UKRAINIAN JOURNAL OF VETERINARY SCIENCES

2026. Volume 17, No. 1 P. 72-91

ISSN 2663-967X;
e-ISSN 2663-9688

Journal homepage: https://veterinaryscience.com.ua/en

DOI: 10.31548/veterinary1.2026.72

UDC 636.028/.09:615.03

Received: 16.10.2025 Revised: 21.01.2026 Accepted: 26.02.2026 Published: 23.03.2026

Assessment of the effect of a biologically active preparation on the development of laboratory animals

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Suggested Citation:
Lyasota, V., Tkachuk, S., Bogatko, N., Bukalova, N., & Bogatko, A. (2026). Assessment of the effect of a biologically active preparation on the development of laboratory animals. *Ukrainian Journal of Veterinary Sciences*, 17(1), 72-91. doi: 10.31548/veterinary1.2026.72.

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Abstract. Investigation of the effects of newly developed drugs containing several compounds on laboratory animals is an important step in scientifically establishing their effective dosage and safety. The purpose of this study was to investigate the effect of the new drug "Imun-depo" on the development of laboratory animals (white mice), their haematological parameters, and the viability of *Tetrahymena pyriformis* ciliate cells. The study material was female white laboratory mice of improved convential type in the amount of 60 animal units (30 units each in the control and experimental groups). The drug was administered to white laboratory mice intragastrically at a dose of 1.0 mL/animal from 14 days of age for 60 days. A set of methods was used to assess the state of the internal environment in the vivarium, the quality of drinking water, the dynamics of body weight and internal organs, behavioural responses of mice, haematological indicators of their body, and the viability of *Tetrahymena pyriformis ciliates*. It was found that the indicators of the internal environment of the vivarium and drinking water were within the limits of regulatory requirements. It was found that the drug at a dose of 1.0 mL/animal had an effect on body weight gain, while the weight of the thymus, thyroid gland, kidneys, liver, and spleen did not change. It was proved that in the blood of white laboratory mice, the haemoglobin content, the number of red blood cells, and the haematocrit level increased (within the reference values). Moreover, the indicators of the leukocyte formula of the mice's blood did not change. In the blood serum, there was an increase (within the reference values) in the content of globulins, total protein, alanine aminotransferase activity, and a decrease in total cholesterol. According to the effect of the "Imun-depo" preparation on the viability of *Tetrahymena pyriformis* ciliate cells, the absence of toxic effects was noted. Scientifically based results of the effect of the "Imun-depo" preparation on the body of white laboratory mice are necessary for further experimental studies with the use of the drug to productive animals as a feed additive to the main diet

Keywords: white mice; keeping conditions; body weight dynamics; morphology and biochemistry of blood; *Tetrahymena pyriformis* ciliates; "Imun-depo" preparation

Introduction

The use of newly created biologically active preparations, which include a number of substances, requires preclinical studies on various types of laboratory animals. The effectiveness of complex drugs on a living organism depends on the amount of each substance in their composition and the possible synergistic effect. The degree of exposure to drugs was assessed by indicators of body weight dynamics, behaviour, haematological parameters of laboratory animals, and also depended on the action of each of the components of the preparation. Therefore, it is extremely important to determine the optimal dose of multicomponent drugs and the first stage is preclinical studies. Biologically active compounds in the composition of such drugs, first of all, have an immunomodulatory effect on the body, the degree of influence of which depends on the dosage of the drug, the state of health of laboratory animals, their age, and sex.

Relevance in the study of the effect of multicomponent biologically active drugs lies in the possible prevention of immunodeficiency and the development of immune responses in the body, which depend on a balanced supply of nutrients to the body. Even a slight deficiency of certain nutrients leads to a violation of immune competence in the body.

Micro- and macronutrients, vitamins, and amino acids have an important regulatory effect on immune mechanisms (Islam & Bellah, 2026). Carotenoids – natural pigments synthesised by plants, microorganisms, and fungi – play an important role in maintaining homeostasis and reactivity of the body (Bohn *et al.,* 2023). Considerable attention of researchers was focused on investigating the effects of carotenoids and vitamin A on adipose tissue metabolism, which determines the balance between energy storage (lipogenesis) and its release (lipolysis). B.V. Aygun *et al.* (2026) found that retinoic acid, β-cryptoxanthin, zeaxanthin, and lycopene contribute to reduced adipogenesis and lipogenesis, inhibit the expression of the peroxisome proliferator-activated gamma receptor (PPARγ), CCAAT/enhancer binding protein (C/EBPα), and sterol regulatory element binding protein 1C (SREBP1-c). As a result, there is a decrease in adipocyte hypertrophy, systemic inflammation, and an increase in mitochondrial activity in cells.

M.S. Abou El-Fetouh *et al.* (2023), when conducting preclinical studies on female albino rats, found that zinc in the composition of the drug affects the functional ability of the immune system, which is manifested by an immunostimulating effect on immunocompetent cells in the body. M.S. Hameed & A.I.A. Al-Ezzy (2024) proved the immunomodulatory activity of vitamins C and E – sodium selenite in studies on laboratory mice intoxicated with sodium nitrate. Sodium selenite and vitamin C have been found to be powerful antioxidants and affect the improvement of kidney function. The effect of drugs on the body, which include sodium selenite, depends on the dose, the size of the compounds of substances, and the combined effect with other biologically active substances. L. Li *et al.* (2024) found that the use of microencapsules with selenium and protein in mice did not lead to toxic effects on the body. Weight gains and growth of organs in mice of the experimental group were observed in comparison with the control group. In addition, the positive effect of selenoprotein was less accumulation in the liver and kidneys. Instead, the use of sodium selenite without proteins led to an increase in selenium content in the kidneys, liver, and other organs. M.K. Basher *et al.* (2024) found that due to the toxic effects of sodium arsenite on the body of female mice, the use of sodium selenite caused a protective effect against haematotoxic, biochemical changes, and toxic effects on organs.

One of the biologically active substances in the body is a water-soluble micronutrient – riboflavin (vitamin B_2), which is considered a precursor of nucleotides, synthesising protein structures of the inner membrane of mitochondria, forming the respiratory chain for the synthesis of ATP (energy) through oxidative phosphorylation, bind to enzymes through complex mechanisms, providing structural stability during the processes of dissimilation and catabolism. E.R. da Silva-Araújo *et al*. (2025) concluded that riboflavin is involved in the regulation of dissimilation and catabolism, ensuring cell growth and development, and the development of proteins, lipids, carbohydrates, and nucleic acids. S. Gomathi *et al*. (2026) proved in experimental studies on female rats whose diet was deficient in riboflavin, weight loss, and developmental delay in their offspring, including weight loss and increased mortality after weaning.

Thus, the study of drugs that include compounds that create an immunomodulatory effect, participate in the processes of energy metabolism, help reduce inflammation, fat deposition, and thereby reduce cholesterol in the body of laboratory mice and rats is an important stage of preclinical studies to obtain appropriate conclusions about their possible further use in productive animals, considering the optimal dose, sex, and age of animals. Thus,

the purpose of this study was to investigate the effect of the "Imun-depo" preparation on the growth and development of white laboratory mice, morphological and biochemical parameters of blood, and the viability of *Tetrahymena pyriformis* ciliates.

Literature Review

Biotechnological, pharmacological, and medical methods are widely used to assess the functional modulation of immune system components. The process of correction of the immune system can be used both in clinical practice and in basic research, the purpose of which is to investigate potential targets for modulating immunity, the body's response to the introduction of certain compounds, and the state of homeostasis. But there are a number of problems in immunomodulation that require the development of new effective and safe therapeutic compounds for the body (Strzelec *et al.*, 2023). S.M. Elhusseiny *et al.* (2022) conducted experimental studies on rats and proved the immunomodulatory activity of aqueous extracts from edible mushrooms of the family *Basidiomycota* that contain carotenoids, enzymes, minerals, proteins, tocopherols, alkaloids, lectins, terpenoids, and volatile compounds. The immunomodulatory effect was confirmed by the results of an increase in the number of lymphocytes, white blood cells, nitric oxide, lysozyme activity, and cytokines in the plasma of experimental animals. R.R. Goel *et al*. (2021) noted that from the list of known endogenous cytokines, interferons involved in the regulation of the immune response are among the most therapeutically useful in the treatment of various diseases. Interferons can be formed by functional units of the body in response to a foreign protein, and participate in cell growth, followed by an immunomodulatory effect.

An important role in immunomodulation belongs to the compound beta-carotene, which is a source of vitamin A, is involved in the regulation of the immune response by restoring the intestinal microbiome. H. Kuang *et al.* (2022) in an experimental study on mice found that had clinical allergic symptoms that beta-carotene is likely to lead to normalisation of intestinal microflora by increasing the number of beneficial bacteria, such as *Clostridiaceae* and reduce pathogenic bacteria, such as *Streptococcaceae*. A. Eroglu *et al.* (2018) pointed out that some of the important carotenoids are α-carotene, β-carotene, and β-cryptoxanthin, which act as precursors (provitamins) of vitamin A, have antioxidant and immunological properties, and serve as proxy biological markers of overall health. In addition, F. Zhou *et al.* (2020) found that retinoic acid, as the most active and biologically available form of vitamin A, can affect the reduction of triglyceride and cholesterol esters in cell cultures and in the body of mice in experimental studies. K.M.M. Koriem & M.S. Arbid (2018) found that an experimentally created favism in an experiment with rats (an acute form of haemolytic anaemia) suppressed blood proteins, and treatment of rats with β-carotene stopped blood cell damage and protein suppression. Furthermore, haematological parameters stabilised and the level of cholesterol in the blood serum of sick rats decreased.

Lipid metabolism and reduction of inflammation in the body depends on the dose and form of administration of drugs with selenium, preclinical studies of which are important for further use in mammals. M.L. Ojeda *et al.* (2022) investigated the effect of low doses of selenite and selenium nanoparticles on adipose tissue deposition, associated insulin secretion, and GPx1, IRS-1, and FOXO3a expression in immature rats. They found that when administered orally to rats with water of different doses of selenite, low levels of supplements contributed to increased adipogenesis, through the pathway of pancreatic peptide hormone signalling

and modulation of LCN2, while the introduction of a small amount of selenite prevented fat accumulation, through reduced pancreatic peptide hormone signalling and promoted cell renewal, reducing the inflammatory process. D. Kumar *et al*. (2025) showed that both low and high doses of sodium selenite applied to laboratory mice resulted in a significant ($p < 0.05$) decrease in haemoglobin concentration and cell mass volume, increased levels of aspartate aminotransferase, alanine aminotransferase, urea, and creatinine, which indicated toxicity to the liver and kidneys. An increased level of lipid peroxidation, enzymes, and a state of oxidative stress were established during repeated oral administration of similar doses of sodium selenite to rats. The micronucleus analysis revealed potential changes that indicated genotoxicity and chromosomal damage. Thus, the results showed the importance of compliance with the administration of certain specific doses of sodium selenite.

R. Li *et al.* (2025) found that arginine is one of the most metabolically adaptive amino acids that can adapt metabolism to environmental influences and changes in the internal environment of the body, providing an energy supply of muscle tissue, protein metabolism, and wound healing. T.S. Fung *et al.* (2025) noted that L-arginine is a key precursor for the synthesis of nitrogen-containing metabolites and promotes the elimination of excess nitrogen (urea) from the body, cellular signalling, and the accumulation of cellular energy. The body does not synthesise enough of this amino acid and the exchange of cellular energy depends on its exogenous intake. Therefore, it is important that animal feeding diets are balanced in the amount of arginine to maintain the health of the body, because its deficiency can contribute to the progression of diseases.

Dosage of drugs and their components remains the main task for achieving a positive effect on the body. Y. Wang *et al.* (2025) proved that a micronutrient such as copper is an important trace element for the animal body, excessive consumption of which leads to the accumulation of copper ions in tissues and organs, and has a toxic effect. Along with this, M.Y. Abbas *et al.* (2025) conducted a study on male white mice using low- and high-dose copper oxide nanomaterials and measured the amount of shaped blood elements and serum lipids, and the activity of liver and kidney enzymes. A high dose of copper oxide led to significant fluctuations in the content of white blood cells, haemoglobin and the number of red blood cells, which led to the conclusion that copper nanoparticles are toxic to the body when using different doses of the substance.

Thus, the analysis of scientific material presented in literature sources indicates that now the study of the effect of biologically active compounds on the body of experimental animals is widely used and is extremely relevant. Furthermore, the dose of the test substance, the age period of the animals used in the experiment, the degree of action of substances on the integral and haematological parameters of the body are considered.

Materials and Methods

The study was conducted in 2025 based on the immunological laboratory of the Bila Tserkva National Agrarian University and the Department of Animal and Food Hygiene named after Prof. A.K. Skorokhodko at the National University of Life and Environmental Sciences of Ukraine, based on the tasks set for scientific research on the topic: "Scientific and practical substantiation for the use of phytogenic feed additives in the production of meat from productive animals" (State registration number 0124U005020). To conduct an experimental study, a new "Imun-depo" preparation was used, which included the following substances: sodium selenite,

potassium selenite, riboflavin, total interferon (dry), L-arginine, zinc sulphate, copper sulphate, 0.25% beta-carotene solution in oil.

The study used 60 animal units of female (body weight at the beginning of the study was 11.0 g) white laboratory mice (*Mus Musculus L*), belonging to the conventional type (*Minimal diseases*). Two groups of laboratory animals were created: a control group (30 units) and an experimental group (30 units). In laboratory mice of the experimental group, the "Imun-depo" preparation at a dose of 1.0 mL/animal was injected through the oral cavity into the stomach using a metal probe. The experiment lasted for 60 days, starting from the 14-day age of white mice.

The experimental study was conducted in four stages. At the first stage, the conditions of keeping, feeding, and giving water to white laboratory mice were investigated. The study of indicators of the internal environment (microclimate) of the room was conducted three times during the research period. The main diet of the control and experimental groups of laboratory mice was formed from fresh crushed wheat and combined feed. Laboratory animals were provided with water from a nipple drinker. Indicators of drinking water for laboratory mice were within the limits of sanitary standards (Order of the Ministry of Health of Ukraine No. 400, 2010) (Table 1).

Table 1. Water quality and safety indicators

Indicators	Unit of measurement	DSanPiN 2.2.4-171-10	Actually
Organoleptic parameters			
Odour (at t = 20°C)	points	⩽ 2	1.7 ± 0.02
Turbidity	mg/dm^3	⩽ 1.0	0.6 ± 0.02
Colour	degrees	⩽ 20.0	15.3 ± 0.07
Taste and aftertaste	points	⩽ 2	1.5 ± 0.17
Physicochemical parameters			
Hydrogen index	pH units	6.5-8.5	6.6 ± 0.04
Overall hardness	mmol/dm^3	⩽ 7.0	6.2 ± 0.24
Chlorides	mg/dm^3	⩽ 250	150.0 ± 4.8
Nitrates	mg/dm^3	⩽ 50	16.3 ± 4.87
Nitrites	mg/dm^3	⩽ 0.5	0.4 ± 0.03
Manganese	mg/dm^3	0.1	0.09 ± 0.002
Sulphates	mg/dm^3	⩽ 250	27.6 ± 1.8
Total Iron	mg/dm^3	⩽ 0.2	0.04 ± 0.01
Epidemic safety indicators			
Common coliforms	CFU/100 cm^3	absent	-
Intestinal helminths	Cells, eggs, larvae, in 50 dm^3	absent	-

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

The room in which the white laboratory mice were kept was typical, built of brick, one-story, the place was well lit by the sun and was located on a flat area. Natural and artificial lighting was used – fluorescent lamps. The flow of fresh air into the room for keeping mice was carried out through a door and a transom. The standards for keeping white laboratory mice in metal cages and the physical indicators of the internal environment of the room in which the laboratory mice were kept met the requirements for sanitary and hygienic condition (SOU 85.2-37-736:2011, 2011) (Table 2). To assess the conditions of keeping white laboratory mice, the following were measured: room temperature

(mercury thermometer, degrees Celsius); relative humidity, % (psychrometric hygrometer VIT-2 (Ukraine)); air velocity, m/s (ball catathermometer (Ukraine)); carbon dioxide concentration, % (gas analyser WT8807 WINTACT (China)); ammonia and hydrogen sulphide, mg/m^3 (Wintact WT8823 gas analyser (China)); illumination, lux (digital lux meter photometer HS1010 (China)). The study of indicators of the internal environment of the room was carried out three times – before the start of the study and on day 30 and day 60.

Table 2. Indicators of the internal environment in the room for keeping laboratory mice

Indicators	Sanitary and hygienic standards	Actual indicator
Temperature, ^{0}C	20-24	22.4 ± 1.13
Relative humidity, %	55	55.6 ± 2.14
Air velocity, m/s	0.3	0.28 ± 0.03
Concentration of CO_2, %	0.15	0.15 ± 0.009
Concentration of NH_3, mg/m^3	10	0.07 ± 0.02
Concentration of H_2S, mg/m^3	5	1.6 ± 0.2
Illumination, lux (1 m above the floor level)	200	198.2 ± 0.35
Photoperiod, h (light: darkness)	12:12	12:12
Air exchange (times/hour)	10-15	11

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

During the second stage of the experiment, the effect of the "Imun-depo" preparation on the behaviour of laboratory mice, the dynamics of body weight, and the weight of internal organs – the liver, thymus, spleen, kidneys and thyroid gland – was investigated. To determine the body weight of the mice, they were weighed on WALCOM LB3002 laboratory scales (Taiwan). Subsequently, the mice were anaesthetised by the intraperitoneal administration of a 2% solution of Xylazine (Netherlands), followed by decapitation using a DCAP guillotine (Germany). The thoracic and abdominal cavities of the mice were then opened using a Surgiwell surgical scalpel (Pakistan) and an Adson-Brown forceps (Pakistan). Internal organs were extracted using an anatomical Adson forceps (Germany) and Asanus MN0654 micro-scissors (Germany). Between extractions, the instruments were washed in physiological saline and dried. The removed organs were placed in Petri dishes filled with physiological saline. Prior to weighing, the internal organs were blotted with filter paper, and the weighing was conducted swiftly to prevent drying of the organs. For weighing, WALCOM LB3002 laboratory scales (Taiwan) were used. Body weight gain in the laboratory mice was compared with the initial body weight at the start of the experiment and at 14, 30, and 60 days of the experiment.

At the third stage of the study, haematological parameters of the body of white laboratory mice were determined. The material for haematological studies was peripheral blood, which was taken on day 60 of the experiment. The following parameters were measured using the DH33 Vet automated haematology analyser (Japan): red blood cell count (RBC, 10^{12}/L), haemoglobin concentration (HGB, g/L), haematocrit level (HCT), white blood cell count (WBC, 10^9/L), neutrophils (NEUT, %), lymphocytes (LYMPH, %), basophils (BASO, %), eosinophils (EO, %), and monocytes (MONO, %). Biochemical parameters of blood serum were evaluated on an automatic biochemical analyser BA-88A Mindray (China). The fourth stage of the

experiment was devoted to the investigation of the effect of the "Imun-depo" preparation on the vital activity of the *Tetrahymena pyriformis* ciliates. A laboratory strain (WH_{14}) was used to conduct the work. Cells of *Tetrahymena pyriformis* ciliates were divided into control and experimental groups, with the number of cells in each group – 10^4 in 1 cm^3 environments. Ciliate cells belonging to the control group were filled with a solution of sea salt with a mass concentration of 0.56%, and the experimental group was filled with a solution of the "Imun-depo" preparation at a dose of 1.0 cm^3 in chemical flasks and placed in a thermostat at a temperature of 37°C. The study was conducted in three repetitions.

The experimental study was conducted in accordance with the ARRIVE recommendations (n.d.) in accordance with Law of Ukraine No. 3447-IV (2006) and 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) and the Order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 (2012). Statistical processing of the results was performed using the table processor "Microsoft Excel". The following were calculated: arithmetic mean and average error (M ± m), confidence level (P) according to Student, and significance criteria – $P < 0.05$; $P < 0.01$; $P < 0.001$.

Results and Discussion

Monitoring of the animal behaviour during experimental studies was a key indicator for identifying adaptive responses to the environment, manifestations of instincts, social interaction, and the ability to perform assigned tasks. Analysis of the parameters of integral behaviour of laboratory mice, such as horizontal and vertical motor activity, "mink reflex", integral activity provides an opportunity to investigate the physiological state of the animal and possible manifestations of anxiety or apathy, the level of activity or stress under the influence of the "Imun-depo" preparation. According to the data obtained (Table 3), no significant changes in body temperature, horizontal and vertical motor activity, integral activity and "mink reflex" between the animals of the experimental and control groups were detected during the use of the "Imun-depo" preparation in white laboratory mice.

Table 3. Indicators of behaviour of laboratory mice after the use of the "Imun-depo" preparation, M ± m, n = 30

Indicators	Experimental group	Control group
Body temperature, °C	38.1 ± 0.36	38.2 ± 1.15
Horizontal motor activity	34.8 ± 1.48	34.5 ± 1.12
Vertical motor activity	18.6 ± 1.2	18.3 ± 1.5
"Mink reflex"	13.6 ± 2.04	13.0 ± 2.02
Integral activity	66.0 ± 1.98	66.3 ± 1.76

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

The body weight of laboratory mice is a key indicator of their health and physiological state. Changes in body weight and internal organs allow analysing the experiment as a whole, critically assessing the keeping conditions, feeding ration, dose of the drug, which may affect the further conduct of the study or reduce its duration. Indicators of body weight and internal organ mass of white laboratory mice after administration of the "Imun-depo" preparation are presented in Table 4.

Table 4. Body weight and internal organ weight of white laboratory mice after administration of the "Imun-depo" preparation, m ± m, n = 30

Indicator	Day of research			
	Beginning of the experiment	14	30	60
Body weight, g	11.0 ± 0.85 11.0 ± 0.64	14.0 ± 0.74 15.0 ± 0.85▲	17.0 ± 0.94 20.0 ± 0.79*	22.1 ± 1.03 25.6 ± 1.08*
Average daily weight gain, mg	–	214.0 ± 1.06 285.0 ± 1.30**	187.5 ± 1.72 312.0 ± 1.56**	170.0 ± 1.94 186.7 ± 1.89**
Thymus weight, mg	–	–	–	31.2 ± 2.89 33.6 ± 2.54
Liver, g	–	–	–	1.12 ± 0.02 1.17 ± 0.03
Spleen, g	–	–	–	0.18 ± 0.04 0.18 ± 0.03
Thyroid gland, mg	–	–	–	4.15 ± 0.02 4.17 ± 0.04
Kidneys, g	–	–	–	0.15 ± 0.06 0.16 ± 0.07

***Note**: * – P < 0.05; ** – P < 0.001 compared to the indicators in the control group mice; ▲ – P < 0.001 compared to the indicator at the beginning of the study. Indicators of the control group are presented in the numerator, the experimental group – in the denominator*
***Source**: compiled by the authors*

As a result of experimental studies, it was found that on the day 14 after administration of the "Imun-depo" preparation in laboratory mice of the experimental group, their body weight increased by 36.3% (P < 0.001) compared to the start of the study, and on day 30 – by 17.6% (P < 0.05), on day 60 – by 15.8% (P < 0.05) compared to the indicators in mice in the control group. The animals actively ate food, were active, and no negative reaction was observed in the mice of the experimental group during intragastric administration of the drug. The average daily weight gain on day 14 increased by 33.2% (P < 0.001), on day 30 – by 66.4% (P < 0.001), and on day 60 of the study – by 9.82% (P < 0.001) compared to the indicator in the control mice. The weight indicators of the studied internal organs of white laboratory mice of the control and experimental groups did not significantly change. According to the physiological state, the studied internal organs corresponded to the physiological norm for sexually mature white laboratory mice.

One of the limiting factors for the body of white laboratory mice, after applying the "Imun-depo" preparation, may be the effect of zinc and copper. On this occasion N.V. Grigorova (2025) pointed out that a deficiency of zinc, magnesium, and copper in the body leads to gradual atrophy of the thymus and to a significant violation of the processes of cellular and humoral immunity. However, as a result of experimental studies, it was found that the introduction of the "Imun-depo" preparation at a dose of 1.0 ml/animal did not cause symptoms of toxic effects on the body of white laboratory mice, due to the combined action of the substances included in the drug. The greatest degree of positive effect on the body of laboratory mice can be created by a 0.25% solution of β-carotene in oil, which is part of the "Imun-depo" preparation. T. Breniere *et al.* (2024) evaluated the effect of tomato supplements of various genotypes and the content of caratinoids on the body condition of experimental mice. The IL6-2 tomato genotype, which

contains a significant amount of β-carotene, was found to reduce obesity in mice and contribute to weight gain.

In the future, the effect of the "Imun-depo" preparation on the indicators of the number of cellular elements in the blood of laboratory mice was determined. The study of the effect of drugs on the blood status of animals plays an important role in predicting possible side effects on morphological and biochemical haematological parameters (Table 5). When evaluating the effect of biologically active drugs, the following risks can be assumed: toxic effects on blood cells and organs, liver and kidney damage, oxidative stress, allergies, gastrointestinal disorders, and overdose. Therefore, the analysis of the effect of the "Imun-depo" preparation is extremely important in conducting preclinical studies.

Table 5. Morphological parameters and leukocyte formula of white mouse blood, M ± m, n = 30

Indicators, measuring units	Control group	Experimental group
Haemoglobin (HGB), g/l	110.56 ± 6.24	127.12 ± 5.02*
Red blood cells (RBC), 10^{12}/l	2.23 ± 0.15	2.85 ± 0.06**
Haematocrit (HCT), %	42.5 ± 1.48	47.9 ± 1.43*
White blood cells (WBC), 10^{9}/l	6.60 ± 0.40	6.80 ± 0.50
Rod neutrophils (NEUT), %	2.90 ± 0.28	3.12 ± 0.19
Segmentonuclear neutrophils (NEUT), %	19.72 ± 1.46	18.63 ± 2.39
Basophils (BASO), %	0.4 ± 0.002	0.4 ± 0.004
Lymphocytes (LYMPH), %	50.0 ± 4.54	51.0 ± 3.22
Eosinophils (EO), %	2.54 ± 0.21	2.12 ± 0.17
Monocytes (MONO), %	5.89 ± 2.38	5.83 ± 1.06

***Note**: * – P < 0.05; ** – P < 0.001 compared to the indicators in the control group mice*
***Source**: compiled by the authors*

According to the obtained morphological parameters of white laboratory blood, it follows that the "Imun-depo" preparation led to the activation of erythrocytopoiesis processes. On day 60 of the study, the concentration of haemoglobin in the blood of mice in the experimental group increased by 15.0% (P < 0.05), and the number of red blood cells – by 27.8% (P < 0.001) compared to mice in the control group. Simultaneously, the haematocrit level increased by 5.4% (P < 0.05) compared to those in the control mice. There was no significant difference in the content of white blood cells, rod-shaped and segmentonuclear neutrophils, basophils, lymphocytes, eosinophils, and monocytes in the blood of mice of the experimental and control groups. Finding out the effect of the "Imun-depo" preparation on the body of laboratory mice, a component attracted attention – copper sulphate, which has bactericidal properties, promotes the digestion of feed nutrients, and improves intestinal function. A.A. Al-Saghee *et al.* (2023) found in an experiment that the addition of copper nanoparticles to rabbits contributed to better feed intake, increased protein content, haematocrit, and haemoglobin levels in the blood compared to other forms of copper. As a result, an increased nutritional value was found when rabbits were fed a diet with added copper.

Thus, the use of the "Imun-depo" preparation at a dose of 1.0 mg/animal intragastrically to white laboratory mice led to the activation of erythrocytopoiesis processes and the maintenance of myeloid and lymphoid chains of haematopoiesis of cells, and also indicated the absence of viral or bacterial diseases, stress, immunodeficiency or the effect of toxins on the body of mice during the study. Along with

morphological parameters of blood, important markers that can be used to confirm the level of metabolic transformations in the body, including in laboratory mice during the experiment, are biochemical parameters of blood serum (Table 6).

Table 6. Morphological parameters and leukocyte formula of white mouse blood, M ± m, n = 30

Indicators, measuring units	Control group	Experimental group
Total protein (T-Pro), g/L	56.3 ± 1.23	60.8 ± 1.06**
Albumins (ALB), %	47.2 ± 3.18	54.0 ± 2.10
Globulins (Glob), %	42.8 ± 0.34	49.6 ± 0.62***
Alanine aminotransferase (ALT), IU/L	24.6 ± 0.6	26.3 ± 0.4*
Aspartate aminotransferase (AST), IU/L	44.8 ± 1.2	45.0 ± 0.03
Total cholesterol, mmol/L	2.56 ± 0.14	2.01 ± 0.12*

***Note**: * – P < 0.05; ** – P < 0.001 compared to the indicators in the control group mice*
***Source**: compiled by the authors*

As can be seen from the table, the effect of using the "Imun-depo" preparation at a dose of 1.0 mg/animal led to an increase in the blood serum of white laboratory mice in total protein by 8.0% (P < 0.01) and globulin by 6.8% (P < 0.001) compared to the indicators in the control mice. It can be assumed that an increase in the content of total protein and globulin (within the physiological norm) are the main factors for increasing the energy of growth and development and increasing the activity of humoural immunity in mice of the experimental group. Therefore, it can be assumed that it is the content of sodium selenite and potassium in the composition of the "Imun-depo" preparation that can lead to an improvement in the state of immunity in laboratory mice. J. Zhang *et al.* (2021) determined the immunomodulatory effects of selenium-rich soy peptides (Se-SPep) in an experiment on mice with cyclophosphamide-induced immunosuppression. They found that injecting selenium into mice maintained proper body weight, total protein levels, albumin, and white blood cells, and stimulated the production of interleukin-2, interferon-gamma, and nitric oxide. These results confirmed that Se-SPep is an effective immunomodulator.

In addition, the content of L-arginine in the composition of the "Imun-depo" preparation affected the improvement of immunity, especially during the period of intensive growth and development of the body. The use of supplements in productive animals containing arginine improved the functional state of the body in other studies. In particular, B. Tan *et al.* (2009) showed that arginine supplementation in pig feeding resulted in a 6.5% (P < 0.05) increase in body weight and skeletal muscle content in the carcass – by 5.5%, while reducing the fat content in the carcass by 11% (P < 0.01). The increase in pig body weight occurred with an increase in protein, glycogen, and fat content in the longest back muscle by 4.8, 42, and 70% (P < 0.05), respectively. Based on the results of the study, researchers concluded that arginine supplements to feeding pigs contributed to weight gain while reducing the accumulation of fat in the carcass. The longest back muscle, which is the most commercially valuable, accumulated protein and other nutrients. A.O. Oso *et al.* (2017) found that feeding arginine supplements to turkeys of different ages and doses affected an increase in the number of red blood cells (P < 0.001), lymphocytes (P < 0.001), and basophils (P < 0.001), total

protein, globulin concentrations, and a decrease in serum enzyme levels. Thus, it was experimentally confirmed that the addition of arginine to feed fattening turkeys led to an increase in haematocrit volume, an improvement in serum parameters, and an increase in the mass of the thymus, spleen and a decrease in the number of salmonella in the small intestine.

In the current study, a significant difference between the amount of albumins in the blood serum of mice of the control and experimental group was not established, although there was some increase in them within the reference values, which may indicate the regulation of oncotic pressure and the transfer of lipids, hormones, and drugs in the bloodstream. The "Imun-depo" preparation also affected the activity of enzymes of the transferase class. It was found that in laboratory mice of the experimental group, the activity of alanine aminotransferase increased by 6.9% ($P < 0.05$), within the physiological norm, compared with the indicator in mice of the control group. However, there was no significant difference in the activity of aspartate aminotransferase between the indicators in the control and experimental mice. An increase in the enzymatic activity of alanine aminotransferase in the blood serum of laboratory mice within the physiological norm indicated activation of the body's energy and plastic needs.

The content of total cholesterol in the blood serum of mice in the experimental group decreased by 21.5% ($P < 0.05$) compared to the indicator in mice in the control group. It is possible to explain the significant decrease in the content of total cholesterol in the blood serum of mice of the experimental group, based on the fact that the composition of the "Imun-depo" preparation includes the essential amino acid L-arginine. B. Tan *et al*. (2012) found that L-arginine is a precursor of nitric oxide and affects the regulation of the metabolism of fatty acids, proteins, amino acids at the cellular level with the emergence of DNA transcription and translation processes, with the stimulation of lipolysis and activation of fatty acid oxidation to carbon dioxide and water. These processes are also associated with the regulation of metabolic pathways to optimise the formation of ATP and the use of energy substrates (glucose, fatty acids) towards reducing the content of triacylglycerols. Therefore, arginine supplementation affected the reduction of fat in subcutaneous tissue, omentum, and around internal organs in diabetic rats and obese rats in the experiment.

In addition, the content of 0.25% β-carotene solution in oil in the "Imun-depo" preparation can help reduce the cholesterol content in the blood serum of laboratory mice. A.M. Abd El-Hady *et al*. (2022) found that a supplement containing β-carotene and zeaxanthin was applied to productive animals (broiler chickens) – *Spirulina platensis*, has lipid-lowering properties. In addition, an increase in the number of red blood cells, haemoglobin, white blood cells, and lymphocytes in the bird's blood was observed ($P < 0.05$) compared to the control indicators. N. Melnikov *et al*. (2022) proved in male mice that vitamin A and carotenoids are involved in the regulation of adipose tissue metabolism and inflammation. The concentration of cholesterol and triglycerides in the blood plasma of mice fed excess fat and supplemented with β-carotene decreased by 30% and 28% compared to mice fed a diet high in fat and supplemented with vitamin A. Thus, the presence of β-carotene in the feed, rather than vitamin A, led to a decrease in the number of monocytes in the bloodstream, which indicated a decrease in inflammation in the body and indicators of fat metabolism in blood plasma.

A rather urgent scientific problem was the investigation of the influence of the environment on the vital activity of both the whole organism and its individual components: functional systems, organs, tissues, and cells.

Conducting research to confirm the degree of environmental impact on the body is a rather complex methodological process, which creates problems for high-performance assessment of the toxicity of numerous chemicals (Sun *et al.*, 2026). One way to solve this problem is to conduct experiments on cell cultures. Their aetiology is limited to a specific organ or tissue, and the range of functions of these cells is quite narrow and specific. Therefore, the use of *Tetrahymena pyriformis*, a representative of protozoa, is unique for such studies. Morphologically, it is a cell, but physiologically, it is a complete organism. These cells are easy to use and do not require special costs for maintaining the vital activity of the culture and conducting research. In addition, due to their short life span and high reproductive capacity, they allow tracking changes in physiological processes over several generations in a short time and are very sensitive to toxins (Yu *et al.*, 2024). The current study monitored the vital activity of *Tetrahymena pyriformis* ciliates under the action of the "Imun-depo" at a dose of 1.0 cm^3 (Table 7).

Table 7. Effect of the drug "Imun-depo" on the vital activity of Tetrahymena pyriformis ciliates, M ± m, n = 3

Observation via:	Control group	Experimental group	Number of dividing cells
1 min	30.60 ± 1.13	30.65 ± 1.10	-
30 min	30.60 ± 1.25	30.59 ± 1.16	-
1 h	30.60 ± 1.10	30.64 ± 1.18	-
2 h	30.50 ± 1.18	30.71 ± 1.12	-
3 h	30.50 ± 1.15	34.15 ± 1.50	8.00 ± 0.06
4 hours	30.60 ± 1.17	34.20 ± 1.60	8.00 ± 0.05
5 h	30.50 ± 1.16	34.29 ± 1.70	8.00 ± 0.06
6 h	30.60 ± 1.23	35.62 ± 1.14*	10.00 ± 0.05
12 h	30.50 ± 1.22	35.92 ± 1.17*	12.00 ± 0.04
24 h	30.60 ± 1.33	35.84 ± 1.14*	14.00 ± 0.09
48 h	30.42 ± 1.26	35.58 ± 1.17*	14.00 ± 1.03
72 hours	30.78 ± 1.32	35.87 ± 1.13*	16.00 ± 1.09
96 h	30.20 ± 1.12	35.17 ± 1.03*	16.00 ± 1.10
120 h	30.40 ± 1.09	35.19 ± 1.24*	16.00 ± 1.08
144 h	30.50 ± 1.22	35.28 ± 1.14*	16.00 ± 1.11
168 h	30.40 ± 1.19	35.18 ± 1.04*	16.00 ± 0.92
192 h	30.20 ± 1.16	35.14 ± 1.32*	16.00 ± 0.84

***Note**: * – P < 0.05 compared to the control group*

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

Consistent with the results obtained, the "Imun-depo" preparation did not cause any deviations from the normal growth and development of the *Tetrahymena pyriformis* ciliates. Three hours after the start of the experiment, active division of ciliate cells of the control and experimental groups was observed. A significant increase in the number of ciliate cells began at 6 hours in the study and lasted until the end of the study – up to 192 hours. Thus, after 6 hours of the experiment, the number of ciliate cells in the bacteriological dish with the drug increased by 16.4%, at 12 hours – by 17.8%, at 24 hours – by 17.1%, at 48 hours – by 17.0%, at

72 hours – by 16.5%, at 96 hours – by 16.4%, at 120 hours – by 15.9%, at 144 hours – by 15.7%, at 168 hours – by 15.7%, and at 192 hours – by 16.3% ($P < 0.05$) compared to the indicators of ciliate cells of the control group.

Investigating the functional parameters of the *Tetrahymena pyriformis* ciliates, it was found that the cells assigned to the control and experimental groups moved in a straight line and energetically. During the entire follow-up period, no slowing of cell growth was detected, which would indicate a decrease in their vital activity due to the presence of toxic substances in the environment. There were no changes in the nature of movement, cell morphology, or the appearance of abnormal shapes. The paramecium cells were densely clustered, moving actively and in a straight line. They were oval and elongated in shape, with a moderate amount of filling. No cell death was observed during the study period. All this gives grounds to assert the harmless effect of the "Imun-depo" preparation at a dose of 1.0 cm^3 per living cell. Slowing down the process of ciliate division with increasing exposure time indicated a time dependence of the stimulating effect of the drug. Thus, according to the results of the study, it was established that the "Imun-depo" preparation activated the vital processes of the ciliary infusoria, based on the increase in dividing cells from 3 to 192 hours of the study. The drug did not have a mutagenic effect on protozoan cells. In a similar study of another immunomodulatory drug, "Biomagn", researchers O.M. Chechet *et al*. (2021) also found no toxic effects on infusoria culture of *Tetrahymena pyriformis* within 30 minutes, with 100% preservation of cell vital activity. The behaviour of this cell population can be an evaluation criterion for an immunostimulating drug, which is important for studying the environment. physiological and medical aspects of the sensitivity and stability of living systems to anthropogenic factors.

Thus, the preclinical experimental study showed that the "Imun-depo" preparation has a positive effect on the physiological state of white laboratory mice, stimulating their growth and development without negatively affecting important haematological and morphological parameters while maintaining 100% survival of mice during the experiment. Simultaneously, the use of the drug did not reveal toxic or mutagenic effects on the cells of *Tetrahymena pyriformis*, which confirms its safety and potential for further use in veterinary and biomedical research. These results open up prospects for the use of "Imun-depo" preparation as an effective and safe immunomodulator in various fields of science.

Conclusions

The effect of the drug "Imun-depo" at a dose of 1.0 mL/animal on body weight and internal organs, morphological and biochemical parameters of the blood of white laboratory mice, and on the vital activity of *Tetrahymena pyriformis* ciliates from 14 to 60 days of the experiment were investigated. The parameters of the internal environment in the room for keeping laboratory mice and the indicators of drinking water for their drinking were evaluated and full compliance with the approved standards was established. Administration of the "Imun-depo" preparation at a dose of 1.0 mL/animal into the stomach of laboratory mice of the experimental group contributed to an increase in their body weight compared to mice of the control group, observed from day 14 (by 36.3% ($P < 0.001$)), on day 30 (by 17.6% ($P < 0.05$)) and up to day 60 of the experiment (by 15.8% ($P < 0.05$)). Furthermore, the average daily weight gain of white laboratory mice in the experimental group on day 14 of the experiment increased by 33.2% ($P < 0.001$), on day 30 – by 66.4% ($P < 0.001$), and on day 60 – by 9.82% ($P < 0.001$) compared to the control. On day 60 of the experiment, a

significant difference in the weight of internal organs of white laboratory mice of the experimental and control groups was not determined. The drug helped to stimulate the development of red blood cells in the body of experimental mice, which was manifested by an increase (within the physiological norm) in the number of red blood cells by 27.8% ($P < 0.001$); an increase in the concentration of oxygen-carrying protein in the body – haemoglobin by 15.0% ($P < 0.05$), and the percentage volume of shaped elements – haematocrit by 5.4% ($P < 0.05$). The effect of the drug on the body of white laboratory mice of the experimental group led to an increase in the total protein content by 8.0% ($P < 0.01$) and globulin by 6.8% ($P < 0.001$) (within the physiological norm), which indicated an increase in the activity of humoural immunity. An increase in the enzymatic activity of alanine aminotransferase in the blood serum of laboratory mice by 6.9% ($P < 0.05$) was established, which indicated stimulation of catabolism and anabolism processes in the body, and activation of enzymatic and hormonal regulation and energy charge of cells. Instead, the total cholesterol content decreased by 21.5% ($p < 0.05$) in the blood serum of white laboratory mice in the experimental group. During the study of the vital activity of the *Tetrahymena pyriformis* ciliates, no toxic effects of the drug "Imun-depo" have been established. In further studies, it is planned to conduct an experiment on the use of the "Imun-depo" preparation with food for productive animals to find out the effect of the drug components on maintaining the physiological status of the body and preventing the risks of morbidity.

Acknowledgements

None.

Funding

None.

Conflict of Interest

None.

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Оцінка впливу біологічно активного препарату на розвиток організму лабораторних тварин

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Анотація. Дослідження дії на організм лабораторних тварин новостворених препаратів, що містять у своєму складі декілька сполук, є важливим етапом наукового обгрунтування їх ефективної дози і нешкідливості. Метою цієї роботи було вивчити вплив нового препарату «Імун-депо» на розвиток організму лабораторних тварин (білих мишей) та їх гематологічні показники, а також життєздатність клітин інфузорії *Tetrahymena pyriformis*. Матеріалом дослідження слугували самки білих лабораторних мишей поліпшеного ковенціального типу у кількості 60 голів (по 30 голів у контрольній і дослідній групі). Препарат білим лабораторним мишам вводили внутрішньошлунково у дозі 1,0 мл/гол. з 14 добового віку впродовж 60 діб. Застосовували комплекс методів для оцінки стану внутрішнього середовища у віварію, якості питної води, динаміки маси тіла та внутрішніх органів, поведінкових реакцій мишей, гематологічних показників їх організму та життєздатності інфузорії *Tetrahymena pyriformis*. З'ясовано, що показники внутрішнього середовища у віварію і питної води, якою напувались миші, знаходились

у межах нормативних вимог. Встановлено, що препарт у дозі 1,0 мл/гол. впливав на прирости маси тіла, а маса тимусу, щитоподібної залози, нирок, печінки та слезінки не змінювались. Доведено, що у крові білих лабораторних мишей збільшувався (у межах референтних значень) вміст гемоглобіну, кількість еритроцитів і рівень гематокриту. При цьому, показники лейкограми крові мишей не змінювались. У сироватці крові відбулося збільшення (у межах референтних значень) вмісту глобулінів, загального білка, активності аланінамінотрансферази та зменшення – загального холестерину. За дією препарату «Імун-депо» на життєздатність клітин інфузорії *Tetrahymena pyriformis* констатували відсутність токсичного впливу. Науково обгрунтовані отримані результати впливу препарату «Імун-депо» на організм білих лабораторних мишей є необхідними для подальшого проведення експериментальних досліджень із застосуванням препарату продуктивним тваринам як кормової добавки до основного раціону

Ключові слова: білі миші; умови утримання; динаміка маси тіла; морфологія і біохімія крові; інфузорія *Tetrahymena pyriformis*; препарат «Імун-депо»