kidney disease in cats and confirms the effectiveness of an integrated approach to the diagnostics and correction of mineral imbalance. The use of individualised dietary strategies in combination with the monitoring of marker indicators can slow down the progression of chronic renal failure and improve the quality of life of animals. Promising areas for further research include studying the long-term results of using different types of phosphate binders, the effect of vitamins D and K on calcium and phosphorus metabolism in the chronic course of nephropathy in cats, and the search for new biomarkers to assess the effectiveness of therapy in the early stages of renal failure in animals.

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Conflict of Interest

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Нутріціологічний вплив кальцію та фосфору на хронічну хворобу нирок у котів: огляд літератури

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Анотація. Актуальність дослідження зумовлена значною поширеністю хронічної хвороби нирок у котів та важливою роллю порушень кальцій-фосфорного обміну в її прогресуванні та формуванні ускладнень, що потребує подальшої уваги науковців для вдосконалення підходів до лікування тварин. Метою цього огляду було узагальнення сучасних наукових даних щодо впливу кальцію та фосфору на перебіг хронічної хвороби нирок у котів та формування рекомендацій щодо нутріціологічної підтримки. Використовувся систематичний підхід до пошуку та аналізу наукової літератури. Проведено аналіз результатів сучасних досліджень з питань діагностики хронічної нефропатії в дрібних м'ясоїдних тварин, клінічних рекомендацій та експертних висновків у галузі ветеринарної медицини. Встановлено, що надлишковий вміст фосфору в раціоні тварин прямо корелює з прискоренням прогресування хвороби, розвитком вторинного гіперпаратиреозу, кальцифікацією м'яких тканин та погіршенням функціонального стану нирок. Водночас надмірне зниження концентрації фосфору може спричиняти гіперкальціємію, втрату апетиту, блювання та інші метаболічні порушення. Оптимальним для раціону тварин із хронічною хворобою нирок є співвідношення кальцію до фосфору в межах 1,1:1-1,5:1. Розглянуто роль сучасних біомаркерів, таких як симетричний диметиларгінін та фактор росту фібробластів-23 у ранній діагностиці та моніторингу хронічної хвороби нирок. Проаналізовано ефективність дієтичної терапії, зокрема застосування фосфатзв'язуючих препаратів, активних форм вітаміну D, пробіотиків, антиоксидантів і засобів для корекції кислотно-лужного балансу. Огляд даних літератури підтверджує, що ефективний нутріціологічний менеджмент є ключовим фактором у контролі хронічної хвороби нирок. Отримані результати підкреслюють важливість розробки оптимальних дієтичних стратегій, що враховують як рівень фосфору, так і співвідношення кальцію до фосфору в сироватці крові. Це дозволить уповільнити прогресування захворювання та покращити якість життя котів із хронічною хворобою нирок

Ключові слова: мінеральний гомеостаз; ветеринарна дієтологія; електролітний обмін; метаболічні порушення; гіперкальцемія; гіперфосфатемія; фосфатбіндери

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