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

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

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

Introduction

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

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

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

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

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

Literature Review

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

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

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

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

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

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

Materials and Methods

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

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

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

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

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

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

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

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

Results and Discussion

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

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

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

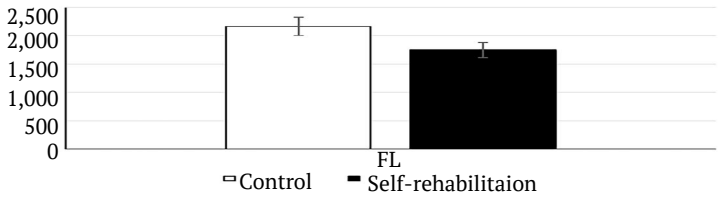

Figure 1. Total phospholipid content (mg/100 g of raw tissue) in liver samples from rats in the experimental group "Self-rehabilitation" with tetracycline-induced fatty hepatosis (M ± m, n = 8)
***Note**: P < 0.05, compared to the values of the corresponding indicator in intact animals in the "Control"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

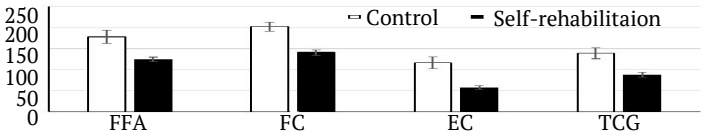

Figure 2. The content of free fatty acids, free and esterified cholesterol, triacylglycerols (mg/100 g of raw tissue) in liver samples of rats in the experimental group "Self-rehabilitation" with tetracycline-induced fatty hepatosis (M ± m, n = 8)
***Note**: P < 0.05, compared to the values of the corresponding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

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

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

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

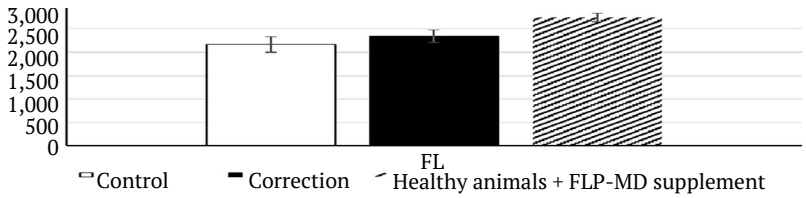

Figure 3. Total phospholipid content (mg/100 g of raw tissue) in liver samples from rats in experimental group II "Correction" and experimental group III "Healthy animals + FLP-MD dietary supplement" with tetracycline-induced fatty hepatosis (M ± m, n = 8)

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

***Sourse**: compiled by the authors*

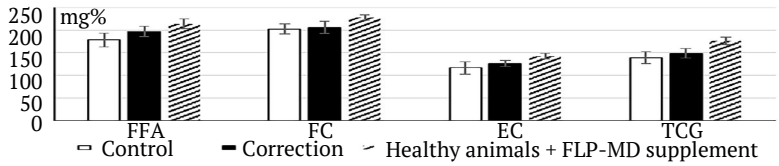

Figure 4. The content of free fatty acids, free and esterified cholesterol, triacylglycerols (mg/100 g of raw tissue) in liver samples from rats in experimental group II "Correction" and experimental group III "Healthy animals + dietary supplement "FLP-MD"" for tetracycline-induced fatty hepatosis (M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

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

***Sourse**: compiled by the authors*

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

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

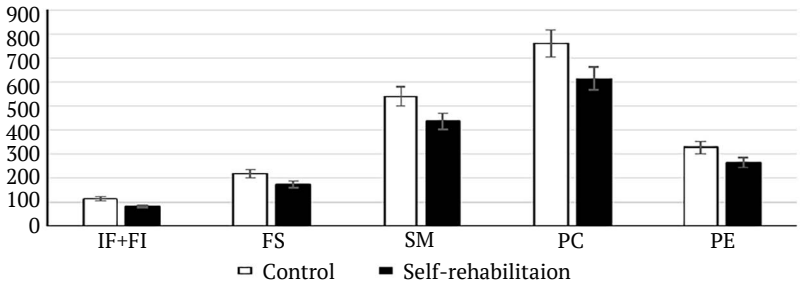

Figure 5. Content of individual phospholipids (mg/100 g of raw tissue) in liver samples of rats in the experimental group "Self-rehabilitation" with tetracycline-induced fatty hepatosis (M ± m, n = 8)
***Note**: P < 0.05, compared with the values of the corresponding indicators in intact animals in the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

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

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

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

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

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

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

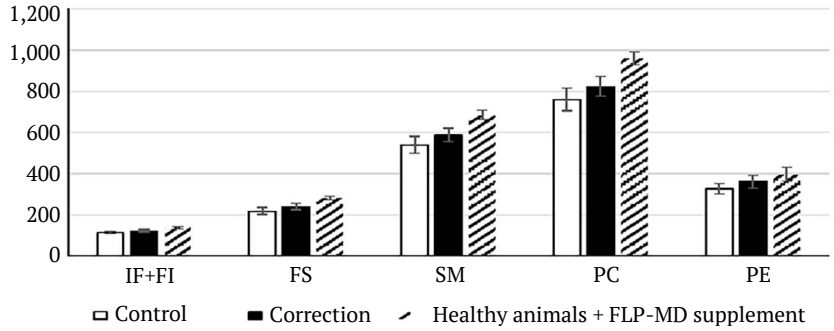

Figure 6. The content of individual phospholipids (mg/100 g of raw tissue) in the liver of rats in experimental group II "Correction" and experimental group III "Healthy animals + dietary supplement "FLP-MD"" for tetracycline-induced fatty hepatosis (M ± m, n = 8)

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

***Sourse**: compiled by the authors*

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

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

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

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

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

Conclusions

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

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Conflict of Interest

None.

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

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

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

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