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Effect of probiotic biologics on morpho-biochemical parameters of broiler chicken blood

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Abstract. In the production of broiler poultry products, market operators use intensive technologies that involve the use of various, environmentally friendly nutraceuticals, in particular, probiotic preparations. In the technology of raising broiler chickens, probiotic preparations are necessary as a means for the prevention and treatment of gastrointestinal diseases, stimulating the growth and increasing the productivity of poultry. The purpose of the study is to investigate the effect of probiotic biologics on haematological parameters when it is fed to broiler chickens. The experiment used 20 broiler chickens, which were given a probiotic from 28 to 42 days in the following amounts: 0.5 g/10 dm^3, 2.0 and 4.0 g/10 dm^3 of water. It was found that the morpho-biochemical parameters of poultry blood corresponded to the physiological standards for broiler chickens of the established age and confirmed the absence of pathophysiological changes in their body. On days 35 and 42 of rearing, an increase in the number of leukocytes from 4.4 to 17.2% and the haemoglobin content by 3.9 and 6.2%, respectively, was observed in the blood of broiler chickens fed the probiotic at a dose of 4.0 g/10 dm^3 compared to the control group. On day 35 of the study, a 1.2-fold increase in total serum protein was observed when drinking a probiotic in poultry, respectively, at doses of 2.0 and 4.0 g/10 dm^3 of water, and on day 42 – 1.0 times for its drinking at a dose of 4.0 g/10 dm^3 of water. It was found that the content of inorganic calcium and phosphorus in the poultry blood serum corresponded to physiological limits, which indicates a sufficient level of mineral nutrition in the body of broiler chickens. The absence of changes in the activity of alanine aminotransferase and aspartate aminotransferase, the content of total lipids, cholesterol, and creatinine in the blood serum of broiler chickens of the experimental groups indicates the hepato- and nephronethoxicity of the probiotic. Based on the results obtained, the drug under study can be recommended to increase the resistance of the poultry body and regulate metabolism

Keywords: organism resistance; balanced feeding; morphological parameters; biochemical indicators; safety

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Introduction

In the process of producing broiler meat, it is important to search for new probiotic drugs that, unlike antibiotics, would have the ability to increase poultry productivity, the activity of natural organism resistance and the reproduction of livestock (Shkodyak *et al.*, 2020). In the context of the development of intensive technologies for raising poultry, it is necessary to use high-quality food substances in feeding and compounds that have inhibitory properties in accordance with pathogenic and opportunistic strains of microorganisms and viruses. Therefore, during 2013-2023, a scientific search was conducted for alternative uses of probiotic and phytobiotic drugs that have an effect on increasing the growth of poultry and animals (Xiong *et al.*, 2023) due to pronounced effects on antioxidant indicators (Gazwi *et al.*, 2022) and immunological activity (Yuan *et al.*, 2023), and this indicates a high resistance of the body of poultry and animals to the action of pathogenic and opportunistic microorganisms.

According to M.I. Sakhatsky & Yu.V. Osadcha (2021), the development and implementation of probiotic preparations for use in the field of broiler chicken rearing is conditioned by the growing problem of antibiotic-resistant microorganisms. Moreover, when raising broiler chickens, it is necessary to take into account the stress factor, which worsens the clinical and biochemical indicators of the blood of broiler chickens for keeping in cages, while reducing the immune status of the bird's body. Probiotics based on live cultures of microorganisms increase the stimulation of biosynthetic processes in the digestive tract and increase poultry productivity (poultry growth, meat yield), affect the improvement of metabolic processes in the body of broiler chickens, and prevent stress. Currently, in accordance with the requirements of the European Community, it is not allowed to use antibiotics when feeding farm animals and poultry (Chaturvedi *et al.*, 2021).

Feeding poultry with probiotic biologics using *C. butyricum* increased the productivity of broiler chickens by improving the morphological characteristics of blood and internal organs and stimulating the development of immunity, which can be an alternative to antibiotics in poultry feed (Yang *et al.*, 2023). The study determined the expression of cytokine genes in peripheral blood mononuclear cells of broiler chickens stimulated by live probiotics. Cytokine genes in peripheral blood mononuclear cells are isolated from whole blood and stimulated by lipopolysaccharides, galactooligosaccharides, and *Lactococcus lactis subsp*. It is established that *L. lactis* has immunostimulating properties of the cytokine gene in peripheral blood mononuclear cells of chickens (Slawinska *et al.*, 2021).

As noted by C.J. Savelli *et al.* (2021), food safety management involves identifying and controlling the risks associated with animal welfare (feeding, health, maintenance, etc.) and correcting actions in the food chain to ensure safe food production. During the production of broiler chicken meat, veterinary medicine specialists and state veterinary medicine inspectors carry out risk-oriented control to obtain safe and high-quality poultry products and animal feeding, in particular, the use of probiotic drugs (Mizernytskyi, 2021). Therefore, the use of a probiotic drug is relevant in poultry farming. The purpose of the study is to experimentally and practically substantiate the effectiveness and safety of using a probiotic biological product in the rearing of broiler chickens and to investigate its effect on the morpho-biochemical parameters of poultry blood.

Literature Review

The development of modern poultry farming in Ukraine is based on the organisational and economic principles of management and optimisation of technological processes during the production of broiler chicken meat, which provides ordinary consumers with valuable food products (Lyasota & Kolodka, 2020). In addition, the use of probiotic drugs is an urgent issue in the treatment and prevention of gastrointestinal infections of viral and bacterial aetiology, and the normalisation of intestinal microflora for the detection of dysbiosis (Delgado-Pando *et al.*, 2019). Obtaining safe meat products from broiler chickens depends on the activity of the intestines of a physiologically healthy bird due to the assimilation of nutrients from the feed that are necessary for poultry growth (Kucheruk & Zasekin, 2018).

The health of poultry depends on the safety and quality of feed, so it is necessary to monitor the feed for the content of toxins that produce harmful microorganisms, namely: *Aspergillus flavus, Aspergillus parasiticus speare, Aspergillus fumigates, Stachybotrys alternans* etc. (Shkodyak *et al.*, 2020). Therefore, it is necessary to stabilise the balance of the bird's intestines by using probiotics that stimulate feeding processes due to the normalisation of microflora, which represents adjuvant-active compounds that enter the blood and stimulate the immune system (Nwaigwe *et al.*, 2020).

Pre-colonisation of the intestines of broiler chickens with probiotic cultures that are considered competitive leads to a decrease in the formation of pathogenic microflora in the intestine, that is, probiotics act as a competitive replacement. This process minimises the occurrence of infectious gastrointestinal diseases in 65-85% of young poultry, and therefore, probiotic preparations have a positive effect on the quality and safety of poultry products (Sarangi *et al.*, 2016). Probiotic preparations for feeding poultry not only increase its productivity, but also meet the needs of the necessary components of the body of ordinary consumers in high-quality and safe nutrition (Podolian, 2016).

Probiotics based on bacilli, in particular, their introduction into special feed mixtures and preparations that are complex, have become widespread in veterinary practice. They are prescribed for the prevention of salmonellosis in poultry during farmstead cultivation. The high efficiency of the use of representatives of the genus *Bacillus* was also established during the prevention and treatment of salmonellosis, purulent, colibacillosis, and staphylococcal infections (Zhou *et al.*, 2015). The use of the probiotic Probiol in the amount of 0.25 g/t of mixed feed when feeding broiler chickens of the cross Ross-308 for 42 days has a positive effect on the morphological and biochemical parameters of the blood. Researchers have found an increase in the level of haemoglobin by almost 15.0%, the content of red blood cells and white blood cells in the blood of poultry, an increase in the content of total protein in the blood serum by almost 5.0% and glucose by 30%. However, these indicators were within the physiological norm for broiler chickens (Podolian, 2017).

It is believed that the addition of probiotics to the poultry diet further increases the titer of antibodies against infectious bursal disease (IBD) and increases the immune response – the response of broiler chickens to this disease. Feed supplements affected the blood counts of broilers on day 35 of rearing. At the same time, the content of total protein, albumins, globulins, cholesterol, and the activity of alanine aminotransferase in blood serum remained unchanged ($P<0.01$). Blood glucose levels were significantly reduced ($P<0.05$), triglycerides increased, and alanine aminotransferase activity also decreased ($P<0.01$) (Rehman *et al.*, 2020). M.M. Khubeiz & A.M. Shirif (2020) found changes in blood counts when feeding chickens natural preparations. An increase in the number of basophils and eosinophils indicated changes in the immune system of chickens under the influence of coriander seed powder. An increase in the number of lymphocytes also indicated a possible improvement in the immune system. The number of basophils and eosinophils increased by 2.5%, and lymphocytes – by 1.5%. The use of probiotic preparations in poultry farming improves their productivity, namely, the increase in carcass weight, the quality and safety of meat and offal, which provides the human body with nutrients and is quickly absorbed.

Materials and Methods

To establish the efficacy and safety of the probiotic biological product Subtiform in the rearing of broiler chickens of the cross SOBB-500, the tests were conducted at the LLC Skibinetska Poultry Farm, Skibintsi village, Tetiiv district, Kyiv Region, during 2022-2023.

At the facilities of the LLC Skibinetska Poultry Farm, the technology used is the rearing of COBB-500 broilers on a floor with deep litter. The broiler chickens are housed in large-sized poultry houses in large single-age batches (up to 50 animal units). The lighting of poultry farm premises is automated; all production processes of poultry rearing in poultry houses are mechanised: feed distribution, water drinking, poultry heating, and droppings cleaning. The basic principle when raising poultry is to control the optimal microclimate in the poultry house: temperature of 28-30°C and humidity level of 70-80%. The poultry farm has developed a special mixed feed for poultry, which includes corn, wheat, several types of meal, a complex of vitamins and minerals, bone meal, fat, yeast, salt, and chalk. To improve the overall nonspecific resistance of the poultry body, broiler chickens were given vitamin preparations for 6-12 days of life in accordance with the requirements of use. In the period from 12 to 14 days of life, broiler chickens were given preventive measures for coccidiosis.

The task was set to experimentally substantiate the feasibility of using and determine the optimal dose of probiotic biologics among the studied doses (0.5, 2.0, and 4.0 g/10 dm^3 of water) for broiler chickens during their feeding. A control group of broiler chickens (birds were not given a probiotic preparation) and 3 experimental groups (20 birds each) were established, which were provided with feed and given probiotics in the specified amounts: experimental group 1 – 0.5 g/10 dm^3 of water; experimental group 2 – 2.0 g/10 dm^3 of water; experimental group 3 – 4.0 g/10 dm^3 of water in the period from 28 to 42 days. Control and experimental groups of poultry were fed nutritious feed according to the established standards, considering the age of broiler chickens. The clinical condition of the birds was monitored daily. Blood samples were taken from six broiler chickens from each group on days 28, 35, and 42 rearing and for further research in the accredited research laboratory of the Department of Veterinary and Sanitary Expertise and Laboratory Diagnostics (certificate of compliance of the measurement system with the requirements of DSTU 10012:2005 (2007) and the inter-faculty laboratory of histochemical and biochemical methods research of the Bila Tserkva National Agrarian University. The effect of probiotic biologics on the morphological parameters of the blood of broiler chickens was investigated: the number of red blood cells and white blood cells counted in the Goryaev chamber, and the haemoglobin concentration was also determined (Levchenko, 2017).

The biochemical parameters of blood serum were determined: the concentration of total serum protein by the Kingel-Weikselbaum biuretic reaction method, the content of albumins by the Nephelometric (turbidimetric) method using a photoelectrocolorimeter (KFK-3); the content of total lipids by the Zlatkis-Zak method, total cholesterol by the

Trinder method by fermentation; creatinine by the Jaffe-Popper method due to deproteinisation with picric acid; the concentration of uric acid – by the colour reaction using phosphor – Wolfram reagent on KFK-3; activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) – by Reitman-Frenkel dinitrophenylhydrasine method; content of triacylglycerols (TAG) and total calcium content – by photometric method, inorganic phosphorus – by reduction of phosphor-molybdenum acid (Levchenko, 2002). Stabilised blood and its serum were used to analyse morpho-biochemical parameters in broiler chickens.

Characteristics of the probiotic biological product Subtiform. Probiotic biological product Subtiform is a drug of symbiotic nature. The composition of the drug includes bacteria of the genus *Bacillus subtilis and Bacillus licheniformis*, the content of which is 2.5 x 10^9 CFU/g, filler – dry milk whey. Immunobiological properties: probiotic preparation is used as an auxiliary agent for the treatment and preventive measures for gastrointestinal infections of bacterial and viral aetiology, stability of intestinal microflora in dysbiosis, growth stimulation, to improve the safety and productivity of livestock and poultry. Probiotic bacteria *Bacillus subtilis* and *Bacillus licheniformis* which are part of the feed additive, show sensitivity to the following antibiotics: gentamicin, neomycin, colistin sulphate, amoxicillin, and streptomycin. When combined with antibiotics with a gram-positive spectrum of action, a partial decrease in the effectiveness of the drug may occur; it can be combined with coccidiostatics.

Experimental studies were conducted in accordance with modern methodological approaches and in compliance with the requirements and national standards, namely DSTU ISO/IEC 17025:2019 (2021) and in accordance with Directive 2010/63/EU (2010), which were approved by the conclusion of the commission on ethics and bioethics of the Faculty of Veterinary Medicine of the Bila Tserkva National Agrarian University dated 12.04.2023. The animals were kept and all manipulations were carried out in accordance with the provisions of the European Convention for the protection of vertebrate animals used for experimental and scientific purposes (1986, March).

The importance of research for their implementation was confirmed by the use of certified equipment, modern methods, and in the process of statistical processing of the results obtained. Variational and statistical processing of the results was carried out using Microsoft Excel and Maple-12 (Maplesoft, 2008) software suites. The Student's t-test was considered in the case of a normal data distribution, and the following differences were assumed statistically significant: $P<0.05$; $P<0.01$, $P<0.001$.

Results and Discussion

According to the clinical examination of broiler chickens, the natural position of their bodies was established, the chickens also reacted to external stimuli, actively moved, and consumed water and feed. The condition of the visible mucous membranes of chickens had a pale pink colour; the condition of the feather cover was marked by cleanliness; the condition of the beak was dry; discharge from the eyes and cloaca was not observed; dryness was found on the surface of the limbs, without visible damage. No wheezing was detected during the breathing acts of broiler chickens. The body temperature corresponded to physiological limits, namely the range of 40.5-42°C. During the testing period, no cases of disease or deaths of broiler chickens were recorded. The results of the studied haematological parameters of broiler chickens for the use of probiotic biologics on days 35 and 42 of rearing in the control and experimental groups are shown in Figures 1, 2.

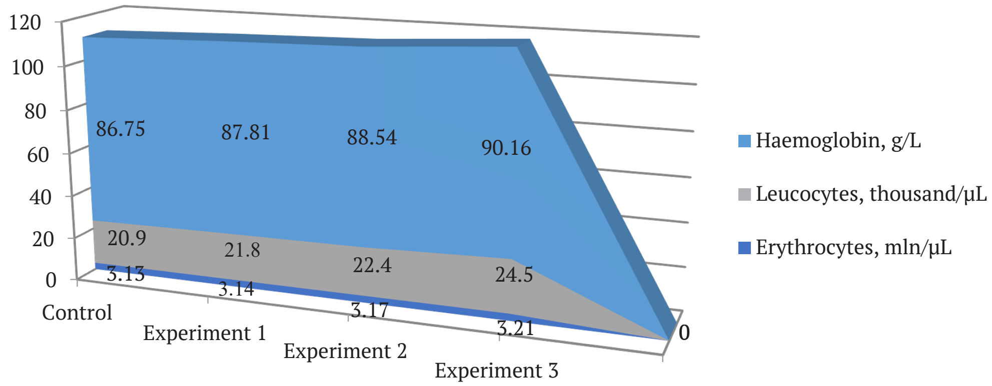

Figure 1. Dynamics of morpho-biochemical parameters of whole blood of broiler chickens on day 35 of rearing

Source: *developed by the author*

The content of red blood cells in the blood of broiler chickens of the 2nd and 3rd experimental groups increased by 1.2% ($P<0.05$) and 2.6%, respectively, according to the indicators of the control group, but these indicators were within physiological limits. In the blood of broiler chickens of the 1st, 2nd and 3rd experimental groups, there was a tendency to increase the number of white blood cells, which indicated an increase in the state of general resistance of the body of broiler chickens and the mobilisation of the corresponding cellular factors, namely on the 35th day of poultry rearing, respectively-by 4.4%, 7.2 and 17.2% ($P<0.05$) in accordance with the indicators of the control group. The haemoglobin content in the blood of poultry of the experimental groups increased, respectively, by 1.2%, 2.1, and 3.9% compared to the control group.

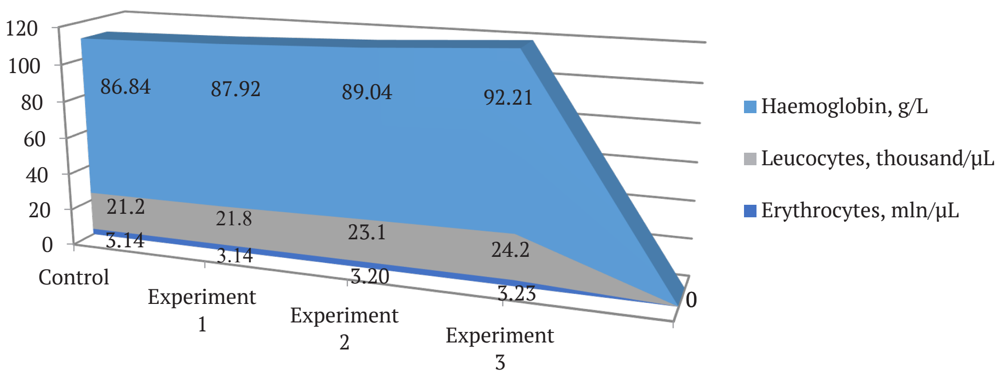

Figure 2. Dynamics of morpho-biochemical parameters of blood of broiler chickens on day 42 of rearing

Source: *developed by the author*

The number of red blood cells in the blood of chickens of the 2nd and 3rd experimental groups increased by 1.9% ($P<0.05$) and 2.9%, respectively, on the 42nd day of rearing compared to the control indicators. In the blood of broiler chickens of the 1st, 2nd and 3rd experimental groups, there was a tendency to increase the number of white blood cells, which indicated an increase in the resistant state of their body and a corresponding increase in cellular factors, namely, on the 42nd day of rearing, respectively – by 2.4%, 9.0 and 14.2% ($P<0.05$) compared to the control group. The studied indicators were within physiological limits. The haemoglobin content in the blood of poultry in the three groups increased by 1.2%, 2.5 and 6.2%, respectively, compared to the control group of broiler chickens.

Thus, the use of probiotic preparations in the production of broiler chickens to produce organic food is a topical issue in the global livestock industry. W. Jiang *et al*. (2017) claim that the use of probiotics exhibits antibacterial properties and immunomodulatory effects on the body of broiler chickens. They had a positive effect on the blood parameters of poultry on the 42nd day of rearing with a significant increase in the number of red blood cells by 2.4% ($P<0.01$), the number of white blood cells by 6.2% ($P<0.01$), and the haemoglobin content by 3.7% compared to the control group of poultry. W. Jiang *et al*. (2017) proved that the introduction of new feed additives into the diet of broiler chickens can cause stress for poultry, and also found that during the rearing of poultry, blood parameters slightly differed with the control group – the number of red blood cells significantly increased by 1.3 times ($P<0.01$), the number of white blood cells by 1.5 times ($P<0.01$), and the haemoglobin content – by 1.2 times ($P<0.01$). When a probiotic drug was introduced into the diet of broiler chickens in combination with thyroxine, the total number of white blood cells increased (11.5×10^3 per 1 μL) relative to the control group of poultry (10.7×10^3 per 1 μL). Researchers have proven an increase in the natural resistance of the body of broiler chickens to the use of probiotics.

According to the obtained haematological parameters, an increase in the level of antibacterial properties of the body protection of broiler chickens of the 3rd experimental group was established on the 35th and 42nd days of rearing when feeding a probiotic biological product at a dose of 4.0 g/10 dm^3. Table 1 shows the biochemical parameters of the blood of broiler chickens of the 1st, 2nd, and 3rd experimental groups when feeding probiotic biologics in the amount of 0.5 g/10 dm^3 of water, 2.0 g/10 dm^3 of water, and 4.0 g/10 dm^3 of water for the 35th day of rearing.

Table 1. Dynamics of biochemical parameters of blood serum of broiler chickens when using a probiotic biological product on day 35 of poultry rearing, M±m, n=6

Indicator	Control group	Day 35 of rearing		
		Experimental group 1	Experimental group 2	Experimental group 3
Total protein, g/L	37.26±1.27	37.35±1.12	43.57±1.44**	44.09±1.39**
Albumins, %	21.56±0.84	22.62±1.02	22.51±1.01	23.01±0.89
Total lipids, g/L	13.19±0.47	13.20±0.47	13.19±0.43	13.23±0.47
Total cholesterol, mmol/L	3.20±0.09	3.19±0.04	3.18±0.05	3.20±0.05
Creatinine, mmol/L	25.71±0.59	26.13±0.34	26.19±0.28	26.25±0.34
Uric acid, mmol/L	0.27±0.04	0.26±0.01	0.21±0.01	0.19±0.01
Alanine aminotransferase, U/L	4.92±0.64	5.42±0.42	5.52±0.29	5.57±0.65
Aspartate aminotransferase, U/L	77.88±1.74	84.16±1.66*	85.33±1.72**	85.68±1.68**
Triacylglycerols, mmol/L	1.84±0.10	1.70±0.09	1.61±0.10	1.44±0.06

Table 1. Continued

Indicator	Control group	Day 35 of rearing		
		Experimental group 1	Experimental group 2	Experimental group 3
Calcium, mmol/L	2.47±0.05	2.61±0.02	2.60±0.02	2.65±0.02
Inorganic phosphorus, mmol/L	2.40±0.03	2.29±0.02**	2.45±0.02	2.47±0.04

Notes: ****P<0.05; **P<0.01; ***P<0.001 compared to the control group of broiler chickens*
Source: *developed by the author*

The total protein content in the blood serum of poultry of the 2nd and 3rd experimental groups increased slightly on the 35th day of rearing, respectively, by 1.17 times (P<0.01) and 1.18 times (P<0.01) compared to the control group. There was no statistically significant difference in the content of albumins and total lipids in the blood serum of poultry with the control group. The content of cholesterol in the blood serum of chickens of experimental groups on the 35th day of rearing was within the reference values. This is due to the fact that cholesterol synthesis is reduced by the action of a probiotic. S. Mookiah *et al.* (2014) proved that feeding broiler chickens a probiotic drug improved the poultry's metabolism, reduced serum cholesterol by 2.9% (P<0.001), and increased the number of white blood cells by 16.0%.

Serum creatinine levels in broiler chickens were not statistically significant compared to the control group (they were within the physiological norm). A.A. Koronowicz *et al.* (2016) state that when using probiotic drugs, the content of total serum protein slightly increased by 1.2% (P<0.01), albumins by 1.1%, and total lipids was within the normal range and at the level of the control group. The results of this study were consistent with the results of Yu.M. Podolian (2017), who found that the serum cholesterol content of broiler chickens fed a probiotic decreased by 7.1%, the total protein content increased by 3.5%, the haemoglobin content increased by 1.1 times, the number of red blood cells increased by 1.2 times, and the number of leukocytes increased by 1.0 times compared to the control.

The concentration of uric acid in the blood serum of chickens of experimental group 1 was within the control values and corresponded to the values of the control group of poultry, and in experimental groups 2 and 3 it slightly decreased. A 1.3-fold decrease in uric acid content (P<0.01) in the blood serum of chickens for setting probiotics is consistent with the results of C. Li *et al.* (2022). The researchers also found that in the control and experimental groups of broiler chickens that were given probiotic drugs, the concentration of urea in the blood serum in its values corresponded to the limits of physiological fluctuations. This confirms the positive effect of probiotics of bacteria of the genus *Bacillus subtilis and Bacillus licheniformis* on the blood parameters of broiler chickens and an increase in their productivity, which affected the improvement of the quality of meat of broiler chickens. According to the conducted study, the activity of the AST enzyme in the blood serum of broiler chickens of the experimental groups significantly increased. In experimental group 1 – by 8.1% (P<0.05), in experimental group 2 – by 9.6% (P<0.01), in experimental group 3 – by 10.0% (P<0.01) compared to the control. Indicators of inorganic phosphorus content in the blood serum of poultry of experimental group 1 significantly decreased by 4.6% (P<0.01) in accordance with the control group. These indicators suggested that there were no violations of the Ca : P ratio, which was in the range from 1.07 to 1.14.

W. Jiang *et al*. (2017) found that when broiler chickens were given probiotics in the blood serum, the content of phosphorus (5.77 mmol/L compared to the control group of poultry – 5.17 mmol/L; P=0.0121) and glucose (256.9 mg/dm^3 compared to the control group – 219.3 mg/dm^3; P<0.0001).

The period of action of the probiotic did not affect the concentration of blood calcium, total protein, albumin, globulins, total cholesterol, triacylglycerols, AST and ALT activity (P<0.05). Table 2 shows the biochemical parameters of the blood serum of broiler chickens of the 1st, 2nd, and 3rd experimental groups when feeding probiotic biologics in the amount of 0.5 g/10 dm^3 of water, 2.0 g/10 dm^3 of water, and 4.0 g/10 dm^3 of water for the 42nd day of rearing.

Table 2. Dynamics of biochemical parameters of blood serum of broiler chickens with the use of probiotic biological product on day 42 of poultry rearing, M±m, n=6

Indicators	Control group	Day 42 of rearing		
		Experimental group 1	Experimental group 2	Experimental group 3
Total protein, g/L	37.26±1.27	37.16±1.57	37.66±2.92	38.36±3.07**
Albumins, %	21.56±0.84	22.64±1.02	22.44±0.95	23.94±1.13
Total lipids, g/L	13.19±0.47	13.18±0.46	13.08±0.51	13.24±0.44
Total cholesterol, mmol/L	3.20±0.09	3.17±0.04	3.18±0.05	2.89±0.08*
Creatinine, mmol/L	25.71±0.59	26.25±0.44	26.51±0.31	26.73±0.36
Uric acid, mmol/L	0.27±0.04	0.17±0.01*	0.15±0.03*	0.14±0.04
Alanine aminotransferase, U/L	4.92±0.64	6.19±0.59	6.36±0.62	6.57±0.65
Aspartate aminotransferase, U/L	77.88±1.74	93.17±1.64***	99.43±1.32***	102.34±0.82***
Triacylglycerols, mmol/L	1.84±0.10	1.64±0.07	1.53±0.09	1.31±0.15
Calcium, mmol/L	2.47±0.05	2.55±0.02	2.57±0.02	2.61±0.02
Inorganic phosphorus, mmol/L	2.40±0.03	2.30±0.01**	2.32±0.03	2.49±0.02*

Notes: ****P<0.05; **P<0.01; ***P<0.001 compared to the control group of broiler chickens*
Source: *developed by the author*

The total protein content in the blood serum of poultry of experimental group 3 on the 42nd day of poultry rearing significantly increased by 1.0 times (P<0.01), the albumin content changed slightly, and in experimental group 3 it slightly increased – by 1.1 times. There is no statistical difference in the amount of albumin content with the indicator of the control group. This indicated that the broiler chickens remained clinically healthy and their bodies maintained proper colloidal and osmotic blood pressure, regulation of water metabolism, the process of binding and transporting lipids, carbohydrates, minerals, and vitamins. Lipids for the body of living things are an energy material. In particular, when 1 g of fat is completely broken down, about 9.6 kcal of energy is released, which is almost 2.2 times more than similar carbohydrates and proteins. The bird's body is provided with energy at the expense of fats due to a lack of carbohydrates. The cell membranes of animal organs and tissues contain a significant amount of lipids (Youssef *et al.*, 2022), and therefore, they also perform a structural function.

The results obtained in this study were consistent with the results of L.W. Chen *et al.* (2021), who found that when feeding broiler chickens probiotics *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae* had a positive effect on intestinal morphological features and regulation of fat metabolism in poultry, an increase in the carcass weight of broiler chickens by 0.771 g, as well as changes in blood serum parameters: an increase in total protein content by 1.4% ($P<0.05$), albumins – by 1.4% ($P<0.01$), reducing cholesterol by 1.1% ($P<0.05$) and uric acid by 1.2% ($P<0.05$), as well as increased activity of alanine aminotransferase by 1.3 times ($P<0.01$) and aspartate aminotransferase by 1.6 times ($P<0.05$). As can be seen from Table 2, there was no statistical difference in the cholesterol content in the blood serum of poultry of experimental groups 1 and 2 with the control group. But in poultry of experimental group 3, this indicator decreased by 1.1 times ($P<0.05$) compared to the control. This is due to the fact that cholesterol synthesis is reduced by the action of a probiotic (Al-Khalaifah *et al*., 2022). The creatinine content in the blood serum of broiler chickens on days 35 and 42 of rearing was within the physiological norm. It was found that creatinine is actively involved in energy processes in cells between mitochondria, and its content in the blood depends on the level of formation and excretion, and this had a positive effect on the muscle development of broiler chickens.

The concentration of uric acid in the blood serum of poultry of experimental groups 1 and 2 decreased by 1.6 times ($P<0.05$) and 1.8 times ($P<0.05$) on the 42nd day of rearing, respectively, compared to the control group. The uric acid content in the blood serum of broiler chickens of the control and experimental groups was within the physiological norm. The results obtained in this paper are consistent with the results of A. Nada *et al.* (2023) that the concentration of uric acid in the blood serum when feeding broiler chickens a probiotic drug decreased by 1.3-1.9% ($P<0.05$). This is indicated by the stability of the balance of microflora in the intestines of poultry, stimulating feeding processes due to the normalisation of microflora, which acts as a source of adjuvant-active substances that enter the blood and stimulate the immune system of broiler chickens. In the future, this leads to an increase in poultry productivity, an increase in the weight of carcasses and internal organs, a decrease in the content of microorganisms in meat and products of slaughter of broiler chickens, and also has a positive effect on the microstructure of muscle tissue, an improvement in the content of amino acid and fatty acid composition of poultry meat.

According to the ALT activity (Table 2) in the blood serum of broiler chickens of the experimental groups, no statistical significance was established with the control group. The results obtained were consistent with data of G. Bazaka *et al.* (2019), who found that probiotic supplements increased serum alanine aminotransferase activity by 2.2% ($P<0.01$) and aspartate aminotransferase 2.0% ($P<0.05$). The content of white blood cells and red blood cells in the blood of broiler chickens increased by 5.6 and 4.8%, respectively. The AST activity in the blood of poultry of experimental groups significantly increased (Table 2). In experimental group 1 – by 19.6% ($P<0.001$), in experimental group 2 – by 27.8% ($P<0.001$), in experimental group 3 – by 31.4% ($P<0.001$) compared to the indicators in the control group. The indicator of AST activity signifies the activation of the formation of substitutable amino acids by reamination during prolonged feeding of the probiotic, and this process is characterised by an increase in muscle tissue weight, which indicates an increase in weight gain in broiler chickens on day 42 of rearing. The content of calcium in the blood serum of poultry on the 42nd day of rearing was at the level of the control group, which indicated that there was no violation of the Ca : P ratio (it was in the range from 1.05 to 1.11).

C. Li *et al.* (2022) found that for feeding broiler chickens a probiotic *Bacillus amyloliquefaciens*

CGMCC18230 during poultry rearing up to 42 days, a higher concentration ($P<0.05$) of phosphorus and alkaline phosphatase activity in the blood serum were observed, but the calcium content did not change. When the experimental groups of broiler chickens were fed the biological product at a dose of 4.0 g/10 dm^3 of distilled water, it was found that their growth improved due to the effect of the probiotic on the activation of processes in the intestine for microbial modulation and increased accumulation of phosphorus and calcium.

C.U. Nwaigwe *et al.* (2020) found that according to the biochemical parameters of the blood serum of poultry, which are significant in assessing the physiological status of the body of broiler chickens, as well as the presence of stress factors and various diseases of poultry, it is possible to assess the state of metabolic processes in the body of broiler chickens. The results obtained in this study regarding the positive effect of probiotic dietary supplements on the morpho-biochemical parameters of the blood of broiler chickens are consistent with the results of other researchers. F.U. Memon *et al.* (2022) found that adding probiotics *Bacillus subtilis* to the diet of broiler chickens had a positive effect on maintaining intestinal homeostasis, modelling its microflora and preventing the development of certain intestinal infections of poultry, as well as increased the immune status and productivity of poultry and improved the quality of slaughter products.

According to the results of experiments that provided for feeding probiotic biologics to broiler chickens in different doses, the biochemical parameters of blood serum on the 35th and 42nd days of their cultivation corresponded to the limits of physiological fluctuations and were characteristic of the corresponding age groups. The results obtained are also consistent with the data of N.R. Sarangi *et al.* (2016) and Yu. M. Podolian (2017). Feeding of the probiotic biological product for 14 days, i.e., from 28 to 42 days of rearing, provided an increase in body weight by 2.0-8.2%, an increase in average daily body weight gain from 96 to 104 g, and feed consumption per 1 kg of weight gain decreased by 1.97-7.55%. According to this study, it was found that feeding probiotic biologics (*Bacillus subtilis* and *Bacillus licheniformis*) to broiler chickens stimulates their productivity.

The results obtained are important for the implementation of the Food Safety System – HACCP (Hazard Analysis and Critical Control Points) at enterprises that process and sell meat and meat products. The basis of this system is the establishment of control over the safety of food products in determining dangerous risks and ensuring the prevention of toxic infections. Providing consumers with safe food products is regulated by national and international laws and regulations. Communicating information to consumers about the implementation of HACCP and traceability systems, as well as its proper functioning, problems, and advantages in implementing these systems throughout the food chain, should provide consumers with an informed choice of a safe and high-quality food product in order to meet the consumer's nutritional needs (Sharma *et al*., 2019). Risk-based control over the technology of feeding broiler chickens is carried out by veterinary medicine specialists, including dosed delivery of probiotic drugs, as well as improving the conditions of keeping poultry, eliminating the occurrence of stress (Vojir *et al*., 2012). The introduction of HACCP and traceability systems at broiler chicken facilities will ensure proper control of poultry rearing with balanced feeding using probiotic biologics and obtaining safe products for slaughtering broiler chickens.

Conclusions

The use of the probiotic preparation in the rearing of broiler chickens for 14 days at the recommended doses of 0.5 g/dm^3, 2.0 and 4.0 g/dm^3 did not reveal any negative changes in the studied morphological and biochemical parameters of the poultry blood. In the blood of broiler chickens of experimental groups 1, 2, and 3, there was a tendency to increase the number of white blood cells on the 35th and 42nd days of rearing, which indicated a sufficient immune status of the body of broiler chickens and an increase in natural resistance against the background of an increase in the number of corresponding cellular factors, namely, on the 35th day of rearing poultry, respectively – by 4.4%, 7.2 and 17.2%; on the 42nd day, respectively, white blood cells – by 2.4%, 9.0 and 14.2% ($P<0.05$) compared to control indicators. The haemoglobin content in the blood of poultry of the experimental groups (on days 35 and 42) increased, respectively, by 1.2%, 2.1 and 3.9% and by 1.2%, 2.5 and 6.2% compared to the control indicators. The content of total protein in the blood serum of poultry of experimental groups 2 and 3 on the 35th day of cultivation slightly increased, respectively, by 1.2 times ($P<0.01$) compared to the control indicators; in experimental group 3 on the 42nd day of cultivation – by 1.0 times ($P<0.01$); the content of albumins in the study groups on the 35th and 42nd days was within the control values. The content of inorganic calcium and phosphorus corresponded to the limits of physiological parameters, which indicated a sufficient level of mineral supply in the control and experimental groups of broiler chickens. The activity of alanine aminotransferase and aspartate aminotransferase, as well as the content of creatinine and total cholesterol in the blood serum of broiler chickens of the experimental groups did not differ from the control group, which indicated the absence of hepatotoxic and nephrotoxic effects of the probiotic drug.

Prospects for further study – to conduct post-slaughter veterinary and sanitary inspection of broiler chicken slaughter products, to establish the biological value of meat and its chemical composition for the use of probiotic biologics.

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Conflict of Interest

None.

References

[1] Al-Khalaifah, H., Al-Nasser, A., Al-Surrayai, T., Sultan, H., Dalal, Al-Attal, D., Al-Kandari, R., Al-Saleem, H., Al-Holi, A., & Dashti, F. (2022). Effect of ginger powder on production performance, antioxidant status, hematological parameters, digestibility, and plasma cholesterol content in broiler chickens. *Animals*, 12(7), article number 901. doi: 10.3390/ani12070901.

[2] Bazaka, G., Sokolyuk, V., Boiko, P., Ligomina, I., & Dukhnytskyi, V. (2019). The effects of Mospilan and Aktara insecticides in the feed on egg production and meat quality of laying hens. *Journal of World's Poultry Research*, 9(4), 233-239. doi: 10.36380/jwpr.2019.29.

[3] Chaturvedi, P., Shukla, P., Giri, B.S., Chowdhary, P., Chandra, R., Gupta, P., & Pandey, A. (2021). Prevalence and hazardous impact of pharmaceutical and personal care products and antibiotics in environment: A review on emerging contaminants. *Environmental Research*, 194, article number 110664. doi: 10.1016/j.envres.2020.110664.

[4] Chen, L.W., Chuang, W.Y., Hsieh, Y.C., Lin, H.H., Lin, W.C., Lin, L.J., Chang, S.C., & Lee, T.T. (2021). Effects of dietary supplementation with Taiwanese tea byproducts and probiotics on growth performance, lipid metabolism, and the immune response in red feather native chickens. *Animal Bioscience*, 34(3), 393-404. doi: 10.5713/ajas.20.0223.

[5] Delgado-Pando, G., Alvarez, C., & Moran, L. (2019). From farm to fork: New strategies for quality evaluation of fresh meat and processed meat products. *Journal of Food Quality*, 2019, article number 4656842. doi: 10.1155/2019/4656842.

[6] Directive 2010/63/EU of the European Parliament and of the council "On the Protection of Animals Used for Scientific Purposes". (2010, September). Retrieved from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:En:PDF.

[7] DSTU ISO 10012:2005. (2007). *Measurement management systems. Requirements for measuring processes and measuring equipment*. Retrieved from http://online.budstandart.com/ua/catalog/doc-page?id_doc=52981.

[8] DSTU ISO/IEC 17025:2019. (2021). *General requirements for the competence of testing and calibration laboratories*. Retrieved from http://online.budstandart.com/ua/catalog/doc-page?id_doc=88724.

[9] European convention for the protection of vertebrate animals used for experimental and scientific purposes. (1986, March). Retrieved from http://zakon4.rada.gov.ua/laws/show/994_137.

[10] Gazwi, H.S.S, Mahmoud, M.E., & Toson, E.M.A. (2022). Analysis of the phytochemicals of Coriandrum sativum and Cichorium intybus aqueous extracts and their biological effects on broiler chickens. *Scientific Reports*, 12, article number 6399. doi: 10.1038/s41598-022-10329-2 27.

[11] Jiang, W., Li, Y., Sun, J., Li, L., Li, J.W., Zhang, C., Huang, C., Yang, J., Kong, G.Y., & Li, Z.F. (2017). Spleen contributes to restraint stress induced changes in blood leukocytes distribution. *Scientific Reports*, 7, article number 6501. doi: 10.1038/s41598-017-06956-9.

[12] Khubeiz, M.M., & Shirif, A.M. (2020). Effect of coriander (*Coriandrum sativum L.*) seed powder as feed additives on performance and some blood parameters of broiler chickens. *Open Veterinary Journal*, 10(2), 198-205. doi: 10.4314/ovj.v10i2.9.

[13] Koronowicz, A.A., Banks, P., Szymczyk, B., Leszczynska, T., Master, A., Piasna, E., Szczepanski, W., Domagala, D., Kopec, A., Piatkowska, E., & Laidler, P. (2016). Dietary conjugated linoleic acid affects blood parameters, liver morphology and expression of selected hepatic genes in laying hens. *British Poultry Science*, 57(5), 663-673. doi: 10.1080/00071668.2016.1192280.

[14] Kucheruk, M.D., & Zasekin, D.A. (2018). Clinical and hematological indicators of broiler chickens under organic cultivation. *Bulletin of the Poltava State Agrarian Academy*, 4, 163-167. doi: 10.31210/visnyk2018.04.25.

[15] Levchenko, V.I. (Ed.). (2017). *Clinical diagnosis of animal diseases.* Bila Tserkva: Bila Tserkva National Agrarian University.

[16] Levchenko, V.I. (Ed.) (2002). *Veterinary clinical biochemistry.* Bila Tserkva: Bila Tserkva National Agrarian University.

[17] Li, C., Cai, H., Li, S., Liu, G., Deng, X., Bryden, W.L., & Zheng, A. (2022). Comparing the potential of *Bacillus amyloliquefaciens* CGMCC18230 with antimicrobial growth promoters for growth performance, bone development, expression of phosphorus transporters, and excreta microbiome in broiler chickens. *Poultry Science*, 101(11), article number 102126. doi: 10.1016/j.psj.2022.102126.

[18] Lyasota, V.P., & Kolodka, A.V. (2020). Hygiene-biotic factors on the application of modern pre- and probiotics in poultry. *Scientific Messenger of Lviv National University of Veterinary Medicine and Biotechnologies. Series: Veterinary Science*, 22(98), 88-93. doi: 10.32718/nvlvet9816.

[19] Memon, F.U., Yang, Y., Zhang, G., Leghari, I.H., Lv, F., Wang, Y., Laghari, F., Khushk, F.A., & Si, H. (2022). Chicken gut microbiota responses to dietary *Bacillus subtilis* probiotic in the presence and absence of *Eimeria* infection. *Microorganisms*, 10(8), article number 1548. [doi: 10.3390/microorganisms10081548](https://doi.org/10.3390/microorganisms10081548).

[20] Mizernytskyi, O. (2021). Use of probiotics in poultry farming. *Modern Poultry Farming*, 1-2(218-219), 12-14.

[21] Mookiah, S., Sieo, C.C., Ramasamy, K., Abdullah, N., & Ho, Y.W. (2014). Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. *Science of Food and Agriculture*, 94(2), 341-348. doi: 10.1002/jsfa.6365.

[22] Nada, A., El-Fateh, M., Amer, M.S., El-Shafei, R.A., Bilal, M., Diarra, M.S., & Zhao, X. (2023). Antioxidative and cytoprotective efficacy of ethanolic extracted cranberry pomace against *Salmonella* enteritidis infection in chicken liver cells. *Antioxidants*, 12(2), article number 460. doi: 10.3390/antiox12020460.

[23] Nwaigwe, C.U., Ihedioha, J.I., Shoyinka, S.V., & Nwaigwe, C.O. (2020). Evaluation of the hematological and clinical biochemical markers of stress in broiler chickens. *Veterinary World*, 13(10), 2294-2300. doi: 10.14202/vetworld.2020.2294-2300.

[24] Podolian, Yu.M. (2016). The effect of probiotics on broiler chickens growth and efficiency. *Biological Bulletin of Bogdan Khmelnitskiy Melitopol State Pedagogical University*, 6(3), 141-148. doi: 10.15421/201680.

[25] Podolian, Yu.M. (2017). Effect of probiotic on hematological parameters of broiler chickens. *Agrarian Science and Food Technology*, 1, 79-83.

[26] Rehman, A., Arif, M., Sajjad, N., Al-Ghadi, M.Q., Alagawany, M., Abd El-Hack, M.E., Alhimaidi, A.R., Elnesr, S.S., Almutairi, B.O., Amran, R.A., Hussein, E.O.S., & Swelum, A.A. (2020). Dietary effect of probiotics and prebiotics on broiler performance, carcass, and immunity. *Poultry Science*, 99(12), 6946-6953. doi: 10.1016/j.psj.2020.09.043.

[27] Sakhatsky, M.I., & Osadcha, Yu.V. (2021). Clinical-biochemical status of hens due to changes of battery cages height location. *Theoretical and Applied Veterinary Medicine*, 9(3), 130-134. doi: 10.32819/2021.93020.

[28] Sarangi, N.R., Babu, L.K., Kumar, A., Pradhan, C.R., Pati, P.K., & Mishra, J.P. (2016). Effect of dietary supplementation of prebiotic, probiotic, and symbiotic on growth performance and carcass characteristics of broiler chickens. *Veterinary World*, 9(3), 313-319. doi: 10.14202/vetworld.2016.313-319.

[29] Savelli, C.J., Simpson, J., & Mateus, C. (2021). Exploring the experiences of members of the international food safety authorities network: An interpretative phenomenological analysis. *Journal of Food Protection*, 84(10), 1683-1697. doi: 10.4315/JFP-21-171.

[30] Sharma, A., Motta, V., & Martinez, L. (2019). Effectiveness of short videos to enhance HACCP information for consumers. *Journal of Foodservice Business Research*, 22(1), 1-14. doi: 10.1080/15378020.2019.1663104.

[31] Shkodyak, N., Zhyla, M., Pyatnychko, O., Avdosyeva, I., & Dmitrotsa, V. (2020). Influence of Bafasal feed additive on morpho-biochemical indices of chickens-broiler's blood. *Scientific and Technical Bulletin* of *State Scientific Research Control Institute of Veterinary Medical Products and Fodder Additives* and *Institute of Animal Biology*, 21(2), 213-218. doi: 10.36359/scivp.2020-21-2.28.

[32] Slawinska, A., Dunislawska, A., Plowiec, A., Gonçalves, J., & Siwek, M. (2021). TLR-mediated cytokine gene expression in chicken peripheral blood mononuclear cells as a measure to characterize immunobiotics. *Genes (Basel),* 12(2), article number 195. doi: 10.3390/genes12020195.

[33] Vojir, F., Schübl, E., & Elmadfa, I. (2012). The origins of a global standard for food quality and safety: Codex Alimentarius Austriacus and FAO/WHO Codex Alimentarius. *International Journal for Vitamin and Nutrition Research*, 82(3), 223-227. doi: 10.1024/0300-9831/a000115.

[34] Xiong, H.H., Lin, S.Y., Chen, L.L., Ouyang, K.H., & Wang, W.J. (2023). The interaction between flavonoids and intestinal microbes: A review. *Foods*, 12(2), article number 320. doi: 10.3390/foods12020320.

[35] Yang, T., Du, M., Zhang, J., Ahmad, B., Cheng, Q., Wang, X., Abbas, Z., Tong, Y., Li, J., Zhou, Y., Zhang, R., & Si, D. (2023). Effects of *Clostridium butyricum* as an antibiotic alternative on growth performance, intestinal morphology, serum biochemical response, and immunity of broilers. *Antibiotics*, 12(3), article number 433. doi: 10.3390/antibiotics12030433.

[36] Youssef, I.M.I., Abdo, I.M.I., Elsukkary, H.F.A., El-Kady, M.F., & Elsayed, M. (2022). Effects of dietary supplementation of chromium methionine chelate on growth performance, oxidative stress, hematological indices, and carcass traits of broiler chickens. *Tropical Animal Health and Production*, 54(5), article number 267. doi: 10.1007/s11250-022-03260-1.

[37] Yuan, P., Xu, H., Ma, Y., Niu, J., Liu, Y., Huang, L., Jiang, S., Jiao, N., Yuan, X., Yang, W., & Li, Y. (2023). Effects of dietary *Galla Chinensis* tannin supplementation on immune function and liver health in broiler chickens challenged with lipopolysaccharide. *Frontiers in Veterinary Science*, 10, article number 1126911. doi: 10.3389/fvets.2023.1126911.

[38] Zhou, X., Jin, E., Li, S., Wang, C., Qiao, E., & Wu, G. (2015). Effects of dietary supplementation of probiotics (*Bacillus subtilis, Bacillus licheniformis*, and *Bacillus natto*) on broiler muscle development and meat quality. *Turkish Journal of Veterinary & Animal Sciences*, 39(2), 203-210. doi: 10.3906/vet-1406-67.

Вплив пробіотичного біопрепарату на морфо-біохімічні показники крові курчат-бройлерів

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Анотація. За виробництва продукції бройлерного птахівництва оператори ринку застосовують інтенсивні технології, що передбачають використання різноманітних, екологічно нешкідливих нутріцевтиків, зокрема, пробіотичних препаратів. У технології вирощування курчат-бройлерів пробіотичні препарати необхідні як засоби для профілактики і лікування шлунково-кишкових захворювань, стимуляції росту та збільшення продуктивності птиці. Мета роботи – дослідити вплив пробіотичного біопрепарату на гематологічні показники за його випоювання курчатам-бройлерам. У досліді використали 20 голів курчат-бройлерів, яким випоювали пробіотик із 28 до 42 доби у кількостях: 0,5 г/10 дм^3, 2,0 і 4,0 г/10 дм^3 води. Встановлено, що морфо-біохімічні показники крові птиці відповідали фізіологічним нормативам для курчат-бролерів встановленого віку та підтверджували відсутність патофізіологічних змін в їх організмі. На 35 та 42 добу вирощування у крові курчат-бройлерів за випоювання пробіотика у дозі 4,0 г/10 дм^3 відзначали збільшення кількості лейкоцитів від 4,4 до 17,2 % і вмісту гемоглобіну – на 3,9 та 6,2 %, відповідно, порівняно з контрольною групою. На 35 добу дослідження за випоювання птиці пробіотика спостерігали збільшення вмісту загального білка у сироватці крові в 1,2 раза, відповідно в дозах 2,0 і 4,0 г/10 дм^3 води, а на 42 добу – в 1.0 раза за його випоювання у дозі 4,0 г/10 дм^3 води. Встановлено, що у сироватці крові птиці вміст кальцію й фосфору неорганічного відповідали фізіологічним межам, що вказує на достатній рівень мінерального живлення організму курчат-бройлерів. Відсутність змін активності аланінамінотрансферази та аспартатамінотрансферази, вмісту загальних ліпідів, холестерину та креатиніну в сироватці крові курчат-бройлерів дослідних груп свідчить про гепато- і нефронетоксичність пробіотику. За результатами роботи досліджений препарат можна рекомендувати для підвищення резистентності організму птиці та регуляції обміну речовин

Ключові слова: резистентність організму; збалансована годівля; морфологічні показники; біохімічні показники; безпечність

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Phosphólipid composition of bile and blood in rats under correction of experimental fatty hepatosis

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Abstract. To date, the aetiology and molecular mechanisms of the development of fatty hepatosis, which is quite common in mammals, have not yet been sufficiently explained. This pathology requires detailed study not only because of functional disorders of the liver and biliary system, but also because of the high probability of dangerous complications – fibrosis, cirrhosis, or hepatocellular carcinoma. The purpose of this study was to determine marker changes in the phospholipid composition of bile and blood in rats with experimental fatty hepatosis and with the use of corrective therapy. Hepatopathology was modelled in *Wistar* laboratory rats by intragastric administration of a 4% solution of tetracycline hydrochloride at the rate of 0.25 g/kg body weight for seven days. Using the method of thin-layer chromatography, the phospholipid components of animal bile and blood were studied. It was found that during experimental fatty hepatosis in rats, there was a decrease in the total phospholipid content in bile, mainly due to a decrease in the level of phosphatidylcholine (by 22.4-27.0%), the total fraction of inositol phosphatide and phosphatidylinositol (by 20.0-27.3%), and phosphatidylethanolamine (by 17.5-25.2%). Conversely, the introduction of milk phospholipids in the form of a dietary supplement "FLP-MD" in sick animals contributed to an increase in bile levels of phosphatidylserine by 67.1-99.8%, inositol phosphatide and phosphatidylinositol by 48.6-57.6%, phosphatidylcholine by 38.8-60.2%, phosphatidylethanolamine by 45.6-57.4%, and sphingomyelin by 30.4-46.3%. In the blood of such rats, a significant decrease in the content of phosphatidylcholine, phosphatidylserine, and sphingomyelin was found, which was not observed after administration of the "FLP-MD" dietary supplement to sick animals. In the case of using the supplement in healthy animals, only a 29.3% increase in the blood content of inositol phosphatide and phosphatidylinositol was noted. The determination of the most sensitive indicators in the phospholipid spectrum of blood and bile reveals the features of changes in molecular processes for the development of fatty hepatosis in animals, and also contributes to preclinical tests of the corrective effectiveness of newly created drugs according to established markers

Keywords: tetracycline hydrochloride; thin-layer chromatography; corrective therapy; dietary supplement "FLP-MD"

Introduction

Fatty liver (fatty hepatosis, steatohepatosis) is a series of liver abnormalities that often begin as simple steatosis and progresses to steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. Due to the growing prevalence of fatty degeneration of liver, it is becoming a serious scientific and clinical problem (Pydyn *et al.,* 2020; Huh *et al.,* 2022). In veterinary practice, animal liver damage, in particular fatty degeneration of liver is noted as often occurring (up to 25%) and has a different aetiology, requires the development of appropriate non-invasive diagnostic methods and effective complex treatment (Imbery *et al.,* 2022; Korolova *et al.,* 2023). Development of fatty hepatosis according to experts (Ota, 2021) is associated with impaired glucose and lipid metabolism in the liver, with hydrophobic bile acid toxicity, macrophage dysfunction and liver stellate cell activation, progressive fibrogenesis, and due to genetic, epigenetic, and environmental factors that contribute to the progression of fibrosis and the risk of hepatocellular carcinoma.

The leading factor of deepening pathological changes in the liver in its fatty hepatosis and the aetiological factor of hepatocellular carcinoma is the accumulation of lipids in liver cells. S. Kartsoli *et al.* (2020) point to the fact that liver tissue samples with fatty degeneration show noticeable changes in the composition of fatty acids and phospholipids, indicating a violation of lipid metabolism as a key factor in the pathogenesis and progression of this disease. Current studies on lipotoxicity and progress in the analysis of the lipid profile, as a leading indicator of pathophysiological mechanisms in fatty hepatosis, show a link between lipid metabolism disorders and chronic inflammation and cell damage. In their studies of liver and serum lipids in patients with this hepatopathology, S. Kartsoli *et al.* (2020) found that as a result of metabolic disorders, several complex lipids, including sphingolipids and other phospholipids, are involved in the lipotoxicity and pathogenesis of steatohepatitis. Among the mechanisms that prevent the transition of fatty hepatosis to hepatocellular carcinoma, D.E. Berardi *et al.* (2022) consider both coordinated autophagic degradation of lipid droplets and stimulation of lipid excretion from hepatocytes.

B. Zhang *et al.* (2021) note that the liver has a pathway for removing various lipid constituents from the body as components of bile. Relatedly, cholesterol and phospholipids synthesised in hepatocytes, together with free fatty acids and triacylglycerols, are secreted to the bile tubules in combination with bile acids and determine the physical and chemical properties of bile. As noted by J.L. Boyer & C.J. Soroka (2021), fatty acids, triacylglycerols, cholesterol and its esters, phospholipids are mandatory lipid components of mammalian bile, but a violation of their ratio can provoke the development of gallstones and damage to the epithelial cells of the bile ducts and the mucous membrane of the gallbladder.

S. Kartsoli *et al.* (2020) note that in the case of fatty hepatosis, the qualitative and quantitative composition of lipids in blood plasma and liver cells changes. First of all, there is a significant accumulation of fatty acids, triacylglycerol, and even phospholipids in the internal environment of the body and a clear correlation between the increase in lipids in the liver parenchyma and in the blood. The search for therapeutic measures that will reduce the intensity of lipid accumulation in the liver and prevent the pathological processes provoked by this accumulation, in particular, increased lipid peroxide oxidation, and inflammation, is an urgent scientific task.

Modern scientific studies using various models of fatty degeneration of liver and statistical analysis of patients' condition indicators in this hepatopathology indicate positive effects in the case of phospholipid-containing drugs and the introduction of phospholipid-containing supplements into the diet of patients with pathology of the hepatobiliary system (Dajani & Popovic, 2020). Essential phospholipids have a hepatoprotective effect, which has been demonstrated in studies on hepatocytes *in vitro* by D. Wupperfeld *et al.* (2022), and in animal models by D. Korolova *et al.* (2023). Phospholipid-containing drugs are actively used in the treatment of patients with fatty liver disease (Yin *et al.,* 2021). Numerous laboratory and clinical studies have investigated the hepatoprotective and therapeutic effects of phospholipids, mainly of plant origin and made from seafood, which are supplemented with omega-3 polyunsaturated fatty acids, food phospholipid-containing egg yolk preparations in combination with fat-soluble vitamins, etc. (Lüchtenborg *et al.,* 2020; Mitrovic *et al.,* 2022).

Affordable and promising in their unique composition and properties are preparations based on milk phospholipids (Gryshchenko *et al.,* 2019). Given the urgency of the problem of finding effective drugs in the treatment of patients with fatty liver degeneration and based on previous studies, the purpose of this study was to investigate the effects of milk phospholipids in the form of dietary supplements "FLP-MD" on the indicators of the total content of phospholipids and five of their individual fractions in the bile and blood of animals with experimental fatty hepatosis.

Literature Review

Fatty liver disease has a number of histological and biochemical features. Among them is the accumulation of lipids in the cells of the liver parenchyma, which is a common feature of pathological conditions of all levels of severity in this disease (Younossi *et al.,* 2016; Gryshchenko, 2017). This liver disease is considered the result of a metabolic syndrome, which, in addition to the accumulation of lipids in the liver tissue, is characterised by abdominal obesity, hyperglycemia, dyslipidemia (Chalasani *et al.,* 2018). It is extremely important to diagnose fatty hepatosis before it develops into steatohepatitis, which can progress to cirrhosis of the liver with complications such as ascites, portal hypertension, and encephalopathy (McGlinchey *et al.,* 2022). The next threatening complication of fatty liver dystrophy and cirrhosis is hepatocellular carcinoma with an unfavourable prognosis.

Non-invasive and minimally invasive tests, including serum biomarkers, are cost-effective and convenient. However, these tests currently have several limitations, such as variability, insufficient accuracy, and risk factors for errors. The limitations and variability of these tests encourage researchers to combine them into diagnostic algorithms to produce more accurate tools (Gryshchenko, 2017; Bassal *et al.,* 2022). Identifying patients with significant fibrosis is important for targeted therapy to prevent disease progression. Effective screening using non-invasive tests can be crucial for patient risk stratification and early diagnosis. Given the need to accumulate information about various biochemical markers of blood in fatty liver degeneration, it is important to conduct a comprehensive analysis of blood composition (Zhou *et al.,* 2019).

The pathogenesis of fatty liver disease is still unclear. It is believed that a number of different damaging factors induce the development of this pathological condition in individuals with a corresponding genetic predisposition (Borrelli *et al.,* 2018). There is also sexual dimorphism of this hepatic pathology, differences in the course and prognosis in people of different sexes (Martin-Grau & Monleon, 2023). Fat disease is one of the main liver diseases, which determines the relevance of its study. The pathological profile includes fatty liver disease and steatohepatitis. However, in clinical practice, there is no reliable non-invasive parameter for detecting characteristic differences between them. With the help of lipidomic analysis, it is possible to distinguish between these two pathological conditions. Moreover, it is in the content of various blood phospholipids that the most striking differences between fatty liver disease and steatohepatitis are revealed (Wang *et al.,* 2021).

Studies of the mechanisms of development of fatty liver disease are carried out simultaneously with the search for effective treatment methods. The course of pathological processes is associated with the state and functioning of cell membranes, and therefore membrane lipids are key structural, signalling, and regulatory components involved in the course of fatty hepatosis in mammals. Therefore, a violation of the ratio of membrane lipids is one of the mechanisms of the pathogenesis of fat degeneration in this organ. In steatohepatosis and steatohepatitis, noticeable changes were found not only in the composition of fatty acids, but also in the total content of phospholipids in the liver parenchyma (Puri *et al.,* 2007). Since these lipids affect many aspects of the pathophysiology of fatty liver disease, they can potentially be prescribed for the treatment of patients (Welch *et al.,* 2022). At the same time, it was found that the effectiveness of drugs in this pathology is also associated with their effect on lipid metabolism and lipid homeostasis (Park *et al.,* 2020).

Later studies have demonstrated changes in the content of various phospholipid fractions in liver biopsies of patients with steatohepatitis, which indicates a violation of their synthesis and is associated with disease progression (Chiappini *et al.,* 2017). The most pronounced statistically significant changes were observed when comparing the content of phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylcholine, and sphingomyelin in the blood plasma of healthy subjects and patients with fatty hepatosis and steatohepatitis (Tiwari-Heckler *et al.,* 2018). In non-toxic concentrations, essential phospholipids (0.1 and 0.25 mg/mL), polyenylphosphatidylcholine and phosphatidylinositol (0.1 and 1 mg/mL) increase the fluidity of the hepatocyte cell membrane, reduce the percentage of apoptosis, and stimulate hepatocellular transport processes that ensure the elimination of substances from liver cells. In combination, this can significantly improve the functional state of the liver (Wupperfeld *et al.,* 2022).

The results of the analysis of the use of phospholipids in the composition of drugs for the treatment of fatty liver disease confirm the improvement in the condition of patients and indicate the need for further study of all aspects of the effectiveness of phospholipid-containing drugs, to clarify the mechanisms of their action (Dajani & Popovic, 2020).

Materials and Methods

The experimental research was conducted during 2021-2022 at the scientific laboratories of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine (NUBIP of Ukraine) and the educational and scientific centre Institute of High Technologies of Taras Shevchenko National University of Kyiv. In the study of the effect of dietary supplements "FLP-MD" based on milk phospholipids on the total content of phospholipids in bile and their five individual representatives: the total fraction of inositol phosphatide and phosphatidylinositol, phosphatidylserine, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, male *Wistar* laboratory rats with a body weight of 200±50 g (n=13) were used. Electronic scales ORION OS-0K22 (ORION ELECTRONICS LTD, Europe) were used for weighing animals.

Laboratory rats were kept in standard vivarium conditions at a temperature 22-24°C, with a 14-hour light period of the day, on a standardised complete diet and with free access to water. The following methods were observed during the experiment: European Convention "For the Protection of Vertebrate Animals Used for Research and other Scientific Purposes" (1986), and Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006, February). All surgical interventions during acute experiments were also performed in compliance with ARRIVE recommendations and were performed without violating the guidelines of Council Directive 2010/63/EU "On the Protection of Animals Used for Scientific Purposes" (2010).

Fatty hepatosis was reproduced according to the author's own method (Gryshchenko *et al.,* 2019). During the modulation of fatty hepatosis, rats were administered a 4% solution of tetracycline hydrochloride using a soft silicone probe at the rate of 0.25 g/kg body weight daily intragastrically for seven days. The rats developed tetracycline-induced fatty hepatosis. The "Self-rehabilitation" group (n=5) was formed from animals that did not receive any therapy under the conditions of antibiotic administration. To determine the corrective efficacy of the liposomal form of a 1% solution of the dietary supplement "FLP-MD", whose main active ingredient is milk phospholipids, on the phospholipid content in bile, rats were orally administered the aforementioned dietary supplement one hour before the administration of tetracycline hydrochloride and additionally for the following two days after the completion of antibiotic administration. The "Correction" group (n=4) was formed from animals treated with tetracycline hydrochloride and dietary supplements "FLP-MD". For animals of this group, the daily dose of dietary supplement"FLP-MD" was 13.5 mg/kg of body weight (Pat. 86516 UA). Rats in the control group ("Control", n=4) were intragastrically injected with an equivalent volume of distilled water. During the study, the animals' body weight was regularly monitored, and drug doses were recalculated consideirng changes in body weight during the experiment. Bile samples were collected in acute experiments on rats with a cannulated bile duct and their phospholipid content was determined. Bile samples were collected under certain conditions. Namely, the day before the acute experiment, the rats were weighed and then kept on a starvation diet with free access to drinking water. Animals were restricted in feeding during the day before surgery during an acute experiment to eliminate possible effects of feed consumption on the formation and secretion of bile.

Immediately before surgery, the animals were intraperitoneally injected with sodium thiopental at a dose of 7 μg/100 g of body weight as anaesthesia. After the animal was put under anaesthesia, the abdominal wall was dissected along a white line and the bile duct was cannulated using a plastic cannula connected to a micropipette. Next, the opening of the abdominal wall was covered with a dressing moistened with saline solution to prevent moisture loss from the dissected abdominal cavity. In the future, bile samples were collected from the immobilised animal in separate plastic containers every 30 minutes for three hours of acute experiment. Thus, at the end of the three-hour acute experiment, six individual half-hour bile samples were obtained. At the third hour of the acute experiment, blood samples were also taken from animals to determine the content of phospholipids in it: total and fractional composition. In samples of bile and native blood stabilised with heparin, after appropriate extraction, a complex of phospholipids characteristic of these biolids was examined, which were divided by thin-layer chromatography on standard "Silufol" plates (Czech Republic) into individual fractions: total inositol phosphatide and phosphatidylinositol, phosphatidylserine, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine (Pat. 99031324 UA). The following mixture was used as a solvent for determining five individual phospholipid fractions in prepared lipid-containing bile samples: chloroform:methanol:water:acetic acid in a ratio of 63:25:4:2.

The results obtained were statistically processed using the "Statistica 5.0" software suite ("StatSoft Inc.", USA). As an indicator of the statistical significance of the differences found between the indicators in different experimental groups, the Student's t-test was calculated in the case of normal distribution of data. The Shapiro-Wilk test was used to determine the nature of the distribution (normal or abnormal). The differences between the two indicators of the compared samples at $P<0.05$, $P<0.01$, and $P<0.001$ were considered statistically significant (Filimonova *et al.,* 2005).

Results and Discussion

A study of the concentration of phospholipids in bile obtained from rats with experimental fatty hepatosis showed that both the total content of phospholipids and their individual fractions – mandatory components of liver secretions – decreased compared to the control

(Fig. 1, Tables 1-5). Thus, the total concentration of phospholipids in the bile of animals with experimental pathology ("Self-rehabilitation" group) was 20.1-25.4% less than the control indicators (50.8-58.7 mg%). The use of dietary supplements "FLP-MD" based on milk phospholipids led to a significant increase in the concentration of total phospholipids in the bile of experimental rats compared to the control. In particular, the content of phospholipids in the bile of animals of the "Correction" group was 79.2-86.2 mg%, which is 40.4-56.1% more than the control indicators. The differences between the indicators of total phospholipids in the bile of laboratory rats from the "Self-rehabilitation" and "Correction" groups are even more significant. Thus, in sick animals that were orally administered dietary supplements "FLP-MD" based on milk phospholipids, the concentration of phospholipids in bile samples increased by 75.8-109.3% compared to rats of the "Self-rehabilitation" group (Fig. 1).

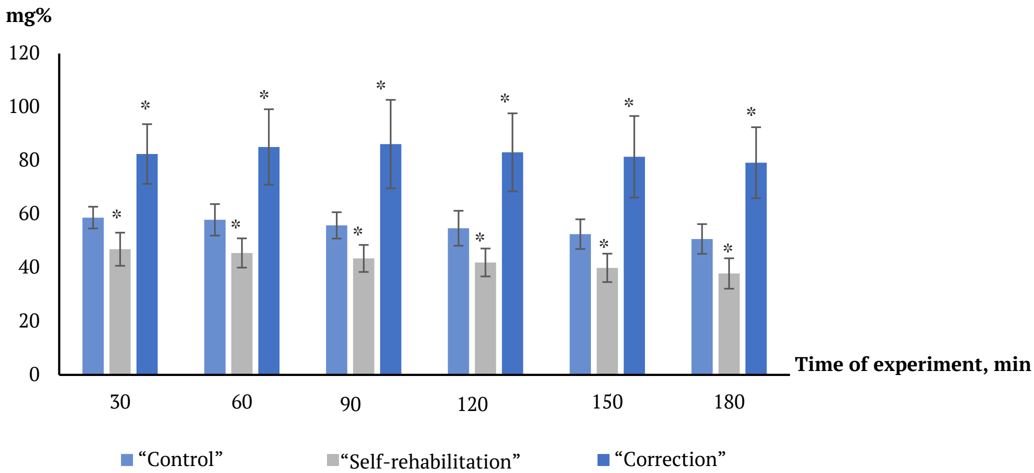

Figure 1. Concentration (mg%) of phospholipids in the bile of rats of the "Control", "Self-rehabilitation", and "Correction" groups

Notes: ** P<0.05, a statistically significant difference compared to the control;*
"Control" – n=4; rats with experimental fatty hepatosis "Self-rehabilitation" – n=5; sick rats that received the dietary supplements "FLP-MD" based on milk phospholipids "Correction" – n=4
Source: *developed by the author*

Consequently, the use of a 1% solution of the liposomal form of dietary supplement "FLP-MD" based on milk phospholipids in an experimentally determined therapeutic dose caused significant changes in the processes of phospholipid secretion in the bile ducts. This was accompanied by a significant increase in the concentration of phospholipids in hepatic secretions. But the question arises whether phospholipids of the dietary supplement "FLP-MD" affect the metabolic and transport processes in hepatocytes, or to a large extent, absorbed by liver cells, transit them and are released to the bile ducts. One of the ways to investigate the molecular mechanism of the effect of milk phospholipids in the form of dietary supplements "FLP-MD" on the phospholipid composition of bile was to determine the spectrum of phospholipids of hepatic secretions in rats of all three experimental groups.

The author's modification of the components of the solvent mixture using the thin-layer chromatography method allowed detecting five phospholipid fractions in the collected rat bile samples. They were evaluated in conjunction with the results of a study of individual phospholipids in rat blood samples from three experimental groups. It was established that in the conditions of simulated fatty hepatosis, the concentration of the total fraction of inositol phosphatide and phosphatidylinositol in the blood of experimental animals was statistically significantly different from the control values only in blood samples from the group of rats receiving only dietary supplement "FLP-MD" based on milk phospholipids (Fig. 2).

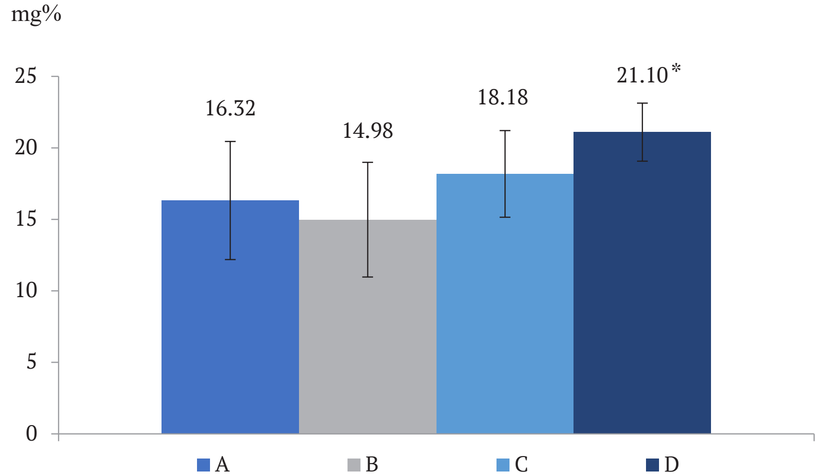

Figure 2. Concentration (mg%) of the total fraction of inositol phosphatide and phosphatidylinositol in the blood of rats of the "Control", "Self-rehabilitation", and "Correction" groups and in clinically healthy animals that received dietary supplement "FLP-MD" based on milk phospholipids

Notes: **P<0.05, a statistically significant difference compared to the control; A – "Control" group (n=4); B – modelling fatty hepatosis ("Self-rehabilitation" group, n=5); C – rats with modelling fatty hepatosis, which were obtained the dietary supplement ("Correction" group, n=4); D – rats, which were obtained only the dietary supplement ("FLP-MD" group, n=4)*

Source*: developed by the author*

In the bile of rats under the conditions of simulated pathology ("Self-rehabilitation" group), a decrease in the concentration of the total fraction of inositol phosphatide and phosphatidylinositol was observed by 20.0-27.3% compared to the control group (Table 1). The applied phospholipids of milk in the form of dietary supplements caused an increase in the concentration of the total fraction of inositol phosphatide and phosphatidylinositol in the bile of rats by 48.6-57.6% in the first two bile samples from six collected in an acute experiment compared to the control indicators and in all the studied samples by 85.8-100.7% compared to the indicators of animals of the "Self-rehabilitation" group (Table 1).

Table 1. Concentration (mg%) of inositol phosphatide and phosphatidylinositol in the bile of rats of the "Control", "Self-rehabilitation", and "Correction" groups

Group of rats / Time of experiment, min	"Control"	"Self-rehabilitation"	"Correction"
30	3.70±0.45	2.96±0.45**	5.50±0.76*###
60	3.60±0.45	2.90±0.44**	5.68±1.10*##
90	3.42±0.40	2.68±0.41**	5.20±1.65#
120	3.28±0.41	2.54±0.43**	4.93±1.55#
150	3.10±0.39	2.38±0.41**	4.63±1.35##
180	3.00±0.39	2.18±0.41**	4.38±1.36##

Notes: **P<0.05, **P<0.01 statistically significant difference compared to the control; #P<0.05, ##P<0.01, ###P<0.001 statistically significant difference in the indicators of the "Correction" group compared to the indicators of the "Self-rehabilitation" group. "Control" group – (n=4); for modelling fatty hepatosis – "Self-rehabilitation" group (n=5); sick rats of the "Correction" group (n=4), which were injected with the dietary supplement "FLP-MD"*
Source*: developed by the author*

The results obtained suggest that the applied correction method promotes the elimination of inositol phosphatide and phosphatidylinositol with bile. At the same time, the concentration of these lipids was characterised by a tendency to increase in the blood of animals with fatty hepatosis when using dietary supplements.

As noted by C.N. Feriod *et al.* (2017), inositol triphosphatide plays a key role in the formation of lipid droplets in hepatocytes and is also involved in the development of fatty liver disease. Together with diacylglycerol, it is produced from membrane phosphatidylinositol in response to regulatory physiological and pathophysiological signals. In the future, this secondary mediator reveals its effects on the course of various intracellular processes. Considering the involvement of inositol triphosphate in the development of fatty liver disease, namely, stimulating the synthesis and accumulation of fat droplets in hepatocytes, the resulting effect of dietary supplements "FLP-MD" prevents the development of fatty degeneration of the liver parenchyma.

Phosphatidylinositol belongs to the minor phospholipid fractions in terms of its content in both blood and bile, which coincides with its own results of chromatographic examination of the corresponding samples of biological material. The authors suggest that the study of the content of this phospholipid fraction in bioliquids will provide a better understanding of the possible links of its metabolic transformations in normal and pathological conditions. In particular, F. de Oliveira Lemos *et al.* (2019) noted that additional studies of the intracellular regulatory pathway inositol triphosphate-calcium may open up prospects for developing pharmacological strategies for the treatment of liver diseases specifically targeting each intracellular receptor isoform of this secondary mediator.

Under the conditions of experimental fatty hepatosis, a significant decrease in the content of phosphatidylserine in the blood of rats by 25.7% compared with its concentration in control samples was noted (Fig. 3). In animals of other experimental groups, this indicator did not differ statistically significantly from the range of control values.

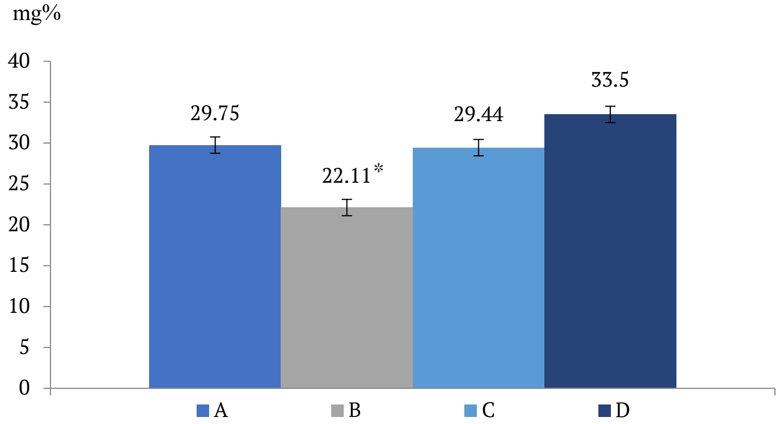

Figure 3. Concentration (mg%) of phosphatidylserine in the blood of rats of the "Control", "Self-rehabilitation", and "Correction" groups and in clinically healthy animals that received dietary supplement "FLP-MD"

Notes: **P<0.05, a statistically significant difference compared to the control; A – "Control" group (n=4); B – modelling fatty hepatosis ("Self-rehabilitation" group, n=5); C – rats with modelling fatty hepatosis, which were obtained the dietary supplement ("Correction" group, n=4); D – rats, which were obtained only the dietary supplement ("FLP-MD" group, n=4)*

Source*: developed by the author*

Under the conditions of simulated pathology ("Self-rehabilitation" group), there was a tendency to reduce the concentration of phosphatidylserine in the bile of rats compared to the control indicators. The introduction of a dietary supplement based on milk phospholipids to rats of the "Correction" group provokes an increase in the concentration of phosphatidylserine in rat bile by 42.0-62.1% compared to the control group. At the same time, in the bile of rats with fatty hepatosis treated with dietary supplements "FLP-MD", the content of phosphatidylserine increased by 67.1-99.8% compared to the indicators in animals of the "Self-rehabilitation" group. Thus, the increase in the content of phosphatidylserine in the bile of animals that were administered dietary supplements "FLP-MD" against the background of experimental pathology exceeded the stimulating effect of the dietary supplement compared to the control group (Table 2).

Table 2. Concentration (mg%) of phosphatidylserine in the bile of rats of the groups "Control", "Self-rehabilitation", "Correction"

Group of rats Time of experiment, min	"Control"	"Self-rehabilitation"	"Correction"
30	4.40±0.67	3.74±0.49	6.25±0.70*##
60	4.27±0.70	3.56±0.46	6.42±1.12*#
90	4.08±0.68	3.44±0.50	6.53±1.36#
120	3.90±0.72	3.26±0.52	6.13±1.11#
150	3.70±0.72	3.02±0.48	5.85±1.12#

Table 2. Continued

Group of rats Time of experiment, min	"Control"	"Self-rehabilitation"	"Correction"
180	3.50 ± 0.67	2.84 ± 0.50	5.68 ± 1.28#

Notes: **P<0.05, a statistically significant difference compared to the control; #P<0.05, ##P<0.01, a statistically significant difference in the indicators of the "Correction" group compared to the indicators of the "Self-rehabilitation" group. "Control" group – (n=4); for modelling fatty hepatosis – "Self-rehabilitation" group (n=5); sick rats of the "Correction" group (n=4), which were injected with the dietary supplement "FLP-MD"*

Source*: developed by the author*

Y. Zhou *et al.* (2021) reported that phosphatidylserine enriched with docosahexaenoic acid has the ability to reduce the likelihood of negative consequences for fatty liver disease and effectively reduces serum levels of total cholesterol, triacylglycerols, non-esterified fatty acids and low-density lipoprotein cholesterol, as well as significantly increases the level of high-density lipoprotein cholesterol. According to the results obtained to determine the effectiveness of dietary supplements "FLP-MD" against the background of experimental liver pathology, an increased intake of phosphatidylserine to bile was noted, which coincided with maintaining its content in the blood at the level of control values. The study of other lipid fractions in the blood and bile is considered for the future. In the conditions of experimental hepatopathology ("Self-rehabilitation" group), the concentration of sphingomyelin in the blood of laboratory animals was characterised by a significant decrease of 1.5 times compared to the control (Fig. 4).

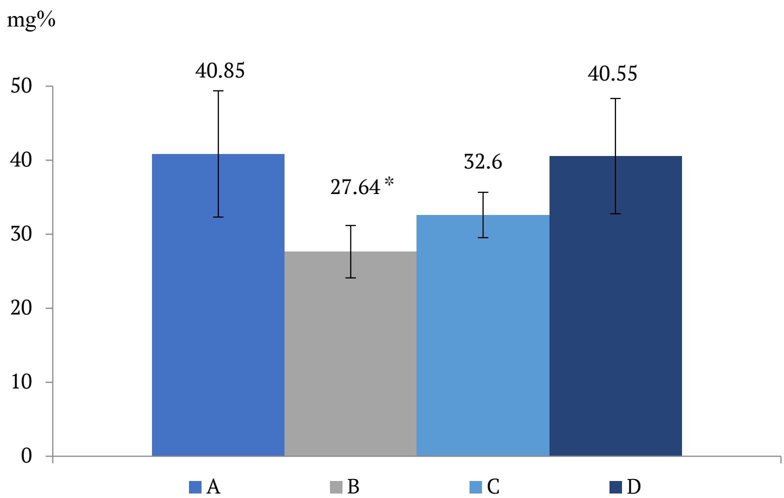

Figure 4. Concentration (mg%) of sphingomyelin in the blood of rats of the "Control", "Self-rehabilitation", and "Correction" groups and in clinically healthy animals that received dietary supplement "FLP-MD"

Notes: **P<0.05, a statistically significant difference compared to the control; A – "Control" group (n=4); B – modelling fatty hepatosis ("Self-rehabilitation" group, n=5); C – rats with modelling fatty hepatosis, which were obtained the dietary supplement ("Correction" group, n=4); D – rats, which were obtained only the dietary supplement ("FLP-MD" group, n=4)*

Source*: developed by the author*

The introduction of the "FLP-MD" dietary supplement ("Correction" group) to sick animals restrained from sharp quantitative changes in this indicator in the blood, which corresponded to the range of concentration values of the control group. And the introduction of dietary supplements to clinically healthy animals did not show a statistically significant effect. S. Tiwari-Heckler *et al.* (2018) reported a significant increase in serum sphingomyelin in patients with fatty liver dystrophy and steatohepatitis compared to the control against the background of metabolic disorders caused by diabetes mellitus, unfavourable PNPLA3 genotype, and other malformations. The inconsistency of the data obtained may be related to the different background clinical conditions of patients, lipid examination of serum rather than whole blood, and differences in methodological approaches (the researchers used liquid chromatography mass spectrometry (LC-MS/MS)).

In the conditions of simulated pathology ("Self-rehabilitation" group), there is a tendency to reduce the concentration of sphingomyelin in the bile of rats compared to the control indicators. However, oral administration of a milk phospholipid supplement to rats ("Correction" group) caused a significant increase in the concentration of sphingomyelin in the bile of rats by 30.4-46.3% compared to its value in animals of the control group and by 50.5-76.6% compared to the indicators in rats from the "Self-rehabilitation" group. Consequently, dietary supplement "FLP-MD" based on milk phospholipids effectively stimulated the flow of sphingomyelin to bile in animals with experimental fatty hepatosis (Table 3).

Table 3. Concentration (mg%) of sphingomyelin in the bile of rats of the "Control", "Self-rehabilitation", and "Correction" groups

Group of rats Time of experiment, min	"Control"	"Self-rehabilitation"	"Correction"
30	5.43±0.56	4.70±0.50	7.08±0.90*##
60	5.30±0.76	4.50±0.50	7.27±1.15*##
90	5.13±0.77	4.36±0.47	7.50±1.36*##
120	4.95±0.75	4.20±0.47	7.15±1.28*##
150	4.70±0.79	3.96±0.44	6.80±1.43*##
180	4.50±0.75	3.68±0.46	6.50±1.23*##

Notes: **P<0.05, a statistically significant difference compared to the control; ##P<0.01, a statistically significant difference in the indicators of the "Correction" group compared to the indicators of the "Self-rehabilitation" group. "Control" group – (n=4); for modelling fatty hepatosis – "Self-rehabilitation" group (n=5); sick rats of the "Correction" group (n=4), which were injected with the dietary supplement "FLP-MD"*
Source*: developed by the author*

In experimental fatty hepatosis, the concentration of phosphatidylcholine in the blood of experimental rats in the "Self-rehabilitation" group experienced a significant decrease of 1.1 times compared to animals in the "Control" group (Fig. 5). Administration of dietary supplements "FLP-MD" based on milk phospholipids ("Correction" group) to animals contributed to the tendency to increase the content of phosphatidylcholine in the blood of sick rats, compared with the control. In addition, the dietary supplement administered to clinically healthy animals ("Dietary supplement" group) also caused a similar trend with the previous group of animals regarding quantitative changes in the blood concentration of phosphatidylcholine (Fig. 5).

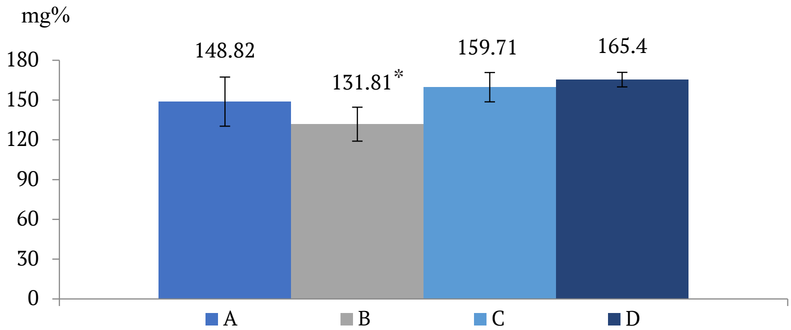

Figure 5. Concentration (mg%) of phosphatidylcholine in the blood of rats of the "Control", "Self-rehabilitation", and "Correction" groups and in animals that received dietary supplement "FLP-MD"

Notes: **P<0.001, a statistically significant difference compared to control; A – "Control" group (n=4); B – modelling fatty hepatosis ("Self-rehabilitation" group, n=5); C – rats with modelling fatty hepatosis, which were obtained the dietary supplement ("Correction" group, n=4); D – rats, which were obtained only the dietary supplement ("FLP-MD" group, n=4)*

Source*: developed by the author*

In the conditions of experimental hepatopathology, a significant decrease in the concentration of phosphatidylcholine in the bile of sick rats ("Self-rehabilitation" group) was observed by 22.4-27.0% compared to its indicators in the control (Table 4). Oral administration of milk phospholipids in the form of the "FLP-MD" dietary supplement to experimental animals caused a significant increase in the concentration of phosphatidylcholine in the bile by 38.8-60.2% compared to the control and by 78.9-119.5% compared to the corresponding indicators in rats of the "Self-rehabilitation" group (Table 4). Consequently, the decrease in phosphatidylcholine content in rat bile caused by tetracycline hydrochloride in the simulation of fatty hepatosis was not only eliminated by the use of dietary supplements "FLP-MD", but also led to an increase in the content of this important phospholipid component in it.

Table 4. Concentration (mg%) of phosphatidylcholine in the bile of rats of the "Control", "Self-rehabilitation", and "Correction" groups

Experimental group Time of experiment, min	"Control"	"Self-rehabilitation"	"Correction"
30	35.98±1.54	27.92±3.89**	49.95±7.14**###
60	35.75±3.33	27.16±3.21**	51.95±8.10**###
90	34.40±2.44	25.86±2.87**	53.10±8.90**###
120	33.98±3.97	25.16±2.90**	51.98±7.85**###
150	32.60±3.03	24.08±3.17**	51.88±8.47**###
180	31.60±3.16	23.06±3.34**	50.63±7.34**###

Notes: ***P<0.01, a statistically significant difference compared to the control; ###P<0.001, a statistically significant difference in the indicators of the "Correction" group compared to the indicators of the "Self-rehabilitation" group. "Control" group – (n=4); for modelling fatty hepatosis – "Self-rehabilitation" group (n=5); sick rats of the "Correction" group (n=4), which were injected with the dietary supplement "FLP-MD"*

Source*: developed by the author*

The study of phosphatidylethanolamine content in the blood and bile of animals with experimental fatty hepatosis is relevant, since the literature notes that this phospholipid is one of the possible pathogenetic factors that leads to the progression of fatty liver disease in existing obesity (Shama *et al.*, 2023). Thus, the researchers noted an increase in the concentration of phosphatidylethanolamine against the background of a clinically established diagnosis of obesity. It is assumed that this effect is associated with a causal cascade of influence on the functional ability of various liver cells. In contrast to the described fact, according to the data presented in the paper by S. Tiwari-Heckler *et al.* (2018), on the contrary, there was a significant decrease in serum phosphatidylethanolamine in patients with fatty liver dystrophy and steatohepatitis compared to the control, which occurred against the background of metabolic disorders and other clinical diagnoses. According to the results of the analysis of blood samples taken from experimental rats with a drug-induced form of fatty hepatosis against the background of tetracycline hydrochloride administration, a significant decrease in the content of phosphatidylethanolamine in the experimental group "Self-rehabilitation" was revealed, which coincides with the data of S. Tiwari-Heckler *et al.* (2018) and is shown in Figure 6. The use of dietary supplements "FLP-MD" ("Correction" group) allowed normalising the level of phosphatidylethenolamine in the blood of experimental animals to values close to the control (Fig. 6).

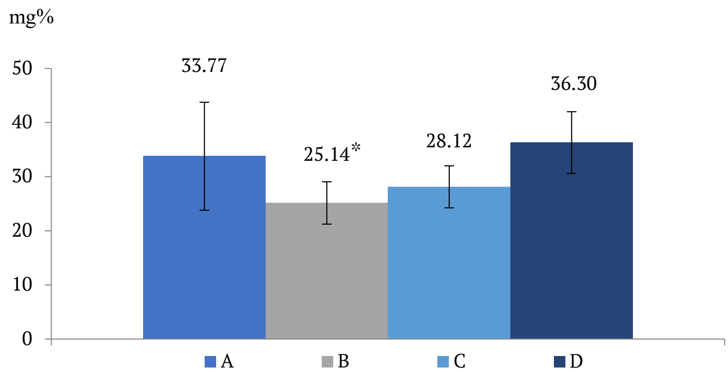

Figure 6. Concentration (mg%) of phosphatidylethanolamine in the blood of rats of the "Control", "Self-rehabilitation", and "Correction" groups and in animals that received dietary supplement "FLP-MD"

Notes: **P<0.05, a statistically significant difference compared to the control. A – "Control" group (n=4); B – modelling fatty hepatosis ("Self-rehabilitation" group, n=5); C – rats with modelling fatty hepatosis, which were obtained the dietary supplement ("Correction" group, n=4); D – rats, which were obtained only the dietary supplement ("FLP-MD" group, n=4)*

Source*: developed by the author*

This contradictory nature of the results of the study of the content of phosphatidylethanolamine in the blood, first of all, is explained by the specifics of experiments and the features of clinical diagnoses in the examined individuals.

Under the conditions of tetracycline-induced hepatosis, a significant decrease in the concentration of phosphatidylethanolamine in rat bile was observed by 17.5-25.2% compared to the control group. The use of milk phospholipids in the form of dietary supplements caused a significant increase in the concentration of phosphatidylethanolamine in the bile of rats compared to the control by 45.6-57.4%, and in comparison with similar data in animals of the "Self-rehabilitation" group, it increased by 80.3-97.5% (Table 5).

Table 5. Concentration (mg%) of phosphatidylethanolamine in the bile of rats of the "Control", "Self-rehabilitation", and "Correction" groups

Experimental group Time of experiment, min	"Control"	"Self-rehabilitation"	"Correction"
30	9.23±0.85	7.60±0.84*	13.70±1.64**###
60	8.98±0.64	7.40±0.84*	13.77±2.62**###
90	8.80±0.63	7.14±0.80*	13.85±3.24**##
120	8.65±0.68	6.82±0.88*	12.93±2.77**##
150	8.45±0.63	6.52±0.81**	12.30±2.83**##
180	8.15±0.58	6.10±0.97**	12.05±2.04**###

Notes: **P<0.05, **P<0.01, statistically significant difference compared to the control; ##P<0.01, ###P<0.001, statistically significant difference in the indicators of the "Correction" group compared to the indicators of the "Self-rehabilitation" group. "Control" group – (n=4); for modelling fatty hepatosis – "Self-rehabilitation" group (n=5); sick rats of the "Correction" group (n=4), which were injected with the dietary supplement "FLP-MD"*
Source*: developed by the author*

According to M. Masoodi *et al.* (2021), changes in blood phospholipid levels are one of the biomarkers of fatty liver disease. According to the results described in this paper, the content of various individual phospholipids in the bile of rats undergoes significantly greater changes than in the blood of animals with experimental fatty hepatosis. This indicates the need for a comprehensive lipid study, considering changes in both mammalian blood and bile. The multidirectional nature of quantitative changes in individual phospholipid fractions can be explained by various mechanisms of influence of these phospholipids on liver function and their participation in the pathogenesis of fatty liver. The use of dietary supplements "FLP-MD" based on milk phospholipids in the experiment statistically significantly changed the content of phosphatidylserine, sphingomyelin, and phosphatidylcholine in the blood of sick animals, which was also noted by other researchers (Gundermann *et al.,* 2016; Dajani & Popovic, 2020; Lüchtenborg *et al.,* 2020). Instead, a significant increase in the concentration of all five phospholipid fractions was observed in the bile of these rats compared not only with animals with experimental fatty hepatosis, but also with controls. The intake of phospholipids in the bile reflects the course of transmembrane transport processes that ensure the movement of these mandatory components of bile to the bile tubules. In conditions of pathology, transport processes are disrupted, which leads to changes in the ratio of lipids in the blood, bile, and liver cells. Scientific sources also emphasise other mechanisms of effects of phospholipid use in the case of fatty liver disease (Ota, 2021; Bassal *et al.,* 2022; Mitrovic *et al.,* 2022). These include a reduction of the intensity of oxidative stress by regulating the activity of enzymes that produce reactive oxygen species, a controlled decrease in the intensity of inflammation and fibrogenesis in the liver (Dajani & Popovic, 2020), an increase in the fluidity of hepatocyte membranes along with a decrease in apoptosis and intensification of hepatocellular transport (Wupperfeld *et al.,* 2022). Both according to the results of the conducted studies and according to other researchers, the effectiveness and systemic effect of phospholipid-containing drugs in fatty liver disease was confirmed. However, it is the milk phospholipids used in the creation of the dietary supplement "FLP-MD" that demonstrate a significant positive effect on the bile secretory function of the liver and show a corrective effect on the concentration of individual phospholipids in the blood of animals during artificial reproduction of fatty hepatosis.

Conclusions

The processes of phospholipid entry into hepatocytes and their secretion into bile are significantly disrupted in the experimental tetracycline-induced form of fatty hepatosis in laboratory rats. Phospholipid components of dietary supplements "FLP-MD" are of animal origin (from milk), and therefore, differ in the most pronounced bioavailability, as evidenced by a significant increase in the content of all certain fractions of phospholipids in the blood of experimental rats. In the future, phospholipids of dietary supplements "FLP-MD", entering the liver, accelerate the restoration of metabolic and transport processes in its cells, contributing to the establishment of a complete composition of bile according to the main biochemical parameters. Considering pathological disorders of lipid homeostasis during experimental fatty liver degeneration, the high effectiveness of dietary supplements "FLP-MD" in normalising the content of phospholipids both in the blood and in the bile of sick animals was established. This gives reason to consider promising the use of dietary supplements "FLP-MD" to correct the structural and functional state of the liver and improve the metabolism of phospholipids for its damage, in particular, with tetracycline antibiotics. When using the supplement on clinically healthy rats, a neutral effect on the studied indicators was observed, with the exception of an increase in the concentration of the total fraction of inositol phosphatide and phosphatidylinositol in the blood by 29.3% compared to the control.

In accordance with the results obtained, it is reasonable to recommend the dietary supplement "FLP-MD" as a drug effective for the development of fatty hepatosis, including for preventing the occurrence of complications in the form of intrahepatic and extrahepatic cholestasis. The latter is explained by the fact that an experimentally determined increase in the content of phosphatidylserine, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine in the bile of sick animals is a favourable sign, since it improves its physical and chemical properties and allows removing phosphatidylinositol from liver cells as a risk factor for increased fatty degeneration of liver tissue.

In the future, it is planned to investigate the features of the lipid and bile acid spectrum of blood and bile in laboratory rats in experimental hepatopathology and to determine the corrective effectiveness of dietary supplements "FLP-MD" based on milk phospholipids in a model experiment.

Acknowledgements

None.

Conflict of Interest

None.

References

[1] Bassal, T., Basheer, M., Boulos, M., & Assy, N. (2022). Nonalcoholic fatty liver disease-a concise review of noninvasive tests and biomarkers. *Metabolites,* 12(11), article number 1073. doi: 10.3390/metabo12111073.

[2] Berardi, D.E., Bock-Hughes, A., Terry, A.R., Drake, L.E., Bozek, G., & Macleod, K.F. (2022). Lipid droplet turnover at the lysosome inhibits growth of hepatocellular carcinoma in a *BNIP3*-dependent manner. *Science Advances,* 8(41), article number eabo2510. doi: 10.1126/sciadv.abo2510.

[3] Borrelli, A., Bonelli, P., Tuccillo, F.M., Goldfine, I.D., Evans, J.L., Buonaguro, F.M., & Mancini, A. (2018). Role of gut microbiota and oxidative stress in the progression of non-alcoholic fatty liver disease to hepatocarcinoma: Current and innovative therapeutic approaches. *Redox Biology,* 15, 467-479. doi: 10.1016/j.redox.2018.01.009/.

[4] Boyer, J.L., & Soroka, C.J. (2021). Bile formation and secretion: An update. *Journal of Hepatology,* 75(1), 190-201. doi: 10.1016/j.jhep.2021.02.011.

[5] Chalasani, N., Younossi, Z., Lavine, J.E., Charlton, M., Cusi, K., Rinella, M., Harrison, S.A., Brunt, E.M., & Sanyal, A.J. (2018). The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Journal of Hepatology,* 67(1), 328-357. doi: 10.1002/hep.29367.

[6] Chiappini, F., Coilly, A., Kadar, H., Gual, P., Tran, A., Desterke, C., Samuel, D., Duclos-Vallée, J.C., Touboul, D., Bertrand-Michel, J., Brunelle, A., Guettier, C., & Le Naour, F. (2017). Metabolism dysregulation induces a specific lipid signature of nonalcoholic steatohepatitis in patients. *Scientific Reports,* 7, article number 46658. doi: 10.1038/srep46658.

[7] Council Directive 2010/63/EU "On the Protection of Animals Used for Scientific Purposes". (2010, September). Retrieved from https://eur-lex.europa.eu/legalcontent/EN/TXT/?uri=celex%3A32010L0063.

[8] Dajani, A.I., & Popovic, B. (2020). Essential phospholipids for nonalcoholic fatty liver disease associated with metabolic syndrome: A systematic review and network meta-analysis. *World Journal of Clinical Cases,* 8(21), 5235-5249. doi: 10.12998/wjcc.v8.i21.5235.

[9] de Oliveira Lemos, F., Florentino, R.M., Melo Lima Filho, A.C., Dos Santos, M.L., & Leite, M.F. (2019). Inositol 1,4,5-trisphosphate receptor in the liver: Expression and function. *World Journal of Gastroenterology*, 25(44), 6483-6494. doi: 10.3748/wjg.v25.i44.6483.

[10] European convention for the protection of vertebrate animals used for experimental and other scientific purposes. (1986). Retrieved from https://rm.coe.int/168007a67b.

[11] Feriod, C.N., Oliveira, A.G., Guerra, M.T., Nguyen, L., Richards, K.M., Jurczak, M.J., Ruan, H.B., Camporez, J.P., Yang, X., Shulman, G.I., Bennett, A.M., Nathanson, M.H., & Ehrlich, B.E. (2017). Hepatic inositol 1,4,5 trisphosphate receptor type 1 mediates fatty liver. *Hepatology Communications,* 1(1), 23-35. doi: 10.1002/hep4.1012.

[12] Filimonova, N.B., Fil, I.O., & Mykhailova, T.S. (2005). Statistical analysis of data according to the principles of science-based medicine. Comparison of groups in quantitative terms. *Medicine of Ukrainian Transport*, 4, 86-93.

[13] Gryshchenko, V.A. (2017). Hematological profile of rats in experimental diclofenac-induced hepatitis. *Ukrainian Journal of Ecology*, 7(3), 78-83. doi: 10.15421/2017_52.

[14] Gryshchenko, V.A., Musiychuk, V.V., Chernyshenko, V.O., Gornytska, O.V., & Platonova, T.M. (2019). Evaluation of biochemical indicators in blood plasma of rats with tetracycline-induced hepatosis and their correction by milk phospholipids. *The Ukrainian Biochemical Journal*, 91(1), 92-99. doi: 10.15407/ubj91.01.092.

[15] Gundermann, K.-J., Gundermann, S., Drozdzik, M., & Mohan Prasad, V.G. (2016). Essential phospholipids in fatty liver: A scientific update. *Clinical and Experimental Gastroenterology*, 5(9), 105-117. doi: 10.2147/CEG.S96362.

[16] Huh, Y., Cho, Y.J., & Nam, G.E. (2022). Recent epidemiology and risk factors of nonalcoholic fatty liver disease. *Journal of Obesity & Metabolic Syndrome*, 31(1), 17-27. doi: 10.7570/jomes22021.

[17] Imbery, C.A., Dieterle, F., Ottka, C., Weber, C., Schlotterbeck, G., Müller, E., Lohi, H., & Giger, U. (2022). Metabolomic serum abnormalities in dogs with hepatopathies. *Scientific Reports*, 12(1), article number 5329. doi: 10.1038/s41598-022-09056-5.

[18] Kartsoli, S., Kostara, C.E., Tsimihodimos, V., Bairaktari, E.T., & Christodoulou, D.K. (2020). Lipidomics in non-alcoholic fatty liver disease. *World Journal of Hepatology,* 12(8), 436-450. doi: 10.4254/wjh.v12.i8.436.

[19] Korolova, D., Gryshchenko, V., Chernyshenko, T., Platonov, O., Hornytska, O., Chernyshenko, V., Klymenko, P., Reshetnik, Ye., & Platonova, T. (2023). Blood coagulation factors and platelet response to drug-induced hepatitis and hepatosis in rats. *Animal Models and Experimental Medicine,* 6(1), 66-73. doi: 10.1002/ame2.12301.

[20] Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty". (2006, February). Retrieved from https://zakon.rada.gov.ua/go/3447-15.

[21] Lüchtenborg, C., Niederhaus, B., Brügger, B., Popovic, B., & Fricker, G. (2020). Lipid profiles of five essential phospholipid preparations for the treatment of nonalcoholic fatty liver disease: A comparative study. *Lipids*, 55(3), 271-278. doi: 10.1002/lipd.12236.

[22] Martin-Grau, M., & Monleon, D. (2023). Sex dimorphism and metabolic profiles in management of metabolic-associated fatty liver disease. *World Journal of Clinical Cases,* 11(6), 1236-1244. doi: 10.12998/wjcc.v11.i6.1236.

[23] Masoodi, M., Gastaldelli, A., Hyötyläinen, T., Arretxe, E., Alonso, C., Gaggini, M., Brosnan, J., Anstee, Q.M, Millet, O., Ortiz, P., Mato, J.M, Dufour, J.-F., & Orešič, M. (2021). Metabolomics and lipidomics in NAFLD: Biomarkers and non-invasive diagnostic tests. *Nature Reviews Gastroenterology & Hepatology*, 18, 835-856. doi: 10.1038/s41575-021-00502-9.

[24] McGlinchey, A.J., Govaere, O., Geng, D., Ratziu, V., Allison, M., Bousier, J., Petta, S., de Oliviera, C., Bugianesi, E., Schattenberg, J.M., Daly, A.K., Hyötyläinen, T., Anstee, Q.M., & Orešič, M. (2022). Metabolic signatures across the full spectrum of non-alcoholic fatty liver disease. *JHEP Reports,* 4(5), article number 100477. doi: 10.1016/j.jhepr.2022.100477.

[25] Mitrovic, M., Sistilli, G., Horakova, O., & Rossmeisl, M. (2022). Omega-3 phospholipids and obesity-associated NAFLD: Potential mechanisms and therapeutic perspectives. *European Journal of Clinical Investigation,* 52(3), article number e13650. doi: 10.1111/eci.13650.

[26] Ota, T. (2021). Molecular mechanisms of nonalcoholic fatty liver disease (NAFLD)/ Nonalcoholic steatohepatitis (NASH). *Advances in Experimental Medicine and Biology*, 1261, 223-229. doi: 10.1007/978-981-15-7360-6_20.

[27] Park, S.-H., Lee, J.-E., Lee, S.M., Lee, J., Seo, C.-S., Hwang, G.-S., & Jung, J. (2020). An unbiased lipidomics approach identifies key lipid molecules as potential therapeutic targets of Dohongsamul-tang against non-alcoholic fatty liver diseases in a mouse model of obesity. *Journal of Ethnopharmacology,* 5(260), article number 112999. doi: 10.1016/j.jep.2020.112999.

[28] Puri, P., Baillie, R.A., Wiest, M.M., Mirshahi, F., Choudhury, J., Cheung, O., Sargeant, C., Contos, M.J., & Sanyal, A.J. (2007). A lipidomic analysis of nonalcoholic fatty liver disease. *Hepatology,* 46(4), 1081-1090. doi: 10.1002/hep.21763.

[29] Pydyn, N., Miękus, K., Jura, J., & Kotlinowski, J. (2020). New therapeutic strategies in nonalcoholic fatty liver disease: A focus on promising drugs for nonalcoholic steatohepatitis. *Pharmacological Reports,* 72(1), 1-12. doi: 10.1007/s43440-019-00020-1.
[30] Shama, S., Jang, H., Wang, X., Zhang, Y., Shahin, N.N., Motawi, T.K., Kim, S., Gawrieh, S., & Liu, W. (2023). Phosphatidylethanolamines are associated with nonalcoholic fatty liver disease (NAFLD) in obese adults and induce liver cell metabolic perturbations and hepatic stellate cell activation. *International Journal of Molecular Sciences,* 24(2), article number 1034. doi: 10.3390/ijms24021034.
[31] Tiwari-Heckler, S., Gan-Schreier, H., Stremmel, W., Chamulitrat, W., & Pathil, A. (2018). Circulating phospholipid patterns in NAFLD patients associated with a combination of metabolic risk factors. *Nutrients,* 10(5), article number 649. doi: 10.3390/nu10050649.
[32] Wang, Z.-H., Zheng, K.I., Wang, X.-D., Qiao, J., Li, Y.-Y., Zhang, L., Zheng, M.-H., & Wu, J. (2021). LC-MS-based lipidomic analysis in distinguishing patients with nonalcoholic steatohepatitis from nonalcoholic fatty liver. *Hepatobiliary & Pancreatic Diseases International*, 20(5), 452-459. doi: 10.1016/j.hbpd.2021.05.008.
[33] Welch, M., Secunda, C., Ghimire, N., Martinez, I., Mathus, A., Patel, U., Bhogoju, S., Al-Mutairi, M., Min, K., & Lawan, A. (2022). Characterization and roles of membrane lipids in fatty liver disease. *Membranes (Basel),* 12(4), article number 410. doi: 10.3390/membranes12040410.
[34] Wupperfeld, D., Fricker, G., Bois De Fer, B., Frank, L., Wehrle, A., & Popovic, B. (2022). Essential phospholipids decrease apoptosis and increase membrane transport in human hepatocyte cell lines. *Lipids in Health and Disease*, 21(1), article number 91. doi: 10.1186/s12944-022-01698-8.
[35] Yin, M., Matsuoka, R., Xi, Y., & Wang, X. (2021). Comparison of egg yolk and soybean phospholipids on hepatic fatty acid profile and liver protection in rats fed a high-fructose diet. *Foods*, 10(7), article number 1569. doi: 10.3390/foods10071569.
[36] Younossi, Z.M., Koenig, A.B., Abdelatif, D., Fazel, Y., Henry, L., & Wymer, M. (2016). Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*, 64, 73-84. doi: 10.1002/hep.28431.
[37] Zhang, B., Kuipers, F., de Boer, J.F., & Kuivenhoven, J.A. (2021). Modulation of bile acid metabolism to improve plasma lipid and lipoprotein profiles. *Journal of Clinical Medicine*, 11(1), article number 4. doi: 10.3390/jcm11010004.
[38] Zhou, J.H., Cai, J.J., She, Z.G., & Li, H.L. (2019). Noninvasive evaluation of nonalcoholic fatty liver disease: Current evidence and practice. *World Journal of Gastroenterology*, 25(11), 1307-1326. doi: 10.3748/wjg.v25.i11.1307.
[39] Zhou, Y., Tian, S., Qian, L., Jiang, S., Tang, Y., & Han, T. (2021). DHA-enriched phosphatidylserine ameliorates non-alcoholic fatty liver disease and intestinal dysbacteriosis in mice induced by a high-fat diet. *Food & Function*, 12, 4021-4033. doi: 10.1039/d0fo03471a.

Фосфоліпідний склад жовчі та крові в щурів за корекції експериментального жирового гепатозу

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Анотація. На сьогодні ще недостатньо з'ясовано етіологію і молекулярні механізми розвитку досить поширеного в ссавців жирового гепатозу. Ця патологія потребує детального вивчення не лише через функціональні порушення печінки та біліарної системи, але й високу ймовірність виникнення небезпечних ускладнень – фіброзу, цирозу або гепатоцелюлярної карциноми. Мета цього дослідження полягала у визначенні маркерних змін фосфоліпідного складу жовчі та крові в щурів за експериментального жирового гепатозу та при застосуванні коригувальної терапії. Моделювання гепатопатології в лабораторних щурів лінії *Wistar* здійснювали шляхом внутрішньошлункового введення 4 %-го розчину тетрацикліну гідрохлориду з розрахунку 0,25 г/кг маси тіла тварини, впродовж семи діб. Завдяки використанню методу тонкошарової хроматографії проведено дослідження фосфоліпідних компонентів жовчі та крові тварин. Встановлено, що за експериментального жирового гепатозу в щурів відбувалося зменшення в жовчі загального вмісту фосфоліпідів, переважно за рахунок зниження рівня фосфатидилхоліну (на 22,4-27,0 %), сумарної фракції інозитолфосфатиду і фосфатидилінозитолу (на 20,0-27,3 %) та фосфатидилетаноламіну (на 17,5-25,2 %). І, навпаки, введення хворим тваринам фосфоліпідів молока у вигляді біодобавки «FLP-MD» сприяло зростанню в жовчі рівня фосфатидилсерину на 67,1-99,8 %, інозитолфосфатиду і фосфатидилінозитолу на 48,6-57,6 %, фосфатидилхоліну на 38,8-60,2 %, фосфатидилетаноламіну на 45,6-57,4 % та сфінгомієліну на 30,4-46,3 %. У крові таких щурів виявлялося вірогідне зменшення вмісту фосфатидилхоліну, фосфатидилсерину і сфінгомієліну, що не відмічалося за введення хворим тваринам біодобавки «FLP-MD». У випадку застосування біодобавки здоровим тваринам відзначали лише збільшення в крові вмісту інозитолфосфатиду і фосфатидилінозитолу на 29,3 %. Визначення найчутливіших показників у фосфоліпідному спектрі крові та жовчі розкриває особливості змін молекулярних процесів за розвитку в тварин жирового гепатозу, а також сприяє проведенню доклінічних випробувань корегувальної ефективності новостворених препаратів за встановленими маркерами

Ключові слова: тетрацикліну гідрохлорид; тонкошарова хроматографія; корегувальна терапія; біодобавка «FLP-MD»

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Effect of probiotic drugs and their metabolites on the microflora of digestive canal of broiler chickens

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Abstract. The relevance of the study is conditioned by the growing demand of the population for safe and organic poultry products. Various microbiological preparations are used as therapeutic and preventive means and alternatives to the use of antibiotics in organic poultry farming. The purpose of the study is to determine the effect of preparations of probiotic microorganisms and

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their metabolites on the optimal composition of the microflora of the digestive canal, the general condition, and the liveability of broiler chickens. The effect of various types of drugs on broiler chickens was investigated: the probiotic LactoPharm LP12, the postbiotic Bacteriosan, and a bacteriocin nisin solution. The qualitative and quantitative composition of the microflora of the digestive canal of broiler chickens was determined by the method of bacteriological cultures. The disc diffusion method, using commercial discs with a minimum inhibitory concentration, revealed sensitivity to antibiotics. The effective effect of the new postbiotic Bacteriosan on the intestines of poultry was confirmed – no coagulase-positive *Staphylococcus aureus* was isolated on the 30th day of the experiment, and the lowest titre of *Escherichia coli* was recorded on days 30, 60, and 81 of the experiment. In terms of the content of lactic acid bacteria in the intestines of broiler chickens, the highest concentration was found in the experimental groups that used the probiotic LactoPharm LP12 and the experimental postbiotic by 21% and 32%, respectively, compared to control group. Treatment of bedding material with prophylactic drugs in poultry houses of experimental groups contributed to air sanitation and a decrease in the microbial background of the room compared to the control. The lowest mortality rate of chickens (-12%) was observed when using probiotics and postbiotics, due to the positive effect on the microbiocenosis of the digestive canal. When using an aqueous solution of nisin, the mortality rate was 14%. Thus, the use of the postbiotic Bacteriosan at a dose of 5 mL/kg of feed and the probiotic LactoPharm LP12 at a dose of 1 g/L of drinking water for seven days with a weekly break during the entire period of poultry rearing was experimentally substantiated, which will allow for effective prevention of infectious diseases and increase the liveability of broiler chickens

Keywords: microbiocenosis; intestine; postbiotic; nisin; organic poultry farming

Introduction

The poultry industry is developing rapidly and is an important component in providing the population with meat products. Organic products, which are characterised by higher quality and safety compared to inorganic ones, are becoming widespread and popular in the world. Domestic production of organic poultry products is controlled by the State Service of Ukraine for Food Safety and Consumer Protection. The establishment of principles and requirements for the organic industry, turnover and labelling of these products is carried out in accordance with Law of Ukraine No. 2496-VII "On the Basic Principles and Requirements for Organic Production, Circulation and Labelling of Organic Products" (2018). Organic poultry farming is a promising industry, driven by increased consumer demand for natural and healthy products, and a fast return on investment period, compared to other livestock industries.

The use of antibiotics is one of the most pressing environmental issues. These drugs are used not only for therapeutic purposes, but also for the prevention of diseases and stimulation of animal growth (*antibiotic growth promoters* (*AGPs*), and their widespread use leads to the development of antimicrobial resistance (Krysiak *et al*., 2021). Antimicrobial-resistant pathogenic strains of microorganisms can spread in the environment and complicate the treatment of poultry, animals, and humans. The ban on the use of antimicrobial agents as growth stimulants (AGP) for animals in the European Union countries came into force in 2006, in accordance with Regulation 1831/2003/EC (Regulation (EU)..., 2003). This event initiated a similar practice in other countries of the world. In recent years, governments and professional organisations have developed a number of legislative documents, requirements, and recommendations based on the concept of "One Health" (Kosenko *et al.,* 2022).

As an alternative to the use of antibiotics in organic animal husbandry and poultry farming, prebiotics, probiotics, and postbiotics that have a positive effect on the body are increasingly being used (Reuben *et al.,* 2021). Prebiotics are used by microorganisms as food, the available forms of which include oligosaccharides, lactulose, and inulin derivatives. Probiotics have a direct effect on the intestinal microbiome by selectively supplying beneficial microorganisms to the bird's digestive canal. Probiotics are living microorganisms that can suppress pathogenic microorganisms in the gastrointestinal tract of animals and poultry, while they are easily combined with other feed additives, while additionally reducing the anthropogenic impact on the environment. Probiotics are most commonly used in clinical practice: bacteria of the genus *Lactobacillus*, *Lactococcus, Bifidobacterium* and *Streptococcus*, as well as yeast *Saccharomyces* (Kothari *et al*., 2019).

The current strategy for raising poultry without antibiotics is aimed at the fastest possible development of proper intestinal microbiocenosis by adjusting the species composition. The immune status and homeostasis of the body depend on the functioning of the digestive system and metabolism. For each animal species, the optimal microbiocenosis will be different in terms of the species and quantitative ratio of microorganisms. There is an increase in the number and diversity of microorganisms of the gastrointestinal tract in the direction of progression from the duodenum to the large intestine. Lacto- and bifidobacteria increase the fermentation of carbohydrates. Butyrate is produced, which is a useful energy substrate for enterocytes and for maintaining the integrity of the intestinal wall (Zhang *et al*., 2021).

Postbiotics are metabolites of probiotics containing live microorganisms. Cell wall components and cytoplasmic extract of many *Lactobacillus* species have been proven to be effective postbiotics (Abd El-Ghany *et al.,* 2022). For the manufacture of postbiotic drugs, non-pathogenic microorganisms are used that are resistant to acid and bile, are able to modulate immune responses, and have a positive effect on intestinal metabolic activity. The advantage of postbiotics is their resistance to acids and environmental conditions, so they can be stored for a long time under different temperature conditions. Studies have confirmed that feeding postbiotics to poultry improves the functioning of the gastrointestinal tract and supports immunity (Zhong *et al*., 2022; Ducatelle *et al*., 2023).

Thus, since organic animal husbandry restricts the use of a whole list of veterinary drugs, alternative means are needed to prevent infectious diseases, and reduce or eliminate the use of veterinary antibacterial drugs. The purpose of the study is to investigate the effect of different types of preparations on the indicators of survival and intestinal microbiota of broiler chickens: probiotic LactoPharm LP12, postbiotic Bacteriosan, and solution of bacteriocin nisin.

Literature Review

Probiotics have a positive effect on the living organism, are able to maintain the homeostasis of the digestive canal of poultry and increase their productivity by changing the composition and function of the intestinal microbiota, as a result, they are increasingly used in poultry farming (Zommiti & Ferchichi, 2021). The most common probiotics include bacteria of the genus *Lactobacillus* (Xu *et al*., 2022).

Lactobacilli are widely represented in the environment and are a component of the normal intestinal microflora in animals. *Lactobacillus* bacteria are able to colonise the epithelial tissues of the poultry intestine and exhibit bactericidal and bacteriostatic effects on foreign microflora due to the synthesis of organic acids (lactic, acetic), hydrogen peroxide, and antibiotic substances. S. Gujvinska & A. Paliy (2018) investigated the biological properties of various strains of lactobacilli and bifidobacteria and found different degrees of inhibitory effect on opportunistic microorganisms in the cultures under study. High antagonistic activity was shown by the following strains from the experimental cultures: *Lactobacillus plantarum 37, Lactobacillus casei 27 N, Lactobacillus plantarum 7-317, Lactobacillus plantarum 22 N., Bifidobacterium adolescentis 3 N, Bifidobacterium adolescentis 47, Bifidobacterium adolescentis 17-316.*The use of experimental strains reduced the number of *Escherichia coli* bacteria, fungi of the genus *Candida*, and the number of microorganisms of the genus *Staphylococcus* (Gujvinska & Paliy, 2018).

A group of researchers from China found that *L. plantarum* ST is a promising probiotic strain that is characterised by resistance in the conditions of the artificial gastrointestinal tract, has high antibacterial activity, and has an antagonistic effect on *Salmonella typhimurium* and *Escherichia coli* (Yang *et al*., 2022). This strain has been found to have a large number of genes involved in carbohydrate transport and metabolism. According to recent studies, inclusion of *L. plantarum* in the diet increases the average daily growth of broiler chickens, increases the content of IgG and IgA in the blood serum, and significantly reduces the number of *Escherichia coli* (Wang *et al*., 2023), which is consistent with the results of other experiments.

Postbiotic is a product of the metabolism of probiotic microorganisms that affects the biological functions of the body. A promising area for creating new postbiotics is the combination of organic acids and bacteriocins (natural antibiotic substances of protein or peptide nature), which are already in industrial production (Kucheruk *et al*., 2018). Nisin is a lantobiotic that is of great interest as an alternative to antibiotics, is characterised by a broad spectrum of action against Gram-positive bacteria and acid-resistant mycobacteria. This antimicrobial drug is synthesised by strains of *Lactococcus lactis* approved for use in the food industry as a preservative by the World Health Organisation and the European Union. A number of studies have established the immunomodulatory effect of nisin on the animal body, which indicates the therapeutic effect of this compound (Malaczewska & Kaczorek-Lukowska, 2021). The expediency of using nisin as a feed additive in animal and poultry feeding requires additional scientific evidence (Kieronczyk *et al*., 2017).

The use of organic acids as antimicrobial agents is promising. When selecting and developing appropriate drugs, the specific effect of various types of acids on pathogenic agents is considered: lactic acid is characterised by greater antimicrobial activity; sorbic acid – antifungal effect, etc. The addition of organic acids to the diet affects the histological structure of the gastrointestinal tract, increases the length of villi, and improves the absorption function of the mucous membrane. Nutrients are better absorbed, which has a positive effect on the growth rate of the poultry. Organic acids, in particular lactic acid, are used in poultry farming to prevent bacterial and fungal contamination. They are able to reduce the pathogenic effects of bacterial infections, such as *Salmonella, Campylobacter, Clostridium perfringens* etc. (Khan *et al*., 2022).

Therefore, normalisation and maintenance of a stable composition of the intestinal microflora of poultry acts as a preventive measure for the occurrence of diseases, since it ensures the full functioning of the digestive, hormonal, and immune systems of the body, the health of poultry and its productive indicators.

Materials and Methods

Testing of microbiological preparations and their metabolites was carried out on the premises of the organic poultry farming enterprise of Ukraine – farm "Dacha" – during 2017-2022. A total of 200 units of broiler chickens of the Cobb-500 cross were selected for the study.

To establish the preventive effect of drugs based on probiotic microorganisms and their metabolites, a production study and a series of laboratory analyses of the microbiological composition of the small intestine of broiler chickens were conducted, the composition of microflora was determined, depending on the composition of the diet and the duration of use of probiotic drugs and their metabolites. The effect of the following drugs was investigated: the probiotic LactoPharm LP12, (LLC "LACTOPHARM" Ukraine), an aqueous solution of nisin, manufactured by "Bio-engineering Co" (China) and postbiotic Bacteriosan. Probiotic LactoPharm LP12 is a drug based on the strain *Lactobacillus plantarum AMT 12*. Morphological features of this strain – gram-positive, immobile bacilli that do not form spores, are placed in the form of filamentous rods or chains.

Postbiotic Bacteriosan was developed and patented by employees of the Department of Veterinary Hygiene named after Professor A.K. Skorokhodko. Experimental drug-postbiotic in 1,000 mL contains: 1 g of nisin (China), 100 mL of 40% lactic acid (Ukraine), and 899 mL of distilled water. Basic physical and chemical properties of the drug Bacteriosan is a clear liquid of light yellow colour, which has a specific smell, and foams slightly when shaken. Before the start of the experiment, isolated rooms were built at the "Dacha" farming enterprise and the territory was fenced off for keeping experimental groups of broiler chickens. The poultry was kept in wooden houses equipped with local heating (underfloor heating and infrared lamps), provided with ventilation and separate fenced paddocks covered with vegetation, at the rate of 4 m^2 for 1 animal. The birds were provided with feeders and a nipple watering system. To protect broiler chickens from predatory birds, a safety net was installed over the paddocks. To determine and compare the effectiveness of the drugs under study in the conditions of organic farming, according to the principle of live weight analogues, four groups were established: one control and three experimental groups, each with 50 animals, respectively (Table 1).

Table 1. Research scheme

Group	Number of animals in a group	Feed composition
Control group	50	Organic feed, without the use of preparations
Experimental 1	50	Organic feed and water were replaced with a probiotic solution *LactoPharm LP12*
Experimental 2	50	Organic feed treated with an aqueous solution of nisin
Experimental 3	50	Organic feed and Bacteriosan

Source: *compiled by the authors*

Chickens of the control group received organic feed without microbiological preparations and their metabolites. No treatment of the bedding material was carried out. Chickens of the first experimental group were fed organic feed, and water was replaced with a solution of the probiotic LactoPharm LP12 in a dosage of 1 g/L twice a day, for 1 hour in the morning and evening for 7 days, then a week break. This scheme was used to feed the probiotic throughout the production period. The probiotic preparation was stored in the refrigerator in a tightly closed container. Additionally, the probiotic LactoPharm LP12 was treated with bedding material once every three days by irrigation, at a dose of 5 mL/m^2. Chickens of the second experimental group were provided with organic feed treated with an aqueous solution of nisin. To prepare the solution, the preparation in an amount of 0.1 g was dissolved in 1 litre of water, the feed was treated at the rate of 5 mL/kg. Chickens of the third experimental group were fed organic feed treated with the postbiotic Bacteriosan at a dose of 5 mL/kg of feed, the drug was prepared *ex tempore*. Drinking was combined with the treatment of bedding material, carried out by spraying, once every three days, at the rate of 5 mL/m^2 of room surfaces and bedding. Combined bedding was used for chickens. When keeping chickens on a warm floor, a gauze cloth was used, which was replaced once a week. The rest of the floor was covered with a layer of pine sawdust.

Before starting the treatment of bedding material and during the study, sanitary and hygienic indicators were determined, such as the microbial count of air, temperature, and humidity in the poultry house. The cleanliness of the air in the poultry house was evaluated by counting the number of microorganisms per 1 m^3. Air samples inside closed spaces were taken by sedimentation. Petri dishes with meat-peptone agar were used, and the exposure time lasted 1-3 minutes. Three air samples were taken from each experimental and control group. The samples were cultured at 37°C for 18 hours, then kept at room temperature for 24 hours. The composition of the microflora of the digestive canal of broiler chickens was determined by taking intestinal samples and conducting microbiological studies on days 30, 60, and 80 of the experiment. Three samples were selected from the control and experimental groups, according to Rules for the selection of samples of pathological material, blood, fodder, water and their forwarding for laboratory research. No 15-14/111 (1997). Determination of the composition of the microflora of the digestive canal of broiler chickens was carried out in the laboratory "Ukrainian Laboratory of Quality and Safety of Agricultural Products", which operates in accordance with the requirements EN ISO/IEC 17025:2017 (2017).

When taking samples to investigate the microflora of the digestive canal of poultry, ligatures were applied to the small intestine, below the duodenum. The intestines were suspended together with their contents by adding an isotonic NaCl solution, swirled in a rotation shaker for 10 minutes and allowed to infuse for another 10 minutes, and then inoculated on nutrient media. The study determined the number of lactic acid microorganisms DSTU ISO 4833:2006 (2007). ISO 15214:1998 (1998), coagulase-positive *Staphylococcus aureus* using Baird-Parker agar medium (DSTU ISO 6888-1:2003 (2004), *Salmonella spp.* (Guidelines "Methods of isolation and identification of salmonella") and *Enterobacteriaceae* (DSTU ISO 6887-1:1999 (2004).

The susceptibility of *Salmonella spp.* to antibiotics was determined by the disc diffusion method, using commercial disks with a minimum concentration of a drug that inhibits the growth of the microorganism. The research was conducted according to the recommendations of EUCAST (European Committee on Antimicrobial Susceptibility Testing) (n.d.) of the European Society for Clinical Microbiology and Infectious Diseases (Giske *et al.,* 2022). The production study using live poultry was carried out in accordance with the ARRIVE guidelines (Percie du Sert *et al.*, 2020).

Staining of isolated microorganisms was performed, followed by determination of morphological features by microscopy. A set of reagents "Gram colouring" (Ukraine) and a laboratory microscope "Granum" (China) were used. Statistical analysis was performed using the Microsoft Excel computer programme. The arithmetic mean (M) and statistical error of the arithmetic mean (m) were determined. The significance of the differences was assessed by Student's t-test. The difference between the indicators was considered significant at $P<0.05$ or 5%, which is acceptable for most biological studies.

Results and Discussion

In order to improve the efficiency of organic meat production, natural preventive measures were tested. It was found that in the first period of growth, chickens are most sensitive to various pathogens. According to L.K. Nolan *et al.* (2020), the immune system of chickens is not fully developed at hatching, so the body is susceptible to various infections. Especially in the first weeks of life, chickens often suffer from bacterial infections, which lead to significant livestock deaths and economic losses. One of the causes of high mortality is *Escherichia coli* infections, which cause enteritis, disrupt the respiratory tract, and cause septicemia.

During the first stage of the study, the number of dead chickens was recorded and the cause of their death was determined. From day 1 to day 30 of the study, a high mortality rate was observed, with the highest number of chickens dying in the control group – 9 animals. In the first experimental group, three chickens died, one of them due to mechanical injury, and one died as a result of gastric obstruction. In the second experimental group, five chickens died, one of them due to gastric obstruction. In the third experimental group, three chickens died, one of them as a result of mechanical injury. The heat also contributed to the deaths of chickens in the experimental groups, as the young organism could not sufficiently carry out proper thermoregulation. M.E. Abd El-Hack *et al.* (2022) in their paper report on the effect of hyperthermia on poultry immunity, a possible immunosuppression that contributes to the occurrence of infectious diseases. Y. Wu *et al.* (2019) note that heat stress affects the homeostasis of the bird's body, increases the colonisation of pathogenic microorganisms, and can lead to increased mortality. T.A. Johnson *et al.* (2019) report that it is possible to reduce the harmful effects of heat stress by using a mixture of probiotics. The results of the studies by various researchers are confirmed by the data of a production trial.

The presence of *Salmonella spp. infection was found in the body of chickens of the control group.* After overcoming the hyperthermic crisis and restoring normal ambient temperature on day 40 of the study, no chicken deaths were recorded in any experimental group. In the control group, bird deaths began on day 20 of the study, and characteristic dysbiotic manifestations were observed.

During the study, in the control group, weakened chickens that were lagging behind in growth and development died first. In the future, clinical signs of the disease began to be observed in other chickens of the control group. The bird had no or reduced appetite, gait became unsteady, and general depression of chickens was documented, they stood with their eyes closed, heads lowered down, and diarrhoea was white or greenish-brown in colour. The manifestation of these clinical signs was exacerbated during periods of high ambient temperature. Considering the high mortality rate in comparison with the experimental groups and clinical signs, a laboratory study of chicken corpses was conducted. According to clinical, pathoanatomical signs and laboratory test results, the chickens were diagnosed with *Salmonella spp.* and *E. coli* of mixed bacterial aetiology. Characteristic pathological changes were found in the heart and intestines of chickens – fibrinous pericarditis, and catarrhal-hemorrhagic enteritis. The heart was enlarged, covered with fibrin in the form of films, and the cavity of the cardiac jacket contained serous-fibrinous exudate with fibrin flakes. The liver was dark brown in colour with a dull surface, enlarged in size, blood vessels and mesentery of the intestine were filled with blood.

To select an effective therapeutic drug for broiler chickens of the control group, the sensitivity of *Salmonella spp.* and *E. coli* from dead birds to antibiotics of different groups was investigated. Sensitivity to chloramphenicol, penicillin, tetracycline, cephalosporins, macrolides, aminoglycosides, and fluoroquinolones was established. The most effective antibiotic was tetracycline. The effect of the postbiotic was quite pronounced, only in the third experimental group of chickens no coagulase-positive *Staphylococcus aureus* was isolated from the intestine (Table 2).

Table 2. Microflora isolated from broiler chickens on the 30th day of the experiment

Group of microorganisms	Group of broiler chickens			
	control	experimental 1	experimental 2	experimental 3
Staphylococcus aureus, CFU/g	3.0×10^3	4.5×10^2	1.0×10^3	Not detected
Enterobacteriaceae	*E. coli* in the liver, stomach, intestines	*E. coli* in the liver, stomach, intestines	*E. coli* in the liver, stomach, intestines	*E. coli* in the liver, stomach, intestines
Bacteria of the *E. coli* group (BECG), CFU/g	1.3×10^8	1.9×10^6	6×10^5	6.7×10^4
Salmonella spp.	Isolated from the liver	Not detected	Not detected	Not detected

Source: *compiled by the authors*

Postbiotic Bacteriosan was created by a synergistic combination of organic acid and bacteriocin, which is obtained naturally from the biomass of probiotic bacteria. High antibacterial activity of postbiotic against *Staphylococcus aureus* presumably, in addition to the nisin content, is associated with the lactic acid content, which according to R.U. Khan *et al.* (2022) is characterised by high antimicrobial activity. A.M. Humam *et al.* (2019) note that antimicrobial metabolites can lower the intestinal pH value and inhibit the proliferation of opportunistic microorganisms in animal feed and intestines. A number of publications report on the inhibitory effect of postbiotics obtained from *Lactobacillus plantarum* on various pathogenic bacteria (Yang *et al*., 2022), including *Salmonella typhimurium* and *Escherichia coli* (Wang *et al*., 2023), which is consistent with the results obtained. The salmonellosis pathogen was not detected in the third experimental group, with the lowest content of *E. coli* bacteria compared to the other experimental groups.

Staphylococcus aureus is a bacterium that is widely distributed in the environment, including air, various surfaces of objects, etc., and is able to develop resistance to antibiotics that are used for treatment. Moreover, *Staphylococcus aureus* is the most pathogenic type of pathogenic staphylococcus species isolated from poultry. Infections associated with *S. aureus* are a global problem of poultry farming, causing economic losses due to diseases and mortality of poultry. The pathogen is dangerous for humans; some strains that produce enterotoxins cause food poisoning. An *in vitro* study by H. Harmentis *et al.* (2020) reports that lactic acid bacteria of the species *Lactobacillus are able to inhibit Escherichia coli, Salmonella enteritidis*, and *Staphylococcus aureus*. In addition, there is a known case of the appearance of a resistant strain of Staphylococcus aureus in the bedding of poultry (Pugazhendhi *et al.*, 2020). In this regard, probiotic, postbiotic, and nisin solution were included in the diet of broiler chickens, and additional treatment of the bedding material was carried out.

It was found that the treatment of bedding material in the poultry house with safe and natural preparations contributes to air sanitation and reduces the microbial background of the room. At the beginning of the experiment, the content of microorganisms in the room air was the same in all groups and amounted to 1.3×10^2 CFU in 1 m^3. The microbial count of air was determined every 10 days, considering the dynamics in the control and experimental groups, it was found that the content of microflora in the experimental and control rooms increases in accordance with the age of chickens, despite periodic cleaning and treatment of bedding material. The experiments of M.C. Oliveira *et al.* (2015) indicate that the treatment of poultry bedding with zeolite, aluminium sulphate, and quicklime does not affect poultry productivity and is economically inexpedient. According to other researchers, there are substances that lower the pH of the bedding, reduce ammonia evaporation, and contribute to an increase in poultry body weight by improving housing conditions. The expediency of processing bedding material requires further investigation. Nowadays, the issue of minimising the harmful effects of animal husbandry on the environment, including poultry farming, is extremely relevant. In organic poultry farming, bedding that has been treated with a probiotic or postbiotic can be used as fertiliser in the soil, unlike traditional poultry farming on bedding with residual amounts of antibiotics.

In the digestive canal of broiler chickens of the first experimental group that received a probiotic *Lactobacillus plantarum*, in comparison with the indicators in chickens of the control and second experimental group, which received a solution of nisin, the number of *Staphylococcus aureus* microorganisms was an order of magnitude smaller. The highest content of *Staphylococcus aureus* was found in the intestines of broiler chickens of the control group – 3×10^3 CFU/g, a fairly high titre was also recorded in the intestines of chickens of the second experimental group – 1×10^3 CFU/g. *Salmonella was also isolated from the liver of the control group of broiler chickens*. The concentration of microorganisms of the *Lactobacillus* group was significantly lower compared to similar indicators of the experimental groups.

In broiler chickens of the control and experimental groups, *Escherichia coli* microorganisms were isolated from the liver, stomach, and intestines. The smallest titre – 6.7×10^4 CFU/g – was found in the digestive canal of chickens from the third experimental group, which received postbiotic with feed, in the control group – 1.3×10^8 CFU/g, respectively. Escherichia coli is well preserved in poultry droppings, dry air is a favourable condition, and it can be found for a long time in the bedding material, on mechanical particles, and dust on various surfaces of poultry houses. Increasing the humidity level helps to reduce the amount of *E. coli* in the room air. Therefore, there is a possibility of re-contamination of livestock with *E. coli* with altered enzymatic properties.

On day 60 of the experiment, the composition of the microflora of the digestive canal of broiler chickens was analysed. *Staphylococcus aureus* was not detected in the control and experimental groups of broiler chickens, which indicates the effectiveness of the therapeutic measures in the control group and the preventive effect of microbiological preparations and their metabolites in the experimental groups. On day 60, microbiological studies revealed the absence of pathogens in the control group and all experimental groups in the intestinal samples of broiler chickens. Thus, the bodies of broiler chickens of the experimental groups overcame it with the help of preventive drugs (Table 3).

Table 3. Microflora isolated from broiler chickens on day 60 of the experiment

Group of microorganisms	Group of broiler chickens			
	control	experimental 1	experimental 2	experimental 3
Staphylococcus aureus	Not detected	Not detected	Not detected	Not detected
Enterobacteriaceae	*E. coli* in the liver, stomach, intestines	*E. coli* in the liver, stomach, intestines	*E. coli* in the liver, stomach, intestines	*E. coli* in the liver, stomach, intestines
Bacteria of the *E. coli* group (BECG), CFU/g	2.3×10^8	7.6×10^7	1.6×10^7	2.1×10^6
Salmonella spp.	Isolated from the liver	Not detected	Not detected	Not detected

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

In the control group of broiler chickens, intense poultry mortality with characteristic clinical symptoms was observed. Therefore, on day 34 of growing, after determining the sensitivity of pathogenic microflora to antimicrobial drugs, a course of antibiotic therapy was started. Chickens of the control group were given the antibiotic tetracycline with drinking water at the rate of 0.5 g/L of water, lasting a course of 7 days. According to the forecast, the poultry of this group were expected to achieve complete body sanitation. The staphylococcal infection was overcome, however *E. coli,* as before, was released from the liver, heart, stomach, and intestines, despite the course of antibiotic therapy. The pathogen of *Salmonella spp.* was isolated from the liver of chickens of the control group, although immediately after completing the course of antibiotic therapy, the clinical condition significantly improved – the manifestation of digestive disorders decreased, appetite improved, and activity increased. The full recovery of the livestock after treatment did not occur. This probably indicates the presence of adaptive properties in microorganisms that are pathogens.

In the control group of broiler chickens, symptomatic signs of salmonellosis were further observed. There were isolated symptoms of *Salmonella spp.* in combination with the clinical picture of escherichiosis. Violation of the microbiological composition in the digestive canal led to dyspeptic phenomena. The progression of diarrhoea led to a significant loss of electrolytes and proteins, which accelerated dehydration and cachexia. Some strains of *E. coli* can colonise and destroy the intestinal epithelium (enteritis). The affected poultry died suddenly or from exhaustion. Due to damage to the intestinal surface by bacteria, toxins and other pathogenic agents, the digestibility of feed components is suppressed and the growth of microorganisms of some species is activated. Violation of the balance between obligate and facultative microflora of the digestive canal of broiler chickens causes a change in the composition of microbial enzymes, disrupts the processes of metabolism and digestion, first the breakdown of polysaccharides, and then proteins and fats. This leads to increased gas formation in the intestines of poultry, fermentation and putrefaction processes begin, and as a result – intoxication of the body. Microorganisms of opportunistic and pathogenic species begin to multiply in the small intestine.

From days 30 to 50 of the study, the highest mortality rate was observed in the control group – 9 animals died. After treatment, poultry mortality decreased. From days 50 to 70 of the experiment, only one case of chicken death from the group was registered. However, starting from days 70 to 80, mortality was again observed – 4 chickens died. Without treatment, the mortality rate would have been higher, with up to 70% of the poultry population dying when affected by *Salmonella spp.* Considering the presence of other infectious agents that deplete the bird's immune system, the prognosis was unfavourable. Three chickens died in the first experimental group, two chickens died in the second experimental group, and three chickens died in the third experimental group. In each group, from 4 to 5% of birds died due to non-infectious pathology – gastric obstruction or mechanical injury. When studying the effect of microbiological preparations and their metabolites on the content of lactic acid bacteria in the intestines of broiler chickens, a significantly higher concentration of lactobacilli was found in samples from poultry of the first and third experimental groups compared to the indicators of the control group throughout the experiment (Table 4).

Table 4. Amount of lactic acid bacteria in the intestines of broiler chickens, LgCFU/g, M±m, n=3

Group of chickens	Day of experiment		
	30	60	81
Control	5.13±0.39	5.09±0.70	4.53±0.13
Experimental 1	7.03±0.22*	5.89±0.07*	5.50±0.10*
Experimental 2	5.71±0.08*	6.07±0.71*	4.23±0.19
Experimental 3	7.10±0.05*	6.15±0.07*	6.00±0.03*

Note: **P<0.05 compared to the control*
Source*: compiled by the authors*

J. Wang *et al.* (2023) report the positive impact of introducing *Lactobacillus plantarum* to the poultry diet, which helps increase immunity, improve digestion and absorption of feed nutrients *L. plantarum* can also produce lactic acid bacteriocins during reproduction. X. Zhang *et al.* (2020) note that lactic acid bacteria, when entering the digestive canal, quickly colonise and compete for nutrients with harmful microorganisms, including *E. coli*. Due to the high concentration of lactic acid in the digestive canal of poultry, the pH value of digestive juices decreases. In the conducted studies, an increase in the number of lactic acid bacteria was observed, while the content of *E. coli* bacteria in the experimental groups decreased, which is fully consistent with the above data. In chickens of the third experimental group, a 38% higher content of lactic acid bacteria was recorded in the intestines on day 30 compared to the control group (Fig. 1).

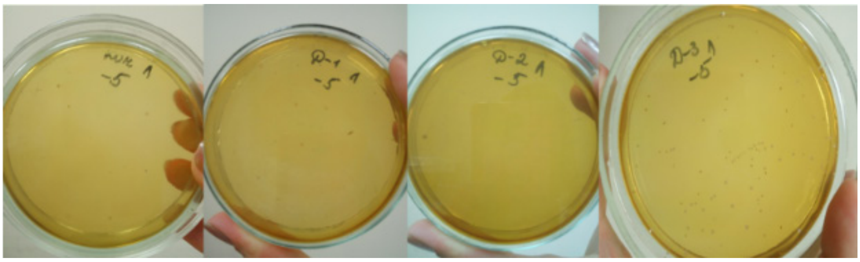

Figure 1. Growth of lactic acid bacteria at a dilution of 1×10^{-5} on day 30 of the experiment

In addition, the experimental postbiotic showed a preventive effect, stopping the attachment of staphylococcus to the intestinal wall of chickens. Broilers have a less robust immune system, their growth rate is fast, and their immune levels do not develop at the same rate, which explains their susceptibility to many infectious diseases. R.U. Khan *et al.* (2022) indicate an improvement in the immune response of broilers when organic acids are introduced to the main diet, which is consistent with the results described in this paper. The mechanism by which organic acids regulate immunity is not fully understood. Improved broiler immune response may be associated with increased numbers *Lactobacillus spp*. in the gastrointestinal tract, which has a positive effect on the immune system of poultry. The antibacterial properties of nisin consist in permeabilising the bacterial cell membrane by forming pores, which leads to leakage of cellular contents and destruction of the cell. It is assumed that lactic acid together with bacteriocin nisin create favourable conditions and stimulate the reproduction of symbiotic microorganisms, in particular lactobacilli, which has a positive effect on the microbiome of the digestive canal of broiler chickens.

On days 60 and 81 of rearing chickens of the first experimental group, a decrease in the average number of lactic acid bacteria was noted by 16% and 22%, respectively, although their content remained at a fairly high level compared to similar indicators in broiler chickens of the control group. It is assumed that this phenomenon is caused by weekly breaks in the use of probiotic drugs. With this scheme of use of microbiological preparations, the microbiocenosis of the digestive canal is stabilised and the digestive process and homeostasis of the bird's body are maintained. In the intestinal samples of broiler chickens of the second experimental group, the number of lactic acid bacteria increased by 6% on day 60 of the experiment compared to the indicators on day 30 of rearing, but on day 81, their content in the intestines of chickens of this group was the lowest compared to other experimental groups. As for the broiler chickens of the third experimental group, it can be argued that the intestinal microbiocenosis is stable, there were slight fluctuations in the content of lactic acid bacteria, the difference between the content of lactobacilli at days 60 and 81 was 2%, respectively, the indicators are quite high, indicating the stable operation of homeostasis processes.

According to the results of laboratory tests, a significant concentration of Escherichia coli in the digestive canal of broiler chickens was established, however, in the first, second, and third experimental groups, no deaths of poultry were observed after 50 days of rearing. Analysis of laboratory tests indicates the corrective effect of preventive drugs (probiotic LactoPharm LP12, bacteriocin nisin, postbiotic Bacteriosan) on the intestinal microflora of broiler chickens, a decrease in the concentration of Escherichia coli bacteria was observed in the experimental groups. On day 60 of the experiment, an increase in the number of *E. coli* bacteria was found in the intestines of broiler chickens of the control and all experimental groups. Presumably, this is due to weather conditions, there was a prolonged increase in ambient temperature from day 40 to 50 of the experiment, which acted as a stress factor and reduced the overall resistance of the broiler chickens. The best preventive effect of the drugs was manifested in the third experimental group. The content of *E. coli* was the lowest in comparison with the control and other experimental groups, as an increase in the number of Escherichia coli can cause the development of colibacillosis.

The absence of coagulase-positive *Staphylococcus aureus* in the digestive tract of broiler chickens on day 81 of the experiment indicates a satisfactory result of treatment with antibiotic drugs in the control group and an effective preventive effect of microbiological preparations on their metabolites. The highest concentration of *E. coli* was isolated from the contents of intestinal and gastric samples in poultry of the control group. The lowest content of *E. coli* bacteria was isolated from the intestines of broiler chickens of the third experimental group, which received the developed new experimental postbiotic. In chickens of the control group, *E. coli* was also isolated from the heart and liver, which may indicate a weakening of the body's immune defence (Table 5).

Table 5. Microflora isolated from broiler chickens on day 81 of the experiment

Group of microorganisms	Group of broiler chickens			
	control	experimental 1	experimental 2	experimental 3
Staphylococcus aureus	Not detected	Not detected	Not detected	Not detected
Enterobacteriaceae	*E. coli* from the heart, liver, stomach, intestines	*E. coli* from the stomach, intestines	*E. coli* from the stomach, intestines	*E. coli* from the stomach, intestines
Bacteria of the *E. coli* group (BECG), CFU/g	8.7×10^7	2.2×10^5	6.3×10^5	2.9×10^4
Salmonella spp.	Detected in the liver	Not detected	Not detected	Not detected

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

In experimental groups of broiler chickens, colibacillosis was not clinically manifested, the birds were active, lively, and had a good appetite. Consequently, these microorganisms did not have an enteropathogenic effect, presumably high concentrations of symbiotic microflora contributed to the competitive displacement of other microorganisms, including opportunistic ones, from the sites of attachment to the intestinal epithelium. Colimorphic bacteria can exchange plasmids, thus developing strains that synthesise endotoxins and cause diseases with clinical signs associated with primary or secondary infection. In the control group, *Salmonella spp was isolated from the liver of chickens.* Some of the chickens in this group were frail, did not consume food and water or had a reduced appetite, and symptoms of dysbiosis were observed. A pathoanatomical autopsy confirmed the presence of characteristic changes in a mixed infection caused by isolated pathogens (*Salmonella spp., E. coli*). At the end of the experiment, the number of microorganisms in the air increased. In the control group, where the bedding material was not treated, the microbial count was 2.6×10^3 CFU/m^3. In the air of the poultry house of the first experimental group, this figure was 1.0×10^3 CFU/m^3, in the second experimental group – 1.3×10^3, and in the third experimental group – 1.1×10^3 CFU/m^3.

During the entire period of the experiment, 12% of chickens died in the first and second experimental groups, and the mortality rate in the second experimental group was 14%. Most chickens died in the control group – 49%. The paddocks of the control and experimental groups of broiler chickens were separated only by a metal net, but no infection of the experimental groups occurred. The use of natural preventive drugs tested in the experiment provided a high percentage of the liveability of chickens of experimental groups in compliance with the requirements for organic poultry rearing. W.A. Abd El-Ghany *et al.* (2022), K. Krysiak *et al.* (2021), and R.U. Khan *et al.* (2022) reported the prospect of using probiotic drugs and their metabolites as therapeutic and preventive agents in poultry farming. The study confirmed the positive effect of the probiotic *LactoPharm LP12*, nisin, and the postbiotic Bacteriosan on the body of broiler chickens. However, probiotics, according to K. Krysiak *et al.* (2021), have certain disadvantages, in particular, sensitivity to temperature and humidity during storage, which can affect their effectiveness. With the use of bacteriocin nisin in the intestines of poultry, a decrease in the concentration of lactobacilli and a lower liveability of chickens were observed compared to other study groups that were treated probiotic and postbiotic. Considering the effect on the microbiome of the gastrointestinal tract, the liveability of chickens, and the conditions and shelf life of the studied microbiological preparations, the postbiotic Bacteriosan turned out to be the most effective and convenient to use.

Therefore, an integrated approach to maintaining the health of poultry, their feeding and maintenance with the use of preventive measures, systematic control of sanitary and hygienic standards and microclimate parameters in poultry houses ensures the successful development of organic poultry farming and obtaining high-quality and safe products.

Conclusions

The production of organic products and the rejection of the use of antibiotic drugs are extremely important and relevant issues. The use of natural preparations for broiler chickens, namely postbiotic Bacteriosan and probiotic *LactoPharm LP12* provided higher poultry liveability compared to the control group. It is established that the use of probiotics *LactoPharm LP12* and Bacteriosan has a preventive effect on the contamination of broiler chickens with *Salmonella spp*. and *Staphylococcus aureus*. In poultry fed a postbiotic, the highest concentration of lactic acid bacteria was recorded in the intestine, ranging from 6.0 to 7.1 LgCFU/g. The drug Bacteriosan has an advantage in terms of storage time and conditions compared to probiotics, which require certain temperature conditions, are less stable, and are unsuitable for long-term storage after dilution. During the production experiment on poultry under the conditions of organic rearing of chickens, the preventive effect of drugs based on probiotic microorganisms was established due to the full formation of microbiocenosis in the digestive canal, since it serves as a powerful link in the body's immune defence. The effect of the postbiotic was quite pronounced on the 30th day of the study, only in the experimental group of chickens treated with Bacteriosan, no coagulase-positive *Staphylococcus aureus was isolated from the intestines.* The use of these drugs can be considered as an alternative to the use of preventive antibiotics. Meanwhile, it is necessary to comply with high sanitary and hygienic standards of production, as housing conditions play an important role in maintaining good poultry health. Treatment of bedding material with postbiotic Bacteriosan and probiotic LactoPharm LP12 is allowed in the presence of poultry. When treating bedding material, conditionally pathogenic microflora is replaced by lactobacilli, which are antagonists, postbiotic exhibits an antimicrobial effect and, as a result, the release of microflora from the bedding into the air decreases. Poultry is not re-contaminated with opportunistic microflora, and the sanitary and hygienic indicators of the indoor microclimate are improved. Postbiotic Bacteriosan and probiotic LactoPharm in combination with the treatment of bedding material is recommended in organic poultry farming, which will facilitate the proper development of the intestinal microbiocenosis of chickens, prevention of dysbiosis and other diseases.

In the future, it is planned to investigate the effect of these natural preparations on the sanitary and hygienic indicators of the microclimate when using various types of bedding and establish the optimal type of bedding material for use in organic poultry farming.

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None.

Conflict of Interest

None.

References

[1] Abd El-Ghany, W.A., Fouad, H., & Quesnell, R. (2022). The effect of a postbiotic produced by stabilized non-viable Lactobacilli on the health, growth performance, immunity, and gut status of colisepticaemic broiler chickens. *Tropical Animal Health and Production*, 54, article number 286. doi: 10.1007/s11250-022-03300-w.

[2] Abd El-Hack, M.E., El-Saadony, M.T., Salem, H.M., El-Tahan, A.M., Soliman, M.M., Youssef, G.B.A., Taha, A.E., Soliman, S.M., Ahmed, A.E., El-Kott, A.F., Al Syaad, K.M., & Swelum, A.A. (2022). Alternatives to antibiotics for organic poultry production: Types, modes of action and impacts on bird's health and production. *Poultry Science*, 101(4), article number 101696. doi: 10.1016/j.psj.2022.101696.

[3] DSTU ISO 6888-1:2003. (2004). *Microbiology of food products and animal feed. Horizontal method of counting coagulase-positive staphylococci (Staphylococcus Aureus and other species). Part 1. Method using Beard-Parker agar medium*. Retrieved from http://online.budstandart.com/ua/catalog/doc-page?id_doc=95735.

[4] Ducatelle, R., Goossens, E., Eeckhaut, V., & Van Immerseel, F. (2023). Poultry gut health and beyond. *Animal Nutrition,* 13, 240-248. doi: 10.1016/j.aninu.2023.03.005.

[5] EN ISO/IEC 17025:2017. (2017). *General requirements for the competence of testing and calibration laboratories*. Retrieved from https://www.iso.org/ru/standard/66912.html.

[6] EUCAST (European Committee on Antimicrobial Susceptibility Testing). (n.d.). Retrieved from https://www.eucast.org/.

[7] European Committee on Antimicrobial Susceptibility Testing. (n.d.). Retrieved from https://www.eucast.org/organization.

[8] Giske, C., Turnidge, J., Canton, R., Kahlmeter, G., & EUCAST Steering Committee. (2022). Update from the European Committee on Antimicrobial Susceptibility Testing (EUCAST). *Journal of Clinical Microbiology*, 60(3), article number e00276-21. doi: 10.1128/jcm.00276-21.

[9] DSTU ISO 4833:2006. (2007). *Microbiology of food products and animal feed. Methods of identifying and counting the number of mesophilic lactic acid microorganisms*. Retrieved from https://files.stroyinf.ru/Index2/1/4293775/4293775303.htm.

[10] Gujvinska, S., & Paliy, A. (2018). Determination of antagonistic and adhesive properties of lactobacterium and bifidobacterium. *Bulletin "Veterinary Biotechnology"*, 80(1), 36-44. doi: 10.15407/microbiolj80.01.036.
[11] Harmentis, H., Marlida, Y., Nur, Y.S., Wizna, W., Santi, M.A., Septiani, N., Adzitey, F., & Huda, N. (2020). Novel probiotic lactic acid bacteria isolated from indigenous fermented foods from West Sumatera, Indonesia. *Veterinary World*, 13(9), 1922-1927. doi: 10.14202/vetworld.2020.1922-1927.
[12] Humam, A.M., Loh, T.C., Foo, H.L., Samsudin, A.A., Mustapha, N.M., Zulkifli, I., & Izuddin, W.I. (2019). Effects of feeding different postbiotics produced by *Lactobacillus Plantarum* on growth performance, carcass yield, intestinal morphology, gut microbiota composition, immune status, and growth gene expression in broilers under heat stress. *Animals*, 9(9), article number 644. doi: 10.3390/ani9090644.
[13] ISO 15214:1998. (1998). *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria – Colony-count technique at 30 degrees C*. Retrieved from https://www.iso.org/ru/standard/26853.html.
[14] DSTU ISO 6887-1:1999. (2004). *Microbiology of food products and animal feed. Preparation of researched samples, initial suspension and tenfold dilutions for microbiological research. Part 1. General rules for preparation of initial suspension and tenfold dilutions*. Retrieved from http://online.budstandart.com/ua/catalog/doc-page?id_doc=95727.
[15] Johnson, T.A., Sylte, M.J., & Looft, T. (2019). In-feed bacitracin methylene disalicylate modulates the turkey microbiota and metabolome in a dose-dependent manner. *Scientific Reports,* 9, article number 8212. doi: 10.1038/s41598-019-44338-5.
[16] Khan, R.U., Naz, S., Raziq, F., Qudratullah, Q., Khan, N.A., Laudadio, V., Tufarelli, V., & Ragni., M. (2022). Prospects of organic acids as safe alternative to antibiotics in broiler chickens diet. *Environmental Science and Pollution Research*, 29, 32594-32604. doi: 10.1007/s11356-022-19241-8.
[17] Kieronczyk, B., Sassek, M., Pruszynska-Oszmalek, E., Kolodziejski, P., Rawski, M., Swiatkiewicz, S., & Jozefiak, D. (2017). The physiological response of broiler chickens to the dietary supplementation of the bacteriocin nisin and ionophore coccidiostats. *Poultry Science*, 96(11), 4026-4037. doi: org/10.3382/ps/pex234.
[18] Kosenko, Yu.M., Ostapiv, N.V., & Zaruma, L.E. (2022). Assignments of the veterinary medicine specialists to comply with the provisions of the procedure for the use of antimicrobial veterinary medicines. *Scientific and Technical Bulletin of State Scientific Research Control Institute of Veterinary Medical Products and Fodder Additives and Institute of Animal Biology*, 23(1), 63-70. doi: 10.36359/scivp.2022-23-1.09.
[19] Kothari, D., Patel, S., & Kim, S. (2019). Probiotic supplements might not be universally-effective and safe: A review. *Biomedicine and Pharmacotherapy*, 111, 537-547. doi: 10.1016/j.biopha.2018.12.104.
[20] Krysiak, K., Konkol, D., & Korczyński, M. (2021). Overview of the use of probiotics in poultry production. *Animals*, 11(6), article number 1620. doi: 10.3390/ani11061620.
[21] Kucheruk, M.D., Zasekin, D.A., & Dymko, R.O. (2018). Microbiological and sanitary-hygienic significance of intestinal eubiozus in agricultural animals. *Ukrainian Journal of Ecology,* 8(2), 287-293. doi: 10.15421/2018_340.
[22] Law of Ukraine No. 2496-VII "On the Basic Principles and Requirements for Organic Production, Circulation and Labeling of Organic Products". (2018, July). Retrieved from https://zakon.rada.gov.ua/laws/show/2496-19#Text.

[23] Malaczewska, J., & Kaczorek-Lukowska, E. (2021). Nisin – a lantibiotic with immunomodulatory properties: A review. *Peptides*, 137, article number 170479. doi: 10.1016/j.peptides.2020.170479.

[24] Nolan, L.K., Barnes, H.J., Vaillancourt, J.-P., Abdul-Aziz, T., & Logue, C.M. (2020). Colibacillosis. In *Diseases of poultry* (pp. 770-830). New Jersey: Wiley-Blackwell. doi: 10.1002/9781119421481.ch18.

[25] Oliveira, M.C., Gonçalves, B.N., Pádua, G.T., Silva, V.G., Silva, D.V., & Freitas, A.M. (2015). Treatment of poultry litter does not improve performance or carcass lesions in broilers. *Revista Colombiana de Ciencias Pecuarias*, 28(4), 331-338. doi: 10.17533/udea.rccp.v28n4a05.

[26] Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E.J., & Würbel, H. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biology*, 18(7), article number e3000410. doi: 10.1371/journal.pbio.3000410.

[27] Pugazhendhi, A., Michael, D., Plakash, D., Krishnamaurthy, P.P., Shanmuganathan, R., Al-Dhabi, N.A., Duraipandiyan, V., Arasu, M.V., & Kaliannan, T. (2020). Antibiogram and plasmid profiling of beta-lactamase producing multi drug resistant *Staphylococcus aureus* isolated from poultry litter. *Journal of King Saud University Science*, 32(6), 2723-2727. doi: 10.1016/j.jksus.2020.06.007.

[28] Regulation (EU) No. 1831/2003 of the European Union. (2003, September). Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32003R1831.

[29] Reuben, R.C., Sarkar, S.L., Roy, P.C., Anwar, A.M., Hossain, A., & Jahid, I.K. (2021). Prebiotics, probiotics and postbiotics for sustainable poultry production. *World's Poultry Science Journal*, 77(4), 825-882. doi: 10.1080/00439339.2021.1960234.

[30] Rules for the selection of samples of pathological material, blood, fodder, water and their forwarding for laboratory research. No 15-14/111. (1997, April). Retrieved from http://surl.li/cexsr.

[31] Wang, J., Yao, L., Su, J., Fan, R., Zheng, J., & Han, Y. (2023). Effects of *Lactobacillus plantarum* and its fermentation products on growth performance, immune function, intestinal pH, and cecal microorganisms of Lingnan yellow chicken. *Poultry Science,* 102(6), article number 102610. doi: 10.1016/j.psj.2023.102610.

[32] Wu, Y., Wang, B., Zeng, Z., Liu, R., Tang, L., Gong, L., & Li, W. (2019). Effects of probiotics *Lactobacillus plantarum* 16 and *Paenibacillus polymyxa* 10 on intestinal barrier function, antioxidative capacity, apoptosis, immune response, and biochemical parameters in broilers. *Poultry Science*, 98(10), 5028-5039. doi: 10.3382/ps/pez226.

[33] Xu, C., Wei, F., Yang, X., Feng, Y., Liu, D., & Hu, Y. (2022). *Lactobacillus salivarius* CML352 isolated from Chinese local breed chicken modulates the gut microbiota and improves intestinal health and egg quality in late-phase laying hens. *Microorganisms,* 10(4), article number 726. doi: 10.3390/microorganisms10040726.

[34] Yang, S., Deng, C., Li, Y., Li, W., Wu, Q., Sun, Z., Cao, Z., & Lin, Q. (2022). Complete genome sequence of *Lactiplantibacillus plantarum* ST, a potential probiotic strain with antibacterial properties. *Journal of Animal Science and Technology*, 64(1), 183-186. doi: 10.5187/jast.2022.e7.

[35] Zhang, L., Liu, C., & Jiang, Q. (2021). Butyrate in energy metabolism: There is still more to learn. *Trends in Endocrinology & Metabolism,* 32(3), 159-169. doi: 10.1016/j.tem.2020.12.003.

[36] Zhang, X., Esmail, G.A., Alzeer, A.F., Valan Arasu, M., Vijayaraghavan, P., Choi, K.C., & Al-Dhabi, A.N. (2020). Probiotic characteristics of *Lactobacillus* strains isolated from cheese and their antibacterial properties against gastrointestinal tract pathogens. *Saudi Journal of Biological Sciences,* 27(12), 3505-3513. doi: 10.1016/j.sjbs.2020.10.022.

[37] Zhong, Y., Wang, S., Di, H., Deng, Z., Liu., J., & Wang, H. (2022). Gut health benefit and application of postbiotics in animal production. *Journal of Animal Science and Biotechnology,* 13, article number 38. doi: 10.1186/s40104-022-00688-1.

[38] Zommiti, M., & Ferchichi, M. (2021). Probiotics and prebiotics in animal feed. In *Probiotics and prebiotics in foods* (pp. 233-261). Cambridge: Academic Press. doi: 10.1016/B978-0-12-819662-5.00011-2.

Вплив пробіотичних препаратів та їх метаболітів на мікрофлору травного каналу курчат-бройлерів

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Анотація. Актуальність дослідження зумовлена зростаючим попитом населення на безпечні та органічні продукти птахівництва. В якості лікувально-профілактичних засобів та альтернативи застосування антибіотиків в органічному птахівництві використовують різні мікробіологічні препарати. Мета роботи – визначити вплив препаратів пробіотичних мікроорганізмів та їх метаболітів на оптимальний склад мікрофлори травного каналу, загальний стан та збереження курчат-бройлерів. Досліджено вплив на курчат-бройлерів різних видів препаратів: пробіотика «LactoPharm LP12», постбіотика «Бактеріосан» та розчину бактеріоцину нізину. Методом бактеріологічних посівів визначено якісний і кількісний склад мікрофлори травного каналу курчат-бройлерів. Диско-дифузійним методом, використовуючи комерційні диски з мінімально інгібуючою концентрацією, виявлено чутливість до антибіотиків. Підтверджено ефективну дію на кишечник птиці нового постбіотика «Бактеріосан» – на 30 добу досліду не виділяли коагулазо-позитивних *Staphylococcus aureus* та зафіксували найнижчий титр *Escherichia coli* під час дослідження на 30 добу, 60 та 81 добу досліду. При дослідженні вмісту молочнокислих бактерій у кишківнику курчат-бройлерів найвища концентрація встановлена в дослідних групах, яким застосовували пробіотик «LactoPharm LP12» і експериментальний постбіотик на 21 та 32%, відповідно, порівняно з контрольною групою. Обробка підстилкового матеріалу профілактичними препаратами в пташниках птиці дослідних груп сприяла санації повітря та зниженню мікробного фону приміщення порівняно з контролем. Встановлена найменша летальність курчат (-12%) при застосуванні пробіотика та постбіотика, внаслідок позитивного впливу на мікробіоценоз травного каналу. При використанні водного розчину нізину показник летальності становив 14%. Таким чином, експериментально обґрунтовано використання в органічному птахівництві постбіотика «Бактеріосан» у дозі 5 мл/кг корму та пробіотичного препарату «LactoPharm LP12» в дозі 1 г/л питної води курсом сім діб, з тижневою перервою впродовж усього періоду вирощування птиці, що дозволить здійснювати ефективну профілактику інфекційних хвороб та підвищить збереженість курчат-бройлерів

Ключові слова: мікробіоценоз; кишечник; постбіотик; нізин; органічне птахівництво

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Effectiveness of group therapy of calves for nonspecific bronchopneumonia by aerosol method

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Abstract. Nonspecific bronchopneumonia of calves causes significant economic damage to farms, and therefore, it is important to develop effective therapeutic drugs, especially for group use. Aerosol-based medicines deserve attention. The purpose of the study was to investigate the effectiveness of the group aerosol method of administering the drug "Calfmin" to calves with nonspecific bronchopneumonia. Analogue calves of two to three months of age, both clinically healthy and patients with nonspecific bronchopneumonia, were involved in the experiment. Standard clinical and haematological research methods were used to determine the diagnosis and monitor the functional state of the calves' bodies. It was found that in the blood of calves with nonspecific bronchopneumonia, an increase in the number of white blood cells was observed by 1.3 times ($P<0.05$) compared to clinically healthy animals. Their blood also showed an increase in the number of stab and segmented neutrophils by 2.0 times ($P<0.05$) and 1.6 times ($P<0.01$), respectively, and a decrease in the number of lymphocytes by 1.3 times ($P<0.001$) and eosinophils by 1.4 times ($P<0.001$), respectively. In sick calves, there was a 1.2-fold decrease in the number of red blood cells in the blood ($P<0.001$) and a 1.2-fold decrease in the haemoglobin content ($P<0.001$) compared to the control. An improvement in the clinical condition and haematological parameters in sick animals was recorded within 3-5 days, a reduction in the duration of therapy by 1.9-2.0 times, and 100% preservation of all sick calves

Keywords: nanoaquachelates; silver; copper; iodine; cobalt; Echinacea purpurea; blood morphology

Introduction

Respiratory diseases are among the most common diseases among different age groups of animals. Most often, such pathologies are identified in young, emaciated, and old animals. Respiratory diseases cause economic damage to the livestock industry, including the death of animals, reduced productivity of sick animals, and low-quality products (Buczinski & Pardon, 2020).

The most common respiratory disease is bronchopneumonia. Bronchopneumonia of young cattle is detected throughout Ukraine, and among the registered cases it ranks second after diseases of the gastrointestinal tract. Every year, up to 20-30% of calves with catarrhal bronchopneumonia are registered in Ukrainian farms. The incidence rate increases in the winter-spring period. The disease is registered in calves, starting from two weeks of age to 2-3 months (Sharandak *et al.*, 2022).

The causes of nonspecific bronchopneumonia in young cattle are a decrease in resistance, immunological reactivity of newborn calves, and the influence of adverse environmental factors. Bacterial opportunistic microflora of the anterior respiratory tract plays a leading role in the development of this pathology. Reduced natural resistance of calves, immunity of young cattle, unjustified means and methods of therapy and prevention are factors contributing to the spread of this disease (Van Driessche *et al.*, 2020). Bronchopneumonia in calves is characterised by the development of pathology not only in the respiratory system, but also throughout the body, since there is a violation of all types of metabolism. This leads to pathological changes in many organs of calves. In order to overcome bronchopneumonia in calves, it is necessary to apply a comprehensive approach to the selection of therapeutic agents and measures (Leenen *et al.*, 2020).

Analysis of literature and research results indicate that nonspecific bronchopneumonia is the subject of constant attention of researchers and veterinary medicine specialists, since this pathology is widespread among calves – from 15 to 25% with 90% preservation. Economic losses from bronchopneumonia consist of a significant percentage of calf deaths, a decrease in the average daily weight gain of the animal (sick calves lag behind in growth and development), the cost of veterinary examination, maintenance, and other care for sick animals (Sharandak *et al*., 2022).

In addition, the effects of bronchopneumonia on the subsequent performance of calves are usually underestimated. It is proved that repair heifers who have suffered subacute or chronic lung diseases gain the necessary body weight more slowly and have disorders in the development of reproductive organs. In other words, it will not be possible to get high milk yields from cows in the future. If the farm creates proper conditions for keeping animals and diseases of young animals (dyspepsia and bronchopneumonia) can be avoided, then reliable promising prerequisites are provided for the optimal age of the first calving of heifers and high milk productivity of cows (Nishi *et al*., 2019).

Nonspecific bronchopneumonia is characterised by inflammation of the bronchi and lungs, accompanied by their filling with catarrhal exudate, a disorder of external and internal gas exchange, the development of respiratory and cardiovascular insufficiency, and a violation of the functions of various organs and systems. The most effective treatment for bronchopneumonia is complex treatment of animals, which is aimed at eliminating violations of the technology of their maintenance and feeding, increasing the body's resistance with the simultaneous use of various means of therapy: antimicrobial, pathogenetic, symptomatic, stimulating, and vitamin (Sharandak *et al.*, 2022).

Treatment of calves for bronchopneumonia is most effective in the initial stages of the disease, when the inflammatory process mainly affects the upper respiratory tract. In the case of a prolonged course of the disease, as oedema, cell infiltration, and sclerosis increases around the inflammatory focus, capillaries are squeezed and desolated in the lungs, as a result of which the penetration of drugs into the area of inflammation becomes more difficult (McGill & Sacco, 2020). Nowadays, the issue of developing and practical use of new highly effective and economically justified treatment and prevention schemes for nonspecific bronchopneumonia of calves is relevant. The advantage of aerosol therapy is that it can be incorporated into existing meat and milk production technologies. It is this approach to the treatment of animals that will allow producing organic livestock products that meet the highest quality standards.

The purpose of the study was to investigate the effectiveness of group therapy of calves with non-specific bronchopneumonia using the aerosol method.

Materials and Methods

The study was conducted in 2017-2021 at the Department of Therapy and Clinical Diagnosis, and Academician Volodymyr Kasyanenko Department of Animal Anatomy, Histology and Pathomorphology at the National University of Life and Environmental Sciences of Ukraine (Kyiv). Experimental trials were conducted on the farm "Podolsky hospodar 2004" of the Khmelnytskyi Oblast, Shepetivka district. Analogue calves of 2-3 months of age, black-and-white breed, were used as the object of research. Animals of the control and experimental groups were kept in the same conditions of keeping, feeding, and care. The study complied with ARRIVE's recommendations and was conducted without violating the Directive 2010/63/EU guidelines on the protection of animals used for scientific purposes (Percie du Sert *et al*., 2020).

Before starting the research, the spread and etiopathogenetic relationships of nonspecific bronchopneumonia of calves in farm conditions were studied. Clinically healthy black-and-white calves and animals suspicious and sick of nonspecific bronchopneumonia were selected for the dispensary examination. Studies were conducted on the clinical condition of young cattle, in particular, determining the habitus of animals, measuring body temperature, calculating the frequency of respiratory movements and heartbeats in one minute, examining the condition of hair, skin, visible mucous membranes, superficial lymph nodes, cardiovascular, respiratory, digestive, urinary, and sexual systems. Standard clinical research methods (examination, palpation, percussion, auscultation, thermometry) were used to study the clinical condition of animals.

Heparin-stabilised blood obtained from the jugular vein (*vena jugularis*) in the morning, before feeding, was used for morphological studies, along with clinical examination of the animals. During morphological studies of blood, the number of red blood cells was determined manually in a hemocytometer with a Goryaev grid; the number of leukocytes – by counting with a small magnification of the microscope in 100 large squares of a hemocytometer with a Goryaev grid; platelets were counted manually in a hemocytometer with a Goryaev grid; the leukogram was derived by counting 200 cells in blood smears stained according to Romanowsky-Giemsa; the hematocrit value – by microcentrifugation according to Shklyar; haemoglobin – by hemiglobincyanide method; the content of haemoglobin in one erythrocyte (CHE) was calculated by mathematical calculations according to the equation.

To conduct experimental studies, three groups of calves were established – analogues of 2-3 months of age, 50 animals each, namely: group 1 – clinically healthy calves; experimental group 1 and group 2 – calves with nonspecific bronchopneumonia. Monitoring of the clinical condition of animals and the effectiveness of group therapy was monitored on the first, third, seventh, ninth, and fifteenth days of treatment. The experiment lasted 30 days. Calves of the control group were not treated with aerosol. Aerosol treatment of calves of the experimental group 1 was carried out by using turpentine at the rate of 0.5 mL/m^3, lactic acid – 0.04 g/m^3, and chlorinated lime – 2 g/m^3. The frequency of aerosol treatment of animals is 1 time per day, the exposure is 45 minutes according to the therapy scheme adopted on the farm. The treatment was carried out every second day, 12 consecutive days. For the prevention of nonspecific bronchopneumonia in calves of experimental group 2, the method of group aerosol treatment was used using the developed experimental preparation "Calfmin" containing silver and copper nanoaquachelates, iodine solution, cobalt lactate (Drobot *et al*., 2013), and echinacea tincture. The drug was applied at a dose of 7 mL per animal with an exposure of 45 minutes. Treatment with this drug was carried out on the first, third, and seventh days of research. To prevent rapid evaporation of aerosol particles and exposure to harmful gases, as well as their long-term retention in a suspended state, a stabiliser was added to the experimental preparation – a 20% glucose solution at the rate of 1 mL/m^3 of the room. Preventive aerosol treatment of calves was carried out in a separate room with carefully closed doors and windows with ventilation turned off (Nedosekov *et al*., 2010).

The obtained results were recorded, and their digital indicators were processed statistically using StatSoft Statistica (2016) software suite, considering the specifics of statistical methods in biomedical research (Horalskyi *et al.*, 2015). The data is presented in tables as x±SD (standard deviation). Differences between the values were determined using the Tukey range test, where the differences were considered reliable at P<0.05, P<0.01, and P<0.001 (considering the Bonferroni correction).

Results and Discussion

According to the conducted propaedeutical studies to investigate the causes of nonspecific bronchopneumonia in calves in the experimental farm "Podilskyi hospodar 2004", it was established that several groups of aetiological factors that act on the body of calves of different age groups in different combinations and combinations lead to the occurrence of this pathology. The first group includes anthropogenic factors, biogeocenosis disorders, and unfavourable factors of intrauterine development that lead to the birth of calves with weakened resistance. Thus, the body temperature decreased by 1-1.5°C compared to the norm. These calves were found to have bradycardia, myopathy, and hypotrophy. The sucking reflex in many newborn calves is weakened with a delay. Most of these calves had dyspepsia, and after some time after getting over dyspepsia, bronchopneumonia developed. This indicates that diseases of young animals in the neonatal period are associated with the anatomical and physiological characteristics of the calves' body.

According to the results obtained, the prevalence of the disease in the "dyspepsia – bronchopneumonia" scheme in newborn calves is due to a violation of the conditions of keeping structural analysis of the diet of breeding cows, it was found that the farm does not always maintain and feeding the breeding stock of cows in different physiological conditions. According to the structure, quality, and completeness of the diet. This is especially relevant for the diet of cows during the interlactation period, where there is a violation of feed quality and a deficiency in the content of macro- and microelements and vitamins.

Nonspecific bronchopneumonia of calves on the farm is caused by the accumulation and accumulation of harmful gases (ammonia, hydrogen sulphide, which were studied organoleptically), low temperature and high humidity in the room. The feed base for calves aged 1.5-2.0 months does not provide a physiological need for protein and vitamins, especially A and C, as well as macro- and microelements, especially iodine and calcium. The increased level of bronchopneumonia in calves is conditioned by the presence of opportunistic microflora in the upper respiratory tract, which under these conditions becomes pathogenic and is the causative agent of nonspecific bronchopneumonia. Drugs were used by the aerosol method, since this method is the most effective for respiratory diseases of animals, since the ways of getting the drug and the causative agent of the disease into the body of sick animals are the same (Sharandak *et al.*, 2022).

According to the conducted study, it was found that mainly calves of 1-3 months of age were ill on the farm, which is 23.1-24.7%; and 7.2-9.4% of calves of 1-2 months of age and 3.4-6.3% of calves of 2-3 months of age died from this pathology. The preservation of calves of 1-3 months of age in this pathology is 90.6%, which indicates a low effectiveness of treatment of animals in the experimental farm.

In a clinical study of calves with nonspecific bronchopneumonia, it is important not to miss the first symptoms of the disease. At the beginning of the disease, attention was paid to animals with symptoms of general depression, decreased appetite, and the appearance of a jerky, sharp, dry, and painful cough. Liquid serous-mucous discharge from the nasal passages of calves and lacrimation were often detected, which on the second or third day changed to catarrhal (they became milky white, became thicker) or even catarrhal-purulent. The presence of liquid serous-mucosal secretions indicated that the role of viral load is not excluded in the aetiology of nonspecific bronchopneumonia of calves, and the change of secretions to catarrhal (or catarrhal-purulent)– that bacterial microflora plays a leading role in the pathogenesis of bronchopneumonia. In all patients with nonspecific bronchopneumonia of animals (100%), at the beginning of the disease, an increase in the number of respiratory movements, the appearance of inspiratory dyspnea and the abdominal type of breathing were observed.

During the clinical examination, cyanosis of the mucous membranes of the oral cavity, conjunctiva and nasal mirror was detected in 100% of calves with nonspecific bronchopneumonia. This indicates a violation of blood circulation and the accumulation in the blood of animals of an excessive amount of reduced haemoglobin, which has a dark, red-blue colour. The accumulation of excessive amounts of reduced haemoglobin in the blood of calves with nonspecific bronchopneumonia is obviously associated with insufficient oxygen saturation of the blood due to inflammatory processes in the lungs. During auscultation of the lungs at the beginning of the disease, most calves showed increased broncho-vesicular respiration. This is due to difficulty in air intake due to the narrowing of airways, a decrease in the elasticity of the alveoli due to infiltration of their walls, and a decrease in the number of alveoli involved in respiratory movements. On the second or third day, wet wheezing was detected, which indicated an accumulation of exudate in the respiratory tract. Crepitation was also noted, which was best listened to at the peak of inspiration.

At the same time, wet wheezing was detected during auscultation of the lungs, which indicates a violation of the elastic properties of the interalveolar membranes, due to the accumulation of exudate in the bronchi. Auscultation of the heart noted a muffling of heart tones and a weakening of the pulse wave. In addition, an increase in the second tone was noted, which is obviously associated with a "shock" of blood during the closure of the semilunar valves of the pulmonary artery. The intensity of the second heart tone in sick calves had a correlation with the speed of blood flow in the small circle of blood circulation, and, consequently, the value of blood pressure, the elasticity of the alveoli and the pressure in the interalveolar membranes.

Chest percussion revealed areas of blunting in the apical and cardiac areas of the lungs. When measuring body temperature in animals, intermittent fever was noted. Almost 90% of calves had a body temperature of 39.5-39.7°C in the morning and 40.7-41.4°C in the evening (Table 1). This can be explained as a protective reaction of the animal's body to the influence of pathological factors, in which leukocytosis, nonspecific and specific protection factors are activated.

Table 1. Clinical parameters of healthy and sick calves with nonspecific bronchopneumonia, M±m, n=7

Indicator	Calf groups	
	clinically healthy	with nonspecific bronchopneumonia
Body temperature, °C	38.9±0.1	40.4±0.1
Pulse rate, beats/min	81.9±1.4	97.1±1.4***
Respiratory rate, movements/min	28.8±0.6	41.8±1.9***

Note: ****P<0.001 compared to data from clinically healthy calves*
Source*: developed by the author*

During the study of the clinical condition of calves, general animal depression, loss of appetite, sharp, dry and jerky cough, and catarrhal exudate discharge from the nasal passages were observed. Sick calves lay down most of the time of the study, and when forced to get up, they showed unsteady gait and muscle tremors. In sick animals, tousled and wet hair was noted. The conjunctiva and nasal mirror in sick calves had a cyanotic tint, which is a sign of impaired hemostasis in the small circle of blood circulation. Shortness of breath in calves was of a mixed type. Hyperthermia was observed in sick calves, and the pulse rate and respiration significantly increased by 1.2 times ($P<0.001$) and 1.5 times ($P<0.001$), respectively, compared to data from clinically healthy animals. Thus, the results of clinical studies indicate the polyaetiology and acute course of bronchopneumonia in calves.

The blood of calves with nonspecific bronchopneumonia is characterised by a significant decrease in haemoglobin content by 1.2 times ($P<0.001$), the number of red blood cells by 1.2 times ($P<0.001$) and a tendency to reduce CHE, hematocrit and a significant increase in platelet count by 1.3 times ($P<0.001$) (Table 2). At the beginning of group therapy in calves with non-specific bronchopneumonia, the number of red blood cells in the blood significantly decreased by 1.3-1.4 times ($P<0.001$) compared to the indicators of clinically healthy animals.

Table 2. Blood parameters of clinically healthy and sick calves with nonspecific bronchopneumonia, M±m, n=7

Indicator	Group of calves	
	clinically healthy	with nonspecific bronchopneumonia
Hematocrit, %	36.42±1.70	33.43±0.53
Haemoglobin, g/L	114.3±1.8	92.3±1.4***
Platelets, 10^9/L	256.0±10.3	331.7±4.7***
Red blood cells, 10^{12}/L	6.8±0.1	5.6±0.2***
Haemoglobin content in one erythrocyte (CHE), pg	16.8	16.5

Note: ****P<0.001 compared to data from clinically healthy calves*
Source*: developed by the author*

Morphological examination of blood showed that the number of white blood cells in the blood of calves suffering from nonspecific bronchopneumonia is 1.3 times significantly higher ($P<0.05$) compared to the data of clinically healthy calves (Table 3). The leukogram of sick calves was characterised by an increase in the number of stab and segmented neutrophils by half ($P<0.05$) and 1.6 times ($P<0.01$), respectively, and a decrease in the number of lymphocytes by 1.3 times ($P<0.001$) and eosinophils by 1.4 times ($P<0.001$) compared to clinically healthy animals (Table 3).

Table 3. Leukocyte count and leukogram parameters of clinically healthy and sick calves with nonspecific bronchopneumonia, M±m, n=7

Indicator	Group of calves	
	clinically healthy	with nonspecific bronchopneumonia
White blood cell count, 10^9/L	6.1±0.1	8.2±0.2*

Table 3. Continued

Indicator	Group of calves	
	clinically healthy	with nonspecific bronchopneumonia
Basophils, %	1	1
Eosinophils, %	2.2±0.2	1.6±0.3*
Stab neutrophils, %	2.1±0.1	4.2±0.4*
Segmented neutrophils, %	25.3±0.6	39.6±1.3**
Lymphocytes, %	62.7±1.2	47.7±0.7***
Monocytes, %	6.7±0.3	5.9±0.4

Note: **P<0.05, **P<0.01, ***P<0.001 compared to data from clinically healthy calves*
Source*: developed by the author*

Neutrophilic leukocytosis, eosinopenia, and lymphocytopenia were observed in the blood of calves with bronchopneumonia. As a result of clinical and morphological studies on the dispensary examination of animals, not only aetiological factors and the spread of the disease in the farm were determined, but also its specificity and the diagnosis was clarified – nonspecific bronchopneumonia of calves. Based on the results of clinical studies on the effectiveness of the drug "Calfmin" and the use of aerosol therapy (Drobot *et al.,* 2013), a significant improvement in the clinical condition of calves with nonspecific bronchopneumonia was established (Table 4).

Table 4. Indicators of body temperature, pulse, and respiratory rate in calves with nonspecific bronchopneumonia when using aerosol therapy, M±m, n=7

Day of experiment	Control group (clinically healthy calves):	Sick calves	
		experimental group 1	experimental group 2
Animal body temperature (day 1)	38.8±0.2	41.0±0.2***	40.9±0.2***
Animal body temperature (day 3)	38.8±0.1	39.5±0.1*	39.1±0.1
Animal body temperature (day 7)	38.9±0.1	39.0±0.1	38.4±0.1
Pulse rate, beats/min (day 1)	78.1±0.3	98.2±1.1***	97.7±1.0***
Pulse rate, beats/min (day 3)	78.9±1.1	87.9±1.1***	84.5±1.1**
Pulse rate, beats/min (day 7)	76.9±1.5	78.2±1.2	76.1±1.2
Respirations per minute (day 1)	29.1±0.1	47.2±0.6***	46.7±1.1***
Respirations per minute (day 3)	29.0±0.8	35.3±1.5***	33.2±1.0**
Respirations per minute (day 7)	28.2±0.5	29.1±1.2	28.3±0.4

Note: **P<0.05, **P<0.01, ***P<0.001 compared to data from clinically healthy calves*
Source*: developed by the author*

Thus, cough in calves with bronchopneumonia of experimental group 2, which were treated with "Calfmin", stopped on the third day. During the treatment of calves with bronchopneumonia, an improvement in their general clinical condition was observed. Thus, on the third day, the animals showed a decrease in body temperature, pulse rate, and respiration compared to the beginning of treatment (Table 4). Table 4 showed that on the seventh day of the study, body temperature, pulse and respiration rates in calves with nonspecific bronchopneumonia of the experimental groups were within the limits of indicators of clinically healthy animals. During the treatment period, the body temperature of the calves of the experimental groups decreased by 1.2 times relative to the data on the first day of the experiment, the heart rate – by 1.3 times, and the respiratory rate – by 1.6 times.

Already on the third day from the beginning of the experiment, the number of red blood cells in the blood of calves that were treated with "Calfmin" during group aerosol therapy significantly increased by 1.3 times (P<0.01) and differed from that in clinically healthy calves. The number of red blood cells in the blood of calves during basic treatment also tended to increase, but on the third day of the experiment it remained significantly lower by 1.1 times (P<0.05) compared to clinically healthy calves. On the seventh day of application of group aerosol therapy, the blood parameters of animals of both experimental groups in terms of the number of red blood cells did not differ from those in clinically healthy calves. At the same time, the number of red blood cells in the blood of calves of experimental group 2 with aerosol use of "Calfmin" increased by 7% compared to animals of experimental group 1 that used basic treatment. The number of platelets in the blood of calves with bronchopneumonia at the beginning of the experiment increased by 1.3-1.4 times (P<0.01) compared to clinically healthy calves (Table 5).

Table 5. Morphological parameters of blood of calves with nonspecific bronchopneumonia using group aerosol therapy, M±m, n=7

Day of experiment	Control group (clinically healthy calves)	Sick calves: experimental group 1	Sick calves: experimental group 2
Number of red blood cells, 10^{12}/L (day 1)	6.7±0.1	5.2±0.4***	5.6±0.2***
Number of red blood cells, 10^{12}/L (day 3)	7.2±0.2	6.3±0.4	7.0±0.2
Number of red blood cells, 10^{12}/L (day 7)	7.6±0.2	7.2±0.2	7.7±0.2
Haemoglobin content, g/L (day 1)	115.2±2.0	98.5±4.9**	98.7±2.9**
Haemoglobin content, g/L (day 3)	121.7±2.0	113.8±2.8*	125.0±1.3
Haemoglobin content, g/L (day 7)	128.0±1.9	118.0±2.8*	132.6±1.1
Hematocrit value, % (day 1)	39.44±1.70	33.43±0.93***	35.14±1.06***
Hematocrit value, % (day 3)	39.67±1.58	37.53±0.99*	39.57±1.05*
Hematocrit value, % (day 7)	40.63±1.60	39.62±1.89	40.43±0.65
Platelet count, 10^9/L (day 1)	256.0±10.3	332.6±20.6**	339.6±14.0***
Platelet count, 10^9/L (day 3)	297.7±6.4	316.7±6.1	305.1±6.9
Platelet count, 10^9/L (day 7)	276.3±12.7	289.4±11.4	273.6±8.9*

Note: **P<0.05, **P<0.01, ***P<0.001 compared to data from clinically healthy calves*
Source*: developed by the author*

On the third day of aerosol group therapy, compared to the first day of the experiment, the number of platelets in the blood of calves treated with basic treatment had a pronounced tendency to decrease, and in the blood of calves treated with "Calfmin" decreased by 10% (P<0.05). At the same time, this indicator in calves of experimental group 1 remained another 7% (P<0.05) higher compared to clinically healthy calves. On the seventh day of application of group aerosol therapy, the blood parameters of calves of both experimental groups in terms of platelet count did not differ from the blood parameters of the control group of animals.

The haemoglobin content in the blood of calves of the experimental groups at the beginning of group aerosol therapy decreased by 1.15-1.17 times (P<0.01) compared to the data of clinically healthy animals. Thus, on the third day, in the blood of calves with nonspecific bronchopneumonia who received basic treatment on the farm, the haemoglobin content significantly increased by 1.2 times (P<0.01), and in comparison with clinically healthy calves decreased by 7% (P<0.05). In the blood of calves of experimental group 1, who were treated with "Calfmin", this indicator also significantly increased by 1.3 times compared to the first day and became as close as possible to the indicators of clinically healthy animals, and in relation to calves of experimental group 1, it increased by 10% (P<0.01) (Table 5). On the seventh day of the study, the concentration of haemoglobin in the blood of calves of both experimental groups did not change in comparison with the indicators of clinically healthy calves. However, the haemoglobin content in the blood of calves of experimental group 2, who were treated with group aerosol therapy using "Calfmin", increased by 11%, compared to calves of experimental group 1, who received basic therapy.

At the beginning of group therapy in calves with nonspecific bronchopneumonia, the hematocrit value decreased by 1.2-1.1 times (P<0.01) compared to that in clinically healthy animals. On the third day of aerosol therapy, the hematocrit value in the blood of calves of both experimental groups significantly increased by 12% (P<0.05), compared to the first day of the experiment. However, on the third day, there was no significant difference in the hematocrit value in the blood of calves of the experimental groups. Group aerosol therapy in calves with bronchopneumonia had a significant effect on the leukogram parameters of animals (Table 6). Before starting group aerosol therapy in the blood of patients with bronchopneumonia calves, the number of white blood cells in the blood was 1.4 times higher (P<0.01), compared to the indicators of clinically healthy calves (Table 6).

Table 6. Leukogram of calves with nonspecific bronchopneumonia based on the results of group aerosol therapy, M±m, n=7

Day of experiment	Control group (clinically healthy calves):	Sick calves	
		experimental group 1	experimental group 2
White blood cell count, 10^9/L (day 1)	6.2±0.1	8.4±0.2***	8.5±0.4***
White blood cell count, 10^9/L (day 3)	5.8±0.2	6.8±0.4*	6.1±0.3
White blood cell count, 10^9/L (day 7)	5.9±0.3	6.3±0.3	6.2±0.1
Basophils, % (day 1)	0.7±0.1	0	0.7±0.1
Basophils, % (day 3)	0	0	0
Basophils, % (day 7)	0	0	0
Eosinophils, % (day 1)	2.2±0.2	1.2±0.1***	1.5±0.1***
Eosinophils, % (day 3)	3.4±1.0	1.4±0.9	3.1±0.3
Eosinophils, % (day 7)	4.5±0.7	1.4±0.8	3.5±0.6

Table 6. Continued

Day of experiment	Control group (clinically healthy calves):	Sick calves	
		experimental group 1	experimental group 2
Stab neutrophils, % (day 1)	2.1±0.4	6.8±1.3	7.2±1.2**
Stab neutrophils, % (day 3)	2.3±0.4	4.3±1.2	3.1±0.6
Stab neutrophils, % (day 7)	2.9±0.4	4.1±0.4	3.4±0.4
Segmented neutrophils, % (day 1)	25.3±0.6	38.1±3.8**	37.3±2.1***
Segmented neutrophils, % (day 3)	25.8±0.9	35.1±0.9***	25.7±2.7
Segmented neutrophils, % (day 7)	26.4±1.2	32.1±1.8*	27.4±1.4
Lymphocytes, % (day 1)	62.7±2.2	48.8±1.9**	48.3±2.1**
Lymphocytes, % (day 3)	63.7±3.1	53.0±2.8*	61.1±2.4
Lymphocytes, % (day 7)	62.4±2.7	55.9±2.5	58.5±1.9
Monocytes, % (day 1)	6.7±0.3	5.2±0.7	5.0±0.5
Monocytes, % (day 3)	7.6 ±0.6	5.2±0.3	7.0±0.8
Monocytes, % (day 7)	7.3±0.8	6.3±0.7	7.2±0.8

Note: **P<0.05, **P<0.01, ***P<0.001 compared to data from clinically healthy calves*
Source*: developed by the author*

On the third day from the beginning of the experiment, the number of white blood cells in the blood of calves of experimental group 1 significantly decreased by 1.2 times (P<0.01), while in calves of experimental group 2, this indicator increased by 1.4 times (P<0.001). There was no significant difference in the number of white blood cells between calves of the experimental groups during this period. On the seventh day of group aerosol therapy, the number of white blood cells in the blood of calves of both experimental groups was as close as possible to the indicators of clinically healthy animals. In relation to the first day of the experiment, the number of white blood cells in the blood of calves of experimental group 1 decreased by 25% (P<0.001), and in the blood of calves of experimental group 2 – by 28% (P<0.001).

Neutrophilic leukocytosis was noted in the leucogram of calves, which was accompanied by an increase in the number of neutrophils by 1.5 times (P<0.001) with a simultaneous decrease in the number of lymphocytes and monocytes by 1.3 times (P<0.01), eosinophils – by 1.9 times (P<0.001) in calves of experimental group 1, and – by 1.5 times (P<0.001) in calves of experimental group 2. Group aerosol therapy of calves of the second experimental group using the drug “Calfmin” on the third day caused a decrease in the number of stab and segmented neutrophils in the blood by 2.4 times (P<0.01) and 1.5 times (P<0.01), respectively, compared with the first day of the experiment. In addition, there was an increase in the number of eosinophils by more than 2.0 times (P<0.001) and lymphocytes by 1.3 times (P<0.01). That is, the leukograms of calves with nonspecific bronchopneumonia who used the “Calfmin” by aerosol method were already as close as possible to the indicators of clinically healthy calves during this period.

Calves of the first experimental group were treated with group aerosol therapy using a farming scheme. The leukogram on the third day was characterised by a tendency to reduce the number of stab and segmented neutrophils by 1.5 times (P<0.001) and by 1.1 times, respectively. An increase in the number of eosinophils by 1.2 times and lymphocytes by 1.1 times

(P<0.05) was also noted compared to the first day of the experiment. In comparison with the leukogram indicators of calves of the control group (clinically healthy animals), calves of the first experimental group did not show significant changes, with the exception of the number of segmented neutrophils, which increased by 36% (P<0.001). This indicates the development of a pathological process of an inflammatory nature and the painful state of their body.

On the seventh day of the experiment, the leukogram of calves that were treated with group aerosol therapy using the drug "Calfmin" almost did not differ from the indicators of clinically healthy animals. The leukogram of calves of experimental group 1, which used the basic method of therapy, was characterised by blood parameters that were as close as possible to those in clinically healthy animals. However, calves of experimental group 1 showed a tendency to reduce the number of eosinophils and lymphocytes and an increase in the number of stab neutrophils. The number of segmented neutrophils in the blood of calves of experimental group 1 exceeded the indicators of clinically healthy animals by 22% (P<0.05), which is a sign of the transition of the inflammatory process to a chronic form.

The effectiveness of using the drug "Calfmin" for the prevention of nonspecific bronchopneumonia in calves has also been proven (Sharandak *et al*., 2022). At the end of the experiment, when using basic aerosol treatment according to the farm scheme, there was a significant increase in the body weight of calves of experimental group 1 by 28% (P<0.001) compared to the initial data, which is 12% (P<0.05) higher than in calves of the control group. The body weight of calves of experimental group 2, which were treated with the drug "Calfmin" in the form of an aerosol, increased by 35% (P<0.001), which is 13.7% (P<0.01) more than the corresponding indicators in animals of the control group. The lower growth energy of the calves of the control group was probably conditioned by the presence of diseases of the gastrointestinal and respiratory tracts in them.

At the beginning of aerosol prevention, the number of red blood cells, haemoglobin content, hematocrit value, and platelet count in the blood of calves in all experimental groups were within the standard indicators. At the end of the experiment, the blood parameters of calves of experimental group 1, which were used for aerosol prevention of nonspecific bronchopneumonia according to the farm scheme, did not differ in their values from the blood indicators of animals in the control. On the third day of the experiment, a significant increase in haemoglobin content and an increase in platelet count by 1.05 times (P<0.05) and 1.10 times (P<0.01) was noted in the blood of calves of experimental group 2, respectively, compared with the indicators in animals of the control group. On the seventh day of the study, the haemoglobin content in the blood of calves of experimental group 2 increased by 1.1 times (P<0.01) compared to the control group. Other blood parameters of calves of experimental group 2 tended to improve at the end of the experiment, but did not have a significant difference compared to the corresponding blood parameters of calves of the control group.

At the beginning of group aerosol prophylaxis, the number of white blood cells in the blood of calves of all groups did not differ and this trend continued throughout the entire period of experimental studies. According to the results of the analysis of the blood leukogram of calves of the experimental groups, an increase in the number of neutrophils by 1.3 times (P<0.001) was established on the first day of the experiment, while a decrease in the number of lymphocytes by 1.1 times (P<0.01) compared to the control group. On the third day of aerosol prophylaxis of calves of experimental group 2 using the drug "Calfmin", the number of neutrophils in the blood decreased by 1.3 times ($P<0.01$) compared to the first day of the experiment, and the number of lymphocytes increased by 1.1 times ($P<0.01$). On the third day of the experiment, the leukogram of calves of experimental group 1, which were treated with aerosol prophylaxis using a farm scheme, was characterised by a tendency to reduce the number of stab and segmented neutrophils. The leukogram of calves in experimental group 1 did not differ from that in the animals of the control group. On the seventh day of group aerosol prophylaxis, the leukogram indicators of calves in both experimental groups did not have significant differences compared to the corresponding indicators in the control.

M.M. Koptev (2011) notes that in healthy animals, the lungs are free of microflora. K. Suzuki *et al*. (2012) and B.T.S. Pantoja *et al*. (2020) reported that this feature is conditioned by the action of protective mechanisms, among which an important role belongs to the ciliated epithelium of the upper respiratory tract, the mucous layer that covers them, surfactant, interferon, lysozyme, micro- and macrophages. Researchers J.B.A. Polanco *et al*. (2020) note that it is this factor that contributes to the occurrence of the disease, when moist cold air irritates trophic nerves. E.R. Schachner (2017) and E. Oliveira *et al*. (2020) report that when an animal overheats, biochemical and biophysical changes occur in its body, manifested by a violation of the cardiovascular system and a decrease in metabolic processes. The bactericidal activity of blood serum and phagocytic activity of white blood cells decreases, which helps to reduce the resistance of lung tissue. This, in turn, contributes to the development of opportunistic microflora and the occurrence of toxicosis, which leads to pneumonia.

B. Wolfger *et al*. (2015) indicate that in the acute course of bronchopneumonia, superficially located lobes of the lungs are first affected. In the initial stages of the disease, interparticle connective tissue is a barrier to the transition of inflammation from the affected areas of the lungs to healthy ones, but in the future, the barrier function of connective tissue is lost. J. Glodek *et al*. (2016) and P. Sharandak *et al.* (2022) note that nonspecific bronchopneumonia is characterised by inflammation of the bronchi and lungs, accompanied by their filling with catarrhal exudate, a disorder of external and internal gas exchange, the development of respiratory and cardiovascular insufficiency, and impaired functions of various organs and systems. This classical picture of the pathogenesis of this pathology was naturally manifested in the study described in this paper.

Researchers expressed that in the affected lobes of the lungs, exudate covers the alveolar epithelium and thereby reduces the lumen of the alveoli and bronchioles (Jodi & Randy, 2020). Therefore, according to K. Hermeyer *et al*. (2012) and N.C. Gaeta *et al*. (2018), part of the respiratory surface of the lungs is excluded from gas exchange and shortness of breath and hypoxia occur, which leads to disruption of tissue and cellular respiration, weakening of oxidative processes and the formation of energy for the vital activity of the body. K Hermeyer *et al*. (2012) and N.C. Gaeta *et al*. (2018) indicate that cardiac activity accelerates, the speed of blood flow increases, as a result of which tissues receive more blood, and with it oxygen. According to K. Dudek *et al*. (2020), all this compensates for impaired breathing, but with the further development of pneumonia and the damage to large areas of the lungs, the compensatory activity of the cardiovascular system weakens, which was also observed in this study

Many researchers and practitioners of veterinary medicine have always paid attention to the causes of bronchopneumonia and its course. P. Sharandak *et al.* (2022) are unanimous in their opinion that pathogenic microflora and unfavourable environmental conditions play a leading role in the development of the disease. However, the opinion of researchers on the issue of primacy is far from ambiguous. In a clinical study of calves with nonspecific bronchopneumonia, it is important not to miss the first symptoms of the disease. The presence of liquid serous-mucosal secretions suggests that the role of viral load is not excluded in the aetiology of nonspecific bronchopneumonia of calves, and the change of secretions to catarrhal (or catarrhal-purulent) indicates that bacterial microflora plays a leading role in the pathogenesis of bronchopneumonia. All animals with nonspecific bronchopneumonia (100%) at the beginning of the disease showed an increase in the number of respiratory movements, the appearance of inspiratory dyspnea and abdominal breathing, which coincides with the results of studies by C. Senthilkumaran *et al*. (2013).

The respiratory rate is a very valuable indicator, since tachypnea always occurs when gas exchange is disrupted due to hypoxemia. Recording of the respiratory rate is an indirect method of analysing the gas composition of blood. Healthy calves older than two weeks should normally have no more than 40 breathing movements per 1 minute. As an exception, respiratory rates of more than 40 per 1 minute are allowed only in animals that are kept indoors at high ambient temperatures. In addition, it is necessary to determine the respiratory rate at a distance, from the passage of the calf house, because this is the only way to get reliable data on the respiratory rate of the animal at rest.

During the conducted studies, the respiratory rate in calves with bronchopneumonia averaged 43 respiratory movements per 1 minute, and in some animals, it reached 50 or even 60 respiratory movements per 1 minute. This makes it possible to diagnose gas exchange disorders in the body of calves due to hypoxemia. The depth (intensity) of breathing in healthy calves is so low that chest movements at a distance are sometimes impossible to distinguish. This is always a favourable prognostic symptom. On the other hand, the easier it is to visually determine the respiratory rate, the more effort the animal spends on breathing movements, which is an unfavourable symptom.

When determining the clinical condition of calves with nonspecific bronchopneumonia, the rhythm of breathing was also evaluated. In the initial phase of the disease for nonspecific bronchopneumonia, the act of inspiration in calves was prolonged and the coefficient was 1:1, or even 1.2:1. This characterised inspiratory dyspnea and indicated a predominance of inflammatory processes in the respiratory tract, rather than in the lung parenchyma. Signs of expiratory dyspnea, which significantly extended the duration of exhalation relative to inhalation (1:1.6-1.8), were found in calves with a prolonged course of bronchopneumonia, which indicated an increase in the volume of intrathoracic gases (the appearance of areas of the lungs with emphysema).

During a clinical examination of calves with nonspecific bronchopneumonia, cyanosis of the mucous membranes of the oral cavity, conjunctiva, and nasal mirror was detected in all (100%) animals. This indicated circulatory disorders and the accumulation of excessive amounts of reduced haemoglobin in the blood of animals, which has a dark colour. The accumulation of excessive amounts of reduced haemoglobin in the blood of patients with nonspecific bronchopneumonia in calves is obviously associated with insufficient oxygen saturation of the blood due to inflammatory processes in the lungs (Amat *et al*., 2019; Buczinski & Pardon, 2020; Di Teodoro *et al*., 2020).

Y. Yamamoto *et al*. (2003) reported that auscultation of the lungs, at the beginning of the disease in most calves, showed broncho-vesicular respiration, which was noted in this study. This is conditioned by difficulty in air intake due to narrowing of the airways, a decrease in the elasticity of the alveoli due to infiltration of their walls, and a decrease in the number of alveoli involved in respiratory movements (Van Driessche *et al*., 2020). On the second or third days, wet wheezing was detected, which indicated an accumulation of exudate in the respiratory tract. Crepitation was also observed, which was best listened to at the peak of inspiration.

The results of morphological studies of the blood of calves with nonspecific bronchopneumonia confirmed the development of an inflammatory process in their body. Neutrophils, especially segmented ones, are known to have the highest phagocytic activity. Pronounced leukocytosis, with a significant increase in the relative and absolute number of these cells in the leukogram of sick calves, indicates activation of defence mechanisms against bacterial infection. A 2.0-fold increase ($P<0.05$) in the relative and absolute number of stab neutrophils in the blood of these animals indicates an acute course of the inflammatory process. Along with the development of neutrophilic leukocytosis (considered as a positive protective reaction of the body), eosinopenia and lymphocytopenia were recorded in sick calves. A decrease in the number of lymphocytes – the main immunocompetent cells in the blood of animals, is interpreted as a decrease in the immune status of the body of calves (Basoglu *et al*., 2016; Braun *et al*., 2018). Thus, lymphocytes (namely B-lymphocytes) are the main producers of plasma cells, which are transformed into immunoglobulins (antibodies) and perform antibacterial protection in the body of animals and humans. Due to intoxication of the animal's body with inflammatory products due to bronchopneumonia, deep violations of the receptor apparatus of lymphocytes occur. A decrease in the immune status of calves' bodies with bronchopneumonia is indicated by a decrease in the number, and sometimes complete absence, of eosinophils in the blood, which were recorded in animals of experimental groups.

The use of aerosol medicines is the most effective in the treatment of respiratory diseases in animals. In this case, direct contact of the drug with the pathogenic microflora of the respiratory tract and the site of the lesion is detected. As a result, the concentration of drugs on the mucous membrane of the respiratory tract is higher compared to intramuscular or oral administration. In this regard, the absorption of the drug was more efficient and faster, which also reduced its consumption by 4 times.

D. Bednarek *et al.* (2005) indicate that complex therapy is the most effective for bronchopneumonia, which is aimed at eliminating violations of the technology of maintenance and feeding, increasing the body's resistance, and includes the simultaneous use of various means of therapy: antimicrobial, pathogenetic, symptomatic, stimulating, and vitamin. The authors recommend using nonsteroidal anti-inflammatory drugs as the main medications. The study investigated the drug "Calfmin", which is a complex with the plant immunomodulator echinacea and silver and copper nanoaquachelates, iodine solution, and cobalt lactate (Drobot *et al*., 2013).

Therefore, the developed method is rational for modern high-tech livestock complexes with a large number of animals and is focused on obtaining high-quality and safe livestock products, the consumption of which in human nutrition should ensure high standards of quality of life. The method of group aerosoletherapy and aerosoleprophylaxis of diseases of young animals in preventive veterinary technologies using a natural remedy in combination with the plant immunomodulator echinacea, will allow agricultural enterprises to obtain organic products that meet the highest quality standards.

Conclusions

In calves with nonspecific bronchopneumonia, which are raised at a modern high-tech enterprise for the production of milk, the following clinical symptoms are observed: significant inhibition of animals (mainly lying down, low appetite, a shaky gait and muscle tremor were noted when forced to get up), visible mucous membranes were cyanotic, which is a sign of impaired gas exchange in the small circle of blood circulation. When examining the respiratory system, mixed shortness of breath, sharp, dry, jerky and painful cough were identified. Exudate discharge was detected in the nasal passages, which is a typical symptom of the disease. Auscultation of the lungs diagnosed the presence of wet wheezing, and chest percussion revealed areas of blunting of the apical and cardiac areas of the lungs. During auscultation of the heart, muffling of heart tones and an increase in pulse wave were noted when examining the arteries. Hyperthermia by 1.0-1.5°C higher than normal, as well as tachycardia and tachypnea, which corresponded to an acceleration of 1.2 and 1.5 times ($P<0.001$), respectively, compared to clinically healthy animals, were noted in sick animals. According to the results of morphological examination of the blood of sick calves, a 1.2-fold decrease in the number of red blood cells ($P<0.001$) and a 1.3-fold increase ($P<0.005$) in the number of white blood cells were noted. Specific changes in the leukogram for bronchopneumonia were established: an increase in the number of stab and segmented neutrophils by 2.0 times ($P<0.005$) and 1.6 times ($P<0.01$), respectively, and a decrease in the level of lymphocytes by 1.3 times ($P<0.001$) and eosinophils by 1.4 times ($P<0.001$) compared to the control group of animals. Characteristic signs of the disease were oligocytopenia, hypochromia, neutrophilic leukocytosis, lymphocytopenia, and eosinopenia. The use of the test agent at a dose of 7.0 mL per 1 animal and exposure of 45 minutes in group therapy of calves with nonspecific bronchopneumonia by aerosol method improved the clinical condition and blood parameters of calves for 3-5 days and reduced the duration of their treatment by 1.9-2.0 times. In the future, it is planned to consider in more detail the immunological parameters of calves' blood for using the proposed method of their treatment in the case of nonspecific bronchopneumonia.

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Conflict of Interest

None.

References

[1] Amat, S., Timsit, E., Baines, D., Yanke, J., & Alexander, T.W. (2019). Development of bacterial therapeutics against the bovine respiratory pathogen *mannheimia haemolytica*. *Applied and Environmental Microbiology*, 85(21), article number e01359-19. doi: 10.1128/AEM.01359-19.

[2] Basoglu, A., Baspinar, N., & Tenori, L., Vignoli, A., & Yildiz, R. (2016). Plasma metabolomics in calves with acute bronchopneumonia. *Metabolomics,*12, article number 128. doi:10.1007/s11306-016-1074-x.

[3] Bednarek, D., Kondracki, M., Friton, G.M., Trela, T., & Niemczuk, K. (2005). Effect of steroidal and non-steroidal anti-inflammatory drugs on inflammatory markers in calves with experimentally-induced bronchopneumonia. *Berliner und Münchener Tierärztliche Wochenschrift*, 118(7-8), 305-308.

[4] Braun, U., Gerspach, C., & Brammertz, C. (2018). The frequency of abnormal ultrasonographic findings in the lungs of 129 calves with bronchopneumonia. *Schweizer Archiv für Tierheilkunde*, 160(12), 737-741. doi: 10.17236/sat00189.

[5] Buczinski, S., & Pardon, B. (2020). Bovine respiratory disease diagnosis: What progress has been made in clinical diagnosis? *Veterinary Clinics of North America: Food Animal Practice,* 36(2), 399-423. doi: 10.1016/j.cvfa.2020.03.004.

[6] Di Teodoro, G., Marruchella, G., Di Provvido, A., D'Angelo, A.R., Orsini, G., Di Giuseppe, P., Sacchini, F., & Scacchia, M. (2020). Contagious bovine pleuropneumonia: A comprehensive overview. *Veterinary Pathology,* 57(4), 476-489. doi: 10.1177/0300985820921818.

[7] Directive 2010/63/EU of the European Parliament and of the council "On the Protection of Animals Used for Scientific Purposes". (2010, September). Retrieved from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:En:PDF.

[8] Drobot, M.V., Tsvilihovskyi, M.I., Dulnev, P.G., & Bereza, V.I. (2013). *Composite drug for therapy, aerosol therapy and aerosol prophylaxis of nonspecific catarrhal bronchopneumonia in calves.* Patent for the invention No. 101574.

[9] Dudek, K., Nicholas, R.A.J., Szacawa, E., & Bednarek, D. (2020). *Mycoplasma bovis* infections – occurrence, diagnosis and control. *Pathogens*, 9(8), article number 640. doi: 10.3390/pathogens9080640.

[10] Gaeta, N.C., Ribeiro, B.L.M., Alemán, M.A.R., Yoshihara, E., Nassar, A.F.C., Marques, L.M., Timenetsky, J., & Gregory, L. (2018). Bacterial pathogens of the lower respiratory tract of calves from Brazilian rural settlement herds and their association with clinical signs of bovine respiratory disease. *Pesquisa Veterinaria Brasileira,* 38(3), 374-381. doi: 10.1590/1678-5150-PVB-5323.

[11] Glodek, J., Adamiak, Z., & Przeworski, A. (2016). Magnetic resonance imaging of reptiles, rodents, and lagomorphs for clinical diagnosis and animal research. *BMC Complementary Medicine*, 66(3), 216-219.

[12] Hermeyer, K., Buchenau, I., Thomasmeyer, A., Baum, B., Spergser, J., Rosengarten, R., & Hewicker-Trautwein, M. (2012). Chronic pneumonia in calves after experimental infection with *Mycoplasma bovis* strain 1067: Characterization of lung pathology, persistence of variable surface protein antigens and local immune response. *Acta Veterinaria Scandinavica*, 54, article number 9. doi: 10.1186/1751-0147-54-9.

[13] Horalskyi, L.P., Khomych, V.T., & Kononskyi, O.I. (2015). *Fundamentals of histological technique and morphofunctional research methods in normal and pathological conditions: Training.* Zhytomyr: Polissya.

[14] Koptev, M.M. (2011). Morpho-functional characteristics of the structural elements of the lungs of rats are normal. *Journal of VDNZU "Ukrainian Medical Stomatological Academy",* 11(4(36)), 92-94.

[15] Leenen, K., Driessche, L., Cremer, L., Masmeijer, C., Boyen, F., Deprez, P., & Pardon, B. (2020). Comparison of bronchoalveolar lavage fluid bacteriology and cytology in calves classified based on combined clinical scoring and lung ultrasonography. *Preventive Veterinary Medicine,* 176, article number 104901. doi: 10.1016/j.prevetmed.2020.104901.

[16] McGill, J.L., & Sacco, R.E. (2020). The immunology of bovine respiratory disease recent advancements. *Veterinary Clinics of North America*: *Food Animal Practice*, 36(2), 333-348. doi: 10.1016/j.cvfa.2020.03.002.

[17] Nedosekov, V.V., Lytvyn, B.L., Polishchuk, V.V., Gomzykov, O.M., & Melnyk, V.V. (2010). *Aerosols in veterinary medicine. The guidelines include material on the use of aerosols in veterinary medicine*. Kyiv.

[18] Nishi, Y., Tsukano, K., Otsuka, M., Tsuchiya, M., & Suzuki, K. (2019). Relationship between bronchoalveolar lavage fluid and plasma endotoxin activity in calves with bronchopneumonia. *Journal of Veterinary Medical Science*, 81(7), 1043-1046. doi: 10.1292/jvms.18-0643.

[19] Oliveira, E., Nascente, E.P., Oliveira, L.P., Neto, J.M.S., Roquete, J.C., Santin, A.P.I., & Moura, V.M.B.D. (2020). Macroscopic and microscopic morphology of the trachea and lungs of giant anteater (Myrmecophaga tridactyla). *Pesquisa Veterinaria Brasileira,* 40(12), 1054-1062. doi: 10.1590/1678-5150-PVB-6608.

[20] Pantoja, B.T.S., Silva, A.R.M., Mondego-Oliveira, R., Silva, T.S., Marques, B.C., Albuquerque, R.P., Sousa, J.C.S., Rici, R.E.G., Miglino, M.A., Sousa, A.L., Franciolli, A.L.R., Sousa, E.M., Abreu-Silva, A.L., & Carvalho, R.C. (2020). Morphological study of larynx, trachea, and lungs of *Didelphis marsupialis* (LINNAEUS, 1758). *Veterinary World*, 13(10), 2142-2149. doi: 10.14202/vetworld.2020.2142-2149.

[21] Percie du Sert, N., Ahluwalia, A., Alam, S., Avey, M.T., Baker, V., Browne, W.J., Clark, A., Cuthil, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Hurst, V., Karp, N.A., Lazic, S.E., Lidser, K., MacCallum, C.J., Macleod, M., Pearl, E.J., Peterson, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E.S., Silberg, S.D., Steckler, T., & Würbel, H. (2020). Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLOS Biology*, 18(7), article number e3000411. doi: 10.1371/journal.pbio.3000411.

[22] Polanco, J.B.A., Mamprim, M.J., Silva, J.P., Inamassu, L.R., & Schimming, B.C. (2020). Computed tomographic and radiologic anatomy of the lower respiratory tract in the red-foot tortoise (Chelonoidis carbonaria). *Wildlife Medicine*, 40(8), 637-646. doi: 10.1590/1678-5150-PVB-6587.

[23] Schachner, E.R., Sedlmayr, J.C., Schott, R., Lyson, T.R., Sanders, R.K., & Lambertz M. (2017). Pulmonary anatomy and a case of unilateral aplasia in a common snapping turtle (*Chelydra serpentine*): Developmental perspectives on cryptodiran lungs. *Journal of Anatomy*, 231(6), 835-848. doi: 10.1111/joa.12722.

[24] Senthilkumaran, C., Clark, M.E., Abdelaziz, K., Bateman, K.G., MacKay, A., Hewson, J., & Caswell, J.L. (2013). Increased annexin A1 and A2 levels in bronchoalveolar lavage fluid are associated with resistance to respiratory disease in beef calves. *Veterinary Research*, 44, article number 24. doi: 10.1186/1297-9716-44-24.

[25] Sharandak, P., Drobot, M., & Druz, N. (2022). Denial of bronchopneumonia by the aerosol method and its influence on calf production indicators. *ScienceRise: Biological Science*, 2(31), 18-23. doi: 10.15587/2519-8025.2022.260754.

[26] Suzuki, K., Higuchi, H., Iwano, H., Lakritz, J., Sera, K., Koiwa, M., & Taguchi, K. (2012). Analysis of trace and major elements in bronchoalveolar lavage fluid of Mycoplasma bronchopneumonia in calves. *Biological Trace Element Research,* 145, 166-171. doi: 10.1007/s12011-011-9180-0.

[27] Van Driessche, L., Vanneste, K., Bogaerts, B., De Keersmaecker, S.C.J., Roosens, N.H., Haesebrouck, F., De Cremer, L., Deprez, P., Pardon, B., & Boyen, F. (2020). Isolation of drug-resistant gallibacterium anatis from calves with unresponsive bronchopneumonia, Belgium. *Emerging Infectious Diseases*, 26(4), 721-730. doi: 10.3201/eid2604.190962.

[28] Wolfger, B., Timsit, E., White, J.B., & Orsel, K. (2015). A systematic review of bovine respiratory disease diagnosis focused on diagnostic confirmation, early detection, and prediction of unfavorable outcomes in feedlot cattle. *Veterinary Clinics of North America Food Animal*, 31(3), 351-365. doi: 10.1016/j.cvfa.2015.05.005.

[29] Yamamoto, Y., Tanaka, A., Kanamaru, A., Tanaka, S., Tsubone, H., Atoji, Y., & Suzuki, Y. (2003). Morphology of aging lung in F344/N rat: Alveolar size, connective tissue and smooth muscle cell markers. *The Anatomical Records Part*, 272A(2), 538-547. doi: 10.1002/ar.a.10172.

Ефективність групової терапії телят за неспецифічної бронхопневмонії методом аерозолю

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Анотація. Неспецифічна бронхопневмонія телят завдає значних економічних збитків фермерським господарствам, а тому актуальним є розробка ефективних терапевтичних препаратів, особливо для групового застосування. Заслуговують на увагу лікарські засоби, що застосовуються аерозольно. Метою роботи було дослідити ефективність групового аерозольного методу застосування телятам препарату «Кальфмін» за неспецифічної бронхопневмонії. У дослід залучали телят-аналогів двох-трьох місячного віку як клінічно здорових, так і хворих на неспецифічну бронхопневмонію. Для визначення діагнозу і контролю за функціональним станом організму телят використовували стандартні клінічні та гематологічні методи дослідження. Встановлено, що у крові телят, хворих на неспецифічну бронхопневмонію, відзначалося підвищення кількості лейкоцитів в 1,3 раза (Р<0,05) порівняно з клінічно здоровими тваринами. В їх крові також виявляли збільшення кількості паличкоядерних і сегментоядерних нейтрофілів в 2,0 рази (Р<0,05) і 1,6 раза (Р<0,01), відповідно, та зменшення кількості лімфоцитів в 1,3 раза (Р<0,001) і еозинофілів в 1,4 раза (Р<0,001), відповідно. В хворих телят відмічали зменшення в крові кількості еритроцитів в 1,2 раза (Р<0.001) і вмісту гемоглобіну в 1,2 раза (Р<0.001) порівняно з контролем. Зафіксували покращення клінічного стану і гематологічних показників у хворих тварин упродовж 3-5 діб, скорочення тривалості терапії в 1,9-2,0 рази та 100% збереженість усіх хворих телят

Ключові слова: наноаквахелати; срібло; мідь; йод; кобальт; ехінацея пурпурова; морфологія крові

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Influence of autonomic nervous system tone on the content of cholesterol and lipoproteins of different density in the blood of cows

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Abstract. The relevance of the study lies in the investigation of the influence of the autonomic nervous system tone on the content of lipids in the body of cows, which is important to consider in order to ensure the high productivity of the dairy herd and balance diets in animal feeding. The purpose of the study was to determine the effect of the tone of the ganglion nervous system on the content of cholesterol and lipoproteins of various densities in the blood of dairy cows of the Ukrainian Black-and-White breed. To determine heart rate variability, an electrocardiograph was used with subsequent measurement of the main indicators according to the Baevsky stress index, which included determining the mode, its amplitude, variation range, autonomous equilibrium index, autonomous rhythm indicator, and stress index. Three experimental groups were formed from experimental cows: normotonics, vagotonics, and sympathotonics. The content of lipoproteins in blood plasma was determined using a Sinnova BS-3000 biochemical analyser (China). According to the results of biochemical analysis, differences in the obtained indicators were established among experimental groups of cows with different tones of the autonomic nervous system. This is due to the fact that activation of the sympathetic nerve branch leads to changes in liver function, which affects the production of glucose in the body. This can lead to increased cholesterol production. In addition, an increased tone of the sympathetic branch can stimulate the activity of enzymes responsible for the synthesis of cholesterol, and lead to a decrease in the level of hormones that regulate its synthesis and transport in the body. In particular, vagotonic animals had the highest blood content of total cholesterol and high-density lipoproteins. Sympathotonics showed the lowest blood values for total cholesterol and high-density lipoproteins. Normotonics occupied an intermediate place among the experimental groups of cows. A detailed study of this issue reveals the features of the influence of the autonomic nervous system tone on the indicators of lipid metabolism in the blood of cows, which is important to consider in production to effectively solve issues related to maintaining their productivity and improving the quality of milk

Keywords: ganglion nervous system; normotonics; vagotonics; sympathotonics; lipids; Ukrainian Black-and-White

Introduction

Lipid metabolism in cattle differs compared to monogastric animal species. Lipids, which are part of cell membranes, are directly involved in the processes of transmembrane active transport of molecules and ions, specific reception on the cell surface, transmission of nerve impulses, etc. Since cell membranes are important regulators of many biochemical processes, changes in the structure, composition, and orientation of membrane lipids cause significant disruption of cellular metabolism. It is quite important to properly balance the diet for highly productive cows. It should also be noted that during the lactation period, the content of lipids in the cow's blood significantly increases compared to the interlactation period. This is especially common before calving. During this period, an increase in blood lipids can be observed, which even goes beyond the normal range (Davis *et al.,* 2019). Despite these changes, they are assessed as a physiological phenomenon, as lipid content decreases and their concentration gradually normalises. Given these features, many factors should be taken into account on modern dairy farms to better balance diets and maintain homeostasis in the body of highly productive animals (Gross *et al.,* 2021). A thorough understanding of this issue plays an important role in countering the growth of excess lipids in the body of cows, which can cause diseases (Ying *et al.,* 2021).

The study of lipid metabolism by O.P. Vergeles *et al.* (2019) focused primarily on the main indicators such as the main lipid depot and factors that have a significant impact on metabolic processes. The liver and subcutaneous tissue are the most important sites in the body where lipolysis and lipogenesis are constantly occurring (Sylvers-Davie & Davies, 2021). These processes are significantly influenced by diet and neurohumoral factors. The content of triglycerides in adipose tissue and the concentration of free fatty acids in the bloodstream depend on the uniform course of lipolysis and lipogenesis. For the transport of lipids in the cow's body, there is a specific list of lipoprotein transport systems. This provides a certain intensity of metabolic processes in other tissues, especially in the muscle and liver. To balance and control homeostasis, the central nervous system corrects the course of metabolic processes, affecting the activity of the corresponding hormones with the involvement of the peripheral nervous system (Basu & Goldberg, 2020).

According to K.R. Feingold (2022), the autonomous nervous system, as one of the components of the peripheral nervous system, has a corrective effect on the intensity of metabolic processes in the body of cows. The liver, with the help of the sympathetic and parasympathetic nervous systems, with the participation of afferent and efferent nerve fibres, provides an opportunity to communicate with regulatory centres in the brain, including the hypothalamus. Hypothalamic anorexic and orexigenic peptides transmit nerve signals to the liver, which provides modulation of lipid content and lipoprotein synthesis (Yang *et al.,* 2022a). Moreover, hormones such as insulin, leptin, glucagon-like peptides, control the concentration of lipids due to their action on peripheral nerves (Wen *et al.,* 2022).

Depending on the individual characteristics of the cow's body, the influence of the autonomous nervous system may vary. It is known that the sympathetic and parasympathetic nervous systems constantly affect the animal, but depending on circumstances, such as stress, one department may prevail over the other, which is the physiological norm. The relationship between these divisions is characterised as a sympathovagal balance, which may vary depending on the tone of the autonomic nervous system. To date, it has been determined that depending on the strength of the influence of the sympathetic or parasympathetic nervous system in the animal's body, it can be characterised as sympathotonia, vagotonia, and normotonia. Based on this, the individual intensity of metabolism in cows may vary. This, in turn, is the basis for studying this issue, for a better understanding of the processes of lipid metabolism in the body and its correction (Umezaki *et al.,* 2021). With this in mind, the purpose of this study is to investigate the effect of the tone of the autonomic nervous system on the content of lipoproteins in the blood of cows of the Ukrainian Black-and-White breed with high dairy productivity.

Literature Review

The most common agro-industrial sector in animal husbandry is the production of meat and dairy products. First of all, it is focused on increasing the number of highly productive cows. For this purpose, work is actively underway to determine the genetic characteristics of the body and the metabolic status of animals. Highly productive cows are sensitive to the conditions of keeping, which affects the ability of the animal body to maintain homeostasis of the internal environment. Most often, the biological material for conducting diagnostic studies of the functional state of the animal's body is blood or its derivatives, such as plasma or serum (Gonçalinho *et al.,* 2021; Razzaghi *et al.,* 2022).

One of the informative characteristics of the physiological status of the body of cattle is the indicators of lipid metabolism. Among the most important components of the blood lipidogram are triacylglycerols and cholesterol. Considerable interest in the need to determine the content of lipids in the body of cows is explained by the fact that this indicator reflects the effectiveness of fat absorption by tissues quite well. This allows predicting the likely quality of meat and milk in the future. Cholesterol also plays an important role in the transport of free fatty acids in the body. With the help of combinations with proteins in the form of lipoproteins of various densities, the necessary energy-valuable lipids are gradually carried through the liver during digestion. Subsequently, they enter the subcutaneous tissue and participate in lipolysis and lipogenesis (Chen *et al.,* 2020*;* Daradics *et al.,* 2021).

Currently, areas are actively developing to improve these processes and ensure a better intake of the necessary amount of free fatty acids in the body of cows. This is due to the fact that to obtain an increase in milk productivity with the required percentage of fat content, it is necessary to ensure effective assimilation of high-energy substances. First of all, this is ensured by adding various feed additives and adjusting the diet in accordance with the needs of cows. However, not only balanced feeding can provide everything necessary for the animal's body. There are many factors influencing the assimilation and use of exogenous lipids in the digestive canal of productive animals (Cai *et al.,* 2022).

Neurohumoral regulation of homeostasis of the animal body is a fairly common question that arises in the study of lipid metabolism. Under the influence of external factors of the animal through various systems, depending on the nature of the influence of exo- or endogenous factors, the body uses or stores the necessary nutrients for its own needs. Correction of these processes is carried out with the help of both the central and peripheral nervous systems. In accordance with this, a certain amount of hormones is released, as well as nerve impulses are transmitted to the target organs to ensure and maintain homeostasis in the mammalian body. A fairly new question in the study of these processes was the determination of the role of the autonomic nervous system in the regulation of lipid metabolism (Rahman *et al.,* 2022). Due to the corresponding parts of the sympathetic and parasympathetic nervous systems, an active response of the body to factors that contribute to the violation of homeostasis in the body is established. Depending on the individual characteristics of the animal's body, the influence of each of these departments may differ in accordance with the tone of the autonomic nervous system.

Materials and Methods

The research was carried out in 2022 on cows of the Ukrainian Black-and-White dairy breed at the dairy farm of LLC "Obriy" (Poltava region, Orzhytskyi district, Vyshneve village). The variational pulsometry study involved 100 clinically healthy cows, 3-4 lactation. In the electrocardiographic study of experimental cows, a single-channel electrocardiograph Heart Mirror IKO (Hungary) was used to record changes in the electrical potentials of the heart. During the variational pulsometry, the animals were at rest. To obtain an electrocardiogram, the animals were fixed in a standing position of the body. At the attachment points of the device's sensors, the skin surface was previously freed from hair, treated with alcohol, and an electrically conductive gel was applied, which allowed avoiding artifacts during heart rate recording.

Three anatomical points were used to perform the variational pulsometry. The first one was placed on the left side between 3-5 intercostal spaces, behind the elbow. In this place, the electrode of the right-hand was placed. The location of the second one was caudal to 1/3 of the jugular sulcus, where the left-hand electrode was placed. The third point was located in the area of the cow's withers, in place of which a neutral tap was fixed. Crocodile clamps were used to secure the electrodes. The teeth on the corresponding clamps were previously blunted to prevent injury to the animal. During the electrocardiographic study, the electrical potentials of the heart in cows were recorded by a galvanometer. Registration and recording of indicators were carried out using a stylus on cardiographic paper. The stylus moved according to changes in the electrical potentials of the heart, which was recorded by the galvanometer. The speed of the tape movement during the recording of cardiac signals was 50 mm/s. The results obtained were calculated using the Microsoft Excel software suite.

The following indicators were determined based on the results of the variability-pulse oximetry study: mode (Mo) – heart rate at the R-R interval among the calculated 100 measurements, which is the most frequent, i.e., a cardiac signal that regularly repeats on the cardiogram; mode amplitude (AMo) – value of the mode indicator in percent, namely the percentage ratio of the mode to the total number of cardiac intervals; variation range (Δx) – difference between the maximum and minimum mode indicators; autonomic balance index (ABI) – value that corresponds to the influence of the parasympathetic or sympathetic nervous system on the animal's body and indicates the degree of action of these departments, calculated when determining the difference in mode amplitude with a variation range; autonomic rhythm index (ARI) – reflects the action of the sympathetic nervous system in the animal's body and is determined by Equation 1:

$$ARI = 1 \div (Mo \times \Delta x). \qquad (1)$$

Stress index – (SI) – indicator that characterises the tension of the animal's body, that is, its stressful state, and the obtained values reflect the state of the autonomic nervous system tone, calculated by the equation:

$$SI = AMo \div (2 \times Mo \times \Delta x). \qquad (2)$$

According to the results of the processed indicators, three experimental groups of cows were formed from 100 animals in accordance with the tone of the autonomic nervous system. The main indicator by which the grouping of experimental groups of cows took place was the stress index, which reflected the state of sympathovagal balance in their body. Animals that showed low values of this indicator based on the results of calculations were classified as vagotonics, with high characteristics – as sympathotonics, and with intermediate values – as normotonics. 5 animals were selected from each group of cows to clarify the features of the autonomic nervous system tone. Thus, cows were divided into three experimental groups: normotonics, vagotonics, and sympathotonics. Blood was collected in sterile syringes in compliance with the rules of asepsis and antiseptics. The content of lipoproteins in blood plasma was determined using a Sinnova BS-3000 biochemical analyser (China), according to the instructions. To confirm the reliability of the results obtained, they were processed using variation statistics. Probable differences in values were determined by the Student's t-test. Designations that reflected the likely differences between the obtained indicators were reflected in the form: $P<0.05$, $P<0.01$, $P<0.001$.

Information on compliance with bioethical standards. The study met ARRIVE's recommendations and was conducted without violating the guidelines of Directive 2010/63/EU of the

European Parliament and of the council "On the Protection of Animals Used for Scientific Purposes" (2010).

Results and Discussion

Based on the results of the analysis of the obtained lipidogram, it should be noted that the obtained values of the studied indicators corresponded to the limits of physiological parameters. If necessary, the body of these cows had sufficient reserves of neutral lipids to maintain the proper level of energy balance and, thereby, ensure the effective functioning of internal organs and tissues. During a biochemical study of the blood plasma of cows of the Ukrainian Black-and-White breed, cholesterol content and lipoprotein concentrations of different densities were obtained (Table 1).

Table 1. Indicators of cholesterol and lipoprotein content of different densities depending on the tone of autonomic nervous regulation (M±m; n=5)

Lipids	Normotonic	Sympathotonic	Vagotonic
Cholesterol, mmol/L	3.50±0.01	3.30±0.05**	3.81±0.02***
HDL, mmol/L	3.03±0.04	2.74±0.05**	3.21±0.04*
LDL, mmol/L	0.39±0.02	0.47±0.02*	0.54±0.03**
VLDL, mmol/L	0.06±0.01	0.09±0.01	0.06±0.01

Notes: *HDL – high-density lipoproteins; LDL – low-density lipoproteins; VLDL – very low-density lipoproteins*
**$P<0.05$, **$P<0.01$, ***$P<0.001$ – relative to the data of the normotonic group*
Source: *developed by the authors*

According to the results obtained, total cholesterol in vagotonic cows was the highest (by 8.85%), and sympathotonics had the lowest content (by 6%), compared to other groups of animals (P<0.01; P<0.001). Normotonics took an average value among the study groups, respectively. Such indicators indicate that sympathotonics have an increased intake of lipids by the body, which is reflected in lower cholesterol values in the blood. This may be conditioned by the fact that animals with a preference for the influence of the sympathetic nervous system in the body are more likely to be exposed to environmental factors. Increased tone of the sympathetic branch of the autonomic nervous system can contribute to an increase in total cholesterol levels in the blood of cows by several mechanisms. The sympathetic branch of the autonomic nervous system stimulates the production of glucocorticosteroids, such as cortisol, which increase blood cholesterol levels. A high tone of the sympathetic branch of the autonomic nervous system can contribute to a violation of lipid metabolism in the body, which also provokes an increase in cholesterol levels in the blood and the mobilisation of fatty acids from fat cells.

In turn, the animal actively uses the body's reserves, which contributes to the processes of lipolysis. Given this, with greater activity of the cardiovascular system, at the same time, the intensity of metabolic processes in the body will increase. Increased activity of the cardiovascular system provides an increase in the supply of oxygen and other nutrients to cells, which leads to increased metabolism and increased consumption of lipids as an energy source. The animal's body increases the production of glucose, which can lead to an increase in the synthesis of triglycerides and total cholesterol. As a result, the course of catabolic reactions will increase to maintain energy balance. An excessive increase in total cholesterol can cause various negative consequences, for example, the development of atherosclerosis. When analysing the established dynamics relative to total cholesterol in the blood, vagotonics were characterised by high indicators. This is conditioned by the fact that the advantage of the parasympathetic nervous system in such cows showed a positive effect on the intensity of lipogenesis in their body. Studies show that activation of the parasympathetic nervous system leads to a decrease in blood glucose levels and the synthesis of triglycerides, which can positively affect the weight gain of animals and their productivity.

It is also worth noting that activation of the parasympathetic nervous system can reduce the production of glucocorticoids, which specifically affect lipid metabolism. Thus, increased parasympathetic nervous system tone can have a positive effect on lipid metabolism and the overall health of cows. In accordance with this, the graph shows high cholesterol levels within the physiological norm (Fig. 1).

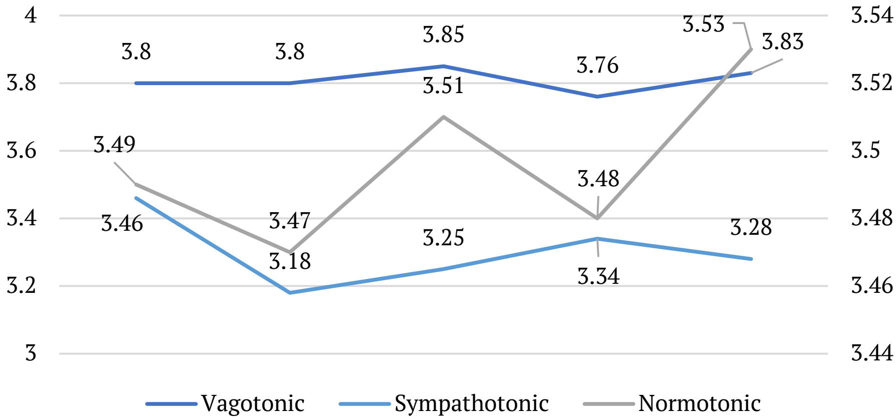

Figure 1. Total cholesterol content in the blood of cows of experimental groups, mmol/L
Source: *developed by the authors*

The HDL content in the blood of animals among the experimental groups was 6% higher in vagotonics than in others, and 9.6% lower in sympathotonics ($P<0.05$; $P<0.01$). Normotonics had average scores compared to cows in the other two groups. According to them, vagotonics have higher HDL levels compared to sympathotonics. High-density lipoproteins can reduce the risk of heart disease by transporting cholesterol from the tissues to the liver for further conversion. Therefore, higher HDL levels in vagotonics may indicate a lower risk of cardiovascular disease compared to sympathotonics. Animals with a sympathovagal balance, where sympathotonia processes predominate, respectively, have a lower content of free fatty acids in the body. In turn, the need for more transport lipoproteins in the bloodstream is not so high, which could affect the HDL content. In addition, animals that are less exposed to stress factors have significantly smaller shifts in body homeostasis. Based on this, the experimental group of sympathotonics showed high activity of the cardiovascular system, which became the basis for the active course of lipolysis processes. These processes often occur under the influence of various factors, including changes in climatic conditions at different times of the year. Depending on the tone of autonomic nervous regulation, the acceleration of metabolic processes occurs at different rates and duration. The autonomic nervous system can affect the speed and duration of metabolic processes in the body, depending on its tone. For example, activating the sympathetic branch of the autonomic nervous system can stimulate metabolism and reduce energy storage in tissues, which can lead to a rapid decrease in blood glucose levels. Activation of the sympathetic branch of the autonomic nervous system can lead to the release of norepinephrine and other catecholamines that stimulate metabolism in tissues, in particular, mobilise fats from complex organic compounds for use as an energy source. This can lead to a decrease in energy storage in the tissues and a rapid decrease in blood glucose levels.

Due to the influence of the tone of autonomic nervous regulation on the processes of lipoprotein metabolism, individual characteristics in the parameters of homeostasis were noted in experimental groups of animals, which is reflected in the HDL content in Figure 2. High HDL values obtained as a result of the study may indicate pathological changes in the body of cows, but considering the fact that sampling was carried out during lactation of cows, these changes could have an adaptive character and be regarded as physiological.

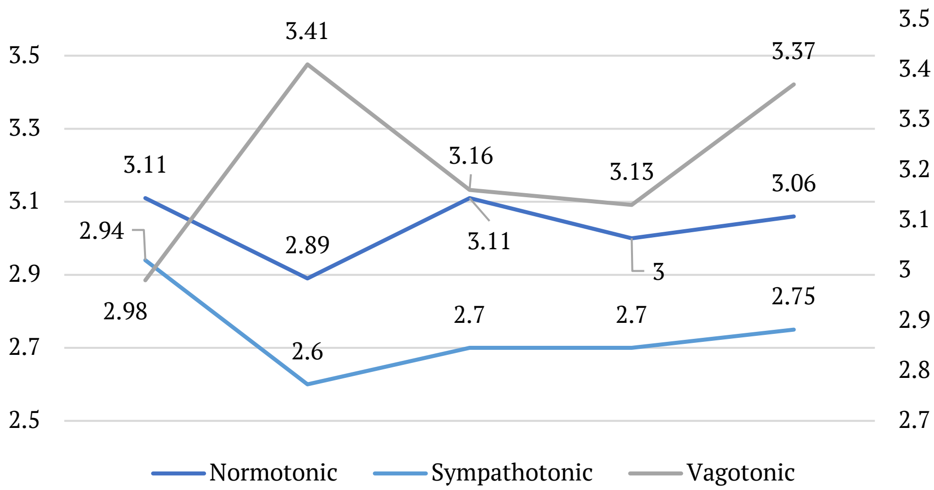

Figure 2. High-density lipoprotein content in cows of experimental groups, mmol/L
Source: *developed by the authors*

Regarding LDL, among the animals of the experimental groups, the lowest content was observed in normotonics. This indicator is 21% less than that of sympathotonics and 38% less than that of vagotonics ($P<0.05$; $P<0.01$). Low LDL levels in experimental groups of animals are explained by the lactation period. Normally, cattle have a much higher LDL than HDL content in their blood. During lactation, an increase in the amount of HDL and a decrease in LDL were noted in the bloodstream due to active processes of milk formation in the mammary gland. During lactation, the LDL content in the blood decreases in cows, and HDL increases. This is conditioned by the fact that during lactation, cows produce more milk, which contains a significant amount of fat. To transport fat to the mammary glands, the cow's body produces more HDL, which can transport fat to the mammary glands. In addition, during lactation, the synthesis of proteins and glucose increases, which contribute to an increase in HDL in the blood. Accordingly, the level of lipids increases, and as a result, the need for HDL synthesis increases, especially for cholesterol transport. In this regard, the quantitative indicators of LDL decrease, which is shown in Figure 3. Among the results obtained for VLDL, there were no significant differences between the animals of the experimental groups.

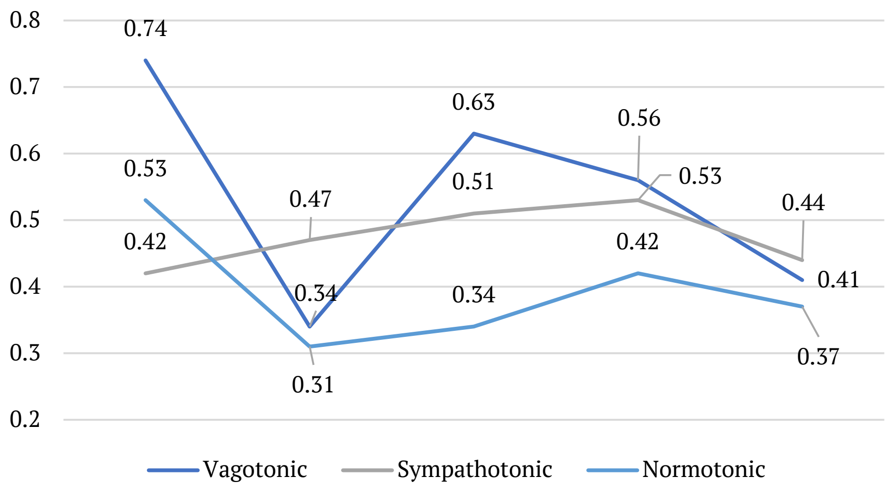

Figure 3. Low-density lipoprotein content in cows of experimental groups, mmol/L
Source: *developed by the authors*

When analysing the results obtained, it should be noted that the influence of the autonomic nervous system tone on lipid content in cows has been studied in more detail by other researchers. Vagotonics may experience a decrease in high-density lipoprotein (HDL) levels and an increase in low-density lipoprotein (LDL) levels. This may be due to a decrease in the activity of enzymes responsible for HDL synthesis and removal, and, conversely, to an increase in the activity of enzymes responsible for LDL synthesis and removal. In addition, there may be a decrease in the intensity of lipid metabolism and an increase in their storage in the form of triglycerides, which can lead to weight gain and the development of obesity. Lipid homeostasis in sympathicotonics may be impaired due to increased activation of the sympathetic branch of the autonomic nervous system. This can lead to an increase in total cholesterol and triglycerides in the blood, a decrease in HDL levels, and an increase in LDL. There is also an increase in lipogenesis activity and a decrease in lipolysis, which contributes to the accumulation of lipids in the body. Cholesterol is an important component of cell membranes and performs a variety of biological functions. It is a precursor for the synthesis of steroids, bile acids, and vitamin D, affects the functioning of the cardiovascular system, in particular, vascular permeability, as well as anti-inflammatory and immunomodulatory processes. However, high blood cholesterol levels may be associated with the risk of developing cardiovascular diseases and other pathologies.

Among the studies, general issues related to the consideration of the participation of the autonomic nervous system on these indicators under different conditions or the use of different feed additives are considered (Yu *et al.,* 2021; Yang *et al.,* 2022b). These papers do not consider the individual characteristics of the animal when describing the autonomic nervous system as an indicator that is important in the development of stable homeostasis of the animal's body. Accordingly, significant or minor changes in the lipidogram are often described under the influence of artificially created stressors or changes in diet (Eder *et al.,* 2021; Ma *et al.,* 2023). There are significant discrepancies when comparing the experimental and control groups, some indicators do not differ or even have significant differences that do not give a clear answer to the question posed (Sammad *et al.,* 2020; Myers *et al.,* 2021). Considering the results obtained, it should be noted that in some researchers, the indicators of high-density lipoproteins were lower compared to low-density lipoproteins (Gonçalinho *et al.,* 2021). This feature is explained by the fact that depending on the period, for example, HDL indicators may increase before calving, which is physiologically normal. High levels of high-density lipoproteins in cows are indicated by T. Takahashi *et al.* (2021), which confirms the reliability of the study and compliance with the physiological norm of the results obtained. Regarding the effect of the autonomic nervous system on lipid metabolism in the body, Y. Liu *et al.* (2020) provide a relevant example regarding the role of the sympathetic nervous system in lipid oxidation. The researchers noted that with the participation of neurohumoral effects on the body, metabolic processes can accelerate or slow down. In stressful situations, the sympathetic branch of the autonomic nervous system is activated in the body, which can lead to an increase in glucose production in the liver and a decrease in glucose consumption by muscles. In addition, increased sympathetic branch tone can contribute to an increase in total cholesterol and low-density lipoproteins in the blood. They repeatedly involve the autonomic nervous system as a control factor. To better understand the need to investigate this issue, it should be noted that research is constantly being conducted to improve the productivity of the dairy herd. Increasing the productivity of a dairy herd is possible by improving the diet, which can affect the work of neurohumoral mechanisms of metabolic regulation, increasing the physical activity of animals, which can cause activation of the sympathetic branch of the autonomic nervous system and contribute to an increase in metabolic processes in the body. On the example of the studies by A. Razzaghi *et al.* (2022) and G.H.F. Gonçalinho *et al.* (2021), the areas of improving the nutritional value of feed and increasing new feed additives are actively developing to obtain a larger volume of quality products. Most processes are tied to the consideration of many factors. The effect of stress on the animal is currently quite significant. Regardless of the aetiology, any negative impact on the cow can affect its productivity. Accordingly, depending on the individual characteristics of each animal, the response to the stress factor may vary. In the future, the recovery period for normal performance will increase significantly. I.C. Okuyucu *et al.* (2023) note that many factors, such as physiological, biochemical, and behavioural mechanisms, must be taken into account.

After analysing the results obtained, it should be noted that lipid metabolism may differ depending on the tone of the autonomic nervous system. On the example of an experimental group of sympathotonics, animals in which the influence of the sympathetic nervous system prevailed had low cholesterol levels. Thus, a decrease in the level of lipids in the blood may indicate the activity of lipolysis processes in the animal's body, which may be associated with an increased energy demand of the body or with an increased tone of the sympathetic branch of the autonomic nervous system. Such processes can help ensure the constancy of lipid homeostasis in the animal's body. The system of lipid homeostasis in animals includes a variety of processes that ensure the preservation of constant levels of lipids in the body. This is achieved through various mechanisms that ensure the production, transport, and use of lipids in the body's cells and tissues. One of these mechanisms is related to the level of lipoproteins of different densities, which transport lipids in the blood. A decrease in lipoprotein levels may indicate that the animal's body consumes lipids to ensure constant homeostasis.

Conclusions

As a result of the conducted studies, it was found that the tone of the autonomic nervous system affects the cholesterol content in the blood of cows of the Ukrainian Black-and-White breed. This is because the sympathetic branch causes changes in the functioning of the liver, which regulates glucose production in the body, and this can lead to increased cholesterol synthesis. In addition, increased sympathetic branch tone can promote the activation of enzymes responsible for cholesterol synthesis and reduce the level of hormones that regulate its synthesis and transport in the body. Among the experimental groups of animals, there is an increase in lipid indicators in vagotonics and a decrease in sympathotonics. This indicates the dependence of the quantitative parameters of lipoproteins and total cholesterol in the blood on the tone of the autonomic nervous system. Normotonics, which are characterised by equilibrium in the sympathovagal balance, occupied an intermediate place in relation to the studied parameters in comparison with animals with a predominant tone of the sympathetic or parasympathetic branches of the autonomic nervous system. This means that normotonics can have certain advantages over animals with an imbalance of the autonomic nervous system, as they are characterised by greater flexibility in responding to changes in the internal and external environment, which can have a positive impact on their health and productivity.

Each animal is special, and its response to the state of sympathovagal balance can be individual. Therefore, to ensure an optimal level of lipid metabolism intensity in the body of productive animals, it is necessary to conduct an individual assessment of the tone of the autonomic nervous system and consider other factors that affect their health and productivity level. Consideration of the results obtained will allow systematically influencing the improvement of consumption and efficiency of assimilation of feed nutrients by animals to obtain the desired productivity. In the future, it is planned to investigate the effect of the tone of the autonomic nervous system on lipid homeostasis in the body of cows depending on the time of year.

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None.

Conflict of Interest

None.

References

[1] Basu, D., & Goldberg, I.J. (2020). Regulation of lipoprotein lipase-mediated lipolysis of triglycerides. *Current Opinion in Lipidology*, 31(3), 154-160. doi: 10.1097/MOL.0000000000000676.

[2] Cai, Y., Wang, Z., Li, L., He, L., Wu, X., Zhang, M., & Zhu, P. (2022). Neuropeptide Y regulates cholesterol uptake and efflux in macrophages and promotes foam cell formation. *Journal of Cellular and Molecular Medicine*, 26(21), 5391-5402. doi: 10.1111/jcmm.17561.

[3] Chen, F., Zhou, Y., Yang, K., Shen, M., & Wang, Y. (2020). NPY stimulates cholesterol synthesis acutely by activating the SREBP2-HMGCR pathway through the Y1 and Y5 receptors in murine hepatocytes. *Life Sciences*, 1(262), article number 118478. doi: 10.1016/j.lfs.2020.118478.

[4] Daradics, Z., Crecan, C.M., Rus, M.A., Morar, I.A., Mircean, M.V., Cătoi, A.F., Cecan, A.D., & Cătoi, C. (2021). Obesity-related metabolic dysfunction in dairy cows and horses: Comparison to human metabolic syndrome. *Life,* 11(12), article number 1406. doi: 10.3390/life11121406.

[5] Davis, A.N., Rico, J.E., Myers, W.A., Coleman, M.J., Clapham, M.E., Haughey, N.J., & McFadden, J.W. (2019). Circulating low-density lipoprotein ceramide concentrations increase in Holstein dairy cows transitioning from gestation to lactation. *Journal of Dairy Science,* 102(6), 5634-5646. doi: 10.3168/jds.2018-15850.

[6] Directive 2010/63/EU of the European Parliament and of the council "On the Protection of Animals Used for Scientific Purposes". (2010, September). Retrieved from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:En:PDF.

[7] Eder, K., Gessner, D.K., & Ringseis, R. (2021). Fibroblast growth factor 21 in dairy cows: Current knowledge and potential relevance. *Journal of Animal Science and Biotechnology*, 12, article number 97. doi: 10.1186/s40104-021-00621-y.

[8] Feingold, K.R. (2022). Lipid and lipoprotein metabolism. *Endocrinology and Metabolism Clinics,* 51(3), 437-458. doi: 10.1016/j.ecl.2022.02.008.

[9] Gonçalinho, G.H.F., Roggerio, A., da Silva Goes, M.F., Avakian, S.D., Leal, D.P., Strunz, C.M.C., & de Padua Mansur, A. (2021). Comparison of resveratrol supplementation and energy restriction effects on sympathetic nervous system activity and vascular reactivity: A randomized clinical trial. *Molecules,* 26(11), article number 3168. doi: 10.3390/molecules26113168.

[10] Gross, J.J., Schwinn, A.C., Müller, E., Münger, A., Dohme-Meier, F., & Bruckmaier, R.M. (2021). Plasma cholesterol levels and short-term adaptations of metabolism and milk production during feed restriction in early lactating dairy cows on pasture. *Journal of Animal Physiology and Animal Nutrition,* 105(6), 1024-1033. doi: 10.1111/jpn.13531.

[11] Liu, Y., Chen, X., Qu, Y., Song, L., Lin, Q., Li, M., & Dong, J. (2020). Central nesfatin-1 activates lipid mobilization in adipose tissue and fatty acid oxidation in muscle via the sympathetic nervous system. *BioFactors,* 46(3), 454-464. doi: 10.1002/biof.1600.

[12] Ma, B.Y., Raza, S.H.A., Hou, S., Wang, Z., Gao, Z.H., Almohaimeed, H.M., & Gui, L.S. (2023). RNA-Seq revealed the effect of adding different proportions of wheat diet on fat metabolism of Tibetan lamb. *Gene,* 851, article number 147031. doi: 10.1016/j.gene.2022.147031.

[13] Myers, M.N., Zachut, M., Tam, J., & Contreras, G.A. (2021). A proposed modulatory role of the endocannabinoid system on adipose tissue metabolism and appetite in periparturient dairy cows. *Journal of Animal Science and Biotechnology*, 12, article number 21. doi: 10.1186/s40104-021-00549-3.

[14] Okuyucu, İ.C., Akdağ, A., Erdem, H., & Nuh, O.C.A.K. (2023). Physiological and pyhsical responses of dairy cattle to heat stress. *Black Sea Journal of Agriculture*, 6(1), 95-103. doi: 10.47115/bsagriculture.1177117.

[15] Rahman, M.S., Einstein, G.P., & Tulp, O.L. (2022). The autonomic, hormonal, and immunological contributions to the metabolism and control of adipose tissue as an organ. *Emerging Trends in Disease and Health Research*, 5, 117-136. doi: 10.9734/bpi/etdhr/v5/3236E.

[16] Razzaghi, A., Ghaffari, M.H., & Rico, D.E. (2022). The impact of environmental and nutritional stresses on milk fat synthesis in dairy cows. *Domestic Animal Endocrinology*, 83, article number 106784. doi: 10.1016/j.domaniend.2022.106784.

[17] Sammad, A., Wang, Y.J., Umer, S., Lirong, H., Khan, I., Khan, A., Ahmad, B., & Wang, Y. (2020). Nutritional physiology and biochemistry of dairy cattle under the influence of heat stress: Consequences and opportunities. *Animals,* 10(5), article number 793. doi: 10.3390/ani10050793.

[18] Sylvers-Davie, K.L., & Davies, B.S.J. (2021). Regulation of lipoprotein metabolism by ANGPTL3, ANGPTL4, and ANGPTL8. *American Journal of Physiology-Endocrinology and Metabolism,* 321(4), E493-E508. doi: 10.1152/ajpendo.00195.2021.

[19] Takahashi, T., Mori, A., Oda, H., Murayama, I., Kouno, M., & Sako, T. (2021). Comparison of cholesterol levels among lipoprotein fractions separated by anion-exchange high-performance liquid chromatography in periparturient Holstein-Friesian dairy cows. *Journal of Veterinary Medical Science,* 83(2), 260-266. doi: 10.1292/jvms.20-0361.

[20] Umezaki, S., Yayou, K.I., Matsumoto, M., & Huang, C.Y. (2021). Comparison of comfortability between tie-stall and stanchion-stall housing through behaviour and autonomic nervous activity. *Animal Behaviour and Management*, 57(1), 12-19. doi: 10.20652/jabm.57.1_12.

[21] Vergeles, O.P., Sheremeta, V.I., & Dzhus, P.P. (2019). Dynamics of lipid exchange in blood donor cows for the use of a biologically active preparation. *Animal Breeding and Genetics*, 58, 80-85. doi: 10.31073/abg.58.11.

[22] Wen, Y., Chen, Y.Q., & Konrad, R.J. (2022). The regulation of triacylglycerol metabolism and lipoprotein lipase activity. *Advanced Biology*, 6(10), article number 2200093. doi: 10.1002/adbi.202200093.

[23] Yang, L., Zhang, L., Zhang, P., Zhou, Y., Huang, X., Yan, Q., Tan, Z., Tang, S., & Wan, F. (2022a). Alterations in nutrient digestibility and performance of heat-stressed dairy cows by dietary L-theanine supplementation. *Animal Nutrition*, 11, 350-358. doi: 10.1016/j.aninu.2022.08.002.

[24] Yang, W., Wang, S., Loor, J.J., Jiang, Q., Gao, C., Yang, M., Tian, Y., Fan, W., Zhao, Y., Zhang, B., & Xu, C. (2022b). Role of sortilin 1 (SORT1) on lipid metabolism in bovine liver. *Journal of Dairy Science*, 105(6), 5420-5434. doi: 10.3168/jds.2021-21607.

[25] Ying, Q., Chan, D.C., Barrett, P.H.R., & Watts, G.F. (2021). Unravelling lipoprotein metabolism with stable isotopes: Tracing the flow. *Metabolism,* 124, article number 154887. doi: 10.1016/j.metabol.2021.154887.

[26] Yu, Y., Qiu, J., Cao, J., Guo, Y., Bai, H., Wei, S., & Yan, P. (2021). Effects of prolonged photoperiod on growth performance, serum lipids and meat quality of Jinjiang cattle in winter. *Animal Bioscience*, 34(9), 1569-1578. doi: 10.5713/ab.20.0750.

Вплив тонусу автономної нервової системи на вміст холестерину та ліпопротеїнів різної щільності в крові корів

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Анотація. Актуальність даної теми полягає у вивченні впливу тонусу автономної нервової системи на вміст ліпідів в організмі корів, що важливо враховувати для забезпечення високої продуктивності дійного стада та збалансування раціонів у годівлі тварин. Мета роботи полягала у визначенні впливу тонусу гангліорнарної нервової системи на вміст холестерину та ліпопротеїнів різної щільності у крові молочних корів української чорно-рябої породи. Для дослідження варіабельності серцевого ритму використовували електрокардіограф із подальшим вимірюванням основних показників за методикою Баєвського, що включало визначення моди, її амплітуди, варіаційний розмах, індекс автономної рівноваги, автономний показник ритму та індекс напруги. З піддослідних корів були сформовані три дослідні групи: нормотоніки, ваготоніки та симпатотоніки. Вміст ліпопротеїнів у плазмі крові визначали за допомогою біохімічного аналізатора Sinnova BS-3000 (Китай). За результатами біохімічного аналізу встановлено відмінності в отриманих показниках серед дослідних груп корів із різним тонусом автономної нервової системи. Це пов'язано з тим, що активізація симпатичної нервової гілки призводить до змін у роботі печінки, яка впливає на вироблення глюкози в організмі. Це може призвести до збільшення вироблення холестерину. Крім того, підвищений тонус симпатичної гілки може стимулювати активність ферментів, що відповідають за формування холестерину, та призвести до зниження рівня гормонів, які регулюють його утворення та транспортування в організмі. Зокрема, у тварин ваготоніків відзначали найбільший вміст у крові загального холестерину та ліпопротеїнів високої щільності. У симпатотоніків виявляли найменші значення в крові вмісту загального холестерину та ліпопротеїнів високої щільності. Нормотоніки займали проміжне місце серед дослідних груп корів. Детальне вивчення цього питання розкриває особливості впливу тонусу автономної нервової системи на показники обміну ліпідів у крові корів, що важливо враховувати на виробництві для ефективного вирішення питань зі збереження їх продуктивності та покращення якісних показників молока

Ключові слова: гангліонарна нервова система; нормотоніки; ваготоніки; симпатотоніки; ліпіди; українська чорно-ряба

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The role of mouse-like rodents in the Chornobyl Exclusion Zone in the spread of blood-borne diseases

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Abstract. A feature of the conducted research is the study of a topical issue – the influence of external and internal factors on the prevalence of pathogens of blood-parasitic diseases. One of the most important external factors of influence is the exposure dose of γ-radiation, since the catching of mouse-like rodents was carried out at the landfills of the drained bed of the cooling reservoir of the Chernobyl Nuclear Power Plant. Therefore, the aim of the work was to study mouse-like rodents for the presence of causative agents of blood-parasitic diseases in natural populations under the influence of radioecological conditions of man-made transformation of the cooling reservoir of the Chernobyl Nuclear Power Plant. Mouse-like rodents of the genera Apodemus and Myodes were studied from three different locations of the cooling pond using parasitological methods. Radiological methods were used to determine the level of contamination of the specified three places where mouse-like rodents were caught using the γ- and β-dosimeter Pripyat RKS 20.3. Examination of blood smears of mouse-like rodents from three experimental polygons of the cooling reservoir of the Chernobyl Nuclear Power Plant revealed the presence of pathogens of blood-parasitic diseases in 93.8% of animals. In particular, such pathogens as *Rickettsia spp.*, *Ehrlichia spp.*, *Bartonella spp.*, *Babesia spp.*, *Hepatozoon spp.*, *Haemobartonella* (Mycoplasma) spp., were identified. and spirochetes. The prevalence of zoonotic agents among mouse-like rodents of the Chernobyl Zone of radioactive contamination, as well as its dependence on the level of radioecological pollution of the respective landfills, was established. Therefore, according to the results of the conducted research, the presence of causative agents of blood-parasitic diseases among mouse-like rodents, which are both intermediate and their definitive hosts, including those from dangerous zoonoses, which, subject to the application of timely and complex preventive measures, will prevent human infection

Keywords: parasitic; radiation level; *Myodes glareolus*; *Apodemus flavicollis; Apodemus agrarius*

Introduction

Since 2014, the Chernobyl Nuclear Power Plant (ChNPP) zone has been undergoing a project to drain the cooling pond (CP). At present, there has been a significant decrease in the water level in the CP with the disappearance of a significant part of the water area, and the transformation of the aquatic ecosystem into a terrestrial ecosystem formed by bottom sediments. According to the literature (Technical and economic feasibility study of the decommissioning of the cooling pond of Chernobyl NPP, 2013), the accidental release resulted in 11.1-10^{13} Bq of ^{137}Cs and 3.7-10 ^{12} Bq of ^{90}Sr entering the cooling pond, which is approximately 0.3% for ^{137}Cs and 0.5% for ^{90}Sr of the total amount released into the environment. The lateral distribution of accidental radionuclides in the drained areas of the ChNPP CP is rather varied due to the unequal depth of the reservoir in different parts (6-20 m) and the fact that the bottom of the reservoir is very uneven and has numerous depressions (IAEA, 2019; Lypska *et al.*, 2022). Due to drainage, active processes of primary succession are taking place in the CP site, i.e., radical changes and formation of new phytocoenoses and faunal communities with high biodiversity. It should be noted that during the period of decommissioning of the cooling pond (2014-2017), the species diversity of phytocoenoses increased from 14 (2001 study) to more than 50 (including 9 woody) plant species (Paskevych & Gorodetskyi, 2018). The presence of various species of predatory mammals was recorded in the drained areas, as well as an increase in the number and species composition of mouse-like rodents, which are the main prey of birds and mammals.

Thus, drained areas of a reservoir are an attractive place for many species of animals to search for food and habitat. It should be noted that mouse-like rodents (Rodentia Muroidea) can be both definitive and intermediate or reservoir hosts in the developmental cycle of many parasitic diseases, including zoonoses, which can lead to infection of other animals and humans too. As they are extremely fertile, heterogeneous and highly dependent on the availability of feed and climatic conditions, as well as the impact of radiation contamination, they can also be carriers of extremely dangerous pathogens such as hantaviruses, *Leptospira*, *Bartonella spp.*, *Anaplasma phagocytophilum*, *Neoehrlichia mikurensis*, *Borrelia burgdorferi* and *Babesia microti* (Jeske *et al.*, 2022). Researchers in Austria have raised questions about the interaction of different pathogens in the host, the countermeasures of the host immune system, the impact of host-pathogen interaction on the host's health, and the spread of infectious agents among wild rodents and from them to other animals or humans. Thus, out of 110 rodents of four species tested in 2008 (40 *Clethrionomys glareolus*, 29 *Apodemus flavicollis*, 26 *Apodemus sylvaticus* and 15 *Microtus arvalis*), specific antibodies to Dobrava-Belgrade hantavirus were detected, Tula hantavirus, lymphocytic choriomeningitis virus, orthopox virus and rickettsial virus, as well as multiple infections involving up to three pathogens (Banović *et al.*, 2022). At the same time, molecular analysis revealed the prevalence of various *Babesia* and *Hepathozoon* species at 0.09% each, and *Sarcocystis* at 0.06% in small and medium mammals in Texas, USA (Modarelli *et al.*, 2020). The four-year period of observation of mouse-like rodents in the Masurian Lakes of Poland also showed the prevalence of such pathogens as *Haemobartonella sp.* (63.9%), *Bartonella spp.* (27.7%), *Babesia microti* (9.0%), *Trypanosoma sp.* (8.4%) and *Hepatozoon lavieri* (3.1%). The prevalence of the latter is closely related to the presence of their transmission vectors, such as fleas (Siphonaptera) and ticks (Ixodida and others), which are often found in populations of mouse-like rodents (Gomes *et al.,* 2018; Grzybek *et al.*, 2020; Ramos *et al.*, 2023). Mouse-like rodents are also important intermediate and paratenic hosts for carnivore parasites, including the important zoonotic agents *Toxoplasma*, *Echinococcus* and *Toxocara*. At the same time, during the study of the *Microtus socialis* species on the territory of the Askania-Nova F.E. Falz-Fein Biosphere Reserve, researchers found only representatives of *Trypanosoma spp.* and *Babesia spp.* (Karbowiak *et al.*, 2002). '*Candidatus* Neoehrlichia mikurensis' causes inflammatory disease in people with underlying medical conditions, but the microorganism also affects immunocompetent individuals who are asymptomatic. It is transmitted in Europe by the *Ixodes ricinus* tick, but rodents can be reservoir hosts (Portillo *et al.*, 2018). Thus, small rodents play an extremely important role in parasite-host relationships and are the main reserves of zoonotic and vector-borne pathogens (Portillo *et al.*, 2018; Baltrūnaitė *et al.*, 2020). Molecular studies, which provide greater sensitivity and specificity in identifying different types of pathogens, should be supplemented by genetic analysis of parasites.

The most common methods for investigating mouse-like rodents for the presence of pathogens of parasitic origin include light microscopy and confirmation of its results using polymerase chain reaction. The latter method allows for the detection of more parasitic pathogens (Baltrūnaitė *et al.*, 2020; Yen *et al.*, 2021).

Studies of the peculiarities of blood-borne parasitic diseases in animals living in radiation-contaminated areas are few or absent. Therefore, it is extremely necessary and relevant to include studies to determine their prevalence among animals in the complex of radioecological and radiobiological monitoring of the water treatment facility at the stage of its decommissioning in order to prevent the occurrence of zoonoses and vector-borne diseases among animals and humans in the Chornobyl Exclusion Zone and adjacent territories.

The aim of the study is to investigate the spread of blood parasites and the state of the blood system of mouse-like rodents in natural populations under the influence of radioecological conditions of the technogenic transformation of the ChNPP CP.

Materials and Methods

Radioecological studies were carried out at three test sites (polygons) of the Chornobyl Exclusion Zone (ChEZ). Test site (polygon) 1 (51°21'38.58 "N 30°8'23.50 "E) is located on the coastline of the Chornobyl Nuclear Power Plant (ChNPP) Cooling Pond (CP) (before hydraulic works). The landfill is dominated by sandy loam and sod-podzolic soils. The territory of site (polygon) 2 (51°22'20.60 "N 30°8'26.94 "E) is located on the drained area of the hot part of the ChNPP CP, bordering the northern part of the distribution dam and the location of the abandoned hydrobiological laboratory. The territory of site (polygon) 3 (51°22'20.60 "N 30°8'26.94" E) is located on the first stage dam of the ChNPP CP and the drained hot part of the CP bed. In the areas of the drained bed of the CP, the soil cover is formed by sands (without silt particles) with a significant coverage of the territory with shells left after the massive death of river dreissena (*Dreissena polymorpha*) colonies. Figure 1 shows the location of the research sites (polygons) on the territory of the ChEZ. The exposure dose rate of γ-radiation and the flux density of β-particles above the soil surface were determined at the animal capture sites using the Prypiat γ- and β-radiometer RKS 20.3. Soil samples were taken at the trapping sites using the envelope technique (four samples in the corners and one sample in the middle of the square). Standard laboratory sample preparation procedure including drying, crushing, and sifting was followed for spectrometric measurements.

Figure 1. Mouse-like rodents sampling test sites (polygons) within the Chornobyl Nuclear Power Plant Cooling Pond **(**Chornobyl Exclusion Zone, Chornobyl, Ukraine)

Canberra-Packard spectrometer (model GX40185, Canberra, USA) was used for measurements of ^{137}Cs in soil samples. ^{90}Sr activity in soil samples has been determined using the method of "thick" samples without radiochemical preparations by β-spectrometer "SEB-50" (SPC Spectr, Ukraine) equipped with plastic scintillation detector.

Mouse-like rodents of various species, which are widely used in radiobiological studies as indicators, were captured in the first half of September 2019 using Sherman traps. The duration of trapping at each site was 3 days with daily inspection of the traps. After capture, the animals were taken to the laboratory of the Institute for Nuclear Research (Kyiv, Ukraine), examined, registered with individual numbers and values of the date and place of capture, species name, sex, and body weight. The species affiliation of individuals was determined by morphological characters. Possibly, ectoparasites (larvae of Ixodes ticks and fleas) from the ears and limbs of animals were collected for the future identification. Ixodes ticks were sampled on the territory of the Chornobyl Radiation and Ecological Biosphere Reserve at 4 main locations: Krasno village, Horodyshche village, Mashevo village and near Lake Hlyboke using the standardised "white flag" method.

After preservation collected ticks using 70% ethyl alcohol, the parasites were placed in a freezer at -20°C until further examination. The systematic classification of ticks included the determination of genus, species, sex, and developmental stage and was based on eidonomic features. The study was carried out at the I.I. Schmalhausen Institute of Zoology of the National Academy of Sciences of Ukraine (Kyiv, Ukraine) using an Axio Imager M1 microscope and photo-recording of the results.

Peripheral blood was collected from the carotid artery under ether anaesthesia. The following parameters were determined on a haematological analyser (Mindray BC-2008 Vet): white blood cell (WBC), red blood cell (RBC), haemoglobin (HGB), haematocrit (HCT), as well as red blood cell parameters mean corpuscular volume (MCV), erythrocyte haemoglobin concentration (MCH), erythrocyte volume distribution (RDW); platelets (PLT), platelet count (PCT), mean platelet volume (MPV), platelet distribution width (PDW).

The blood smears were prepared from a drop of blood, dried and delivered for further examination to the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine. They were fixed and stained using a Leukodif 200 (LDF 200) dye kit according to the manufacturer's instructions. Each smear was examined under the immersion system of a Micros Viola MC30 microscope (×10 eyepiece and ×100 objective).

To assess the level of parasitic infection in mice, the smear was examined in 100 fields of view. The results were used to calculate average intensity of infection (II), the number of parasites per infected animal on average; extensiveness of infection (EI), the number of infected animals to the number of examined animals in percentage terms. The experimental part of the work was carried out in accordance with the "General Ethical Principles for Experiments on Animals" (2018) and in compliance with the international requirements of the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986).

Results and Discussion

Based on the radiometric studies, it was established that the experimental sites differ significantly in radiation conditions, namely, the highest exposure dose rate of γ radiation (EDRγ) was recorded at polygon 2 and the β-particle flux density at polygon 1 (Table 1).

Table 1. EDRγ and β-particle flux density (beta) at the points of capture of mouse-like rodents (50 points at each site)

Site (polygon)	PEDγ, mR/h			Beta, imp cm^{-2} min^{-1}		
	Average	Standard deviation	Range	Average	Standard deviation	Range
1	0.37	0.07	0.29-0.51	688	232.18	435-1200

Table 1. Continued

Site (polygon)	PEDγ, mR/h			Beta, imp cm^{-2} min^{-1}		
	Average	Standard deviation	Range	Average	Standard deviation	Range
2	0.22	0.03	0.07-0.40	115	43.98	72-250
3	0.93	0.44	0.7-1.20	254	141.03	102-620

Source: *authors' development*

The data presented in Table 1 show that the spatial distribution of EDRγ and the flux density of β-particles at the test sites vary in a wide range. According to the average values of EDRγ, the landfills are in the range: 3>1>2, and by the density of β-particle flux above the soil surface 1>2>3. The density of soil contamination at test site (polygon 1) with ^{137}Cs was in the range of 8.16÷10.23 MBq/m^2, ^{90}Sr – 5.44÷9.35 MBq/m^2, ^{241}Am – 0.72÷0.91 MBq/m^2. At test site (polygon) 2, the density of soil contamination was much lower, namely ^{137}Cs – 1.32÷2.20 MBq/m^2, ^{90}Sr – 0.76 ÷ 1.25 MBq/m^2, ^{241}Am – 0.15÷0.21 MBq. As for the 3rd polygon, it occupies an intermediate position in terms of radionuclide contamination density: ^{137}Cs – 3.35÷6.51 MBq/m^2, ^{90}Sr – 1.33÷4.74 MBq/m^2, ^{241}Am – 0.22÷0.54 MBq/m^2. The density of soil contamination with ^{137}Cs at polygon 1 was 5.6 and 1.9 times higher than at polygons 2 and 3, respectively, and ^{90}Sr – 7.5 and 2.5 times higher.

Among the captured mouse-like rodents (Rodentia Muroidea), representatives of the genera Apodemus and Myodes were identified. Samples of animals at the polygons were as follows: polygon I – 14 (*M. glareolus* – 7, *A. flavicollis* – 7), polygon II – 11 (Apodemus – 5, *A. agrarius* – 5, *M. glareolus* – 1)) and polygon III – 8 (*A. flavicollis* – 6, *A. agrarius* – 2) animals.

A total of 68 ticks of the family Ixodidae were collected. The species identification of the parasites revealed that the captured individuals belonged to two species: *Dermacentor reticulatus* and *Ixodes ricinus*, with the former dominating. It accounted for 98.5% of all parasites. According to the literature, the *Dermacentor reticulatus* tick is involved in the spread and transmission of pathogens such as *Babesia spp*. (*B. microti* and *B. canis*) and *Rickettsia spp.* (*R. rickettsii*, *R. slovaca*, *R. raoultii*), (Raoult *et al.*, 2002). The developmental cycle of these pathogens is closely related to mouse-like rodents, mainly as reservoirs of pathogens. Moreover, the detection of *B. microti* in *D. reticulatus* was associated with the species of the tick's host rodent. *B. microti* was more common in *Microtus spp.* than *Apodemus spp.* (Dwużnik *et al.*, 2019). Studies have also established the possibility of *Dermacentor reticulatus* and *Ixodes ricinus* ticks being involved in the transmission of *Hepatozoon spp.* pathogens, despite the fact that *Rhipicephalus sanguineus* was previously considered the only transmission vector (Hornok *et al.*, 2013; Hamšíková *et al.*, 2016).

Blood smears revealed the presence of blood-borne pathogens in 93.8% of the animals studied. Among the pathogens of these diseases were found: *Babesia spp.*, rickettsiae (*Ehrlichia spp.*, *Mycoplasma spp*. (*Haemobartonella spp.*), *Spirochaeta spp*.

The study of the species composition of parasites in blood smears of animals revealed the pathogens *Babesia spp*. (56.6%), *Rickettsia spp*. (53.3%), *Ehrlichia spp*. (23.3%). For the first time in Ukraine, the pathogen *Hepatozoon spp*. was recorded in blood smears of 5 animals (Fig. 2). Representatives of the species *A. flavicollis* – 3 cases and *M. glareolus* – 2 cases. These animals lived at the polygon I. It should be noted that animals from the experimental polygon I, in a number of generations, permanently lived in this area and received the highest radiation load compared to other experimental groups.

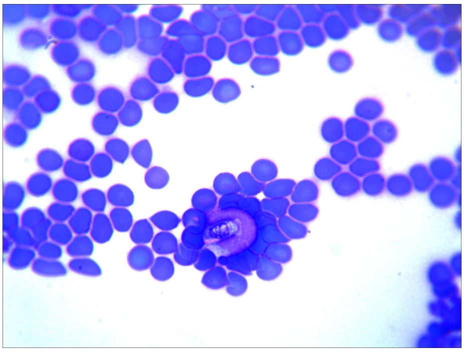

Figure 2. *Hepatozoon spp.* in a blood smear fixed and stained with Leukodif 200 (LDF 200) dye set from a mouse of the species *Apodemus flavicollis* (magnification ×1000)
Source: *authors' development*

The high extensiveness of the infestation in animals from the experimental polygons was recorded: 1 – 100%, 2 – 90.9%, 3 – 87.5%. In mouse-like rodents infected with blood parasites, almost all species were dominated by individuals affected by 2-3 species of parasites. Thus, in 56.6% of the examined animals, the presence of several species of parasites in one host was detected, namely, in polygons 1, 2 and 3 – 71.4%, 44.4% and 66.7%, respectively. The structure and specificity of the parasitofauna of mouse-like rodents in the experimental polygon I was almost equally determined by the red (*M. glareolus*) and yellow-throated mice (*A. flavicollis*), in II – by *A. agrarius* (63.6%), and in III – by *A. flavicollis* (75%), however, the few species present at the landfills also contribute to the prevalence of blood-borne diseases. Table 2 presents the results of studies of the prevalence of blood-borne pathogens in different species of rodents at the research sites.

The presence of hyperinvasive individuals in the host population may be the result of a certain synergy of parasites of different species, when high infection with any species leads to a decrease in host immunity and makes it more accessible to other parasites.

Table 2. Pathogens of blood-borne diseases in some species of mouse-like rodents in three experimental polygons

Species of animals and the presence of blood-borne pathogens in microscopic examination (%)			
Pathogens of blood-borne diseases	*Myodes glareolus* (n=8, mainly polygon I)	*Apodemus flavicollis* (n=15, I, II i III polygons)	*Apodemus agrarius* (n=6, mainly polygon II)
Rickettsia spp.	87.5	46.7	16.7
Ehrlichia spp.	25.0	13.3	16.7
Bartonella spp.	12.5	0	0
Babesia spp.	37.5	66.7	50.0
Hepatozoon spp.	25.0	20.0	0
Haemobartonella (Mycoplasma) spp.	12.5	33.3	50.0
Spirochaetes	12.5	0	16.7

Source: *authors' development*

The presented data indicate the presence of epidemically dangerous pathogens on the drained areas and the Chornobyl Exclusion Zone coastline. Given that since 2018, a significant increase in the number of animals has been recorded at the polygons II and III, which is likely due to both migration processes and comfortable conditions for animal reproduction, namely due to the availability of a rich food base, these areas may become foci of parasitic diseases in the future.

The average intensity of infection (AI) (the number of all parasites per infected animal) was determined: at the polygon I, this indicator was 2.12 for *M. glareolus* and 2.0 for *A. flavicollis*; at the polygon II – *A. flavicollis* (1.8) and *A. agrarius* (1.6); at polygon III – *A. flavicollis* (1.8) and *A. agrarius* (1.7). Authors did not find any significant differences in the values of intensity of infection (II) for different sites and species. In the red mole (*M. glareolus*), Authors observed 7 species of blood parasitic pathogens with the dominance of *Rickettsia spp.* and in the yellow-throated and field mice – 5 and 7, respectively, with the dominance of *Babesia spp*. It should be noted that in the study populations, individuals of different age groups had unequal (uneven) infection, the highest parasitic load was in young individuals, which may be due to intrauterine infection of the fetus during pregnancy. Data are confirmed by C.M.R. Turner (2009), where *Hepatozoon* and *Grahamella spp*. were detected mainly in young individuals of mouse-like rodents, while older animals were susceptible to infections caused by *Babesia* and *Trypanosoma spp.* This statement is supported by the facts of infection of pregnant females and those that have already given birth. There were no sex differences in the presence of parasitic pathogens in the groups of experimental animals.

Data on the prevalence of blood-borne diseases among such species of mouse-like rodents as *M. glareolus*, *A. flavicollis* and *A. agrarius* reveal for the first time the prevalence of such pathogen as *Hepatozoon spp.* in Ukraine. The highest prevalence of this agent (25%) is similar to the data of Hungarian (17% among *M. glareolus* species) and Lithuanian researchers, where it is 32% and is the most common among such rodent species as *Myodes glareolus* and *Microtus agresti* (Rigó *et al*, 2016; Baltrūnaitė *et al*., 2020). However, according to the results of microscopic studies, the prevalence of this pathogen among mouse-like rodents in the Chornobyl radioactive contamination zone is lower than the data obtained by Lithuanian researchers, where this figure was 40.8%, and according to the polymerase chain reaction, it reached 100% (Baltrūnaitė *et al*., 2020). At the same time, the pathogen was isolated from ectoparasites: Ixodes ticks and fleas of the species *Megabothris turbidus* (Rothschild), *Ctenophthalmus assimilis* (Taschenberg) and *Ctenophthalmus agyrtes* (Heller) (Rigó *et al*., 2016), which indicates their direct involvement in the life cycle.

Authors did not detect *Hepatozoon spp.* in *A. agrarius*, which also coincides with the data of the microscopic examination of internal organs in rodents from the Estonian study and may indicate the absence of a certain arthropod species as ectoparasites that transmit this pathogen or the absence of circulation of this pathogen in the bloodstream during blood sampling. According to the results of a recent study, *Hepatozoon spp.* was found in such rodent species as *Clethrionomys rufocanus, Clethrionomys rutilus, Clethrionomys glareolus* and *Microtus agrestis*. And it was not detected in such species as *Microtus arvalis, Apodemus agrarius* and *Apodemus flavicollis* (Laakkonen *et al*., 2001; Jeske *et al*., 2022). However, the highest prevalence (25%) of *Hepatozoon spp.* was found in *M. glareolus* according to results of research. These data coincide with the results of Lithuanian researchers (Baltrūnaitė *et al*., 2020). According to Austrian scientists, pathogens such as *Leptospira borgpetersenii, Bartonella spp., Borrelia miyamotoi* and *Neoehrlichia mikurensis* were found in striped field mice (*Apodemus agrarius*), while *Babesia, Anaplasma, Ehrlichia and Rickettsia spp.* were not detected (Jeske *et al*., 2022).

The parasitemia did not exceed 1-3 parasites per 100 microscope fields of view (in most cases, single agents were observed in the smear), which, in authors opinion, indicates carriage rather than clinical course. This suggests that mice mainly play the role of reservoirs of blood-borne pathogens. The basis for this assumption was the results of haematological studies, as well as the presence of small rounded and filamentous forms of parasites in the smears. Table 3 shows the main indicators of peripheral blood of different species of mouse-like rodents.

Table 3. Peripheral blood parameters of different species of mouse-like rodents

Indicator	Animal specie		
	Myodes glareolus (n=8, mainly polygon I)	*Apodemus flavicollis* (n=15, I, II and III polygons)	*Apodemus agrarius* (n=6, mainly polygon II)
Leukocytes, 10^9/L	5.86±0.99	4.22±0.47	5.2±0,86
Lymphocytes, % 10^9/L	59.7±2.72 3.45±0.61	46.91±3.67 1.99±0.3	54.5±3.55 1.9±0.32
Granulocytes, % 10^9/L	33.26±2.07 2.03±0.38	46..3±4.03 1.98±0.30	37.63±4.09 2.83±0.49
Eosinophils, % 10^9/L	3.88±0.91 0.23±0.05	6.59±1.55 0.28±0.06	1.88±0.72 0.1±0.04
Monocytes, % 10^9/L	6.33±0.34 0.34±0.06	6.47±0.57 0.27±0.03	7.87±1.59 0.47±0.16
Erythrocytes, 10^{12}/L	8.23±0.45	8.19±0.33	8.95±0.47
Haemoglobin, g/L	121.25±2.18	133.5±5.16	142.2±5.04
Hematocrit, %	39.57±0.94	43.78±1.81	48.48±2.77
Mean red blood cell volume (MCV)	48.87±2.18	53.53±0.9	54.37±2.06
Mean haemoglobin content per red blood cell (MHC)	14.95±0.74	16.22±0.24	15.93±0.39
Red blood cell distribution width (RDW-SD)	17.07±0.48	15.31±0.27	18.02±1.01
Erythrocytes pathology (% of animals): Anisocytosis	12.5	6.7	-
Joly bodies	25.0	66.7	50.0
Polychromatophilia of erythrocytes	12.5	40.0	50.0
Thrombocytes, 10^9/L	332.62±40.8	407.4±4.92	384.83±41.25

Source: *authors' development*

It was found that most of the leukogram indicators had only a tendency to change (increase/decrease): an increase in the percentage of eosinophils and monocytes against the background of a decrease in lymphocytes. However, it should be noted that in all experimental animals, an increase in the absolute number of leukocytes, granulocytes and monocytes was observed compared to the data of non-irradiated controls (Lypska *et al.,* 2020), while eosinophils had only a tendency to increase in experimental animals from polygons I and II. The presence of a blood-borne parasitic infection did not lead to a significant decrease in erythrocytes in the peripheral blood, but it should be noted that in all blood smears, erythrocyte pathology was recorded: Joly bodies and nuclear erythrocytes, anisocytosis, polychromatophilia, which indicates a violation of the maturation of erythroid cells and may be due to the presence of pathogens of infectious and protozoan diseases in the body. A significant number of polychromatophiles were detected in the blood smears of 9 animals, which is 28.1%. Among them were 6 blood smears from animals of the species *Apodemus flavicollis*, 1 from *Myodes glareolus* and 2 from *Apodemus agrarius*. Thus, the results of studies have provided new data on the prevalence of pathogens of various diseases among mouse-like rodents, which require further detailing using modern methods (Colle *et al*., 2019; Baltrūnaitė *et al*., 2020; Alabí *et al*., 2021). Also, for the first time, the influence of radiation factors such as EDRγ and the flux density of β-particles on the circulation of *Rickettsia spp., Ehrlichia spp., Bartonella spp., Babesia spp., Hepatozoon spp., Haemobartonella (Mycoplasma) spp.* and spirochaetes in the population of mouse-like rodents *Myodes glareolus, Apodemus flavicollis* (Melchior) and *Apodemus agrarius* (Pallas) was revealed.

Conclusions

Thus, the species composition of parasites and their abundance is a direct reflection of the peculiarities of the biology of mouse-like rodents: their number, specificity of feeding, behavioural reactions and distribution, on the other hand, characterises their hosts as first-order conspecifics with a wide ecological valence, which is an important link in the food chains of terrestrial ecosystems and the spread of parasitic pathogens.

In the red mole (*M. glareolus*), authors observed 7 species of blood parasitic pathogens with the dominance of *Rickettsia spp.* and in the yellow-throated and field mice – 5 and 7, respectively, with the dominance of *Babesia spp. Hepatozoon sp.* in Ukraine firstly was registered in mouse-like rodents *M. glareolus*.

The presence of a blood-borne parasitic infection did not lead to a significant decrease in erythrocytes in the peripheral blood. But it should be noted that in all blood smears, erythrocyte pathology was recorded. A significant number of polychromatophiles were detected in the blood smears of 9 animals, which is 28.1%. Among them were 6 blood smears from animals of the species *Apodemus flavicollis*, 1 from *Myodes glareolus* and 2 from Apodemus.

The studies revealed an increase in the number and species diversity of both parasites and their hosts, which indicates an intensive development of blood-borne diseases in elementary (newly formed) populations in the areas around the ChNPP CP and is a possibility of developing an unfavourable epidemiological situation. In this regard, there is a need for further studies of the distribution of blood-borne pathogens and species diversity in natural populations of the ChNPP Zone. At the next stage of research, authors plan to confirm the results of the microscopic examination using polymerase chain reaction and appropriate set of primers for each of the pathogens.

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Conflict of Interest

None.

References

[1] Alabí, A.S., Monti, G., Otth, C., Sepulveda-García, P., Perles, L., Machado, R.Z., André, M.R., Bittencourt, P., & Müller, A. (2021). Genetic diversity of *Hepatozoon spp*. in rodents from Chile. *Revista Brasileira de Parasitologia Veterinária,* 30(4), article number e012721. doi: 10.1590/S1984-29612021082.

[2] Baltrūnaitė, L., Kitrytė, N., & Križanauskienė, A. (2020). Blood parasites (*Babesia, Hepatozoon and Trypanosoma*) of rodents, Lithuania: Part I. Molecular and traditional microscopy approach. *Parasitology Research*, 119(2), 687-694. doi: 10.1007/s00436-019-06577-3.

[3] Banović, P., Díaz-Sánchez, A.A., Simin, V., Foucault-Simonin, A., Galon, C., Wu-Chuang, A., Mijatović, D., Obregón, D., Moutailler, S., & Cabezas-Cruz, A. (2022) Clinical aspects and detection of emerging rickettsial pathogens: A "one health" approach study in Serbia, 2020. *Frontiers in Microbiology*, 12, article number 797399. doi: 10.3389/fmicb.2021.797399.

[4] Colle, A.C., Mendonça, R.F.B., Maia, M.O., Freitas, L.D.C., Witter, R., Marcili, A., Aguiar, D.M., Muñoz-Leal, S., Labruna, M.B., Rossi, R.V., & Pacheco, R.C. (2019). Molecular survey of tick-borne pathogens in small mammals from Brazilian Amazonia. *Revista Brasileira de Parasitologia Veterinária*, 28(4), 592-604. doi: 10.1590/S1984-29612019086.

[5] Dwużnik, D., Mierzejewska, E.J., Drabik, P., Kloch, A., Alsarraf, M., Behnke, J.M., & Bajer, A. (2019). The role of juvenile *Dermacentor reticulatus* ticks as vectors of microorganisms and the problem of 'meal contamination'. *Experimental and Applied Acarology*, 78(2), 181-202. doi: 10.1007/s10493-019-00380-6.

[6] Environmental impact assessment of the drawdown of the Chernobyl NPP cooling pond as a basis for its decommissioning and remediation. (2019). Vienna: IAEA.

[7] European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes. (1986). Retrieved from https://rm.coe.int/168007a67b.

[8] General Ethical Principles for Experiments on Animals. (2018). Retrieved from http://surl.li/kcehf.

[9] Gomes, L. de A., Moraes, L.A., Aguiar, D.C.F., Dias, H.L.T., Ribeiro, A.S.S., Rocha, H.P. do C.R., Nunes, M.R.T., & Gonçalves, E.C. (2018). Genetic diversity of *Hepatozoon spp.* in *Hydrochoerus hydrochaeris* and *Pecari tajacu* from eastern Amazon. *Ticks and Tick-Borne Diseases*, 9(2), 314-318. doi: 10.1016/j.ttbdis.2017.11.005.

[10] Grzybek, M., Tołkacz, K., Alsarraf, M., Dwużnik, D., Szczepaniak, K., Tomczuk, K., Biernat, B., Behnke, J.M., & Bajer, A. (2020). Seroprevalence of tick-borne encephalitis virus in three species of voles (*Microtus spp.*) in Poland. *Journal of Wildlife Diseases,* 56(2), 492-494. doi: 10.7589/2019-02-048.

[11] Hamšíková, Z., Silaghi, C., Rudolf, I., Venclíková, K., Mahríková, L., Slovák, M., Mendel, J., Blažejová, H., Berthová, L., Kocianová, E., Hubálek, Z., Schnittger, L., & Kazimírová, M. (2016). Molecular detection and phylogenetic analysis of *Hepatozoon spp*. in questing *Ixodes ricinus* ticks and rodents from Slovakia and Czech Republic. *Parasitology Research*, 115(10), 3897-3904. doi: 10.1007/s00436-016-5156-5.

[12] Hornok, S., Csörgő, T., de la Fuente, J., Gyuranecz, M., Privigyei, C., Meli, M.L., Kreizinger, Z., Gönczi, E., Fernández de Mera, I.G., & Hofmann-Lehmann, R. (2013). Synanthropic birds associated with high prevalence of tick-borne rickettsiae and with the first detection of *Rickettsia aeschlimannii* in Hungary. *Vector-Borne and Zoonotic Diseases,* 13(2), 77-83. doi: 10.1089/vbz.2012.1032.

[13] Jeske, K., Herzig-Straschil, B., Răileanu, C., Kunec, D., Tauchmann, O., Emirhar, D., Schmidt, S., Trimpert, J., Silaghi, C., Heckel, G., Ulrich, R.G., & Drewes, S. (2022). Zoonotic pathogen screening of striped field mice (*Apodemus agrarius*) from Austria. *Transboundary and Emerging Diseases,* 69(2), 886-890. doi: 10.1111/tbed.14015.

[14] Karbowiak, G., Wita, I., & Czaplinska, U. (2004). Protozoan parasites in the blood of the social vole (Microtus socialis) in Askania. *Vestnik Zoologii*, 18, 186-187.

[15] Laakkonen, J., Sukura, A., Oksanen, A., Henttonen, H., & Soveri, T. (2001). Haemogregarines of the genus Hepatozoon (Apicomplexa: Adeleina) in rodents from northern Europe. *Folia Parasitologica,* 48(4), 263-267. doi: 10.14411/fp.2001.043.

[16] Lypska, A.I., Nikolaev, V.I., Shytiuk, V.A., Burdo, O.O., & Vyshnevskyi, D.O. (2022). Radioecological studies on the drained bed areas of the Chornobyl nuclear power plant cooling pond. *Nuclear Physics and Energy*, 23(4), 263-270. doi: 10.15407/jnpae2022.04.263.

[17] Lypska, A.I., Rodionova, N.K., Ryabchenko, N.M., Burdo, O.O., Ganja, O.B., Vyshnevskyi, D.O., & Ishiniva, H. (2020). Assessment of the state of natural populations of small of small rodents from transformed ecosystems of the ChNPP exclusion zone. *Nuclear*, 21(4), 328-337. doi: 10.15407/jnpae2020.04.328.

[18] Modarelli, J.J., Westrich, B.J., Milholland, M., Tietjen, M., Castro-Arellano, I., Medina, R.F., & Esteve-Gasent, M.D. (2020). Prevalence of protozoan parasites in small and medium mammals in Texas, USA. *International Journal for Parasitology: Parasites and Wildlife,* 21(11), 229-234. doi: 10.1016/j.ijppaw.2020.02.005.

[19] Paskevych, S.A., & Gorodetskyi, D.V. (2018). Characteristics of vegetation and animal population on the sites of the dried bottom of the ChNPP cooling pond. *Problems of Chernobyl Exclusion Zone*, 18, 99-102.

[20] Portillo, A., Santibáñez, P., Palomar, A.M., Santibáñez, S., & Oteo, J.A. (2018). 'Candidatus *Neoehrlichia mikurensis*' in Europe. *New Microbes and New Infections*, 22, 30-36. doi: 10.1016/j.nmni.2017.12.011.

[21] Ramos, C.J.R., de Souza Franco, C., da Luz, S.P., Marques, J., de Souza, K.M., do Nascimento, L.F.N., das Neves, G.B., Moreira, R.S., & Miletti, L.C. (2023) First record of *Trypanosoma evansi* DNA in *Dichelacera alcicornis* and *Dichelacera januarii* (Diptera: Tabanidae) flies in South America. *Parasites and Vectors*, 16, article number 4. doi: 10.1186/s13071-022-05562-7.

[22] Raoult, D., Fournier, P.-E., Abboud, P., & Caron, F. (2002). First documented human *Rickettsia aeschlimannii* infection. *Emerging Infectious Diseases*, 8(7), 748-749. doi: 10.3201/eid0807.010480.

[23] Rigó, K., Majoros, G., Szekeres, S., Molnár, I., Jablonszky, M., Majláthová, V., Majláth, I., & Földvári, G. (2016). Identification of *Hepatozoon erhardovae* Krampitz, 1964 from bank voles (*Myodes glareolus*) and fleas in Southern Hungary. *Parasitology Research,* 115, 2409-2413. doi: 10.1007/s00436-016-4992-7.

[24] Technical and economic feasibility study of the decommissioning of the cooling pond of Chernobyl NPP, Vol.1, reg. No. 0112U005382 . (2013). Chernobyl: Institute of Problems of Safety of Nuclear Power Plants (IPSNPP).

[25] Turner, C.M.R. (1986). Seasonal and age distributions of *Babesia*, *Hepatozoon*, *Trypanosoma* and *Grahamella* species in *Clethrionomys glareolus* and *Apodemus sylvaticus* populations. *Parasitology,* 93(2), 279-289. doi: 10.1017/S0031182000051453.

[26] Yen, W.-Y., Stern, K., Mishra, S., Helminiak, L., Santiago, S.-V., & Kim, H.K. (2021). Virulence potential of *Rickettsia amblyommatis* for spotted fever pathogenesis in mice. *Pathogens and Diseases*, 79(5), article number 24. doi: 10.1093/femspd/ftab024.

Роль мишоподібних гризунів Чорнобильської зони відчуження у поширенні кровопаразитарних хвороб

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Анотація. Особливістю проведеного дослідження є вивчення актуального питання – впливу зовнішніх і внутрішніх факторів на поширеність збудників кровопаразитарних хвороб. До одного з найважливіших зовнішніх факторів впливу належить експозиційна доза γ-випромінювання, оскільки відлов мишоподібних гризунів здійснювався на полігонах осушеного ложа водоймища-охолоджувача Чорнобильської атомної електростанції. Тому метою роботи було дослідити мишоподібних гризунів на наявність збудників кровопаразитарних хвороб у природних популяціях під впливом радіоекологічних умов техногенної трансформації водоймища-охолоджувача Чорнобильської атомної електростанції. Мишоподібні гризуни родів *Apodemus* і *Myodes* були досліджені з трьох різних місць водойми-охоложувача з використанням паразитологічних методів. Радіологічними методами було визначено рівень забрудненості зазначених трьох місць відлову мишоподібних гризунів з використанням γ- і β-дозиметра Прип`ять RKS 20.3. Дослідженням мазків крові мишоподібих гризунів із трьох експериментальних полігонів водоймища-охолоджувача Чорнобильської атомної електростанції виявили наявність збудників кровопаразитарних хвороб у 93,8 % тварин. Зокрема, ідентифіковано таких збудників, як *Rickettsia spp., Ehrlichia spp., Bartonella spp., Babesia spp., Hepatozoon spp., Haemobartonella (Mycoplasma) spp.* і спірохети. Встановлено поширеність збудників зоонозів серед мишоподібних гризунів Чорнобильської зони радіоактивного забруднення, а також її залежність від рівня радіоекологічної забрудненості відповідних полігонів. Отже, за результатами проведених досліджень встановлено наявність збудників кровопаразитарних хвороб серед мишоподібних гризунів, які є як проміжними, так і їх дефінітивними хазяями, у тому числі з небезпечних зоонозів, що, за умови застосування своєчасних і комплексних превентивних заходів, дозволить не допустити зараження ними людини

Ключові слова: паразитарні хвороби; рівень опромінення; *Myodes glareolus*; *Apodemus flavicollis; Apodemus agrarius*

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SteE regulation of Th1/Th2 cytokines expression in chickens during *S. Pullorum* infection

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Abstract. Nowadays, timely monitoring of zoonotic agents, including salmonellosis, which are caused by various serovars of the family Salmonella, is relevant. Attention should be paid to the study of cytokine levels in combination with immunological studies. This helps clarify the pathogenesis of infectious diseases and develop preventive measures. The main purpose of study was to detect the process of regulating Th1/Th2 cytokines expression in chickens infected with salmonellosis. The field strain of *S. Pullorum* CVCC 530 was used in the research. The *steE* deletion mutant (Δ*steE*) and *steE*-complemented Δ*steE*:*steE* (Δ*steE*+*steE*) strains were constructed in the WT strain using the λ-Red recombination method. Chickens were orally infected with WT, Δ*steE*, and Δ*steE*+*steE* strains (1×10^9 CFU/individual). The effect of *steE* on the host immune response remains unknown. Compared with the group infected with the WT or Δ*steE*+*steE* strain, IL-12 and IFN-γ mRNA transcript levels were significantly higher, while IL-10 mRNA expression was significantly reduced in the liver and bursa infected with the Δ*steE* strain; IL-4 showed a dramatically reduced transcription level, but IL-18 mRNA expression was significantly increased in the Δ*steE* strain – spleen, cecum, and heart; IL-10 mRNA expression was significantly reduced in the spleen and cecum infected with the Δ*steE* strain. These results suggest that *steE* may regulate the Th1/Th2 cytokine response balance in chickens infected with *S. Pullorum* and provide new insights into the pathogenesis of salmonellosis for the treatment of persistent infection

Keywords: poultry; immune system; pathogenesis; immunity; infectious diseases

Introduction

Poultry breeding is widely developed throughout the world. The concentration of a large number of poultry in a limited area creates favourable conditions for the emergence and spread of infectious diseases among the livestock of poultry farms. Salmonellosis, which is accompanied by damage to the gastrointestinal tract and septicaemia, is one of the poultry diseases that causes concern among veterinary medicine specialists. According to L. Zhike *et al.* (2021), salmonellosis agents pose a threat to human health because salmonellosis is a zoonotic disease.

Infection of poultry with salmonellosis can occur both from the parent flock and during the cultivation of commercial broiler stock. In most cases, given the relatively short life span of broilers, *S. pullorum* infection in most cases occurs vertically from the parent flock. Infection of poultry with the causative agent of salmonellosis can occur through the alimentary route, as well as aerogenic and transovarian (through the egg). T.I. Fotina & T.V. Sergeychik (2022) proved that an important point for the occurrence of the disease is the presence of sources of infection. Poultry, rodents, other farm animals, feed, wild animals, poor cleaning and disinfection, disposal of poultry, and workers and visitors can be infected.

Researchers substantiated that the key point in preventing the development of salmonellosis is strict compliance with veterinary and sanitary requirements and rules in poultry farms. Researchers also indicate that the disease worsens in the presence of stress factors (Zhike *et al.*, 2021). B. Alosaimi (2020) draws attention to the fact that cytokines, which are produced by almost all cells of the body for the purpose of intercellular interaction and regulation of biochemical processes, are an important factor influencing the immune reactions of poultry. Cytokine imbalance is important in the pathogenesis of infectious diseases.

H.C. Webster (2022) proved that the important properties of cytokines are: pleiotropic action, the presence of overlapping and doubling phenomena in a single regulatory network. Induction conditions are created, that is, when one cytokine acts on others and regulates the expression of cytokine receptors.

Cytokines regulate specific immune reactions and the immune response to the pathogenic properties of pathogens of infectious diseases, including salmonellosis.

In this regard, the development of methods of prevention of poultry salmonellosis is urgent. The purpose of the study is the experimental reproduction of salmonellosis in chickens and the investigation of the process of regulating the expression of Th1/Th2 cytokines under the conditions of infection of chickens with the field strain *S. Pullorum* CVCC 530 (WT) using a deletion mutant. At the second stage, the immune response of chickens was investigated. In connection with the urgency of the problem and the solution of the set goal, the tasks of research were to study the effect of cytokines Th1/Th2 on the development of inflammatory processes in the spleen, liver, tissue bag and small intestine of chickens, under the conditions of their infection with the causative agent of salmonellosis.

Literature Review

Researchers P. Li *et al.* (2022) reported that *Salmonella Pullorum* (*S. Pullorum*) is an important host-specific pathogen that can cause white diarrhoea with high mortality in young chicks at 2-3 weeks of age. An infected adult chicken does not have a number of clinical symptoms. According to S. Geng *et al.* (2019), *S. Pullorum* causes not only horizontal but also vertical transmission through eggs, which seriously threatens severe economic losses and the healthy development of poultry in some developing countries.

M. Wang *et al.* (2020) concluded that pathogens avoid elimination by the immune system and avoid causing excessive harm to the host, which are two essential elements of bacterial systemic infection. N. Naseer *et al*. (2022) identified the encoded *Salmonella* type III secretion system (T3SS), *Salmonella* pathogenicity islands-1 and -2 (SPI-1 and SPI-2) that deliver effector proteins to host cells. Different effector molecules have different biological activities. N. Foster *et al.* (2021) demonstrated that some effector proteins can manipulate host pathways that promote intracellular survival and replication of *Salmonella* in host cells. For example, researchers from different years (Li *et al.,* 2016; Geng *et al.,* 2019; Zhou *et al.,* 2021) reasoned that spvB and spvC can reduce autophagy or apoptosis of host cells by regulating the expression of inflammatory cytokines, while sseL may enhance intracellular survival induced by other signalling pathways. I.E. Brodsky (2020) proved that *SteE* as an anti-inflammatory effector protein is a gene associated with *Salmonella* virulence and is encoded in *Salmonella* prophase Gifsy-1, which can contribute to persistent *Salmonella* infection by regulating the host's innate immune response in macrophages. And J. Dong *et al.* (2021) determined that nuclear factor κB (NF-κB) is involved in the inflammatory response and can also promote the development of Th cells. Researchers Q.J. Wu *et al.* (2018), S.L. Clay *et al.* (2020) state that in the normal host immune system, Th1/Th2 cells do not differentiate, but the high expression of inflammatory cytokines causes disorder in case of *Salmonella* infection. X. Kang *et al.* (2022) demonstrated that some host-adapted *Salmonella* serovars can suppress the innate immune response and create an infection-friendly environment for long-term survival in host cells, such as *S. Pullorum*. *SteE* has been reported to be closely associated with colonisation of bacterial invasion in mice (Jaslow *et al.,* 2018). Previous study (Liu *et al.,* 2018) showed that *steE* was associated with persistent infection and virulence of *S. Pullorum* Among them, steE promotes intracellular replication of *Salmonella* in host cells. However, the role of *steE* prior to *S. Pullorum* infection on host immune responses remained largely unknown. In this regard, *S. Pullorum*-infected chickens were used as an in vivo model to investigate the effect of *steE* on the host's immune response.

Materials and Methods

The study was carried out during 2022-2023 at the China Veterinary Culture Collection, Beijing, China, and the Laboratory of Innovative Technologies, Safety and Quality of Livestock Products of Sumy National Agrarian University, Ukraine.

Bacterial strains

The wild-type strain of *S. Pullorum* CVCC 530 (WT) was obtained from the China Veterinary Culture Collection, Beijing, China. The *steE* deletion mutant (Δ*steE*) and *steE* complemented Δ*steE*:*steE* (Δ*steE*+*steE*) strains were constructed in WT strain by using the λ-Red recombination method, and stored in laboratory (Liu *et al.,* 2018). All strains were grown overnight in Luria-Bertani medium at 37°C with shaking prior to use in chicken infection.

Chicken infection assay

40 Hy-Line white chicks (2-day-old) were obtained from the Henan Animal Incubation Centre (China). This study was approved by the Animal Care and Ethics Committee of Henan Institute of Science and Technology. All animal studies were conducted in accordance with Directive 2010/63/EU of the European Parliament and of the Council "On the Protection of Animals Used for Scientific Purposes" (2010) approved by the Commission on Ethics and Bioethics of the Faculty of Veterinary Medicine of the Sumy National Agrarian University (protocol No. 3 dated 12.21.2021). The chicks were randomly divided into four groups (n=10). The chicks in experimental groups were orally infected with WT, Δ*steE* and Δ*steE*+*steE* strains (1×10^9 CFU/individual), and normal control group were orally infected with PBS as previously method and dose described (Liu *et al.,* 2018). At 3 days post-infection (dpi), the liver, spleen, bursa, cecum, and heart tissues of each chicken from each group was collected and stored at -80°C for further analysis.

qRT-PCR assay

Total RNA extraction was prepared using TRIzol reagent (Invitrogen, USA) and then reverse transcribed to cDNA synthesis using a PrimeScript RT reagent Kit (TakaRa, Japan). qRT-PCR was performed using SYBR Premix Ex Taq™ || (TakaRa, Japan) according to the manufacturer's instructions. Primer sequences were: IL-12 forward: 5′-TGCCTTACTTTCATTACTTTCCTTTG-3′reverse: 5′-TTTAGC TGGTGTCTCATCGTTCC-3′; IL-18 forward: 5′-GTTCCCAAAGACATTCCT GG-3′, reverse: 5′-GGCCAAGAACATTCCTTGTT-3′; IFN-γ forward: 5′-CTTTG GAGTTGAAGGCAGTGTGG-3′, reverse: 5′-TCTGGGTTGTGGGGTTTGTGAG-3′; IL-4 forward: 5′-AGTGAATGACATCCAGGGAGAGG-3′, reverse: 5′- CTGACGCATGTTGAGGAAGAGACTT-3′; IL-10 forward: 5′- CGCTGTCACC GCTTCTTCAC-3′, reverse: 5′- GGCTCACTTCCTCCTCCTCATC-3′; and β-actin forward: 5′-TATTGCTGCGCTCGTTGTTGAC-3′, reverse: 5′-GATACCTCTTTTG CTCTGGGCTTC-3′. The PCR (polymerase chain reaction) programme was set to 95°C for 30s followed by 40 cycles of denaturation at 95°C for 5s, annealing at 59°C for 30s, and extension at 72°C for 30s. Relative mRNA expression level of each gene was determined using the 2-ΔΔCT method, and β-actin was the housekeeping gene. Each experiment was performed in triplicate.

Statistical analysis

All results are presented as mean ± standard deviation (SD) for at least three independent experiments. Quantitative data was analysed by one-way ANOVA using GraphPad Prism software suite, version 8.0 (Graph Pad Software Inc., San Diego, CA, USA). Statistical significance was expressed as *P<0.05 and **P<0.01.

Results and Discussion

The study has found that *SteE* changes the mRNA of inflammatory cytokines levels in spleen of infected chickens *by S. Pullorum*. At the first stage of research, *SteE* level was evaluated. It was found to alter mRNA of inflammatory cytokines levels in spleen of chickens during *S. Pullorum* infection. The mRNA levels of IL-18, IL-12 were much higher in spleen of the Δ*steE* group than that in the WT or Δ*steE*+*steE* strain group at 3 dpi (Fig. 1). Regarding the mRNA anti-inflammatory cytokines levels, the IL-10, IL-4, were substantially decreased in the spleen of Δ*steE* group compared to the WT or Δ*steE*+*steE* groups, whereas the mRNA IFN-γ level was not significantly different.

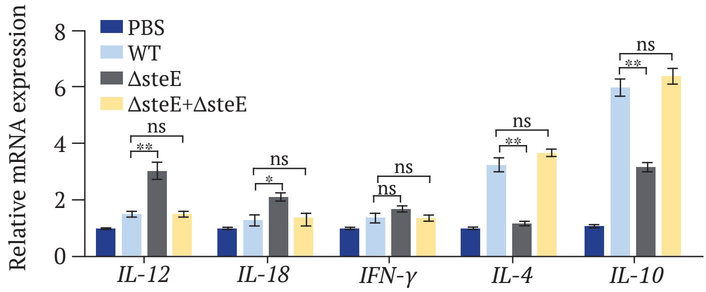

Figure 1. *SteE* changes the mRNA inflammatory cytokines levels in spleen of chickens infected with *S. Pullorum*

Source: *developed by the authors*

SteE changes the mRNA inflammatory cytokines levels in liver of chickens infected with *S. Pullorum*. During the second stage of experimental research, it was established that *SteE* changes the mRNA inflammatory cytokines levels in liver of chickens infected with *S. Pullorum*. The mRNA levels of IFN-γ, IL-12 were much higher in liver of the Δ*steE* group than that in the WT or Δ*steE*+*steE* at 3 dpi, but the mRNA level of IL-18 had no significant difference (Fig. 2). The mRNA level of IL-10 was much lower in liver of the Δ*steE* group than in the WT or Δ*steE*+*steE* groups, whereas the mRNA level of IL-4 had no significant difference.

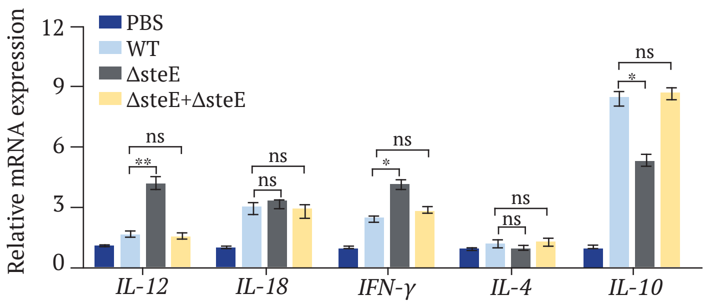

Figure 2. *SteE* changes the mRNA inflammatory cytokines levels in liver of chickens infected with *S. Pullorum*

Source: *developed by the authors*

SteE changes the mRNA inflammatory cytokines levels in bursa of chickens infected with *S. Pullorum*. Authors were given the task of investigating *SteE* changes mRNA inflammatory cytokines levels in bursa of chickens infected with *S. Pullorum*. The mRNA levels of IFN-γ, IL-12 were much higher, but no difference was detected in IL-4, IL-18 in bursa of the Δ*steE* group than in the WT group or Δ*steE*+*steE* at 3 dpi (Fig. 3). The mRNA level of IL-10 was much lower in bursa of the Δ*steE* group than in the WT or Δ*steE*+*steE* groups.

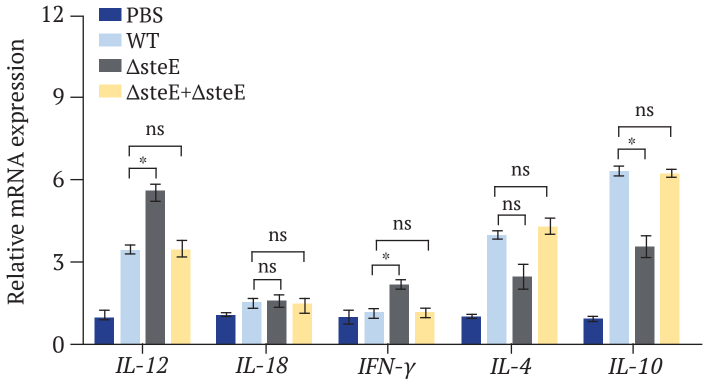

Figure 3. *SteE* changes the mRNA of inflammatory cytokines levels in bursa of chickens infected with *S. Pullorum*

Source: *developed by the authors*

SteE changes the mRNA of inflammatory cytokines levels in cecum of chickens infected with *S. Pullorum*. Experimental studies of the fourth stage proved that SteE changes mRNA of inflammatory cytokines levels in cecum in chickens infected with *S. Pullorum*. The mRNA level of IL-18 was much higher, but no difference was detected in IFN-γ, IL-12 in cecum of the Δ*steE* group than in the WT or Δ*steE*+*steE* groups at 3 dpi (Fig. 4). The mRNA levels of IL-10, IL-4 were much lower in cecum of the Δ*steE* group than in the WT or Δ*steE*+*steE* groups.

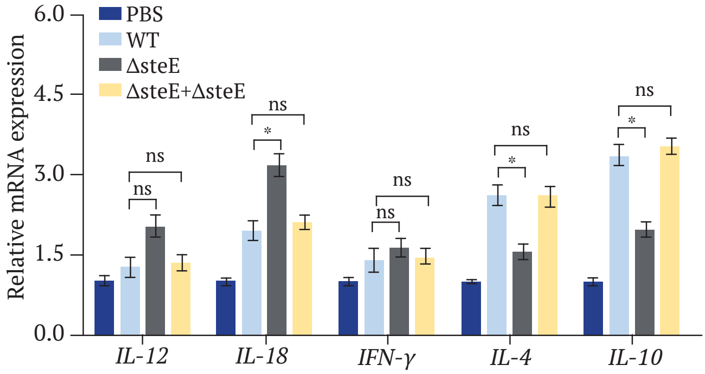

Figure 4. *SteE* changes the mRNA of inflammatory cytokines levels in cecum of chickens infected with *S. Pullorum*

Source: *developed by the authors*

SteE changes the mRNA of inflammatory cytokines levels in heart of chickens *with S. Pullorum* infection. The mRNA level of IL-18 was higher, but no difference was detected in IFN-γ, IL-12, IL-10 in heart of the Δ*steE* group than in the WT or Δ*steE*+*steE* groups at 3 dpi (Fig. 5). The mRNA level of IL-4 was much lower in hearts of the Δ*steE* group than in the WT or Δ*steE*+*steE* groups.

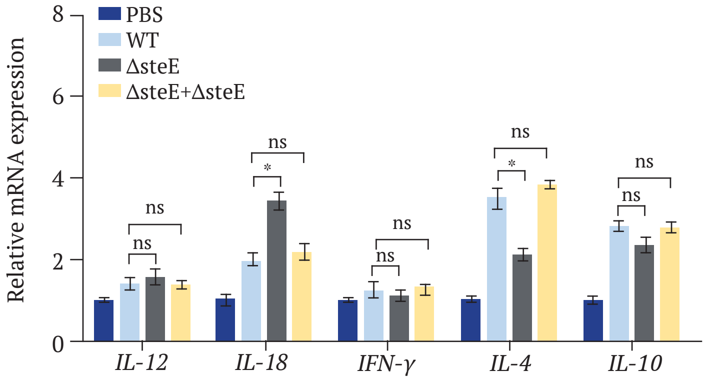

Figure 5. *SteE* changes the mRNA of inflammatory cytokines levels in heart of chickens infected with *S. Pullorum*

Source: *developed by the authors*

Salmonella is an intracellular pathogen that causes great harm to human and livestock health worldwide, with complex and diverse antigenicity and serotypes. Y. Hu *et al.* (2019) demonstrated that among common serotypes, *S. Pullorum* causes systemic lethal disease with high mortality in chickens within 2-3 weeks. An infected adult chicken exhibits abnormalities of the reproductive tract without serious clinical symptoms, leading to chronic or recessive infection. *S. Pullorum* causes significant economic losses to chicken farms worldwide, especially in developing countries. Therefore, T.I. Fotina & T.V. Sergeychik (2022) state that it is very important to control the spread of *S. Pullorum* in poultry farms.

S. Pullorum can spread horizontally and vertically to progeny, which is unlikely to be eliminated. Thus, accurate and rapid pathogen diagnosis is important for the control and eradication of *S. Pullorum*. At present, the detection of pathogenic bacteria depends on culture methods and biochemical identification, which are time-consuming, have a long detection cycle and low sensitivity, and cannot meet the needs of social development. Thus, Zh. Liu *et al.* (2018) suggest the need to develop an accurate and simple detection method for *S. Pullorum* serotype diagnosis.

Studies by Z. Lin *et al.* (2017) found that *Salmonella* can colonise the intestine and spleen and directly accept macrophages as target cells. After infection with *S. Pullorum*, the bacterium can not only escape the destruction of intracellular active substances, but also proliferate and spread within macrophages. SteE is required for *Salmonella* replication and virulence in macrophages. Thus, it was hypothesised that the pathogenic mechanism of *S. Pullorum* infection in chickens may be the same as in HD-11 cells.

In this study, steE was selected as a research gene based on the λ-Red recombination system to construct the *S. Pullorum* Δ*steE* strain. The results showed that the growth and biochemical characteristics of *S. Pullorum* and *S. Pullorum* Δ*steE* strains are similar, which is consistent with a previous result, but steE is not essential for the growth and metabolism of *S. Pullorum* (Liu *et al.*, 2018). The results showed that *steE* would reduce the colonisation ability and virulence of *S. Pullorum* in HD-11 cells. *S. Pullorum*-induced apoptosis of HD-11 cells is a specific virulence mechanism that may facilitate the spread of bacteria between cells. Recent studies have shown that deletion of *S. Pullorum* SPI-2 significantly reduced chicken pathogenicity, which is consistent with steE belonging to the SPI-2 effector protein. In addition, T.H.M. Pham *et al.* (2020) reported that *steE* can drive macrophages to polarise to the M2 type and enhance the ability of the *Salmonella* infection-susceptible state. Therefore, it was suspected that steE might be associated with *Salmonella* virulence. The results of *S. Pullorum* infection of HD-11 cells also showed that *steE* enhances the intracellular viability of *S. Pullorum* and promotes HD-11 cell apoptosis.

When determining the virulence, it was found that the deletion of steE had an effect on reducing the pathogenicity of *S. Pullorum* in chickens, and this proved the important role of steE in the virulence of *S. Pullorum*. In addition, the presence of bacterial colonies in the organs of chickens showed that the general trend of change in *S. Sullorum* is similar to that of the Δ*steE* strain. The presence of colonies of WT strains and Δ*steE* strains in the spleen, caecum, and bursa of chickens reached its maximum peak on day 3, and the number of WT strains and strains Δ*steE* in the liver of chickens reached its maximum peak on day 4. During the process of infection, the number of Δ*steE* in defferent organs of the initial stage of infection after *Salmonella* infection in chickens that first increased, reached the peak at the middle infection stage, and then decreased at the later infection stage. The amount of Δ*steE* strains in organs of chicken was always much lower than of WT strain. And, the amount of colonisation of the Δ*steE* strains in organs of chicken was always lower than of the WT strains. The results of the study indicate that steE can promote *S. Pullorum* colonisation in organs of chicken and contribute to *S. Pullorum* virulence.

Recent studies have shown that deletion of SPI-2 reduces the virulence of *Salmonella*, which has been attributed to the fact that SPI-2 is a DNA fragment acquired externally during *Salmonella* evolution. I. Panagi *et al.* (2020) reported that deletion of steE significantly attenuated mouse spleen colonisation and reduced *S. Typhimurium* virulence. SteE has been considered as an important effector in host organs for persistent *Salmonella* infection. *SteE* was encoded by the *Salmonella* prophage Gifsy-1, which was reported to activate the signal transducer and activator of transcription 3 signalling pathway and then produce the anti-inflammatory cytokine IL-10, thus promoting *Salmonella* replication in cells and increasing bacterial colonisation *in vivo*. S.L. Jaslow *et al.* (2018) identified many factors that affect the accuracy of the final results. On the one hand, the interval between organ collections was relatively long, and the exact time of penetration of bacteria into different organs cannot be precisely determined. However, only by reducing the interval between organ harvests it is possible determine the time at which bacteria settle in chicken organs to reach a peak. In addition, there were other factors affecting the amount of *S. Pullorum* in the testing process, such as equipment, reagents, cleanliness of the Petri dish, and errors caused by the operation, etc.

Inflammatory cytokines are one of the most important regulators of host-cell interaction in acute systemic disease (Alosaimi *et al.,* 2020). After *S. Pullorum* infection in chickens, the cells of the host will secrete Th1/Th2 cytokines, which regulate the balance of the internal environment and resist the damage of external harmful substances (Wu *et al.,* 2018). *SteE* increased the bacterial virulence, translocated into the cytoplasm of the host cell through T3SS-2, and caused the severe disseminated infection of other extra-intestinal tissues, such as the spleen and liver in mouse infected with *Salmonella* (Pham *et al.,* 2020). Here, it was found that *steE* positively regulated the balance of Th1/Th2-related cytokines in tissues of chicken infected with *S. Pullorum*.

Salmonella as an intracellular pathogen, can regulate host immune response via T cell activation (Cerny & Holden, 2019). IL-12 is a pro-inflammatory cytokine that plays an important role in the host's defence against intracellular pathogen infection (Yang *et al.,* 2018). IFN-γ production by Th1 cells and initiated by IL-12 and IL-18, induced the oxidative effect and improved the ability to suppress intracellular bacteria growth (Withanage *et al.,* 2005). A previous study showed that *sspH2* significantly decreased the mRNA expression of Th1-related cytokines (IL-12 and IFN-γ) in tissues of mice infected with *S. Enteritidis* (Shappo *et al.,* 2020). Furthermore, *S. Pullorum* decreased the mRNA expression levels of pro-inflammation cytokine (IL-12, IL-18, and IFN-γ) compared with *S. Enteritidis* in infected macrophages (Tang *et al.,* 2018).

In the current study, *steE* increased the Th1-related cytokines expression (IL-12, IL-18, and IFN-γ) in the chicken tissues infected with *S. Pullorum*, which was consistent with the previous report. *SteE* might be involved in Th1-mediated tissue injury and elimination of pathogen during *S. Pullorum* infection. Anti-inflammatory cytokines are mainly related to clear pathogens and tissue repair after *Salmonella* infection. L-10, as an important anti-inflammatory medium, can inhibit the expression of various pro-inflammatory factors induced by Th1 cell, and also participate in Th2 cell-mediated anti-inflammatory response (Rasquinha *et al.,* 2021). Recent studies have found that *steE* can induce the production of anti-inflammatory cytokine IL-10 in the spleen of mouse infected with *Salmonella*, but it has no relevant data on other tissues (Jaslow *et al.,* 2018). Moreover, the persistent infection of *Mycobacterium tuberculosis* needs the participation of IL-10, which indicates that IL-10 contribute to long-term systemic infection of intracellular bacteria (Park *et al.,* 2021). Another study found that the persistent infection of *Salmonella* needs the support of IL-10, which is associated with the Th1/Th2 balance (Liu *et al.,* 2018). Induced the expression of IL-10 contributed to limit the Th1 cytokines and escape the immune response of host cells (Webster *et al.,* 2022). In the present study, *steE* obviously increased the IL-10 mRNA transcript level during the infection phase. Therefore, *steE* may be more conducive to the repair of damaged tissues by promoting the anti-inflammatory or Th2 cytokines expression during early stages of infection.

The imbalance of Th1/Th2 cytokine is an important indicator of pathogens invasion (Abebe, 2019). Recent studies show that *S. Pullorum* was inclined to regulate host immunity toward a Th2-like immune response in poultry (Tang *et al.,* 2018). In this study, results demonstrated that *steE* inhibited the Th1 immune response, but promoted the Th2 immune response. Furthermore, *steE* activity promoted anti-inflammatory M2 phenotype and facilitated *Salmonella* invasion into host cells, leading to increased pathogen persistence (Jaslow *et al.,* 2018). *SteE* have the function of immune response of transforming different types, which is beneficial to intracellular survival of *Salmonella* (Panagi *et al.,* 2020). The previous study showed that *steE* can also increase the virulence of *S. Pullorum* and aggravate the outcome of host infection (Liu *et al.,* 2018). Thus, researchers speculate that *steE*-inhibited inflammatory response could be related to M2 phenotype, which was beneficial to evade immunity and persistent infection of *S. Pullorum*. However, it needs to be further confirmed how *steE* regulates the cytokine balance of Th1/Th2 in *S. Pullorum*-infected chicken. It has been established that the results obtained coincide with the findings of other researchers and confirm the influence of *steE* regulation of Th1/Th2 cytokine expression in chickens infected with *S. Pullorum*.

Conclusions

It was established that IL-12 and IL-18 mRNA levels were much higher in spleen of the Δ*steE* group than in group of WT or Δ*steE*+*steE* strains at 3 dpi. Regarding the mRNA anti-inflammatory cytokines levels IL-4, IL-10 were strong reduced in spleen of the ΔsteE compared with the WT or Δ*steE*+*steE* groups, while the mRNA level of IFN-γ had no significant difference.

It was noted that IL-12, IFN-γ mRNA levels were much higher in liver of the Δ*steE* group than in the WT or Δ*steE*+*steE* groups at 3 dpi, but the IL-18 mRNA level had no significant difference. The mRNA level of IL-10 was much lower in liver of Δ*steE* group than of the WT or Δ*steE*+*steE* groups, and the mRNA level of IL-4 had no significant difference.

It was determined that IL-18 mRNA level was much higher, but no difference was found in IL-12, IFN-γ in bursa of Δ*steE* group than in WT or Δ*steE*+*steE* groups at 3 dpi. IL-10, IL-4 mRNA levels were much lower in bursa of Δ*steE* group than in WT or Δ*steE*+*steE* groups. It is argued that IL-18 mRNA level was much higher, but no difference was found in IL-12, IFN-γ in cecum of Δ*steE* group than in WT or Δ*steE*+*steE* groups at 3 dpi. IL-4, IL-10 mRNA levels were much lower in cecum of Δ*steE* group than in WT or Δ*steE*+*steE* groups. *The* mRNA IL-18 level was much higher, but no difference was detected in IL-12, IFN-γ, IL-10 in hearts of Δ*steE* group than in the WT or Δ*steE*+*steE* groups at 3 dpi. The mRNA level of IL-4 was much lower in the hearts of Δ*steE* group than in the WT or Δ*steE*+*steE* groups.

It has been shown that steE can modulate the Th1/Th2 immune response in *S. Pullorum*-infected chickens. And steE enhanced the anti-inflammatory response induced by a strong Th2 immune response. Overall, this study extends to further elucidating the *S. Pullorum* and the host immune response interaction, providing new insights into *Salmonella* pathogenesis for the treatment of persistent *Salmonella* infection, without the use of antibacterial drugs, which will provide the opportunity to obtain high-quality and safe poultry products.

Acknowledgements

None.

Conflict of Interest

None.

References

[1] Abebe, F. (2019). Synergy between Th1 and Th2 responses during *Mycobacterium tuberculosis* infection: A review of current understanding. *International Reviews of Immunology,* 38(4), 172-179. doi: 10.1080/08830185.2019.1632842.

[2] Alosaimi, B., Hamed, M.E., Naeem, A., Alsharef, A.A., AlQahtani, S.Y., AlDosari, K.M., Alamri, A.A., Al-Eisa, K., Khojah, T., Assiri, A.M., & Enani, M.A. (2020). MERS-CoV infection is associated with downregulation of genes encoding Th1 and Th2 cytokines/chemokines and elevated inflammatory innate immune response in the lower respiratory tract. *Cytokine*, 126, article number 154895. doi: 10.1016/j.cyto.2019.154895.

[3] Brodsky, I.E. (2020). JAK-ing into M1/M2 polarization SteErs *Salmonella*-containing macrophages away from immune attack to promote bacterial persistence. *Cell Host & Microbe,* 27(1), 3-5. doi: 10.1016/j.chom.2019.12.007.

[4] Cerny, O., & Holden, D.W. (2019). *Salmonella* SPI-2 type III secretion system-dependent inhibition of antigen presentation and T cell function. *Immunology Letters,* 215, 35-39. doi: 10.1016/j.imlet.2019.01.006.
[5] Clay, S.L., Bravo-Blas, A., Wall, D.M., MacLeod, M.K.L., & Milling S.W.F. (2020). Regulatory T cells control the dynamic and site-specific polarization of total CD4 T cells following *Salmonella* infection. *Mucosal Immunology,* 13(6), 946-957. doi: 10.1038/s41385-020-0299-1.
[6] Directive 2010/63/EU of the European Parliament and of the council "On the Protection of Animals Used for Scientific Purposes". (2010, September). Retrieved from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:En:PDF.
[7] Dong, J., Xu, O., Wang, J., Shan, C., & Ren, X. (2021). Luteolin ameliorates inflammation and Th1/Th2 imbalance via regulating the TLR4/NF-κB pathway in allergic rhinitis rats. *Immunopharmacology and Immunotoxicology*, 43(3), 319-327. doi: 10.1080/08923973.2021.1905659.
[8] Foster, N., Tang, Y., Berchieri, A., Geng, S., Jiao, X., & Barrow, P. (2021). Revisiting persistent *Salmonella* infection and the carrier state: What do we know? *Pathogens*, 10(10), article number 1299. doi: 10.3390/pathogens10101299.
[9] Fotina, T.I., & Sergeychik, T.V. (2022). Monitoring of risk factors on farms to keep chicken broilers. *Bulletin of Sumy National Agrarian University. The Series: Veterinary Medicine*, 1(56), 31-36. doi: 10.32845/bsnau.vet.2022.1.5.
[10] Geng, S., Wang, Y., Xue, Y., Wang, H., Cai, Y., Zhang, J., Barrow, P., Pan, Z., & Jiao, X. (2019). The SseL protein inhibits the intracellular NF-κB pathway to enhance the virulence of *Salmonella* Pullorum in a chicken model. *Microbial Pathogenesis,* 129, 1-6. doi: 10.1016/j.micpath.2019.01.035.
[11] Hu, Y., Wang, Z., Qiang, B., Xu, Y., Chen, X., Li, Q., & Jiao, X. (2019). Loss and gain in the evolution of the *Salmonella enterica* serovar Gallinarum biovar Pullorum genome. *mSphere,* 4(2), article number e00627-18. doi: 10.1128/mSphere.00627-18.
[12] Jaslow, S.L., Gibbs, K.D., Fricke, W.F., Wang, L., Pittman, K.J., Mammel, M.K., Thaden, J.T., Fowler Jr., V.G., Hammer, G.E., Elfenbein, J.R., & Ko, D.C. (2018). *Salmonella* activation of STAT3 signaling by SarA effector promotes intracellular replication and production of IL-10. *Cell Reports*, 23(12), 3525-3536. doi: 10.1016/j.celrep.2018.05.072.
[13] Kang, X., Zhou, X., Tang, Y., Jiang, Z., Chen, J., Mohsin, M., & Yue, M. (2022). Characterization of two-component system CitB family in *Salmonella* Pullorum. *International Journal of Molecular Sciences,* 23(17), article number 10201. doi: 10.3390/ijms231710201.
[14] Li, P., Zhang, M., Hao, G., & Sun, S. (2022). Research note: Hypervirulent arthritis-causing *Salmonella* Pullorum isolated from Chinese native chicken breeds significantly decreased growth performance of chicks. *Poultry Science*, 101(2), article number 101575. doi: 10.1016/j.psj.2021.101575.
[15] Li, Y.Y., Wang, T., Gao, S., Xu, G.M., Niu, H., Huang, R., & Wu, S.Y. (2016). *Salmonella* plasmid virulence gene *spvB* enhances bacterial virulence by inhibiting autophagy in a zebrafish infection model. *Fish and Shellfish Immunology*, 49, 252-259. doi: 10.1016/j.fsi.2015.12.033.
[16] Lin, Z., Tang, P., Jiao, Y., Kang, X., Li, Q., Xu, X., Sun, J., Pan, Z., & Jiao, X. (2017). Immunogenicity and protective efficacy of a *Salmonella* Enteritidis *sptP* mutant as a live attenuated vaccine candidate. *BMC Veterinary Research*, 13, article number 194. doi: 10.1186/s12917-017-1115-3.
[17] Liu, Q.Q., Zhong, D., Zhang, X., & Li, G.Z. (2018). IL-10 targets Th1/Th2 balance in vascular dementia. *European Review for Medical and Pharmacological Sciences*, 22(17), 5614-5619. doi: 10.26355/eurrev_201809_15826.

[18] Naseer, N., Egan, M.S., Reyes Ruiz, V.M., Scott, W.P., Hunter, E.N., Demissie, T., Rauch, I., Brodsky, I.E., & Shin, S. (2022). Human NAIP/NLRC4 and NLRP3 inflammasomes detect *Salmonella* type III secretion system activities to restrict intracellular bacterial replication. *PLOS Pathogens*, 18(1), article number e1009718. doi: 10.1371/journal.ppat.1009718.

[19] Panagi, I., Jennings, E., Zeng, J., Günster, R.A., Stones, C.D., Mak, H., Jin, E., Stapels, D.A.C., Subari, N.Z., Pham, T.H.M., Brewer, S.M., Ong, S.Y.Q., Monack, D.M., Helaine, S., & Thurston, T.L.M. (2020). *Salmonella* effector SteE converts the mammalian serine/threonine kinase GSK3 into a tyrosine kinase to direct macrophage polarization. *Cell Host & Microbe,* 27(1), 41-53. doi: 10.1016/j.chom.2019.11.002.

[20] Park, H.S., Back, Y.W., Jang, I.T., Lee, K.I., Son, Y.J., Choi, H.G., Dang, T.B., & Kim, H.J. (2021). *Mycobacterium tuberculosis* Rv2145c promotes intracellular survival by STAT3 and IL-10 receptor signaling. *Frontiers in Immunology,* 12, article number 666293. doi: 10.3389/fimmu.2021.666293.

[21] Pham, T.H.M., Brewer, S.M., Thurston, T., Massis, L.M., Honeycutt, J., Lugo, K., Jacobson, A.R., Vilches-Moure, J.G., Hamblin, M., Helaine, S., & Monack, D.M. (2020). *Salmonella*-driven polarization of granuloma macrophages antagonizes TNF-mediated pathogen restriction during persistent infection. *Cell Host & Microbe*, 27(1), 54-67. doi: 10.1016/j.chom.2019.11.011.19.

[22] Rasquinha, M.T., Sur, M., Lasrado, N., & Reddy, J. (2021). IL-10 as a Th2 cytokine: Differences between mice and humans. *The Journal of Immunology*, 207(9), 2205-2215. doi: 10.4049/jimmunol.2100565.

[23] Shappo, M.O.E., Li, Q., Lin, Z., Hu, M., Ren, J., Xu, Z., Pan, Z., & Jiao, X. (2020). SspH2 as anti-inflammatory candidate effector and its contribution in *Salmonella* enteritidis virulence. *Microbial Pathogenesis,* 142, article number 104041. doi: 10.1016/j.micpath.2020.104041.

[24] Tang, Y., Foster, N., Jones, M.A., & Barrow, P.A. (2018). Model of persistent *Salmonella* infection: *Salmonella enterica* serovar pullorum modulates the immune response of the chicken from a Th17-type response towards a Th2-type response. *Infection and Immunity,* 86(8), article number e00307-18. doi: 10.1128/IAI.00307-18.

[25] Wang, M., Qazi, I.H., Wang, L., Zhou, G., & Han, H. (2020). *Salmonella* virulence and immune escape. *Microorganisms*, 8(3), article number 407. doi: 10.3390/microorganisms8030407.

[26] Webster, H.C., Gamino, V., Andrusaite, A.T., Ridgewell, O.J., McCowan, J., Shergold, A.L., Heieis, G.A., Milling, S.W.F., Maizels, R.M., & Perona-Wright, G. (2022). Tissue-based IL-10 signalling in helminth infection limits IFNγ expression and promotes the intestinal Th2 response. *Mucosal Immunology*, 15, 1257-1269. doi: 10.1038/s41385-022-00513-y.

[27] Withanage, G.S., Wigley, P., Kaiser, P., Mastroeni, P., Brooks, H., Powers, C., Beal, R., Barrow, P., Maskell, D., & McConnell, I. (2005). Cytokine and chemokine responses associated with clearance of a primary *Salmonella enterica* serovar Typhimurium infection in the chicken and in protective immunity to rechallenge. *Infection and Immunity Journal*, 73(8), 5173-5182. doi: 10.1128/IAI.73.8.5173-5182.2005.

[28] Wu, Q.J., Zheng, X.C., Wang, T., & Zhang, T.Y. (2018). Effects of oridonin on immune cells, Th1/Th2 balance and the expression of BLys in the spleens of broiler chickens challenged with *Salmonella* pullorum. *Research in Veterinary Science*, 119, 262-267. doi: 10.1016/j.rvsc.2018.07.008.

[29] Yang, R., Yang, E., Shen, L., Modlin, R.L., Shen, H., & Chen, Z.W. (2018). IL-12+IL-18 cosignaling in human macrophages and lung epithelial cells activates cathelicidin and autophagy, inhibiting intracellular *Mycobacterial* growth. *The Journal of Immunology*, 200(7), 2405-2417. doi: 10.4049/jimmunol.1701073.

[30] Zhike, L., Fotina, T., Petrov, R., Klishchova, Z., & Fotin, A. (2021). Isolation, identification and analysis of drug resistance of *Salmonella Pul-lorum*. *Ukrainian Journal of Veterinary and Agricultural Sciences*, 4(1), 33-38. doi: 10.32718/ujvas4-1.07.
[31] Zhou, L., Li, Y., Gao, S., Yuan, H., Zuo, L., Wu, C., Huang, R., & Wu, S. (2021). *Salmonella spvC* gene inhibits autophagy of host cells and suppresses NLRP3 as well as NLRC4. *Frontiers in Immunology*, 12, article number 639019. doi: 10.3389/fimmu.2021.639019.

SteE регуляція експресії цитокінів TH1/TH2 у курчат за *S. Pullorum* інфекції

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Анотація. На сьогодні актуальним є своєчасне проведення моніторингу збудників зоонозів, у тому числі сальмонельозів, які викликаються різними сероварами родини *Salmonella*. Особливу увагу слід приділити визначенню рівнів цитокінів у комплексі з імунологічними дослідженнями. Це допомагає уточнити патогенез інфекційних захворювань та розробити профілактичні засоби. Метою досліджень було вивчення процесу регуляції експресії цитокінів TH1/TH2 у курчат за умов зараження сальмонельозом. У дослідженнях використовували польовий штам *S. Pullorum* CVCC 530. Делеційний мутант *steE* (Δ*steE*) і штами Δ*steE*:*steE* (Δ*steE*+*steE*), доповнені *steE*, були сконструйовані в штамі WT за допомогою методу рекомбінації λ-Red. Курчат перорально інфікували штамами WT, Δ*steE* та Δ*steE*+*steE* (1×10^9 КУО/особину). Тотальну екстракцію РНК готували за допомогою реагенту TRIzol, синтез кДНК проводили з використанням набору реагентів PrimeScript RT. qRT-PCR проводили за допомогою SYBR Premix Ex TaqTM. Вплив *steE* на імунну відповідь господаря залишається невідомим. Порівняно з групою, інфікованою штамом WT або Δ*steE*+*steE*, рівні транскриптів мРНК IL-12 та IFN-γ були значно вищими, тоді як експресія мРНК IL-10 була суттєво знижена в печінці та бурсальній сумці, інфікованій штамом Δ*steE*; IL-4 показав різко знижений рівень транскрипції, але експресія мРНК IL-18 була значно підвищена в штамі Δ*steE* – селезінка, сліпа кишка та серце; експресія мРНК IL-10 була істотно нижчою в селезінці та сліпій кишці курчат, інфікованих штамом Δ*steE*. Результати свідчать про те, що *steE* може регулювати баланс цитокінової відповіді Th1/Th2 у курчат, інфікованих *S. Pullorum*, та уточнити патогенез збудника сальмонельозу для лікування персистуючої інфекції

Ключові слова: птиця; імунна система; патогенез; імунітет; інфекційні захворювання

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Morphological criteria for identifying coronavirus infection in companion animals

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Abstract. Due to the high level of urbanisation in Ukraine, during the years 2021-2023, there is a tendency for a rapid increase in the number of companion animals in large cities – dogs and cats. Humanity has not yet finally eliminated the SARS-CoV-2 coronavirus pandemic, as world scientists have established the involvement of these animals in its spread, which causes concern among specialists and requires a comprehensive investigation. The purpose of the scientific study was to determine marker changes in haematological parameters and haematopoiesis parameters under the influence of coronavirus infection in dogs and cats. For this purpose, spectrophotometric and refractometric methods of haematological studies were used: morphological and biochemical parameters of blood, the functional state of erythro- and leukocytopoiesis. Considering changes in blood morphological parameters, the main parameters of the identity of the pathogenic effect of coronavirus on the body of dogs and cats were established, namely: a decrease in the number of lymphocytes, glucose content, and protein coefficient. There was a significant increase in the activity of alkaline phosphatase in the blood and α-amylase, the content of total bilirubin, and the erythrocyte sedimentation rate. In particular, it has been experimentally established that the use of leukocyte indices increases the information content of the general blood test for coronavirus infection, and additional analysis of integral changes based on leukocyte blood parameters allows determining not only the state of reactivity of the body, but also the extent of endogenous intoxication. The results of changes in blood morphological parameters are somewhat comparable to those in humans, which indicates the need for an in-depth study of the genetic potential of coronavirus pathogens in companion animals and humans at the molecular and biological levels. Experimentally determined marker changes in haematological parameters for coronavirus infection in dogs and cats can be useful for its timely diagnosis and prediction of the severity of the disease

Keywords: coronavirus; erythrocytopoiesis; leukocytopoiesis; stabilised blood; blood serum; morphological and biochemical parameters

Introduction

Nowadays, in addition to solving problematic issues regarding the nature of the occurrence of SARS-CoV-2 and its pandemic properties and ways to overcome them, even more complex issues have arisen – its zoonotic nature and spread among pets (Kannekens-Jager *et al.,* 2022). Cases of natural coronavirus 2 infection with severe acute respiratory syndrome (SARS-CoV-2) in companion animals after contact with humans are rare. Infections in animals are usually subclinical or associated with transient respiratory or gastrointestinal diseases. At the same time, European researchers report that among companion animals, namely 2% of samples from dogs and 7.7% from cats are positive in the polymerase chain reaction (PCR) to SARS-CoV. Given the established transmission of the virus from humans to dogs and cats, the role of these animals in the spread of the virus is indisputable (Rotstein *et al.,* 2022).

Companion animals have the closest contact with humans, creating ample opportunities for common infectious diseases. According to the 2021-2022 national survey of pet owners, 70% of all U.S. households have a companion animal. Pets can have an impact on lowering blood pressure in humans, reducing the impact of stress factors, and reducing the rate of heart disease. Traditionally, the most common companion animals are dogs and cats

(Khan *et al.,* 2022). Recently, viral diseases have been increasingly diagnosed and one of the main pathogens is the coronavirus, which is registered quite often in companion animals (Felten *et al.,* 2022). Among mammals, pigs, cattle, dogs, cats and rodents are most often affected by the virus (Goralskii *et al.,* 2019).

Canine and feline coronaviruses are a group of acute infectious diseases caused by a virus of the family *Coronaviridae*, which is enveloped with a complex RNA genome and belongs to the subfamily *Orthocoronaviridae,* genus *Alphacoronavirus*. Coronaviruses are characterised by variable tropism to tissues and the ability to easily overcome interspecific barriers, causing diseases with noticeable differences (Felten & Hartmann, 2019; Sha *et al.,* 2022).

Coronavirus infections in dogs are clinically manifested in a respiratory form and sometimes with complications in the form of severe respiratory syndrome with an acute or asymptomatic course. In a third of sick animals, coronavirus causes damage to the gastrointestinal tract, which is manifested by the development of infectious gastroenteritis. The intestinal form has a latent course, but due to complications involving helminths or bacteria, the disease can be lethal (Vlasova *et al.,* 2022; Pandit *et al.,* 2023).

Coronavirus infections in cats have two different forms – feline intestinal coronavirus and infectious peritonitis. Feline infectious peritonitis (FIP) is extremely common. This is a relatively new disease, which is named due to a specific clinical symptom – peritonitis. One of the most sensitive to infectious coronavirus peritonitis is a domestic cat. Usually, cats suffer from infectious peritonitis quite seriously, possibly due to the initiation of multiple organ failure by the coronavirus, which usually leads to the death of the animal. Infectious peritonitis of coronavirus origin occurs in many members of the feline family (*Felidae*), even the wild fauna of Africa. This disease is one of the five most common infectious diseases of cats in many countries of the world (Guan *et al.,* 2020).

Coronaviruses exhibit unique molecular mechanisms of transcription and recombination. Due to the relatively high mutation rate, it was RNA-positive viruses that evolved. Due to biomolecular methods developed over the past two decades, new strains, serotypes, and subtypes of the virus have been identified. Given their virological characteristics, these viruses are capable of recombinant mutation and infection of several cell species and types (Krentz *et al.,* 2021).

According to the World Health Organisation, laboratory research methods provide more than 70% of diagnostic information about the patient's condition, and it is the blood test that is one of the fastest and most affordable diagnostic methods (Semotyuk & Kolesnyk, n.d.). Blood is a marker of the state of metabolism and the body as a whole, i.e., it reflects the functioning of all organs and systems. Accordingly, with any effect on the body, certain morphological changes occur in hemocytopoiesis. It is by quantitative and qualitative changes in haematological parameters that it is possible to assert the reactive nature provoked by an infectious agent, and to detect pathological processes at an early stage of the disease, even before the manifestation of clinical symptoms. A qualified interpretation of these indicators significantly increases the diagnostic value of a blood test (Jaroensong *et al.,* 2022).

At the initial stages of the pathological process, a complex of pathochemical shifts is formed, the generalisation of which occurs due to the intensive intake of toxic products from the primary lesions, which contributes to the synthesis of allergic antibodies, changes in immunological reactivity, and a decrease in non-specific resistance of the body. At the same time, leukogram indicators reflect the general nature and direction of haemostatic processes. That is why the calculation of leukocyte indices based on leukogram indicators determines the effectiveness of the body's adaptive reactions and establishes the immunological reactivity of various parts of the immune system, making it possible to predict the course of the disease (Osadcha, 2021).

The purpose of the study is to establish identification parameters based on the determination of morphological parameters of blood, and indicators of erythrocytopoiesis and leukocytopoiesis in cats and dogs with coronavirus infection.

Materials and Methods

The research was conducted during 2020-2022 at the Faculty of Veterinary Medicine at the National University of Life and Environmental Sciences of Ukraine, and at the premises of veterinary clinics "Bahira" (Zhytomyr) and "Victoria" (Kyiv).

The study included pedigree and non-pedigree dogs and cats with coronavirus infection. During the research, the basic rules of GLP (Good laboratory practice) (Order No. 944..., 2009) and the provisions of the "General Ethical Principles of Animal Experiments", adopted by the National Congress on Bioethics (Kyiv, 2001) (On the Protection..., 2021) were observed. The entire experimental part of the research was carried out in accordance with the requirements of the international principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (1986), "Rules for conducting work using experimental animals", approved by Order of the Ministry of Health of Ukraine No. 281 (2000), in accordance with Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006). For the euthanasia of animals, medications were used to ensure a quick and painless death, in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (1986).

Diagnostic tests to confirm coronavirus were performed using VetExpert rapid test systems in dogs (CPV/CCV Ag, solid-phase immunochromatographic analysis for qualitative detection of the antigen Canine Parvovirus/Coronavirus) and in cats (FCoV Ag, solid-phase immunochromatographic analysis for qualitative antigen detection Feline Coronavirus) produced in Poland. Diagnostic studies for the presence of coronavirus antigen in dogs and cats were carried out on the premises of a private veterinary laboratory of the LLC "Bald" (Kyiv) using LLC "XEMA" (Ukraine) diagnostic preparations for detection of antibodies; Asan Korea, Megacor (Austria) and Agrolabo (Italy) – for detection of antigen along wigh enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) by Astrovir (Ukraine), Biocorp (Ukraine), DNA-Technology (Ukraine), Huarian Biologi (Taiwan).

For the diagnosis of coronavirus infection in veterinary clinics, diagnostic test systems based on solid-phase ELISA were used, which usually have sensitivity and specificity at the level of at least 95% and allow diagnostics without specialised skills and laboratory conditions in a fairly short period of time. In case of ambiguous results obtained during the immunochromatographic diagnostic test to clarify the diagnosis, biological material was sent to the laboratory for more specific studies using ELISA and PCR.

Morphological and biochemical parameters of blood were studied using a biochemical analyser "BioChem SA" (USA) using reagents of the company *High Tehnology, Inc*. (USA). To determine the deviation from the morphological parameters of the blood of clinically healthy animals, a control group of dogs and cats was established in the amount of 10 animals each. During the experimental study, 24 dogs and 27 cats were diagnosed with coronavirus infection. Biological material (stabilised blood and blood serum) was collected from infected animals and subjected to haematological studies. Blood for the study was taken from the superficial forearm vein *v. Anterbrachium*, the medial saphenous vein or the subcutaneous vein of the calf *v.* Saphena.

General clinical blood test – the number of red blood cells and white blood cells was determined by the melange method in a chamber with a Goryaev grid; haemoglobin content in the blood – by the hemiglobincyanide method; haematocrit volume – by microcentrifugation according to Shklyar. Based on the results obtained, red blood indices were calculated – mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and mean cell volume (MCV).

Separate biochemical studies of blood serum were performed using the following methods: total protein was determined refractometrically, urea concentration – by colour reaction with diacetylmonooxime, aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) by the Reitman-Frenkel method, creatinine concentration – by the Jaffe reaction. The calculation of integral intoxication indices was carried out in accordance with the methodological recommendations "Integral haematological indices for assessing the degree of endogenous intoxication in dogs" (Goralskii *et al.*, 2019). Digital data were processed biometrically using generally accepted methods of variation statistics using STATISTICA 6.0 and Microsoft Excel 2007 software suites. The probability of differences between indicators was evaluated according to the Student's t-test. Three degrees of probability were calculated $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

Results and Discussion

Given that certain morphological parameters of blood can confirm the development of pathological processes provoked by viral agents, a study was conducted to investigate the degree of destabilisation of the body of dogs and cats under the influence of coronavirus. Morphological parameters of the blood of clinically healthy animals formed the basis for creating reference values. Considering the results of previous studies (Radzikhovskyi *et al.*, 2021) and foreign researchers (Decaro & Buonavoglia, 2008; Addie *et al.*, 2020), outbred or mixed-breed animals were most likely to contract coronavirus infection. This was taken into account when distributing animals to the control group. The results of the conducted studies are presented in Tables 1-3.

Table 1. Morphological parameters of blood in dogs and cats with coronavirus infection, M±m

Indicator		Control group, clinically healthy dogs (n=10)	Dogs infected with coronavirus (n=24)	Control group, clinically healthy cats (n=10)	Cats infected with coronavirus (n=27)
RBC, 10^6/μL		6.1±0.4	5.2±1.3	8.5±0.6	5.9±0.4**
HCT, %		45.4±2.4	35.2±3.8*	38.3±1.5	31.8±1.4**
PLT, 10^3/μL		252.3±19.7	343.6±34.1*	240.2±15.2	255.8±15.9
WBC, 10^3/μL		9.4±0.2	4.6±0.9***	8.4±1.2	16.6±1.8***
NEU, %	stab	3.1±0.2	1.6±0.4**	2.8±0.7	8.8±1.3***
	segmented	58.5±1.4	73.6±4.8**	46.2±3.2	61.2±4.1**
EO, %		1.8±0.3	3.1±0.8	1.9±0.5	1.7±0.4
MON, %		3.1±0.2	1.8±0.9	1.2±0.4	8.1±1.3***

Table 1. Continued

Indicator	Control group, clinically healthy dogs (n=10)	Dogs infected with coronavirus (n=24)	Control group, clinically healthy cats (n=10)	Cats infected with coronavirus (n=27)
LYM, %	35.1±1.3	18.4±3.2***	31.7±2.1	22.7±2.3*
ESR, mm/hr	4.4±0.5	7.2±1.1*	3.9±0.3	17.7±1.7***

Notes: **P<0.05; **P<0.01; ***P<0.001 compared to the control group of animals*
Source*: developed by the authors*

According to the results of hematologic examinations, erythropenia was diagnosed in the affected dogs and cats (Table 1). There was a decrease in the number of red blood cells in the blood of dogs by 15%, and in cats – by 31% (P<0.01) compared to the indicators of the corresponding control group. This fact indicates that the viral agent – coronavirus causes haemolysis of red blood cells, which was also established by F. Riemer *et al.* (2016), and leads to the development of anaemia. Along with this, it may indicate iron deficiency, which was confirmed by erythropenia and a decrease in haemoglobin concentration in animals of the study groups compared to the physiological limit (Table 1). In the blood of both dogs and cats, there was a significant decrease in the haematocrit volume, respectively, by 32.5% (P<0.05) and by 17% (P<0.01) compared to the control group, which also proves the presence of anaemia.

Coronavirus generally affects homeostasis in the body of sick animals and provokes the development of haemolytic anaemia. In addition, sick dogs showed a significant increase in the number of platelets in the blood by 36% (P<0.05) compared to the control group. In cats, the number of blood plates increased by 6.5% compared to the control group. Along with the development of thrombocytosis in these animals, the presence of inflammatory processes provoked by an infectious agent was confirmed.

T. Castro *et al.* (2013) reported leukopenia in puppies with coronavirus. Similar changes were found in the blood of adult dogs, namely, a likely decrease in the number of white blood cells by 2 times (P<0.001) compared to the corresponding control, which can occur against the background of immune suppression and the latent course of the disease. N.C. Pedersen (2014) and E. Gülersoy & M. Maden (2021) indicated that leukocytosis was observed in cats with coronavirus infection. As a result of the conducted studies, leukocytosis in cats (an increase in the number of white blood cells by 2 times) characterises a severe course of the disease with periodic waves of exacerbation. O.O. Melnyk (2020) noted that a similar trend in the development of leukocytosis in severe cases of coronavirus infection was recorded in humans. At the same time, leukocytosis was detected in 11.4% of patients with severe disease compared to 4.8% of patients with mild or moderate disease. Changes in the number of white blood cells were also noted on the quantitative indicators of stab neutrophils. Thus, in dogs, their number decreased by 48% (P<0.01), and in cats, it increased by 3 times (P<0.001) compared to the corresponding control. The development of segmented neutrophil leukocytosis with coronavirus infection was established. This was confirmed by a 26% increase in the number of segmented neutrophils in the blood of dogs (P<0.01) and a 32% increase in the number of segmented neutrophils in cats (P<0.01) compared to the control group. The consequence of this process is a shift in the leukocyte formula to the right. In dogs with coronavirus infection with simultaneous eosinophilia (an increase in the number of eosinophils by 72%), the presence of an infectious and toxic process was established, while in cats there was only a tendency to slightly decrease the number of eosinophils compared to the corresponding control.

Important macrophages of the body – monocytes – respond to the presence of a viral antigen and monocytopenia has been observed in sick dogs. In the blood of such dogs, the number of monocytes decreased by 42%, and in cats, it increased by 6 times (P<0.001). S. Chen *et al.* (2019) and E. Gülersoy & M. Maden (2021) also characterised monocyte tropism for coronavirus in cats. The likely decrease in the number of lymphocytes in the blood of dogs by 48% (P<0.001), and in cats by 28% (P<0.05) compared to control animals, is probably caused by low molecular weight proteins – interferon, which are synthesised in response to viral infection. The rate of erythrocyte sedimentation in the blood of dogs increased by 1.6 times (P<0.05), and in cats – by 4.5 times (P<0.001) compared to the same indicator in healthy animals. This fact confirms the development of an inflammatory process in the body of sick animals.

According to biochemical parameters, it is possible to determine qualitative changes in the blood, get information about the state of health of the animal and the functioning of many organs, and identify pathological processes at an early stage. Therefore, at the next stage of the study, changes in blood biochemical parameters and indicators of the state of erythrocytopoiesis in animals with coronavirus infection were analysed (Table 2).

Table 2. Erythrocytopoiesis and biochemical parameters of blood serum in dogs and cats with coronavirus infection, M±m

Indicator	Control group, clinically healthy dogs (n=10)	Dogs infected with coronavirus (n=24)	Control group, clinically healthy cats (n=10)	Cats infected with coronavirus (n=27)
MCH, pg	23.6±1.6	25.3±1.8	15.6±1.3	14.6±1.4
MCHC, g/dL	33.9±1.4	38.5±1.7*	34.7±1.5	49.6±2.3***
MCV, fL	67.1±3.7	69.1±0.9	45.1±2.3	46.4±1.1
HGB, g/dL	145.0±12.5	131.1±27.2	132.3±8.3	105.5±4.3**
Total protein, g/L	71.1±1.8	39.6±6.8***	70.2±1.4	74.8±4.1
Albumins, g/L	36.5±0.5	11.3±0.1***	31.5±1.2	27.3±1.2*
Globulin, g/L	37.6±1.2	17.7±1.7***	36.8±1.4	40.7±1.9
Protein coefficient	0.97±0.04	0.63±0.10**	0.90±0.02	0.67±0.05***
Creatinine, µmol/L	97.1±3.9	48.8±14.2**	90.2±6.5	112.7±8.6*
Urea, mmol/L	5.3±0.2	4.2±0.4*	7.2±0.5	10.7±1.4*
Glucose, mmol/L	4.2±0.2	3.4±0.6	4.8±0.2	4.2±0.3
γ-GTP, U/L	4.1±0.2	4.9±0.4	3.1±0.7	5.2±0.7*
ALP, U/L	34.6±2.7	310.8±27.2***	27.2±1.7	68.8±10.1***
Total bilirubin, µmol/L	4.3±0.3	10.2±0.9***	4.4±0.3	14.1±2.2***
ALT, U/L	46.6±3.3	36.8±6.4	40.4±3.1	84.5±9.9***
AST, U/L	50.1±3.8	26.7±6.1**	21.6±2.5	57.8±8.9***
De-Ritis Ratio, ratio	1.2±0.2	0.7±0.02*	1.1±0.4	0.7±0.3
α-Amylase, U/L	1,635.2±76.7	1,918.6±96.7*	720.2±25.3	1,828.2±116.9***

Notes: **P<0.05; **P<0.01; ***P<0.001 compared to the control group of animals*

Source*: developed by the authors*

According to the data given in Table 2, an increase in the erythrocytopoiesis index was found, which characterises the intensity of maturation of red blood cells in the bone marrow and their saturation with haemoglobin. Thus, the mean concentration of haemoglobin in one red blood cell in dogs increased by 14% ($P<0.05$), and in cats – by 43% ($P<0.001$) compared to the corresponding control. Along with this, there was a decrease in the concentration of haemoglobin in the blood serum of dogs by 10%, and in cats – by 21% ($P<0.01$), which proves the development of anaemia in their body.

Albumin content (Table 2) in the blood serum of dogs decreased by 3 times ($P<0.001$), and in cats by 13% ($P<0.05$), in comparison with the indicators of the corresponding control groups. The content of total protein and globulins in the blood serum of dogs decreased, respectively, by 44% ($P<0.001$) and 53% ($P<0.001$), and in cats, a tendency to increase these indicators was observed. According to the above-mentioned changes in protein metabolism indicators, impaired liver function under the influence of a viral agent was established. S. Paltrinieri *et al.* (2002) and T. Castro *et al.* (2013) also indicate a decrease in serum albumin levels in dogs. N.C. Pedersen (2014) reported changes in protein metabolism indicators under the influence of coronavirus antigen on the liver of dogs. A significant decrease in the protein coefficient in the blood serum of dogs by 35% ($P<0.01$), and in cats by 26% ($P<0.001$) emphasises the infectious aetiology of liver damage.

To determine the effectiveness of renal excretory function under the influence of coronavirus infection, the final nitrogen-containing products of protein metabolism – creatinine and urea – were examined in the blood of companion animals. In particular, the concentration of creatinine in the blood reflects the filtration capacity of the kidneys. With coronavirus infection, the creatinine content in the blood serum of dogs decreased by 2 times ($P<0.01$), compared to healthy animals, which may indicate changes in muscle mass due to exhaustion of the body. At the same time, in sick cats, the creatinine content increased by 25% ($P<0.05$), which may indicate the development of renal failure.

The concentration of urea in the blood serum may indicate a violation of the hepatorenal system. This indicator is of key importance in the diagnosis of diseases of both the kidneys and liver. In dogs, the concentration of urea decreased by 21% ($P<0.05$), and in cats, on the contrary, it increased by 49% ($P<0.05$) compared to the control group. It is the concentration of urea that can be used to establish a balance between its synthesis in the liver and excretion by the kidneys. High indicators of urea concentration in the blood serum indicate pathology in the kidneys, which is associated with a violation of the outflow of urine from the organ, and low indicators suggest a violation of urea synthesis by the liver.

According to the results of the conducted studies, it was found that the concentration of glucose in the blood serum of dogs was marked by a tendency to decrease by 19%, and in cats – by 13% compared to healthy animals. The development of hypoglycaemia in sick animals may be a consequence of the occurrence of hepatorenal syndrome and damage to the gastrointestinal tract. When the level of glucose in the blood of animals is low, there is a compensatory increase in gluconeogenesis, which is primarily characteristic of the liver.

Alanine aminotransferase (ALT) is a biochemical marker of liver diseases. The activity of this enzyme decreased in the blood serum of dogs by 21%, which may indicate structural and functional changes in hepatocytes. Along with a simultaneous significant increase in the serum bilirubin level (2.4 times), this indicates the development of complications from the hepatobiliary system. In contrast, in the blood serum of cats, ALT activity increased by 2 times ($P<0.001$) compared to the control, which indicates a pathological state of the liver and the development of cholestasis.

Aspartate aminotransferase (AST) – an enzyme, the largest amount of which is produced by heart and liver cells. Thus, in dogs, its activity decreased by 47% ($P<0.05$), compared with the control, which indicates damage to myocardial and liver cells. And in the blood serum of cats, an increase in the activity of this enzyme was noted by 2.7 times ($P<0.001$), which indicates structural and functional changes in myocardiocytes and hepatocytes.

Tendency to increase the activity of γ-glutamyltranspeptidase (γ-GTP) in the blood serum of dogs (by 20%), and in cats, a significant increase in its activity by 67% ($P<0.05$) compared to the corresponding control, against the background of a simultaneous increase in the content of total bilirubin in dogs by 2.4 times ($P<0.001$), and in cats – by 3.2 times ($P<0.001$), and a significant increase in the activity of alkaline phosphatase (ALP) in dogs – 9 times ($P<0.001$), and in cats – 2.5 times ($P<0.001$) indicates liver pathology, the development of obstruction of the bile ducts and impaired bile outflow.

The functional activity of the pancreas is characterised by α-amylase (Amyl, α-AM) (Sulehria *et al.,* 2020). This indicator, according to F. Riemer *et al.* (2016), is a marker of the acute phase of pancreatitis. In the blood serum of dogs with coronavirus, α-AM hyperemesis was observed – an increase in activity by 17% ($P<0.05$), and in cats – 2.5 times ($P<0.001$) compared to the corresponding control. This fact may also indicate the development of pancreatic complications in sick animals (Jaroensong *et al.,* 2022).

O.O. Melnyk (2020) and D.A. Lysenko *et al.* (2021) note that biochemical changes in animal blood serum, namely: increased α-AM activity, total bilirubin content, and alkaline phosphatase activity partially coincide with changes in the body in people with severe coronavirus infection.

Regarding the statistical diagnostic formula for the main morphological and biochemical changes in the blood of sick animals, the results obtained coincide with the findings of other researchers. In coronavirus infection in both dogs and cats, the most commonly observed changes are: an increase in the number of platelets and segmented neutrophils (Hazuchova *et al.*, 2017); an increase in the erythrocyte sedimentation rate and mean cell haemoglobin (Stranieri *et al.*, 2018); activity of γ-GTP, ALP, and α-AM; total bilirubin content and a decrease in the number of red blood cells and lymphocytes (Simsek *et al.*, 2022); haematocrit volume, haemoglobin concentration, albumin and glucose content, and protein coefficient value (Hazuchova *et al.*, 2017).

The main cells of the body's immune response to the presence of a foreign agent are white blood cells and their derivatives. As a result of the conducted studies, certain differences in the effect of coronavirus on the body of dogs and cats were established, namely, leukopenia was characteristic of dogs, and leukocytosis was characteristic of cats. A similar trend was observed in relation to stab lymphocytes and monocytes.

For a deeper understanding of the pathogenic effect of the virus on leukocytopoiesis, integral indicators of blood leukocytes were determined, which can be used to find not only the degree of reactivity of the body, but also to assess the level of endogenous intoxication, which is important for predicting the course of the disease. Ultimately, infectious agents in animal organisms cause intoxication and, as a result, are characterised by increased catabolism processes, and insufficient liver and kidney function (Table 3).

Table 3. Indicators of integral haematological indices in dogs and cats with coronavirus infection, M±m

Indicator	Control group, clinically healthy dogs (n=10)	Dogs infected with coronavirus (n=24)	Control group, clinically healthy cats (n=10)	Cats infected with coronavirus (n=27)
Lii	2.2±0.4	1.4±0.03	3.3±0.7*	1.6±0.04
Rrn	10.3±1.2***	2.1±0.1	2.2±0.5*	1.1±0.08
Blsi	2.3±0.3**	1.5±0.2	4.0±0.35***	1.9±0.04
Li	0.74±0.01**	0.96±0.07	0.20±0.02***	0.54±0.01
Irle	13.4±0.7**	16.7±0.8	6.0±0.2**	9.3±1.03
Ai	1.1±0.1***	2.4±0.1	0.7±0.01***	1.7±0.1
Ii	32.5±3.1	34.8±2.8	23.2±2.5**	39.8±4.2
Hi	0.40±0.01***	0.70±0.02	0.24±0.003***	0.60±0.01
Irlm to ESR	1.7±0.03***	8.4±0.9	2.9±0.1***	9.9±0.3

Notes: **P<0.05; **P<0.01; ***P<0.001 compared to the control group of animals*

Source*: developed by the authors*

Analysing the results of integral leukocyte indices for coronavirus infection in dogs and cats (Table 3), certain identities have been established, namely, an increase in such indicators in the blood as: leukocyte intoxication index (Lii) by 2 times (P<0.05) in dogs, which coincides with the study by L. Goralskii *et al.* (2019) and by 57% in cats (Radzikhovskyi *et al.*, 2021); reactive neutrophil response (Rrn) in dogs by 2 times (P<0.05), and in cats by 5 times (P<0.001), and the leukocyte shift index (Blsi) in dogs by 2 times (P<0.001), and in cats by 53% (P<0.01). T. Jaroensong *et al.* (2022) note that this indicates the development of endogenous intoxication against the background of immunosuppression and impaired immunoreactivity.

A significant addition to the state of immunoreactivity of the body shows a decrease in the blood value of such indicators as: leukocyte index (Li) in dogs by 63% (P<0.001), and in cats by 23% (P<0.01); lymphocyte-eosinophil ratio index (Irle) in dogs by 35% (P<0.01), and in cats by 20% (P<0.01); allergisation index (Ai) in dogs by 55% (P<0.001), and in cats by 59% (P<0.001); immunoreactivity index (II) in dogs by 42% (P<0.01), and in cats by 7%; the Harkavy's index (hi) in dogs by 60% (P<0.001), and in cats by 43% (P<0.001); and the ratio of lymphocytes and monocytes to the rate of erythrocyte sedimentation (irlm to ESR) in dogs by 70% (P<0.001), and in cats by 80% (P<0.001). A.R. Fehr & S. Perlman (2015) in their paper indicate that a decrease in these indicators indicates an inflammatory process due to the persistence of the virus. F. Riemer *et al.* (2016) noted that this trend indicates immunosuppression for lymphopenia, and L. Goralskii *et al.* (2019) indicated brain hypoxia for such indicators, because erythropenia and the dominance of delayed-type hypersensitivity processes were observed. L. Goralskii *et al.* (2019) noted the pathogenic effect of the virus on the indicators of "white blood" in viral diseases in dogs, and the results of studies by F. Riemer *et al.* (2016) point to similar changes in cats.

Summing up the results of this study, there is a need for a comprehensive laboratory diagnosis of coronavirus infection, since changes in the morphological parameters of blood indicate the complex pathogenesis and multiple organ effects of the virus, which complicates treatment in the early stages of the disease.

Conclusions

Based on the results of comprehensive studies of blood parameters for coronavirus infection in companion animals, the following changes were established: an increase in the value of erythrocyte sedimentation rate from 1.5 to 4.5 times; the number of segmented neutrophils by 26-32%; mean cell haemoglobin concentration from 14 to 43%; total bilirubin content from 2.4 to 3.2 times; alpha-amylase activity from 17% to 2.5 times; γ-glutamyltranspeptidase from 20 to 67%; alkaline phosphatase from 2.5 to 9 times. It is necessary to consider a decrease in the value of such indicators as protein coefficient by 26-35%, glucose concentration by 13-20%; albumins by 14% and up to 3 times; haemoglobin content by 10-21%; haematocrit volume by 17-23%; the number of red blood cells by 15-30% and lymphocytes by 29-48%. Therefore, according to the conducted complex morphological and biochemical studies of the blood of companion animals, a certain identity of changes in coronavirus infection can be traced, which indicates a complex pathogenesis of the disease. Based on the parameters of statistical calculation of integral leukocyte indices, it was established that, being in the animal's body, the coronavirus contributes to the development of endogenous intoxication, depletion of the immune system, impaired immunoreactivity with the dominance of the cellular immune system and delayed-type hypersensitivity processes. In the blood of sick animals, the indicators of intoxication indices increased: Lii – from 57% to 2 times; Rrn – from 2 to 5 times; Blsi – from 53% to 2 times. As a result of the inflammatory process against the background of immune system suppression, there is a tendency to an incomplete immune response and a deficiency of cytokines of lymphocytic origin due to a decrease in non-specific reactivity indicators, such as: Li – from 23 to 63%; Irle – from 20 to 35%; Ii – from 7 to 42%; Hi – from 43 to 60%. By investigating the pathological effects of the virus on the body of dogs and cats, it is possible to improve the understanding of the pathobiology of coronavirus, which will allow establishing early intravital diagnostic markers. Thus, a certain set of changes in the morphological and biochemical parameters of blood can be considered a characteristic criterion in the differential diagnosis of coronavirus infection in companion animals. Further study is planned to investigate pathomorphological features of the impact of coronavirus on companion animals, which will expand diagnostic capabilities at different stages.

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Conflict of Interest

None.

References

[1] Addie, D.D., Curran, S., Bellini, F., Crowe, B., Sheehan, E., Ukrainchuk, L., & Decaro, N. (2020). Oral Mutian®X stopped faecal feline coronavirus shedding by naturally infected cats. *Research in Veterinary Science,* 130, 222-229. doi: 10.1016/j.rvsc.2020.02.012.

[2] Castro, T.X., Cubel Garcia, R.C.N., Gonçalves, L.P.S., Costa, E.M., Marcello, G.C.G., Labarthe, N.V., & Mendes-de-Almeida, F. (2013). Clinical, hematological, and biochemical findings in puppies with coronavirus and parvovirus enteritis. *Canadian Veterinary Journal*, 54(9), 885-892.

[3] Chen, S., Liu, D., Tian, J., Kang, H., Guo, D., Jiang, Q., Liu, J., Li, Z., Hu, X., & Qu, L. (2019). Molecular characterization of HLJ-073, a recombinant canine coronavirus strain from China with an ORF3abc deletion. *Archives of Virology*, 164, 2159-2164. doi: 10.1007/s00705-019-04296-9.

[4] Decaro, N., & Buonavoglia, C. (2008). An update on canine coronaviruses: Viral evolution and pathobiology. *Veterinary Microbiology*, 132(3-4), 221-234. doi: 10.1016/j.vetmic.2008.06.007.

[5] European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes. (1986, March). Retrieved from http://zakon4.rada.gov.ua/laws/show/994_137.

[6] Fehr, A.R., & Perlman, S. (2015). Coronaviruses: An overview of their replication and pathogenesis. *Methods in Molecular Biology*, 1282, 1-23. doi: 10.1007/978-1-4939-2438-7_1.

[7] Felten, S., & Hartmann, K. (2019). Diagnosis of feline infectious peritonitis: A review of the current literature. *Journals Viruses*, 11(11), article number 1068. doi: 10.3390/v11111068.

[8] Felten, S., Klein-Richers, U., Unterer, S., Bergmann, M., Leutenegger, C.M., Pantchev, N., Balzer, J., Zablotski, Y., Hofmann-Lehmann, R., & Hartmann, K. (2022). Role of feline coronavirus as contributor to diarrhea in cats from breeding catteries. *Viruses*, 14(5), article number 858. doi: 10.3390/v14050858.

[9] Goralskii, L., Radzikhovsky, N., Dyshkant, O., Dunaievska, O., & Sokulskiy, I. (2019). Experimental study of tropism in cultivated canine coronavirus in the small intestine of puppies. *Regulatory Mechanisms in Biosystems*, 10(4), 489-496. doi: 10.15421/021972.

[10] Guan, X., Li, H., Han, M., Jia, S., Feng, B., Gao, X., Wang, Zh., Jiang, Y., Cui, W., Wang, L., & Xu, Y. (2020). Epidemiological investigation of feline infectious peritonitis in cats living in Harbin, Northeast China from 2017 to 2019 using a combination of an EvaGreen-based real-time RT-PCR and serum chemistry assays. *Molecular and Cellular Probes,* 49, article number 101495. doi: 10.1016/j.mcp.2019.101495.

[11] Gülersoy, E., & Maden, M. (2021). Effects of gs-441524 on clinical and hematochemical parameters of cats with effusive fip over 60 days follow-up. *Assiut Veterinary Medical Journal*, 67(171), 40-51. doi: 10.21608/AVMJ.2021.205169.

[12] Hazuchova, K., Held, S., & Neiger, R. (2017). Usefulness of acute phase proteins in differentiating between feline infectious peritonitis and other diseases in cats with body cavity effusions. *Journal of Feline Medicine and Surgery*, 19(8), 809-816. doi: 10.1177/1098612X16658925.

[13] Jaroensong, T., Piamwaree, J., & Sattasathuchana, P. (2022). Effects of chemotherapy on hematological parameters and $CD4^{+}/CD8^{+}$ ratio in cats with mediastinal lymphoma and seropositive to feline leukemia virus. *Animals*, 12(3), article number 223. doi: 10.3390/ani12030223.

[14] Kannekens-Jager, M.M., *et al.* (2022). SARS-CoV-2 infection in dogs and cats is associated with contact to COVID-19-positive household members. *Transboundary and Emerging Diseases,* 69(6), 4034-4040. doi: 10.1111/tbed.14713.

[15] Khan, M.A.A., Schoene, K., Cashman, J., Abd, El., El Wahed, A.A., & Truyen, U. (2022). Evaluation of a simple ultrafiltration method for concentration of infective canine parvovirus and feline coronavirus from cell culture supernatants. *Journal of Virological Methods*, 310, article number 114628. doi: 10.1016/j.jviromet.2022.114628.

[16] Krentz, D., *et al.* (2021). Curing cats with feline infectious peritonitis with an oral multi-component drug containing GS-441524. *Viruses,* 13(11), article number 2228. doi: 10.3390/v13112228.

[17] Law of Ukraine No. 3447-IV “On the Protection of Animals from Cruelty”. (2006, February). Retrieved from https://www.globalanimallaw.org/downloads/database/national/ukraine/library64.pdf.

[18] Lysenko, D.A., Andrushko, I.I., & Gunko, I.P. (2021). Hematological parameters of peripheral blood as prognostic factors in patients with COVID-19 (Literature review). *Reports of Vinnytsia National Medical University*, 25(1), 175-180. doi: 10.31393/reports-vnmedical-2021-25(1)-31.
[19] Melnyk, O.O. (2020). *Nonvirological laboratory markers in the context of the disease COVID-19*. Retrieved from https://median.kiev.ua/ua/publikatsii-likariv/31-nevirusologicheskie-laboratornye-markery-v-kontekste-zabolevaniya-covid-19.
[20] Order No. 944 "Good Laboratory Practice GLP". (2009, December). Retrieved from https://ips.ligazakon.net/document/re17348?an=48.
[21] Order of the Ministry of Health of Ukraine No. 281 "On Measures to Further Improve Organizational Forms of Work with the Use of Experimental Animals". (2000, November). Retrieved from https://zakononline.com.ua/documents/show/210966___515024.
[22] Osadcha, Yu. (2021). Nonspecific reactions of chickens under the influence of a technological stressor. *Agrarian Bulletin of the Black Sea Region*, 101, 16-22. doi: 10.37000/abbsl.2021.101.03.
[23] Paltrinieri, S., Comazzi, S., Spagnolo, V., & Giordano, A. (2002). Laboratory changes consistent with feline infectious peritonitis in cats from multicat environments. *Journal of Veterinary Medicine. Series A*, 49(10), 503-510. doi: 10.1046/j.1439-0442.2002.00494.x.
[24] Pandit, R., Ipinmoroti, A.O., Crenshaw, B.J., Li, T., & Matthews, Q.L. (2023). Canine coronavirus infection modulates the biogenesis and composition of cell-derived extracellular vesicles. *Biomedicines,* 11(3), article number 976. doi: 10.3390/biomedicines11030976.
[25] Pedersen, N.C. (2014). An update on feline infectious peritonitis: Diagnostics and therapeutics. *The Veterinary Journal*, 201(2), 133-141. doi: 10.1016/j.tvjl.2014.04.016.
[26] Radzikhovskyi, N., Dyshkant, O., Tolokevich, O., & Moshkivsky, V. (2021). Epizootological features coronavirus infection in cats. *Scientific and Technical Bulletin of the State Scientific Research Control Institute of Veterinary Medical Products and Fodder Additives and Institute of Animal Biology,* 22(2), 317-322. doi: 10.36359/scivp.2021-22-2.37.
[27] Riemer, F., Kuehner, K.A., Ritz, S., Sauter-Louis, C., & Hartmann, K. (2016). Clinical and laboratory features of cats with feline infectious peritonitis – a retrospective study of 231 confirmed cases (2000-2010). *Journal of Feline Medicine and Surgery,* 18(4), 348-356. doi: 10.1177/1098612X15586209.
[28] Rotstein, D.S., *et al.* (2022). Investigation of SARS-CoV-2 infection and associated lesions in exotic and companion animals. *Veterinary Pathology*, 59(4), 707-711. doi: 10.1177/03009858211067467.
[29] Semotyuk, S.L., & Kolesnyk, I.V. (n.d.). *Laboratory diagnosis of thyroid pathology*. Retrieved from https://omdc.zhitomir.ua/blog/view/83-laboratorna_diagnostika_tireoidnoi_patologii/.
[30] Sha, X., Li, Y., Huang, J., Zhou, Q., Song, X., & Zhang, B. (2022). Detection and molecular characteristics of canine coronavirus in Chengdu city, Southwest China from 2020 to 2021. *Microbial Pathogenesis*, 166, article number 105548. doi: 10.1016/j.micpath.2022.105548.
[31] Simsek, A., Kochan, A., Yesilmen Alp, S., Sayin Ipek, D.N., & Icen, H. (2022). Serum calprotectin levels in dogs with diarrhea. *Acta Scientiae Veterinariae*, 50, article number 119992. doi: 10.22456/1679-9216.119992.
[32] Stranieri, A., Giordano, A., Paltrinieri, S., Giudice, C., Cannito, V., & Lauzi, S. (2018). Comparison of the performance of laboratory tests in the diagnosis of feline infectious peritonitis. *Journal of Veterinary Diagnostic Investigation,* 30(3), 459-463. doi: 10.1177/1040638718756460.

[33] Sulehria, M.U., Ahmad, S.S., Ijaz, M., Mushtaq, M.H., Khan, A.Y., & Ghaffar, A. (2020). Molecular evidence and hematological alterations associated with the occurrence of coronavirus in domestic dogs in Pakistan. *Tropical Biomedicine*, 37(4), 963-972. doi: 10.47665/tb.37.4.963.
[34] Vlasova, A.N., Diaz, A., Damtie, D., Xiu, L., Toh, T.-H., Lee, J.S.Y., Saif, L.J., & Gray, G.C. (2022). Novel canine coronavirus isolated from a hospitalized patient with pneumonia in East Malaysia. *Clinical Infectious Diseases*, 74(3), 446-454. doi: 10.1093/cid/ciab456.

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Анотація. У зв'язку з високим рівнем урбанізації в Україні, протягом 2021-2023 років відмічається тенденція до стрімкого збільшення у великих містах чисельності тварин-компаньйонів – собак і котів. Людство ще остаточно не ліквідувало пандемію коронавірусної інфекції SARS-CoV-2, як світові науковці встановили участь цих тварин у її поширенні, що викликає занепокоєння у фахівців і потребує всебічного вивчення. Мета наукового дослідження полягала у визначенні маркерних змін гематологічних показників та параметрів гемопоезу за впливу на організм собак і котів коронавірусної інфекції. Для цього у роботі використовували спектрофотометричні та рефрактометричні методи гематологічних досліджень: морфологічних і біохімічних показників крові, функціонального стану еритро- і лейкоцитопоезу. Враховуючи зміни морфологічних показників крові, встановлено основні параметри ідентичності патогенного впливу коронавірусу на організм собак і котів, а саме: зменшення кількості лімфоцитів, вмісту глюкози та величини білкового коефіцієнта. Водночас відзначалось істотне підвищення в крові активності лужної фосфатази та α-амілази, вмісту загального білірубіну й величини швидкості осідання еритроцитів. Зокрема, експериментально встановлено, що використання лейкоцитарних індексів підвищує інформативність загального аналізу крові за коронавірусної інфекції, а додатковий аналіз інтегральних змін на основі лейкоцитарних показників крові дозволяє визначати не лише стан реактивності організму, але й величину ендогенної інтоксикації. Результати щодо змін морфологічних показників крові певною мірою є співставними з такими в людей, що свідчить про необхідність глибокого вивчення ще й генетичного потенціалу збудників коронавірусу в тварин-компаньйонів і людини на молекулярно-біологічному рівні. Експериментально визначені маркерні зміни гематологічних показників за коронавірусної інфекції у собак і котів можуть бути корисними для своєчасного її діагностування та прогнозування ступеня важкості перебігу захворювання

Ключові слова: коронавірус; еритроцитопоез; лейкоцитопоез; стабілізована кров; сироватка крові; морфологічні та біохімічні показники

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