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Morphometric features of the duodenal wall in piglets during different periods of postnatal and neonatal ontogenesis under the influence of the 'Globigen Jump Start' feed additive

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SOM: Project administration; Formal Analysis; Writing — original draft.

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The morphological parameters of the small intestinal mucosa, such as villi size and crypts, play a key role in the formation of the intestinal tube's absorption surface. The number of goblet and enterochromaffin cells indicates the epithelium's functional condition in terms of mucus secretion and production of catecholamines and hormones (serotonin, dopamine). That is why it is important to study and determine certain morphometric parameters of the duodenal wall in piglets during different periods of neonatal and postnatal development, especially during stress situations, namely weaning and transition to protein feeding. The article presents the resulting effect studies of 'Globigen Jump Start' feed additive on the histo-morphological parameters of the piglets' duodenal mucosa on day 7, 14 and 28 of life. A positive effect on mitigating weaning stress was manifested by a decrease in the quantitative and linear indicators of histoarchitectonics of intestinal wall's individual morphological components. A significant increase in goblet cells, especially in the experimental group, had a positive impact on the mucopolysaccharide synthesis. The piglets' gastrointestinal tract physiology involves a complex interaction between the central nervous system, meta-sympathetic nervous system, APUD system, and endocrine system. Due to these systems, the information is transmitted according to the direct and reverse communication mechanisms in the regulation of the gastrointestinal tract function. An increase in the number of enterochromaffin cells and their nuclei diameter in the experimental group of piglets indicated an increased synthesis of catecholamines and hormones. It has an extremely positive effect on the immune and physiological status of piglets, thus emphasizing the crucial role of serotonin in neuronal metabolism and the formation of stress resistance. The increase in the thickness of the duodenal wall muscle layer, in our opinion, occurred due to the active peristalsis, which was enhanced by the action of some catecholamines, which were synthesized in a slightly larger amount under the influence of nutrients making up the 'Globigen Jump Start' feed additive.

Key words: piglets, duodenum, villi, crypts, goblet cells, enterochromaffin cells, yeast, egg powder

Introduction

The development of the gastrointestinal tract (GIT) in pigs is a complex physiological process that begins in a prenatal period with the subsequent genesis in a post-natal one. It is known that colostrum and sow's milk contain biologically active substances that exert a positive impact on the GIT development, allowing it to adapt to feeding changes. At the same time, due to insufficient secretion of digestive enzymes in early weaned piglets, the roughage cannot be digested quickly in the gastrointestinal tract, which partially leads to the intestinal barrier destruction [11]. As reported by Pluske in his research [22], such physiological changes affect the small intestine's absorption capacity, growth, and development of piglets. Hampson [10] found the villi height in the small intestine decreased by about 25–35% during the first 24 hours after the weaning and remained at this level for up to five days. In non-weaned piglets, on the contrary, the correlation in the villi height was insignificant [37]. The same researchers also noted the elongation of crypts in the weaned piglets, which was recorded during the first 11 days after the feeding change [36].

A decrease in the digestive enzymes' activity on the brush border of enterocytes is also known to decrease in the gastrointestinal tract of the piglets after weaning [11]. Lalles et al. [17] reported a decrease in lactase and aminopeptidase activity within 15 days after weaning, while maltase activity decreased within 2 days with a subsequent increase by the 15th day after weaning. In addition, a number of authors found a short-term decrease in pancreatic secretion up to 15 days after weaning. These and some other changes can affect the small intestine's functional activity, its secretory and barrier functions, and the feed digestion intensity [29, 30, 31].

Modern pig breeding technologies and multifertility of sows provide for early weaning of piglets, mainly at the age of 21 to 28 days. The strategy of gradually transitioning piglets from milk to roughage has been used to reduce the stress of weaning. Accordingly, the piglets are accustomed to consuming various feed additives from the first week of life [18]. For instance, when using yeast as a feed additive, the productivity and growth stimulation improved due to the presence of relatively high levels of protein, vitamins, biologically active substances, amino acids, energy, and trace elements in yeast.

Chowdhury and Knabe [5] investigated the effect of *S. cerevisiae* yeast and aspartic acid on breeding boars and revealed an increase in their productivity. White et al. [35] demonstrated an increase in lactobacilli number and a decrease in bifidobacteria number in the intestine of weaned piglets when feeding yeast, while Stanley et al. [27] recorded a decrease in the population of coliforms. Sauerwein et al. explained in their work [25] that the preservation of normal intestinal morphology occurred due to the binding and reduction of pathogen colonization in the gastrointestinal tract, which helped improve the intestinal mucosa integrity and enhanced the immune system activity.

To reduce feeding-related stress and improve piglet health, egg powder obtained from hens sensitized to certain pathogens is added to feed additives, serving as a source of specific immunoglobulins, vitamins, omega-3 fatty acids, antioxidants, and trace elements. One of the main proteins contained in egg powder is ovomucin and the enzyme lysozyme [1]. In addition, egg powder contains such amino acids as tryptophan, alanine, valine, lysine, and methionine. The synergistic effect of these bioactive nutrients helps improve the piglets' growth, immunity, and health.

Many studies prove that lysozyme enhances the intestinal function and protects it from enteropathogenic strains of *Escherichia coli* in newborn piglets [15]. Ovomucin and its derivatives have anti-inflammatory, antioxidant, and immunomodulatory properties [1, 33, 39].

It is known that the physiology of piglets' GIT involves a complex interaction between the central nervous system, the metasympathetic nervous system, the APUD system, and the endocrine system, through which information is transmitted by direct and reverse communication mechanisms regulating the tract's function. Enterochromaffin cells (ECs) of the gastrointestinal tract play an important role in this complex process, performing the function of forming catecholamines, hormones, and affecting intestinal motility and secretion [9, 24]. Research by some scientists has shown that enterochromaffin cells express sensory receptors, the activation of which promotes the release of 5-HT serotonin receptors with subsequent stimulation of intestinal contractility [7].

Anatomically, the small intestine of pigs is divided into three parts: the duodenum (accounting for approximately 4–4.5% of the total small intestine volume in adult pigs), the jejunum (approximately 88–91%) and the ileum (about 4–5%) [8]. Histologically, the intestinal mucosa is divided into four main membranes: mucosal, submucosal, muscular, and serous. The mucosa consists of the epithelial layer, a mucosal lamina propria, and a muscular lamina propria. The intestinal epithelium is characterized by a folded surface, which is manifested at different levels of organization by intestinal folds, forming finger-like protrusions called villi with their own vascular, muscular, and nervous apparatus. At the base of the villi, the epithelial layer forms protrusions known as Lieberkuhn crypts (intestinal glands). The microvilli increasing the absorption surface are located on the luminal surface of enterocytes. It should be noted that the morphology of the gastrointestinal villi differs throughout the intestinal tract of animals, depending on the intestines function. The villi length increases from the duodenum to the middle of the jejunum and decreases towards the distal ileum. Similarly, the crypts also vary in size and composition along the intestine: they are deeper in the duodenum and jejunum and less deep in the ileum [6]. On the villi surface, three types of cells are localized, which derive from stem cells located at the crypt base: enterocytes (94%), goblet cells (up to 5%), and enteroendocrine cells (about 1%) [20, 21]. Small intestine enterocytes are divided into absorptive cells, goblet cells,

Pannet cells, and undifferentiated enterocytes. The enzymatic activity of enterocytes begins when they reach the basal third of the villus axis, while the absorptive function begins when they reach the apex and the upper third of the villus. Goblet cells are secretory which means they secrete viscous mucus; their basal activity increases when the cells come into contact with substances that cause secretion. The third type of cells, namely enteroendocrine cells, produce catecholamines, hormones that play an important role in the gastrointestinal tract functioning [9].

Physiological changes in the mucosa associated with absorption and barrier function are usually expressed by its morphological parameters. Thus, qualitative functional and morphological parameters of the small intestinal mucosa, such as the size of villi and crypts, and the activity of brush border enzymes, can provide additional information about the functional condition of the animal organism [23]. Other functional and morphological parameters, such as the number of goblet and enterochromaffin cells, are the parameters indicating the epithelium condition and the mucous membrane function as a barrier between the intestinal lumen and body tissues.

The **aim of our work** was to define individual morphometric parameters of the piglets' duodenum during different periods of neonatal and postnatal ontogenesis and determine the number of goblet and enterochromaffin cells under the influence of the 'Globigen Jump Start' feed additive.

Materials and Methods

The study of the 'Globigen Jump Start' feed additive effectiveness among the weaned piglets was carried out at the *Barkom* farm in the Lviv region. For this purpose, two groups of ten piglets each of the Large White breed were formed, which were fed with the feed additive from 7 to 28 days of life. During the experiment, piglets of the control group received a standard nutrient-balanced diet, and piglets of the second experimental group were fed 2 kg of the 'Globigen Jump Start' additive per 1 t of feed. The additive contains dry yeast and egg powder enriched with immunoglobulins (manufactured by *EW Nutrition GmbH*, Germany). The main feed was given in the form of pellets, which met the needs of piglets in all nutrients. During the experiment, the piglets were kept in the same conditions with free access to feed and water. Weaning was performed at 28 days of age. On days 14 and 28, three animals from each group were euthanized and material was taken for histological examination.

To study the cyto- and histoarchitectonics of the duodenum, its fragments were fixed in a 10% neutral aqueous solution of formalin and Bouin's fluid, dehydrated through the ascending series of alcohols, and embedded in paraffin blocks after I and II chloroforms. Sections of 7 μm thickness were made from the obtained blocks on a microtome MS-2, which were mounted on a glass slide, and stained with hematoxylin and eosin after drying [19].

To detect enterochromaffin cells, the diazo reaction was used as a staining method. Sections were deparaffinized and saturated with water. For 30 seconds, the sections were treated with a dilute solution (1 mg/ml) of stabilized 5-nitroanisidine diazotate (Fast Red salt B) in 0.1 M veronal acetate buffer (pH 9.2). They were thoroughly washed in running water. The nuclei were stained with Mayer's hematoxylin for 6 min followed by rinsing in running water for 30 min. After that, the sections were dehydrated in alcohols, enlightened in xylene, and embedded in balsam. On histological preparations, the granules of argentaffin cells were stained orange-red, nuclei — dark blue, and cytoplasmic structures — yellow [19].

The McManus PAS reaction was used to detect goblet cells. Sections were deparaffinized, saturated with water, and oxidized with 0.5% aqueous iodic acid solution for 2 min followed by rinsing in distilled water. They were treated with Schiff's reagent for 10 min and rinsed in running water for 10 min. The nuclei were counterstained with Mayer's hematoxylin for 3 min and rinsed thoroughly with running water. The sections were dehydrated in alcohols, enlightened in xylene, and embedded in balsam. The mucoproteins and neutral mucopolysaccharides were intensively stained in purple-red color.

Using the *Aperio Image Scope* software, the villi height, crypt depth, circular and longitudinal muscle layer thickness, and the number of goblet and enterochromaffin cells per 0.45 mm² in 5 fields of view were determined on histological specimens. Statistical processing of the results was performed using a one-factor ANOVA analysis of variance with Bonferroni correction. For this purpose, the StatPlus program (*Analyst Soft Inc.*, USA) was used. The results are presented as Mean $\pm$ SD. Differences between groups of animals were considered statistically significant at $P < 0.05$, $P < 0.01$, $P < 0.001$. Finished histological preparations were photographed using a *Leica DM-2500* microscope (Switzerland) with a *Leica DFC450C* camera and *Leica Application Suite Version 4.4* software. During the research, the requirements of ethical treatment with animals used in experimental studies were fully observed (Strasburg, 1986; Kyiv, 2002), and the research methodology itself was approved by the bioethical commission in the Institute of Animal Biology (National Academy of Sciences of Ukraine; Protocol no. 93-01 dated June 3, 2021).

Results and Discussion

Investigating the histo-morphometric parameters of the duodenum in the piglets' on day 7 of life, which consumed exclusively milk from the sow, it was found that the villi height was quite high and amounted to 696.35 μm , the crypt depth was 240.72 μm , consequently, the ratio of the villi height to the crypt depth was 2.89. It should be noted the thickness of the muscle membrane's outer longitudinal layer was 180.25 μm , and that of the inner circular layer — 146.96 μm .

Studying the morphological structure of the piglets' duodenum wall during the changes in feeding, we noted some differences in its morphometric parameters when introducing roughage into the diet and using the 'Globigen Jump Start' feed additive. Thus, in piglets of the control group, which consumed a standard nutrient-balanced diet from the 7th day of life, a decrease in the villi height by 29.53% was noted compared to 7 days before feeding. Whereas in piglets of the experimental group, which were fed with the 'Globigen Jump Start' additive, an insignificant decrease in the villi height was observed, compared to the same indicator on the 7th day (by 22.04 μm) and a significant increase in comparison with the control group on the 14th day by 37.42% respectively (table 1).

A similar trend was observed with the crypt depth parameters. In piglets on day 14 of the experiment in the control group, the crypt depth was 314.13 μm , while in the experimental group it was 413.02 μm , which is 31.48% higher than in the control one. On the 28th day of the experiment, piglets of both groups showed slight differences in the villi height: 654.51 μm in the control group and 675.32 μm in the experimental one. The crypt depth in the control group was significantly higher ($P<0.01$) compared to that on day 14 and equaled to 528.96 μm . In the experimental group, the crypt depth was 423.03 μm , i.e. literally unchanged. The total thickness of the duodenal wall muscle layers during the experiment also correlated a bit, which probably happened due to the beginning

of roughage consumption. On day 7 of life, before feeding additive, the muscle wall was 327.21 μm , on day 14 — 327.93 μm in the control group and 404.44 μm in the second experimental group; on day 28 — 426.76 μm and 405.25 μm in the control group and experimental one respectively (table 1).

Some correlation was also found when counting the number of goblet and enterochromaffin cells of the duodenal mucosa on the area of 0.45 mm² (fig. 1). According to table 2, the number of goblet cells in piglets on day 7 of life amounted to 80 cells. A slightly lower number of them was found on day 14 with the introduction of dry feed and the 'Globigen Jump Start' additive, namely 67.8 and 71.0 cells respectively. At the same time, on day 28 of the experiment, a significant increase in the number of goblet cells was observed, compared to day 14 in both the control and experimental groups ($P<0.05$). In the control group, the number of goblet cells was 94.6, and in the experimental one — 96.3 cells.

Analyzing the number of enterochromaffin cells, it was noted that 7-day-old piglets had the lowest number of them, namely 7.8 cells. On day 14, in both groups, their increase was noted, by 2.56% and 7.69% respectively, compared to day 7 (fig. 2). A significant increase in the number of these cells was noted on day 28 of the experiment. Actually, the number of enterochromaffin cells was 9.8 in the control group and 12.4 in the experimental one ($P<0.05$).

Table 1. Morphometric parameters of the piglets' duodenal wall in the control and experimental groups fed with the 'Globigen Jump Start' additive (n=5, M $\pm$ SD)

Morphological structures	7 th day	14 th day		28 th day	
	Before feeding	Control group	Experimental group	Control group	Experimental group
Villi height, μm	696,35 $\pm$ 43,94	490,67 $\pm$ 30,22**	674,31 $\pm$ 25,05	654,51 $\pm$ 14,81***	675,32 $\pm$ 12,44
Crypt depth, μm	240,72 $\pm$ 11,49	314,13 $\pm$ 21,54**	413,02 $\pm$ 10,03***	528,96 $\pm$ 20,12***	423,03 $\pm$ 18,35
Villi height to crypt depth ratio (V:C)	2,89	1,56	1,63	1,24	1,59
Thickness of the muscle membrane's outer longitudinal layer	146,96 $\pm$ 1,97	143,27 $\pm$ 2,09	194,02 $\pm$ 6,97***	166,31 $\pm$ 3,90***	160,08 $\pm$ 3,07
Thickness of the muscle membrane's inner circular layer	180,25 $\pm$ 2,45	184,66 $\pm$ 3,34	210,42 $\pm$ 4,94***	260,45 $\pm$ 12,76***	245,17 $\pm$ 10,54
Muscle membrane thickness	327,21 $\pm$ 2,12	327,93 $\pm$ 2,07	404,44 $\pm$ 4,23	426,76 $\pm$ 7,24	405,25 $\pm$ 5,23

Note. Here and further: * — $P<0.05$, ** — $P<0.01$, *** — $P<0.001$ compared to the previous day of the experiment.

Table 2. The average number of enterochromaffin and goblet cells in the duodenal mucosa of piglets fed with the 'Globigen Jump Start' additive (n=5, M $\pm$ SD)

Parameters	7 th day	14 th day		28 th day	
	Before feeding	Control group	Experimental group	Control group	Experimental group
Number of goblet cells	80,0 $\pm$ 7,47	67,8 $\pm$ 5,38	71,0 $\pm$ 4,73	94,6 $\pm$ 8,35*	96,3 $\pm$ 5,36*
Number of enterochromaffin cells	7,8 $\pm$ 0,86	8,0 $\pm$ 0,70	8,4 $\pm$ 1,02	9,8 $\pm$ 0,73	12,4 $\pm$ 1,69*
Enterochromaffin cells' nucleus volume	108,19 $\pm$ 5,80	109,72 $\pm$ 3,51	110,8 $\pm$ 2,17	111,3 $\pm$ 3,43	112,1 $\pm$ 1,78

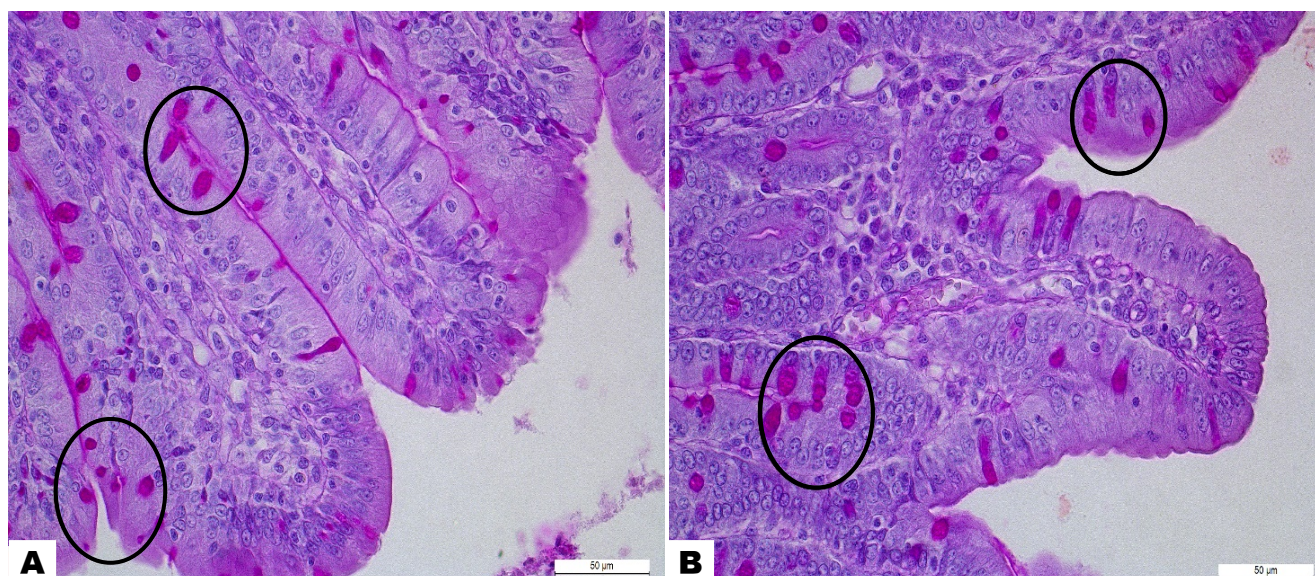


Fig. 1. Duodenal mucosa of piglets on day 14 of the experiment. A — control group, B — experimental group. Goblet cells are marked in a circle. PAS reaction

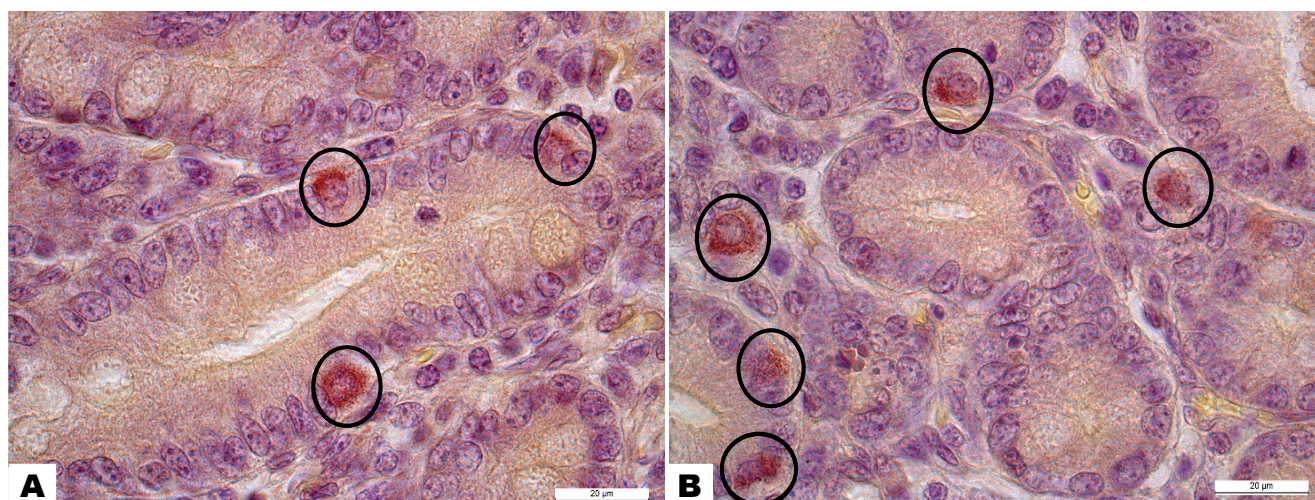


Fig. 2. Piglets' duodenum on day 14 of the experiment. a — control group, b — experimental group. Enterochromaffin cells are marked in a circle. Diazo staining method

The intestine performs a variety of functions, including providing a primary site for digestion and absorption of nutrients, as well as acting as a selective barrier against exogenous harmful substances. It is the integrity of the intestinal mucosa that is a key to the digestion and absorption of nutrients in piglets. Intestinal morphology, including villi height, crypt depth, and the ratio of villi height to crypt depth, clearly reflects the intestinal mucosa condition and its function. A decrease in villi height and crypt depth indicates a certain dysfunction of the mucous membrane, namely a decrease in its absorption capacity [32, 37]. Conversely, higher values of villi height and crypt depth indicate a better intestinal function. Changes in the structure of villi crypts are usually noted in weaned piglets.

Researchers described villi destruction, crypt hyperplasia, and intestinal mucosal atrophy, which destroyed

the barrier function of the intestinal mucosa [13, 28, 30, 31]. For example, the study by Bomba et al. [3] 21 showed that 5 days after weaning (33 days of age), the villi height and crypt depth in the piglets' ileum were significantly lower than before weaning (28 days of age). Similarly, Hu et al. [14] showed that a decrease in villi height was caused by weaning, during which villi height and crypt depth were lower on days 3 and 7 after weaning, compared to the pre-weaning stage. In addition, Boudry et al. reported [4] that weaning stress caused long-term structural changes in the piglet ileum and villi height was still significantly lower on the 15th day than before weaning.

Additionally, it was shown that weaning stress caused a decrease in the relative weight of the small intestine, with the total weight on the 15th day after weaning being only 50% of the pre-weaning weight. Taken together, early

weaning and abrupt transition to roughage usually lead to morphological damage to the intestinal wall in piglets, namely a decrease in villi height, crypt depth, and relative intestinal weight. Therefore, in order to optimize the absorption of feed nutrients in piglets, it is necessary to minimize the impact of weaning stress.

Some morpho-functional differences in certain cytostructural and histostructural morphometric parameters of the duodenal wall in piglets during the neonatal and postnatal periods of development using the 'Globigen Jump Start' additive convincingly indicate a positive effect on mitigating the weaning stress. This was manifested by a decrease in the quantitative and linear indicators of histoarchitectonics of certain morphological components in the intestinal wall. It should be noted that the components of the feed additive have a positive effect on the synthesis of mucopolysaccharides, which is manifested by a significant increase in goblet endocrinocytes, especially in the experimental group.

It is known that mucins are synthesized mainly by goblet cells in the intestine [2, 16, 26]. Thus, any factors that affect goblet cell differentiation will also affect intestinal mucin secretion. Some authors report that weaning stress damages the differentiation of secretory cells, which leads to a decrease in mucin secretion [34, 38]. For example, Hedemann and Jensen [12] indicated that early weaning not only led to a decrease in intestinal mucin secretion, but also changed the structure of mucin glycosylation, weakening the function of the intestinal barrier and increasing the likelihood of developing intestinal infection.

Similarly, Yang et al. reported [38] that the MUC2 gene was negatively regulated in weaned piglets, suggesting that weaning stress destroyed barriers in the intestinal tract. Normal secretion and expression of mucins are essential for maintaining intestinal barrier function. When intestinal mucin secretion decreases, the mucosal layer of the intestinal mucosa becomes thinner and pathogens can easily pass through it. Moreover, those pathogens that compete with normal microflora on the intestinal mucosa surface for adhesion sites can destroy the normal microbial barrier. Finally, such pathogenic microorganisms as *Salmonella* and *Shigella* can destroy the mechanical barrier of the intestinal mucosa, inducing apoptosis of intestinal epithelial cells and disrupting the distribution of proteins between intestinal mucosal cells. In addition, changes in mucin secretion and expression can cause inflammation and damage the immune barrier of the intestinal mucosa [9].

Our studies have shown an increase in the number of goblet cells in the duodenum of piglets in the experimental group, both on day 14 and 28 of the experiment. Thus, on day 14, the number of goblet cells in the second experimental group increased by 4.71% compared to the control group, and by 1.79% on day 28, respectively. An increase in the number of enterochromaffin cells and the diameter of their nuclei in the experimental group indicated an increased synthesis of catecholamines and hormones, which had a very positive effect on the immune

and physiological status of piglets, also emphasizing the crucial role of serotonin in neuronal metabolism and stress resistance formation.

The thickness of the duodenal wall muscle layer, in our opinion, increases due to the active peristalsis, which is enhanced by the action of some catecholamines, which are synthesized in a slightly larger amount under the influence of nutrients that make up the 'Globigen Jump Start' feed additive. The very fact of changes in the morphometric parameters of the villi height in the control group, namely a significant decrease on day 14, can be explained by a sharp change in the diet, which manifested itself morphologically and functionally. A slight change in the villi height on day 14 of the experiment should be considered a manifestation of the protective effect caused by the 'Globigen Jump Start' additive.

The increase in the crypt depth on day 14 indicates a high proliferative activity of enterocytes, slightly lower morphometric parameters in a control group indicate a lower proliferative activity, which was manifested by a decrease in the villi height, compared to the values obtained during the experiment. Analysing the indicators of crypt depth on day 28 in the control group of piglets, we can outline an interesting trend that was manifested by a slight increase in crypt depth, compared to day 14 of the experiment. This means the absence of the negative effect caused by weaning stress and the mild effect of switching to another feeding type, which provided the relative stability of linear morphometric parameters. Comparing the crypt depth in the control group between days 14 and 28, we revealed their increase, which is associated with the improved proliferative and regenerative activity of the epithelium. This should be regarded as a compensatory mechanism of a somewhat delayed type, compared to the experiment in which the following dynamics were observed: relative constancy of villi height and relatively stable morphometric parameters of crypt depth compared to day 14, which is a normal physiological condition for the intestinal mucosa under a minor feeding stress.

According to the results of histo-morphometric studies of the piglets' duodenal mucosa during different periods of postnatal and neonatal ontogenesis under the influence of the 'Globigen Jump Start' feed additive, a significant increase in the villi height was revealed in the experimental group, compared to the control one on day 14 by 37.42%, with the height's gradual leveling on day 28 of the experiment, indicating the mitigation of weaning stress.

The increase in morphometric parameters, namely, the number of enterochromaffin cells, goblet cells, and intestinal wall muscle thickness in piglets of the experimental group fed with the 'Globigen Jump Start' additive, compared to the control group, indicates an increased synthesis of catecholamines, hormones, and mucopolysaccharides, which positively affect the immune and physiological status of piglets stimulating their growth.

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Морфометричні особливості стінки дванадцятипалої кишки поросят у різні періоди постнатального та неонатального онтогенезу за впливу кормової добавки «Глобіген джамп старт»

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Морфологічні параметри слизової оболонки тонкого відділу кишечника, такі як розміри ворсинок і крипт відіграють ключову роль у формуванні абсорбційної поверхні кишкової трубки. Кількість келихоподібних та ентерохромафінних клітин вказують на функціональний стан епітелію, стосовно секреції слизу та продукції катехоламінів та гормонів (серотонін, дофамін). Саме тому актуальним є вивчення та визначення окремих морфометричних показників стінки дванадцятипалої кишки поросят у різні періоди неонатального та постнатального розвитку, особливо в період стресу-відлучення та переходу на білковий тип годівлі. У статті представлені результати досліджень впливу кормової добавки «Глобіген джамп старт» на гісто-морфологічні параметри слизової оболонки дванадцятипалої кишки поросят на 7, 14 та 28 добу життя. Встановлено позитивний вплив на пом'якшення стресу-відлучення, що проявлявся зниженням кількісних та лінійних показників гістоархітекtonіки окремих морфологічних компонентів кишкової стінки. Вірогідне збільшення келихоподібних ендокриноцитів, особливо в дослідній групі, позитивно впливало на синтез мукополісахаридів. Фізіологія шлунково-кишкового тракту поросят передбачає складну взаємодію між центральною нервовою системою, метасимпатичною нервовою системою, APUD-системою, ендокринною системою, за допомогою яких відбувається передача інформації за механізмами зворотного та прямого зв'язку в регулюванні функції шлунково-кишкового тракту. Збільшення кількості ентерохромафінних клітин, діаметру їх ядер в дослідній групі поросят вказувало на посилений синтез катехоламінів та гормонів, які вкрай позитивно впливають на імунний та фізіологічний статус поросят, а також надважливу роль серотоніну в нейрональному обміні та формуванні стресостійкості. Збільшення товщини м'язового шару стінки дванадцятипалої кишки, на нашу думку, відбувалось за рахунок активної перистальтики, що посилювалась під дією деяких катехоламінів, які синтезувались в дещо більшій кількості під впливом нутрієнтів, з котрих складається кормова добавка «Глобіген джамп старт».

Ключові слова: поросята, дванадцятипала кишка, ворсинки, крипти, келихоподібні клітини, ентерохромафінні клітини, дріжджі, яєчний порошок



From Chilean saltpeter to modern agriculture: navigating nitrate toxicity in ruminants through compartmental modeling

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OIY: Conceptualization; Project management; Methodology; Formal analysis; Research; Resources; Data curation; Writing — original draft, review & editing; Visualization.

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Nitrate/nitrite poisoning is a significant issue in ruminant livestock health, with historical roots predating the widespread use of nitrogen fertilizers. This review explores the various factors contributing to nitrate toxicity, including natural and anthropogenic sources, metabolic pathways, mechanisms of action, and the variability in reported data. The importance of compartmental modeling in understanding nitrate metabolism dynamics is emphasized. These models provide a framework for simulating the complex processes involved in nitrate intake, conversion, absorption, distribution, and excretion, ultimately informing effective mitigation strategies. The goal of this article is to provide a comprehensive overview of nitrate/nitrite poisoning in ruminants and highlight the role of compartmental modeling in safeguarding animal health, optimizing agricultural practices, and ensuring food safety in the context of modern agriculture.

Key words: nitrate toxicity, ruminants, compartmental modeling, methemoglobinemia, feed management

Introduction

Nitrate/nitrite poisoning has been documented long before the widespread use of nitrogen fertilizers. Acute nitrate poisoning in cattle was reported as early as 1895 [11]. All plants contain some nitrates, and their concentration can be influenced by climatic factors such as cold and drought [14]. The use of nitrogen fertilizers also impacts nitrate levels in plants, with high amounts being a significant factor in nitrate accumulation [3]. Early spring growth usually contains more nitrates than summer plants due to rapid growth [10]. Roots and stems of plants generally have higher nitrate levels than leaves and seeds [10].

Excessive use of poultry litter or animal manure as fertilizers can lead to nitrate accumulation in soil, subsequently affecting plant feeds consumed by herbivores [20]. Nitrates can reach dangerous levels in ponds, shallow wells, or streams due to agricultural runoff and nitrogen fertilizers. Industrial wastewater is another source of

nitrates [5]. Plants irrigated with treated wastewater, rich in nitrogen, can accumulate large amounts of nitrates [9].

Ruminant animals, such as cattle and sheep, are sensitive to nitrate poisoning. Bacteria in their rumen convert nitrates to nitrites [19]. Nitrates can be absorbed through the ruminant epithelium via a $\text{Cl}^-/\text{HCO}_3^-$ exchange mechanism [1]. Nitrate toxicity in ruminants occurs when high nitrate levels in feed overwhelm the animal's digestive system, causing nitrate-to-nitrite conversion to exceed nitrite-to-ammonia conversion, which is part of amino acid and protein synthesis [7]. Nitrate itself has low toxicity compared to nitrite, which causes the formation of methemoglobin (MetHb), a molecule with very limited oxygen-carrying capacity. Methemoglobinemia, resulting from MetHb formation, is a primary adverse effect. MetHb gives blood a brown-black color [18]. Species differences in MetHb formation rates are mainly due to the degree and speed of nitrate-to-nitrite reduction, which is highest in ruminants, lower in horses, and lowest in other monogastric species.

The mechanism of action (MoA) of nitrates and nitrites includes several effects in agricultural and domestic animals, such as increased oxidative stress, thyroid function suppression, and lowered blood pressure. Other effects (e.g., depletion of vitamins A and E, abortions, and fertility impacts) remains to be elucidated [4]. High nitrite levels in the blood cause vasodilation, blood pressure drop, and circulatory shock.

Different authors from various countries report varying nitrate/nitrite content analysis results. This diversity can lead to confusion and misinterpretation of the data. Furthermore, literature sources often employ different methods for nitrite detection, each with varying accuracy and sensitivity, which can further complicate result interpretation. Therefore, standardized recommendations and a unified understanding of laboratory methods are essential for comprehending the impact of nitrates on animal organisms [15].

To address these challenges and enhance our understanding of nitrate metabolism in ruminants, the development and application of compartmental models are crucial. These models provide a framework for simulating the complex processes of nitrate intake, conversion to nitrite, absorption, distribution, and excretion within the animal's body [8].

The overarching goal of this article is to present a comprehensive review of nitrate/nitrite poisoning in ruminants, encompassing its historical context, sources of exposure, metabolic pathways, mechanisms of action, and the variability in reported data. Additionally, we aim to highlight the importance of compartmental modeling in understanding nitrate metabolism and its potential for informing evidence-based interventions to safeguard animal health and productivity. This is of paramount relevance in the context of modern agriculture, where nitrogen fertilizer use and intensive animal production practices can inadvertently elevate nitrate levels in the environment, posing a risk to livestock. By unraveling the complexities of nitrate toxicity and leveraging modeling approaches, we can strive towards sustainable agricultural practices that prioritize animal welfare and minimize the adverse impacts of nitrate exposure.

Results and Discussion

1. Physicochemical properties of nitrates, nitrites, and certain specific salts, and their use in animal husbandry, agriculture, and the food industry

On January 19, 2020, the Law of Ukraine "On Feed Safety and Hygiene" came into effect, stipulating that any person can use feed additives provided that such an additive is registered in Ukraine. However, the state registration procedure for feed additives was only unblocked on September 1, 2021. To prevent feed shortages, amendments to the Law of Ukraine "On Feed Safety and Hygiene" were made through the draft Law no. 3672, allowing the unre-

stricted use of feed additives registered in the European Union. According to the EU Feed Additives Register under Regulation (EC) no. 1831/2003, sodium nitrite (E 250) is a permitted feed additive. Additionally, sodium nitrate (E 251) and potassium nitrate (E 252) are included in the list of food additives approved for use in food products in Ukraine and are part of the EU Feed Additives Register as per Annex II of Regulation (EC) No 1333/2008.

In acidic conditions, nitrite can form N-nitroso compounds (NOCs), including genotoxic and carcinogenic N-nitrosamines, by reacting with certain secondary amines in feed or endogenously in the stomachs of animals.

The physicochemical properties of nitrates, nitrites, and certain specific salts used in food products, feed, and fertilizers are shown in table 1.

The physicochemical properties of these compounds, such as solubility in water and their oxidizing nature, make them versatile in various applications. In the food industry, nitrates and nitrites are used as preservatives to prevent the growth of harmful bacteria and to maintain the color and flavor of processed meats. In agriculture, these compounds are essential components of fertilizers, contributing to the growth and health of plants. Additionally, in animal husbandry, they are used as feed additives to enhance animal growth and health.

Nitrates are ubiquitous in the environment and play a crucial role in the nitrogen cycle, forming significant deposits, particularly as sodium nitrate (NaNO_3) in specific regions. The most notable industrial deposit is associated with the accumulation of caliche in the Atacama Desert, Chile, which has led to NaNO_3 being sometimes referred to as Chilean saltpeter.

The use of nitrates is extensive. They are employed in animal feeds and food products, notably in the production of fertilizers and food preservatives. Generally, nitrates are highly soluble in water and serve as vital nutrients for plants, being present in all plant species, especially in green leafy vegetables. Sodium nitrate and potassium nitrate are particularly significant for feeds and food products.

Sodium nitrate (NaNO_3) is a white, crystalline, slightly hygroscopic powder that serves as an antimicrobial agent, preservative, and color fixative. Sodium nitrate is used in the treatment of cyanide poisoning, in combination with sodium thiosulfate, recommended only for severe cases of cyanide poisoning. Sodium nitrate is also utilized in fertilizers, pyrotechnics, and in the glass industry [2].

Potassium nitrate (KNO_3): this white crystalline powder or transparent prisms have a cooling, salty, pungent taste. It is used as a preservative and in combination with nitrite salts in curing mixtures (e.g., sodium chloride solutions) for meat to develop and fix the color of the meat, inhibit microbial growth, and develop characteristic flavor profiles. In veterinary medicine, potassium nitrate is used as a diuretic for pigs, cattle, and horses.

Two other nitrates widely used, especially as fertilizers, are calcium nitrate ($\text{Ca}(\text{NO}_3)_2$) and ammonium nitrate (NH_4NO_3). Anhydrous calcium nitrate is colorless and hygroscopic, readily absorbing moisture to form

Table 1. Physicochemical properties and applications of nitrates, nitrites, and certain specific salts

Compound	Empirical formula	Properties	Applications
Nitrate			
Nitrate Sodium (Sodium Nitrate, E 251)	NaNO ₃	White crystalline solid, highly soluble in water, oxidizing agent	Used as a preservative in food, feed additive, fertilizer
Nitrate Potassium (Potassium Nitrate, E 252)	KNO ₃	White crystalline solid, highly soluble in water, oxidizing agent	Used in food preservation, fertilizers, and fireworks
Nitrate Calcium (Calcium Nitrate, anhydrous)	Ca(NO ₃) ₂	White granular or crystalline solid, highly soluble in water	Used in fertilizers, concrete additives, and cold packs
Nitrate Calcium (Calcium Nitrate, tetrahydrate)	Ca(NO ₃) ₂ · 4H ₂ O	White crystalline solid, highly soluble in water, contains water of crystallization	Used in fertilizers, wastewater treatment, and cold packs
Nitrate Ammonium (Ammonium Nitrate)	NH ₄ NO ₃	White crystalline solid, highly soluble in water, strong oxidizer	Used in fertilizers, explosives, and in veterinary medicine
Nitrite			
Nitrite Sodium (Sodium Nitrite, E 250)	NaNO ₂	White to slightly yellowish crystalline powder, soluble in water, can act as both an oxidizing and reducing agent	Used as a food preservative, curing agent, feed additive, and in pharmaceuticals
Nitrite Potassium (Potassium Nitrite)	KNO ₂	White to yellow crystalline powder, soluble in water, strong reducing agent	Used as a curing agent in the food industry, in pharmaceuticals, and in the production of heat transfer salts, dyes, and other chemicals.

tetrahydrates. Ammonium nitrate is a colorless crystalline salt used in fertilizers and explosives.

Nitrite (NO₂⁻): the nitrite anion is present in inorganic nitrite salts. Nitrite naturally occurs in the environment as part of the nitrogen cycle, typically in very low concentrations. It is formed naturally by nitrifying bacteria as an intermediate stage in nitrate formation.

The most important nitrites in feeds and food are sodium nitrite (NaNO₂) and potassium nitrite (KNO₂).

Sodium nitrite (NaNO₂): this compound appears as a white or slightly yellow crystalline powder, hygroscopic, with a melting point of 270°C, and decomposes above 320°C. It slowly oxidizes to sodium nitrate in the air. Sodium nitrite has various applications, including the production of numerous compounds. In medicine and veterinary practice, it has been used as a vasodilator, bronchodilator, and as an antidote for cyanide poisoning. In veterinary medicine, it is applied topically on the teats of dairy cows post-milking to prevent mastitis. Sodium nitrite is permitted as a food additive (E 250) to stabilize the color of cured fish and meat and to inhibit the growth of *Clostridium botulinum*, the bacterium that produces botulinum toxin.

Nitrate levels and toxicity in unified metrics

Reading reports on nitrate levels in various literatures reveals different parameters for determining nitrate contamination. The results of nitrate analysis can be misleading due to differences in the methods of presenting the results. In chemical analysis of nitrates, the actually determined element is oxidized nitrogen. However, values can be reported as a percentage of nitrates (NO₂⁻), nitrate-nitrogen (N–NO₃), or as a percentage of potassium nitrate (KNO₃) concentration.

Elevated nitrogen doses and nitrate accumulation in forage are presented as concentration of nitrate nitrogen N–NO₃ [6]. Authors [12], accumulation of N–NO₃ above 0.07% in dry matter is considered harmful, 0.07–0.2% can lead to poisoning in cattle, and more than 0.25% can be lethal. Other authors determined nitrate concentrations as concentrations of NO₃⁻ ions [16]. Similarly, nitrate concentrations can be determined as concentrations of potassium nitrate (KNO₃) [21].

For unification and interpretation of the results presented in the literature, formulas are used for converting between different measurement units:

$$\begin{aligned}
 \text{KNO}_3 &= \text{N–NO}_3 \times 7.22 \\
 \text{KNO}_3 &= \text{NO}_3^- \times 1.63 \\
 \text{NO}_3^- &= \text{N–NO}_3 \times 4.43 \\
 \text{NO}_3^- &= \text{KNO}_3 \times 0.613 \\
 \text{N–NO}_3 &= \text{KNO}_3 \times 0.139 \\
 \text{N–NO}_3 &= \text{NO}_3^- \times 0.226
 \end{aligned}$$

Thus, for accurate interpretation of results in the literature, it must be clearly stated in which units the nitrate concentration was determined. Predominantly, nitrate analysis and information on safe concentrations in feed and water are presented as the concentration of NO₃⁻ ions (mg per kg dry matter). However, in modern literature, results can also be found in percentages or parts per million (ppm). The following formulas are provided for conversion to interpret the results:

- 1000 mg/kg = 0.1%
- 750 mg/kg = 0.075%
- 0.1% = 1000 ppm
- 2100 ppm = 2100 mg/kg
- 300 ppm = 0.3 mg/L (for water)

Calculation examples:

$$0.1\% \text{ N-NO}_3 = 0.443\% \text{ NO}_3;$$

$$0.1 \times 4.43 = 4430 \text{ ppm NO}_3 = 4430 \text{ mg/kg}$$

$$0.44\% \text{ NO}_3 = 0.099\% \text{ N-NO}_3;$$

$$0.44 \times 0.226 = 994 \text{ ppm N-NO}_3 = 994 \text{ mg/kg}$$

Almost all feeds contain some amount of nitrates. Since herbivores, especially ruminants, consume diets with high levels of nitrates, they are potentially among the most sensitive species to nitrite poisoning due to their inherent microbial ability to convert nitrates to nitrites. However, under normal circumstances, problems do not arise because the resulting nitrite is quickly removed through bacterial fermentation for anabolism, forming ammonia, amino acids, and proteins.

2. Sensitivity of methods for determining nitrates: spectral analysis, nitrite meter, and electrode analysis

The determination of nitrate levels is essential for assessing environmental and agricultural impacts. Various methods are employed to measure nitrates, each differing in sensitivity, accuracy, and application. Here, we discuss the sensitivity of three common methods: spectral analysis, nitrite meters, and electrode analysis.

Spectral analysis

Spectral analysis, including techniques such as UV-Vis spectrophotometry, is widely used for nitrate determination [23]. This method is based on the absorption of light by nitrate ions at specific wavelengths. The advantages of spectral analysis include:

- **High sensitivity:** UV-Vis spectrophotometry can detect nitrates at low concentrations, typically in the range of 0.1 to 5.0 mg/L.
- **Precision:** this method provides precise and repeatable results, making it suitable for laboratory use.
- **Non-destructive:** spectral analysis is a non-destructive technique, preserving samples for further analysis.

However, spectral analysis requires clear, colorless samples and proper calibration to avoid interference from other absorbing species.

Nitrite Meter

Nitrite meters are portable devices designed for field use to measure nitrate levels directly. These meters use colorimetric or electrochemical principles to provide quick and easy readings. Key features include:

- **Moderate sensitivity:** nitrite meters can detect nitrates in concentrations ranging from 0.5 to 100 mg/L, depending on the device.
- **Portability:** these meters are compact and easy to use in various settings, including on-site field measurements.
- **Rapid results:** nitrite meters offer fast results, often within minutes, making them suitable for real-time monitoring.

While convenient, nitrite meters may have limitations in terms of accuracy and potential interferences from other ions present in the sample.

Electrode Analysis

Electrode analysis, such as using ion-selective electrodes (ISEs), is another method for determining nitrate concentrations [17]. This technique measures the potential difference generated by nitrate ions in a solution. Its benefits include:

- **High sensitivity:** ISEs can detect nitrates at very low concentrations, often below 0.1 mg/L.
- **Specificity:** ion-selective electrodes are designed to respond specifically to nitrate ions, reducing the risk of interference from other ions.
- **Versatility:** electrode analysis can be performed in various sample types, including soil, water, and plant tissues.

However, electrode analysis requires regular calibration and maintenance to ensure accurate readings. It may also be influenced by the ionic strength and pH of the sample.

Each method for determining nitrate levels has its strengths and limitations. Spectral analysis offers high sensitivity and precision but is best suited for laboratory environments. Nitrite meters provide rapid and portable measurements but may sacrifice some accuracy. Electrode analysis combines high sensitivity and specificity but requires careful calibration and maintenance.

The choice of method depends on the specific requirements of the analysis, including the desired sensitivity, accuracy, and practicality for field or laboratory use. By understanding the capabilities and limitations of each method, researchers and practitioners can select the most appropriate technique for their needs.

The impact of consuming feeds with significant nitrate concentrations on animals depends on various factors related to the animals' condition.

Ruminants can tolerate a wide range of nitrate levels depending on several factors. Factors that make nitrates less toxic include:

1. **Gradual adaptation:** animals can be conditioned to consume higher amounts of nitrate-rich feed if the increase is gradual.
2. **Health status:** healthy animals are less susceptible to adverse effects than animals with poor health.
3. **Adequate carbohydrates:** an adequate supply of carbohydrates (grain) allows animals to consume more nitrates, as carbohydrates enhance the conversion of nitrates to microbial protein.

Factors that make nitrates more toxic include [13]:

1. **Rapid dietary changes:** a sudden change in diet can trigger nitrate poisoning.
2. **Parasitism or anemia:** conditions causing anemia or parasitism increase susceptibility.
3. **Multiple sources of nitrates:** nitrates in more than one dietary component (e.g., water and feed).

4. Rate of feed consumption: the speed at which feed is consumed can influence toxicity.

Regardless of the nitrate levels present in feeds, there are two types of potential toxicity that cause concern:

1. **Acute or lethal toxicity:** this occurs when daily nitrate concentrations ingested by the animal exceed the conversion rate to nitrites, leading to rapid nitrite accumulation.
2. **Chronic or non-lethal toxicity:** this occurs when prolonged exposure to nitrate-rich feed results in nitrite accumulation and absorption into the bloodstream, causing chronic effects.

These effects depend on the duration of consumption of toxic feed. Specifically, the table 2 presents various effects based on nitrate concentration per kg of body weight ingested daily for cattle.

3. A compartmental model for nitrate metabolism in ruminants: relevance and potential insights

3.1. Relevance of modeling nitrate metabolism

The study of nitrate metabolism in ruminants is crucial due to the significant role these animals play in agriculture and food production. Ruminants, such as cattle and sheep, are particularly sensitive to nitrate toxicity because of their unique digestive systems. The bacteria present in the rumen can reduce nitrates to nitrites, which, in excessive amounts, can lead to methemoglobinemia, a condition that affects the oxygen-carrying capacity of the blood [22].

Modeling nitrate metabolism in ruminants provides several important benefits:

Risk assessment and management: by understanding how nitrates are metabolized and accumulated in ruminants, farmers and veterinarians can better manage feed and forage to prevent nitrate toxicity. This is particularly important in regions where nitrate levels in plants and water can fluctuate significantly.

Optimizing feed composition: a compartmental model allows for the simulation of different dietary

scenarios, helping in the formulation of feeds that minimize nitrate accumulation while ensuring nutritional adequacy. This can lead to the development of feeding strategies that enhance animal health and productivity.

Environmental impact assessment: modeling can help assess the environmental impact of nitrate usage in agriculture. By understanding how nitrates are processed in ruminant systems, it is possible to predict the fate of nitrates excreted in manure, which can contribute to nitrate pollution in soil and water.

Guiding agricultural practices: insights from the model can inform agricultural practices, such as the timing and amount of fertilizer application, to ensure that nitrate levels in forage crops remain within safe limits. This can lead to more sustainable farming practices that protect both animal health and the environment.

Public health and food safety: ensuring that nitrate levels in meat and dairy products are within safe limits is essential for public health. Modeling nitrate metabolism helps in understanding how nitrates and their metabolites accumulate in animal tissues, which is vital for food safety regulations and standards.

3.2. Compartmental model for nitrate metabolism in ruminants

Compartmental Model:

- **Rumen compartment C_0 :** nitrite concentration in the rumen (mg)
- **Rumen compartment C_1 :** this compartment represents the rumen, where nitrates are ingested through contaminated feed or water. In the rumen, nitrates are rapidly reduced to nitrites by microbial action. Nitrate concentration in the rumen (mg)
- **Blood compartment C_2 :** this compartment represents the bloodstream, where absorbed nitrites are distributed. Nitrite concentration in the blood (mg)
- **Metabolism and excretion compartment C_3 :** this compartment represents the metabolism and excretion of nitrates and nitrites from the body. Nitrite concentration in the tissues (mg)

Table 2. Effects of nitrate concentration on cattle

Total daily dose (mg NO_3^- /kg body weight)	Total daily dose (mg $\text{N}-\text{NO}_3^-$ /kg body weight)	Total daily dose (mg KNO_3 /kg body weight)	Effect
<97.7	<22.0	<97.7	Generally safe under all conditions.
97.7–195.3	22.0–44.0	97.7–195.3	Safe for most animals. May be unsafe for pregnant and very young animals.
195.3–293	44.0–66.1	195.3–293	Potentially unsafe for pregnant and young animals. May cause reduced appetite, slow growth, abortions, and decreased milk production. Research suggests a decrease in milk production of approximately 15.9 mg NO_3^- /kg body weight.
293–390.7	66.1–88.2	293–390.7	Unsafe level of intake for all animals. Reduced appetite, vitamin A deficiency, abortions, and overall reduced productivity.
>390.7	>88.2	>390.7	Acute toxicity possible in most animals. In dairy cattle, 45.4 mg NO_3^- /kg body weight can result in 50% mortality.

The kinetic equations for this compartmental model can be described as follows:

1. Rumen (Nitrite) :

$$dC_0 / dt = k_{conv} * C_1 - k_{01} * C_0$$

dC_0 / dt — the rate of change of nitrite concentration in the rumen over time;

$k_{conv} * C_1$ — the rate of nitrite formation in the rumen due to the conversion of nitrate.

2. Rumen (Nitrate):

$$dC_1 / dt = -k_{12} * C_1 - k_{conv} * C_1$$

dC_1 / dt — the rate of change of nitrate concentration in the rumen over time;

$-k_{12} * C_1$ — the rate of nitrate transfer from the rumen to the blood;

$-k_{conv} * C_1$ — the rate of nitrate conversion to nitrite within the rumen.

3. Blood:

$$dC_2 / dt = k_{12} * C_1 + k_{01} * C_0 - k_{23} * C_2$$

dC_2 / dt — the rate of change of nitrite concentration in the blood over time;

$k_{12} * C_1$ — the rate of nitrite entering the blood from the rumen (after nitrate conversion);

$k_{01} * C_0$ — the rate of nitrite entering the blood directly from the rumen;

$-k_{23} * C_2$ — the rate of nitrite absorption from the blood into the tissues.

4. Tissues:

$$dC_3 / dt = k_{23} * C_2 - k_{30} * C_3$$

dC_3 / dt — the rate of change of nitrite concentration in the tissues over time;

$k_{23} * C_2$ — the rate of nitrite absorption from the blood into the tissues;

$-k_{30} * C_3$ — the rate of nitrite excretion/metabolism from the tissues.

Description:

- These differential equations describe the dynamics of nitrate and nitrite concentrations in different compartments within the ruminant animal's body.
- The model accounts for the conversion of nitrate to nitrite in the rumen, the transfer of nitrite from the rumen to the blood, the absorption of nitrite from the blood into tissues, and the excretion/metabolism of nitrite from tissues.
- The rate constants (k_{12} , k_{23} , k_{30} , k_{conv}) determine the intensity of each process and can be adjusted based on experimental data or physiological parameters of the animals.

This model is a simplified representation of a complex biological process. For more accurate results, it's essential to consider individual animal characteristics, their diet,

and other factors that can influence nitrate metabolism. Using the NumPy-based SciPy library in code written in Python, the proposed system of ordinary differential equations (ODEs), which are the core of our compartmental model, was solved. The results are shown in fig.

The figure displays the change in nitrite concentration within the rumen over a 24-hour period. The model demonstrates the conversion of nitrate to nitrite in the rumen and the subsequent transfer of nitrite to the bloodstream. The initial spike in nitrite concentration is due to the rapid conversion of nitrate, followed by a gradual decrease as nitrite is absorbed into the blood.

$k_{12} = 0$: this eliminates the direct transfer of nitrate from the rumen to the blood, preventing its dilution and potential detoxification in the liver.

$k_{conv} = 0.9$: this significantly increases the rate of conversion of nitrate to nitrite in the rumen, leading to a rapid accumulation of nitrite.

$C_{10} = 400.0$: the high initial dose of nitrate provides ample substrate for the rapid conversion to nitrite.

The combination of these factors results in a scenario where nitrite rapidly accumulates in the rumen and then overwhelms the animal's ability to detoxify it, leading to a dangerous spike in blood nitrite levels and potential fatality.

Given the provided parameters and initial conditions, let's analyze the expected outcome of the simulation in terms of nitrate and nitrite levels in a ruminant animal. With the modified parameters, the animal is exposed to a high initial dose of nitrate (400 mg) and a rapid conversion rate of nitrate to nitrite ($k_{conv} = 0.9$). Simultaneously, the direct transfer of nitrate from the rumen to the blood is blocked ($k_{12} = 0$), while the absorption of nitrite from the blood to tissues and its excretion are relatively slower ($k_{23} = 0.1$, $k_{30} = 0.2$). Since the animal received a lethal dose of nitrate, we can expect a rapid accumulation of nitrite in the rumen due to the high conversion rate and lack of direct nitrate transfer. This will subsequently lead to a significant increase in nitrite levels in the blood, exceeding toxic thresholds, and potentially causing acute toxicity. To confirm this, let's run the simulation without plotting the graph and examine the peak nitrite concentrations in the blood.

With the modified parameters, the simulation results in a maximum nitrite concentration in the blood of 256.56 mg. This high level of nitrite in the blood, likely exceeding the toxic threshold, would be consistent with a lethal dose of nitrate leading to acute toxicity in the ruminant animal.

3.3. Insights from the model

A compartmental model for nitrate metabolism in ruminants can provide detailed insights into several key aspects:

- 1. Kinetics of nitrate reduction:** the model can elucidate the rates at which nitrates are reduced to nitrites and further to ammonia in the rumen. Understanding these kinetics is essential for predicting the conditions under which toxic levels of nitrites might accumulate.

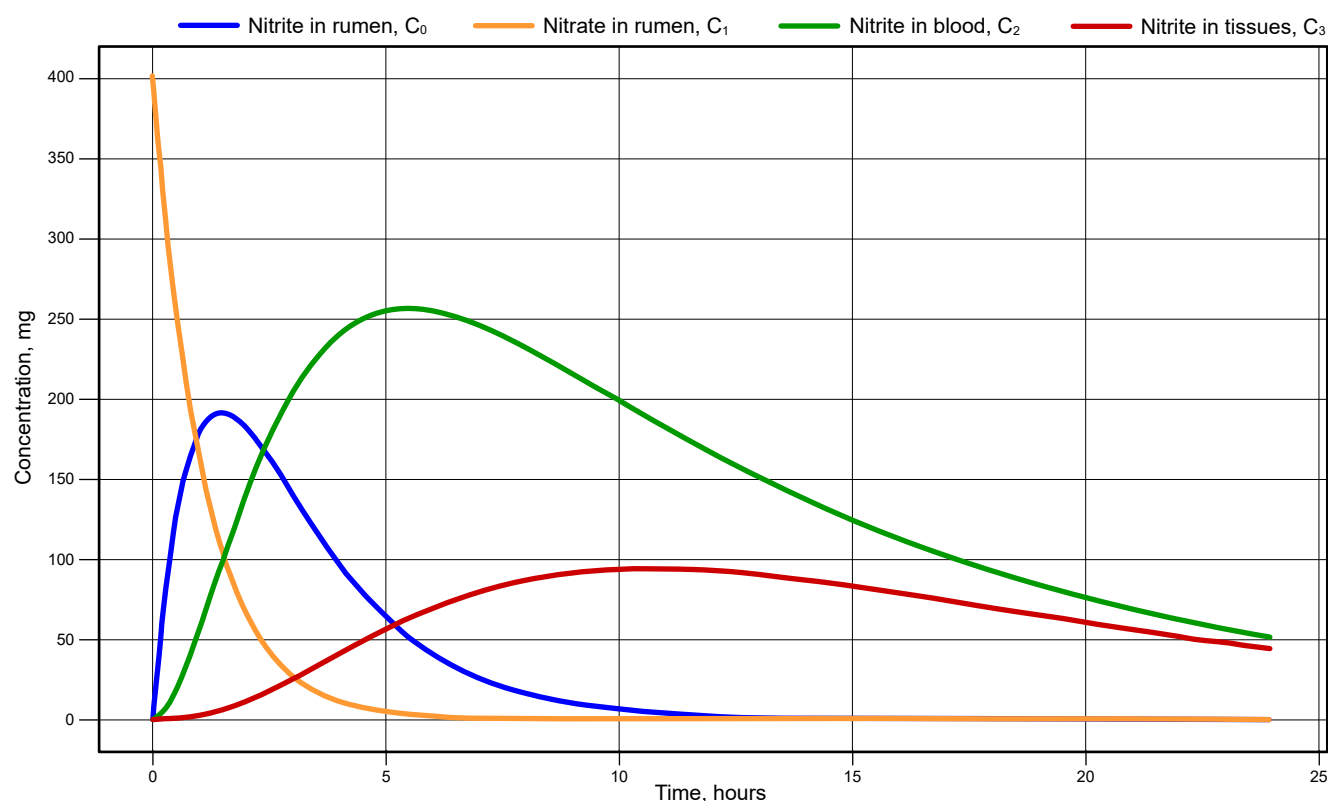


Fig. Nitrite concentration in the rumen over time (results of the proposed model implemented in Python)

- 2. Distribution of nitrate metabolites:** by modeling different compartments within the animal's body (e.g., rumen, blood, tissues), it is possible to track the distribution and concentration of nitrate metabolites. This helps in identifying critical points where interventions can be made to reduce toxicity.
- 3. Impact of diet and environment:** the model can simulate how different dietary components and environmental conditions (such as water nitrate levels and forage type) affect nitrate metabolism. This can guide the selection of optimal feeding practices and environmental management.
- 4. Inter-species variability:** The model can be adapted to study different ruminant species, allowing for comparisons and the development of species-specific recommendations. This is particularly useful for mixed farming operations.
- 5. Predictive tool for management decisions:** by integrating real-time data and scenario analysis, the model can serve as a predictive tool for making informed management decisions. This can help in preemptively addressing potential issues related to nitrate toxicity.

In summary, a compartmental model for nitrate metabolism in ruminants is a valuable tool for improving animal health, optimizing agricultural practices, and ensuring food safety. By providing detailed insights into the complex processes involved in nitrate metabolism, the model supports the development of effective strategies for managing nitrates in ruminant production systems.

Nitrate/nitrite poisoning poses a significant threat to ruminant livestock, necessitating a comprehensive understanding of its multifaceted nature. This article has provided an in-depth overview of the historical context, diverse sources of exposure, intricate metabolic pathways, and varied mechanisms of action associated with nitrate toxicity. The complexity of this issue is further compounded by the variability in reported nitrate/nitrite levels and the diversity of analytical methods employed.

Compartmental modeling emerges as a crucial tool in navigating these complexities. By simulating the dynamic processes of nitrate intake, conversion to nitrite, absorption, distribution, and excretion, these models offer valuable insights into the factors that influence nitrate toxicity in ruminants. This knowledge can be leveraged to develop evidence-based interventions, optimize feeding practices, and guide agricultural management decisions to mitigate the risks associated with nitrate exposure. Ultimately, the integration of research, modeling, and practical application holds the promise of fostering sustainable agricultural practices that prioritize animal welfare, environmental health, and food safety.

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Від чилійської селітри до сучасного сільського господарства: дослідження нітратної токсичності в жуйних тварин за допомогою компартментального моделювання

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Отруєння нітратами/нітридами є значною проблемою для здоров'я жуйних тварин, з історичними коренями, що передують шірокому використанню азотних добрив. Цей огляд досліджує різні фактори, які сприяють нітратній токсичності, зокрема природні та антропогенні джерела, шляхи метаболізму, механізми дії та варіабельність у наявних даних. Підкреслюється важливість компартментального моделювання для розуміння динаміки метаболізму нітратів. Ці моделі надають основу для моделювання складних процесів, пов'язаних зі споживанням, перетворенням, абсорбцією, розподілом та виведенням нітратів, що зрештою сприяє розробці ефективних стратегій пом'якшення наслідків. Метою цієї статті є надання всебічного огляду отруєння нітратами/нітридами в жуйних тварин та підкреслення ролі компартментального моделювання для збереження здоров'я тварин, оптимізації сільськогосподарських практик та забезпечення безпеки харчових продуктів у контексті сучасного сільського господарства.

Ключові слова: нітратна токсичність, жуйні тварини, компартментальне моделювання, метгемоглобінемія, управління кормами



Biochemical blood profile of red deer (*Cervus elaphus*), reared in the conditions of Polissya Volyn

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Authors' Contributions:

HR: Conceptualization; Project management; Methodology; Formal analysis; Research; Data curation; Writing — original draft, review & editing; Visualization.

Declaration of Conflict of Interests:

None to declare.

Ethical approval:

All the work with experimental animals was performed in accordance with the rules of bioethics in compliance with the European Convention "On the Humane Treatment of Laboratory Animals", "General Principles of Animal Experiments" and in accordance with "The Regulations on the Use of Animals in Biomedical Experiments".

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It was found that the biochemical parameters of blood of red deer (*Cervus elaphus*) reared in the conditions of Polissya Volyn, in general, correspond to the main patterns characteristic of this population, although they have some fluctuations. Thus, we have shown that the content of total protein, its fractional composition, concentration of glucose, urea and creatinine in the blood serum of English deer blood line and Latvian crossbred deer were within the reference values characteristic of this species of wildlife. It was found that the activity of ALT and GGT was higher in animals of English origin, and AST and ALP — in Latvian crossbred deer. The content of Ca, P, Mg and Na ions in the blood was also higher, respectively, by 9.4, 28, 22.7 and 4%. It was found that against the background of stress caused by physical method of capturing animals for blood sampling, the level of total protein and its albumin fraction. The concentration of urea was by 22.5%, glucose — by 18.4% and creatinine by 6.5% higher than in the group of animals with sedation. Under technological stress in deer, the activity of ALT and AST in the blood increases (by 11 and 5.5%) and ALP and GGT (by 9.1 and 20.0%), respectively. The study of blood biochemical parameters in young animals (3–4 months old) revealed that the content of total protein in females is 6.4% higher than in males. At the same time, the activity of AST activity in the blood serum of males was 17.4% higher than in young females. The concentration of Ca ions in males was 32.4% higher than in females, and concentration of P, on the contrary, was lower in males. The ratio of Ca to P in the blood of males was 1.57, and in females — 1.30. It was found that the content of total protein in animals increased with age and was the highest in adult red deer. While the concentration of the studied macronutrients (Ca, P, Na, K) had a higher level in the blood of young animals. While the concentration of the studied macronutrients (Ca, P, Na, K) had a higher level in the blood of young animals.

Key words: deer, stress, blood, biochemical parameters

Introduction

The intensification of hunting management in Ukraine was conditional nature even before the full-scale invasion of the Russian Federation. Unfortunately, in terms of the level of development of the hunting industry, we were inferior even to the countries of Eastern Europe [12]. Among the main problems that hinder the sustainable development of

the industry are: low and ineffective level of immunization of wild animals; death of animals due to the spread of dangerous Infectious diseases; deterioration of the habitat of game animals as a result of climate change and negative anthropogenic activities; use of pesticides and use of modern mechanisms in agriculture in violation of environmental requirements; insufficient environmental requirements; insufficient scientific and methodological support. Today,

as a result of military aggression, there are also significant losses of biodiversity and destruction of natural habitats of wildlife species, pollution of territories by explosive ordnance, violation of natural ecosystems, soils, water bodies and water bodies and water management systems [10]. Therefore, for the sustainable and efficient use of hunting fauna requires the development and application of a number of urgent and promising measures for the conservation, reproduction and maintenance of favorable condition of biodiversity of natural ecosystems, adaptation to climate change and pollution, which leads to the degradation of natural biocenoses. An important aspect of this action plan is to ensure the restoration and of such an action plan is to ensure the restoration and maintenance of wildlife populations in accordance with the indicators of their optimal number and sex and age structure of wildlife populations. And, in particular, the breeding of wild game ungulates, including red deer, in semi-free conditions, taking into account the results of in-depth study of biology, ethology and ecology of animals to improve their comfort zone [5].

The physiological processes and functions of an animal are constantly influenced by environmental factors. The animal's body and blood, in particular, react in a special way react to each environmental factor in a special way [4, 13, 14]. Knowledge of the range of reference values of blood parameters and their determination for individual species and their determination for individual game species is key to monitoring individual and herd health as part of the overall environmental monitoring. The indicators characterizing the state of wild populations of animals and red deer, in particular, may be influenced by the habitat, housing conditions, breed, sex, age, physical activity, diseases, stress, etc [14, 17].

The method of animal immobilization is also important [2, 11, 15]. It is believed that the very stress to which deer are exposed during blood sampling can significantly affect the can significantly affect the results obtained [3]. At the same time, there are few reports in the scientific literature, there are few reports on the reference biochemical parameters of blood of red deer, as well as the effects of anesthesia or muscle relaxation. The published data differ significantly [1]. Most of the described indicators are obtained from animals, when tranquilizers were used.

The aim of this stage of work was to find out the impact of blood sampling methods on red deer for the study of biochemical parameters against the background of different conditions of animal husbandry, their age, sex and population type.

Materials and Methods

The experiment was conducted in the conditions of the farm "Amila", which is geographically located in Western Polissya (Radovychi village, Kovel district, Volyn region) and specializing in the production of region) and specializes in red deer breeding since 2017. The farm breeds deer of three populations (English, Hungarian and Eastern

European) and two crossbreeds (Latvian and Lithuanian). The system of red deer rearing in the farm conditions involves a whole range of biotechnical, zootechnical and veterinary sanitary measures [9, 16, 18]. It usually includes, in addition to assessing the general condition of the of the animal, determination of its body weight (at a certain stage of development), coprological examination of feces for the presence of helminth eggs or larvae and selective blood tests for morphological and biochemical parameters.

To perform the experimental part of the work 7 groups of deer (n=10) were selected. In particular, groups D1–D3 were formed from females of the English population, aged 2–3 years (enclosed — the area of the fenced watering from watering troughs; existing feeding systems and provision of agrotechnical and veterinary measures). Deer of experimental groups D1 and D2 were subjected to prophylactic deworming with *Ivomec*®, at the rate of 1 ml per animal, subcutaneously. Animals of group D3 were not dewormed. Animals in groups D4 (English population) and D5 (Latvian crossbreed) were also 2–3 years old. Groups D6 (males) and D7(females) — fawns of the English population of 3–4 months of age, which were kept in pens with females (mothers) in fenced areas.

We used two methods of contact with deer to collect blood samples. Thus, from deer D1 and D2 groups and young animals of D6 and D7 groups, blood for research was collected after driving them through the appropriate system of mazes from the transition corridors and fixing them in a special box. It is worth noting that with this form of capture wild animals are subjected to technological stress, which is likely to have an impact on the results obtained.

Animals of D3, D4 and D5 of the experimental groups, which were kept in a semi-free were subjected to a medical method of immobilization. To create the effect of sedation and muscle relaxation and 30-minute immobilization of deer, we used the xylazine-containing drug *Xyla* (Interchemie Verken "De Adeyaar" Esti AS, Estonia). It was administered to animals using a special DAN inject gun in a dose of 1 ml per 100 kilo. The time from drug administration to the start of blood sampling was 30–45 min.

Blood for the study was taken from deer by puncture of the jugular vein. Blood was collected in separate vacuum tubes "Vacutest" (red lid, with silicon dioxide as an activator, using a double-sided needle "Voles"). Were taken 10 ml of blood to obtain serum. Biochemical analysis of serum was performed using a *Humalyzer 3000* analyzer and standard certified test kits "Human Diagnostics Worldwide" (Germany).

Results and Discussion

During the study of biochemical parameters of red deer blood, we confirmed the data of many scientists that they are relatively stable constants, characteristic of individual population subfamilies, may vary depending on geographical habitat, age, sex, physiological condition, type and nature of feeding (feeding), time of year [4, 13].

It has been established that they are largely subject to fluctuations depending on the specifics of blood sampling from animals. Stress factors usually disrupt the established homeostasis in this species of wildlife and can provoke numerous disorders, including slower growth rates, metabolic disorders, immunodeficiency's, and often cause various diseases [8].

The main stress factors in livestock production, including among deer when rearing them both in free (aviary) and semi-free conditions (farm), are considered to be changes in feeding regime, regrouping, introduction, climatic and temperature factors etc.

In the course of our research, we found that against the background of a stressful factor (running animals through long mazes to a stationary fixation box) in the blood serum of deer of the first experimental group (D1) increased the content of total protein ($P<0.05$) and its albumin fraction ($P<0.05$), compared to similar indicators of animals of the third experimental group, which were immobilized with a xylazine-containing preparation (table 1).

According to some researchers [1], this trend is apparently the result of the following the use of sedatives can change the permeability of capillaries and cause, to a certain extent, hemodynamic to a certain extent, hemodilution, loss of proteins and especially albumin.

As a result of experimental studies, we noted that the concentration of urea in the blood serum of deer of group D1 was higher than that of group D3 by 1.4 mmol/L or 22.5%. It is important that the data characterizing the content of the studied substance in deer of group D1 slightly exceeded the reference values for this species of wildlife. It is known that the concentration of urea in the blood of animals can be subject to significant fluctuations and depends on the time of year and the structure of the probable diet. The observed increase in the level of urea in the blood serum of deer (group D1 and D2) is likely to be the

result of catabolic processes that occurred during animal feeding, concentrated feed mixtures on the farm. In our studies, the level of urea in the blood of deer subjected to chemical immobilization was slightly lower than that of the physical method of capturing animals.

Similar changes were observed in the concentration of glucose in the blood serum of deer. Its content is entirely related to animal feeding, but other factors, such as stress. We have found that the glucose concentration in the blood of D3 deer which were reared in farm conditions, under *Xyla* sedation was 18.4% ($P<0.05$) lower than that of animals of group D1. Some scientists who have studied such patterns tend to believe that the depression of the central and efferent nervous system, against the background of the use of adrenergic drugs, may be accompanied by the release of insulin and the glucose output from the liver [1, 14].

We have found that the level of creatinine in the blood serum of deer, against the background of technological stress associated with intense movement and muscle exertion, tended to increase (by 6.5%) in animals of group D1 compared to the analogues of group D3. In this series of experiments, we also analyzed the biochemical parameters of deer blood against the background of preventive deworming. According to the results of the spring (2021) parasitological examination of red deer feces of different ecotypes, mainly eggs of strongyles of digestive tract infections (*Trichostrongylus axei*, *Ostertagia ostertagia*, *Coope-ria ancophora*, *Chabertia ovina*) and larvae of strongyles of respiratory tract infections (*Muellerius capillaris*), which are also characteristic of other ungulate species, were found [6, 7]. According to the experimental scheme, deer of group D1 were treated with an anthelmintic, while group D2 was not. The method of catching animals for blood sampling was the same. The deer were driven to a stationary box and biomaterial was obtained during fixation.

Table 1. Dynamics of some biochemical parameters of blood serum of red deer under different methods of their immobilization ($M\pm m$, $n=10$)

Parameter	Reference values	Animal group		
		D1	D2	D3
Protein total, g/L	63.6–66.3	68.2 $\pm$ 2.7	62.8 $\pm$ 4.1 ¹¹	65.7 $\pm$ 7.8*
Albumin, g/L	35.7–37.5	37.9 $\pm$ 1.4	33.9 $\pm$ 3.0 ¹¹	35.4 $\pm$ 4.0*
Globulins, g/L	27.2–29.5	30.3 $\pm$ 3.3	28.9 $\pm$ 2.6	30.3 $\pm$ 3.4
α -globulin, g/L	10.1–10.9	10.5 $\pm$ 0.9	11.9 $\pm$ 0.7	11.2 $\pm$ 0.3
β -globulin, g/L	4.8–5.7	5.6 $\pm$ 0.6	4.6 $\pm$ 0.4	5.9 $\pm$ 0.8
γ -globulin, g/L	11.8–13.5	14.2 $\pm$ 1.0	12.4 $\pm$ 0.9	13.2 $\pm$ 0.5
A/G ratio	—	1.25 $\pm$ 0.08	1.17 $\pm$ 0.09	1.17 $\pm$ 0.07
Glucose, mmol/L	8.5–9.7	11.3 $\pm$ 0.6	10.3 $\pm$ 0.6	9.2 $\pm$ 0.5*
Urea, mmol/L	5.1–6.0	7.6 $\pm$ 0.44	7.7 $\pm$ 0.9	6.2 $\pm$ 0.5
Creatinine, μ mol/L	147.6–159.4	174.3 $\pm$ 6.5	184.4 $\pm$ 11.4 ¹¹	163.6 $\pm$ 14.4**

Note. Reference values are given according to O. Rosef et al. for animals immobilized with a neuroplegic agent. In this and other tables:

* — $P<0.05$; ** — $P<0.01$ (D3 vs. D1); ¹ — $P<0.05$; ¹¹ — $P<0.01$ (D2 vs. D1)

As a result of the studies (table 1), it was found that in non-dewormed deer of group D2 against the background of an apparently chronic development of the pathological process caused by helminths, the protein synthesizing function was suppressed. The content of total protein in the blood serum of animals of this group was 8% lower ($P<0.05$) than in animals treated with *Ivomek*. At the same time, the observed decrease in total protein was mainly due to a decrease in its albumin fraction ($P<0.01$). The concentration of glucose in the blood of non-dewormed animals tended to decreased, and the creatinine level increased slightly.

It is well known that the activity of certain enzymes is an indicator of stress in animals. We found that the activity of ALT, AST, ALP, and GGT had significant deviations in blood obtained from animals subjected to different immobilization methods (table 2). Thus, in deer under chemical sedation with *Xyla*, the activity of transaminases (ALT and AST) was lower by 11.0 and 5.5% ($P<0.01$; $P<0.05$) than in animals subjected to exhaustive running to the fixation box through the corridor system.

A similar trend was characteristic of alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT). Against the background of technological stress in the blood of deer of group D1 the concentration of these enzymes exceeded that of animals of group D3 by 9.1 and 20.0% ($P<0.05$). It was noted that the studied indicators of enzyme activity in animals of group D3 (under chemical sedation) were within the limit values determined for this group of adult wild animals. As for the indicators of animals of group D1 animals that went beyond average values, we agree with the opinion of some authors [3, 8],

that the reference constants of biochemical digital expressions have significant differences in deer with different methods of capturing animals (physical fixation or anesthesia) and require additional research.

The electrolyte balance of blood and tissues in the body of deer is extremely important, as it ensures appropriate osmosis, metabolic processes, bone growth and is the basis of the body's homeostasis. For this species of animal, it is also peculiar in that it promotes the growth of horns (antlers). We studied the concentration of Calcium, Phosphorus, Magnesium, Sodium and Potassium ions in the blood serum (table 3). It was found that their levels were within the reference values determined for red deer. At the same time, there were no significant deviations in animals subjected to different methods of immobilization were not noted.

According to many scientists, the course of metabolic processes in the body of deer depends on many factors. We have previously described the results of research on deer body weight depending on the population. It was shown that the difference in body weight between animals of the same sex and age was up to 36 kg. In particular, the animals of the Latvian crossbreed were not only heavier in weight compared to the English population, but also taller, and more massive. The males had more horny processes etc.

In our opinion, it was interesting to find out the biochemical parameters of blood in deer of different lines that were raised on a farm in the Western Polissya of Ukraine. For this purpose, blood of animals from two populations: English and Latvian crossbreeds were studied (table 4).

Table 2. The activity of individual enzymes of red deer blood serum under physical and chemical method of their immobilization ($M\pm m$, $n=10$)

Parameter	Reference values	Animal group		
		D1	D2	D3
ALT U/L	51.7–56.7	60.2 $\pm$ 3.7	63.4 $\pm$ 6.2	53.6 $\pm$ 4.0**
AST U/L	55.0–63.3	66.9 $\pm$ 4.0	74.4 $\pm$ 5.2 ¹	63.2 $\pm$ 6.6
de Ritis ratio (ALT/AST)	1.06–1.12	1.11 $\pm$ 0.08	1.17 $\pm$ 0.06	1.18 $\pm$ 0.1
ALP U/L	195.5–252.1	240.4 $\pm$ 16.8	264.8 $\pm$ 9.8	220.4 $\pm$ 10.4*
GGT U/L	18.4–22.4	24.0 $\pm$ 0.8	30.2 $\pm$ 1.1	20.0 $\pm$ 2.2

Table 3. Concentration of macronutrients in the blood serum of red deer ($M\pm m$, $n=10$)

Parameter	Reference values	Animal group		
		D1	D2	D3
Ca, mmol/L	1.89–1.99	2.12 $\pm$ 0.18	2.05 $\pm$ 0.34	1.90 $\pm$ 0.40
P (inorganic), mmol/L	1.12–1.36	1.44 $\pm$ 0.1	1.36 $\pm$ 0.1	1.32 $\pm$ 0.3
Mg, mmol/L	0.46–0.50	0.54 $\pm$ 0.06	0.52 $\pm$ 0.04	0.54 $\pm$ 0.06
Na, mmol/L	136.7–142.2	141.7 $\pm$ 4.8	140.4 $\pm$ 10.2	136.6 $\pm$ 8.8
K, mmol/L	5.5–6.3	6.4 $\pm$ 0.64	6.4 $\pm$ 0.4	6.1 $\pm$ 0.8

Table 4. Biochemical parameters of blood serum of red deer of the English population and Latvian crossbreeds ($M \pm m$, $n=10$)

Parameter	Reference values	Animal group	
		D4	D5
Protein total, g/L	63.6–66.3	64.4±2.8	66.6±6.6
Albumin, g/L	35.7–37.5	36.1±4.2	34.2±3.8
Globulins, g/L	27.2–29.5	26.3±2.2	32.4±4.0
α-globulin, g/L	10.1–10.9	10.2±0.8	12.9±0.6
β-globulin, g/L	4.8–5.7	4.4±0.4	5.6±0.4
γ-globulin, g/L	11.8–13.5	11.7±0.5	19.9±0.6
A/G ratio	1.31–1.27	1.37±0.08	1.06±0.08
Glucose, mmol/L	8.5–9.7	8.8±0.6	9.2±0.2
Urea, mmol/L	5.1–6.0	5.6±0.2	5.8±0.4
Creatinine, μmol/L	147.6–159.4	154.4±8.2	162.4±6.0

Table 5. Serum enzyme activity of red deer from different populations ($M \pm m$, $n=5$)

Parameter	Reference values	Animal group	
		D4	D5
ALT U/L	51.7–56.7	64.1±3.6	60.2±4.4
AST U/L	55.0–63.3	68.2±6.8	69.4±7.0
de Ritis ratio (ALT/AST)	1.06–1.12	1.06	1.15
ALP U/L	195.5–252.1	240.4±14.6	264.2±10.8
GGT U/L	18.4–22.4	22.8±1.4	20.2±0.8

Table 6. Electrolyte composition of blood serum of red deer of different origin ($M \pm m$, $n=5$)

Parameter	Reference values	Animal group	
		D4	D5
Ca, mmol/L	1.89–1.99	1.90±0.44	2.08±0.32
P (inorganic), mmol/L	1.12–1.36	1.39±0.3	1.78±0.4*
Mg, mmol/L	0.46–0.50	0.44±0.08	0.54±0.06
Na, mmol/L	136.7–142.2	136.6±18.2	142.0±8.8
K, mmol/L	5.5–6.3	5.8±0.40	5.4±0.34

As a result of the experiment, it was stated that deviations in such indicators such as the content of total protein, its fractional composition, concentration glucose, urea, creatinine were insignificant and generally within the average values, recommended for red deer as reference. The activity of serum enzymes in the blood of deer of two subpopulations are presented in table 5. It was found that the activity of AST and ALP was higher in animals of group D5, and ALT and GGT in deer of group D4, although all obtained results were within the reference range.

The electrolyte composition of the blood serum of deer from the English population and the Latvian crossbreed was slightly different (table 6). It was found that the concentration of calcium ions, Phosphorus, Magnesium and Sodium ions in animals of group D5 (Latvian crossbreed) was comparable to with those of deer of group D4 was 9.4, 28.0, 22.7, and 4.0% higher than in the D4 group.

Tables 7, 8 and 9 show the results of biochemical studies of blood serum of young, 3–4 month old fawns, depending on sex. It was found that the content of protein total protein content in females was 6.4% ($P<0.05$) higher

Table 7. Biochemical parameters of blood serum of young red deer ($M \pm m$, $n=5$)

Parameter	Reference values	Animal group	
		D6	D7
Protein total, g/L	56–70	54.4±4.4	57.9±6.8*
Albumin, g/L	24–33	34.2±2.6	33.6±5.0
Globulins, g/L	—	20.2±1.8	24.3±3.2
α -globulin, g/L	—	10.4±0.6	8.2±0.8
β -globulin, g/L	—	3.2±0.2	6.0±0.4
γ -globulin, g/L	—	6.6±0.6	10.1±0.6
A/G ratio	—	1.69±0.04	1.38±0.06
Glucose, mmol/L	4.7–8.0	5.4±0.6	5.2±0.2
Urea, mmol/L	3.6–7.5	4.2±0.4	4.0±0.4
Creatinine, μ mol/L	63–93	112.4±7.2	88.6±6.0*

Note. In this and the following table, reference values are given for fawns without the use of tranquilizers.

Table 8. Serum enzyme activity of young red deer depending on sex ($M \pm m$, $n=5$)

Parameter	Reference values	Animal group	
		D6	D7
ALT U/L	7.0–54.0	46.6±3.0	44.2±4.2
AST U/L	30.0–91.0	40.4±5.2	34.4±5.0**
de Ritis ratio (ALT/AST)	4.29–1.69	0.87±0.02	0.78±0.02
ALP U/L	129–475	312.4±26.0	300.6±14.8
GGT U/L	7–44	40.1±1.4	36.4±3.8

Table 9. Macronutrient composition of blood serum of young males and females of red deer ($M \pm m$, $n=5$)

Parameter	Reference values	Animal group	
		D6	D7
Ca, mmol/L	1.89–1.99	2.86±0.26	2.16±0.44*
P (inorganic), mmol/L	1.12–1.36	1.82±0.4	1.66±0.2*
Mg, mmol/L	0.46–0.50	0.62±0.05	0.56±0.08
Na, mmol/L	136.7–142.2	174.4±12.2	180.2±20.2
K, mmol/L	5.5–6.3	7.8±0.5	7.0±0.6

than in males. At the same time, according to the analysis of fractional composition of protein in the blood of females revealed a higher level of globulins, in particular α -globulins. The determined ratio of albumin to globulins (A/G) was also lower in them. Of the other studied indicators, the value of creatinine concentration in blood serum of young females was significantly lower, by 21.2% ($P<0.05$).

The activity of the studied enzymes in the blood of young deer was within the reference values. However, it was noted that the activity of AST in the blood of males was by 17.4% ($P<0.01$) higher than in females.

The analysis of the macronutrient composition of the blood serum revealed that the concentration of calcium ions in males was 32.4% ($P<0.05$) higher than in females, and Phosphorus, on the contrary, prevailed in males. The ratio of Ca to P in males was at the level of 1.57, and in females — 1.30.

So, summarizing, we conclude that a higher level of protein in the blood serum of adult deer (table 1), compared to the same indicator in young individuals of this species (table 7) is obviously the result of catabolic changes in their body. We cannot exclude possible anabolic

abnormalities in metabolism in adult animals, against the background of estrogen action during the rut (blood sampling was carried out at the end of September).

We found that in the blood of young deer, compared to adults, the following levels were higher levels of Ca, P, Na, and K ions compared to adults. Authors report similar results in their studies [3]. It is believed that young deer during this period of postnatal development is characterized by high activity of osteoblasts and osteoclasts in tissue remodeling, which leads to an increased need for macro- and microelements.

Comparing the activity of the studied enzymes in adult and young animals it was found that in fawns the activity of GGT and ALP was higher and the activity of AST was lower.

1. The biochemical parameters of deer blood undergo significant fluctuations against the background of technological stress. When taking blood from deer immobilized by the use of sedative Xyla, the results obtained are within the reference values determined for this species of wildlife.

2. The results of biochemical studies of blood in red deer did not have significant deviations at both the population and individual levels.

3. According to the results of biochemical studies of blood of non-dewormed deer the probable development of a chronic inflammatory process in animals of this group was established, which confirms the importance of preventive deworming.

4. The biochemical profile of blood of young deer has certain peculiarities and is determined by morphological changes and functional development of their organism in this period of postnatal development.

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Біохімічний профіль крові оленів (*Cervus elaphus*), вирощених в умовах Полісся Волині

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З'ясовано, що біохімічні показники крові благородних оленів (*Cervus elaphus*), вирощених в умовах Полісся Волині, загалом відповідають загальним закономірностям, характерним для цієї популяційної підродини, хоч і мають певні коливання. Ми виявили, що вміст загального протеїну, його фракційний склад, концентрація глюкози, сечовини і креатиніну в сироватці крові оленів англійської популяції та латвійського кросбрїду була в межах референтних величин, характерних для цього виду диких тварин. При цьому встановлено, що активність АлАТ і ГГТ була вищою у тварин англійського походження, а АсАТ і ЛФ — в оленів латвійського кросбрїду. В останніх був вищим і вміст у крові іонів Са, Р, Mg та Na — відповідно, на 9,4; 28; 22,7 і 4%. Підтверджено дані інших дослідників щодо залежності біохімічних показників крові в оленів від способу знерухомлення. Встановлено, що на тлі стресу, спричиненого фізичним способом відлову тварин для забору крові, в сироватці крові оленів зростає рівень загального протеїну та його альбумінова фракція. Концентрація сечовини була вищою на 22,5%, глюкози — на 18,4% а креатиніну — на 6,5%, ніж у групі тварин за медикаментозної седатії. За технологічного стресу в оленів у крові зростає активність АлАТ і АсАТ — на 11 і 5,5%, та ЛФ і ГГТ — на 9,1 і 20,0% відповідно. За дослідження біохімічних показників крові у молодих тварин (3–4 міс.) з'ясовано, що вміст протеїну загального в самочок на 6,4% вищий, ніж у самців. Водночас активність АсАТ у сироватці крові самців була на 17,4% вищою, ніж у молодих самок. Концентрація іонів Са у самців перевищувала аналогічний показник у самочок на 32,4%, а Р — навпаки, була нижчою в самців. Співвідношення Са до Р в крові самців було на рівні 1,57, а в самок — 1,30. Встановлено, що вміст загального протеїну у тварин з віком зростає і був найвищим в дорослих оленів. Концентрація досліджуваних макроелементів (Са, Р, Na, К) мала вищий рівень у крові молодих тварин.

Ключові слова: олені, стрес, кров, біохімічні показники



Дослідження дії пробіотики *Lactobacillus casei* B 7280 за різної тривалості застосування на резистентність організму бджіл

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KTM: Investigation.

TMM: Writing — original draft.

PAZ: Data curation; Visualization.

KVV: Methodology.

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Declaration of Conflict of Interests:

None to declare.

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Науковий супровід сучасного бджільництва спрямований на отримання безпечної екологічної продукції, розроблення засобів і методів стимуляції розмноження та підвищення стійкості бджіл до різних збудників хвороб, а також на захист від несприятливих умов довкілля. Існує тенденція до використання нових ефективних засобів натурального походження, механізми дії яких відрізняються від синтетичних речовин і препаратів активацією захисних реакцій організму на фізіологічному рівні. Пробіотик *Lactobacillus casei* IMV B-7280 має ефективну стимулюючу дію на фізіологічні процеси за різних екологічних та експериментальних умов життєдіяльності бджіл. Його фізіологічний вплив пов'язаний з нормалізацією кишкової бактеріальної мікрофлори та участю в модуляції захисних реакцій організму. Тому метою досліджень було визначити вплив пробіотичного препарату класу *L. casei* B-7280 на вміст білка, продуктів перекисного окиснення та активність каталази у гомогенатах тканин організму бджіл за різної тривалості застосування. Бджоли контрольної (С) групи отримували підгодовлю з 60% цукрового сиропу в кількості 1 мл/групу/добу. Дослідні групи додатково отримували розчин пробіотики *L. casei* B-7280 у концентрації 10⁶ КУО/мл: дослідна група 1 (E1) — щодобово; дослідна група 2 (E2) — через добу; дослідна група 3 (E3) — через кожні 3 доби; дослідна група 4 (E4) — раз на тиждень. Тривалість випоювання сиропу та пробіотики — 4 тижні. Встановлено збільшення вмісту загального білка у тканинах цілого організму бджіл в групах E1–E3, але ці різниці невірні, що може свідчити про відсутність суттєвого впливу пробіотики *L. casei* B-7280 на концентрацію протеїнів у тканинах бджіл. Каталазна активність тканин організму бджіл зросла на 79.80% і 38.07% у групах E1, E2 (P<0.05) щодо контролю, а для бджіл E1 групи — на 52.75% (P<0.05) порівняно з підготовчим періодом. Вміст ГПЛ знижувався на 10.00%, 9.23% та 10,38% (P<0.05) у групах E1, E2 і E3 відповідно щодо контрольної групи. Вміст ТБК-активних продуктів мав тенденцію до зменшення у дослідних групах E1, E2, E3, а в групі E4 — на 14.98% (P<0.01) щодо підготовчого періоду. Результати досліджень показали, що додавання *L. casei* B-7280 впливало на антиоксидантну дію в організмі бджіл за різних термінів його згодовування, що супроводжувалося зниженням вмісту ТБК-активних продуктів (МДА) і гідроперекисів ліпідів в гомогенатах тканин організму бджіл усіх дослідних груп, окрім E3, та впливало на життєздатність бджіл.

Ключові слова: бджоли, пробіотик, протеїн, продукти перекисного окиснення, активність каталази

Вступ

В Україні створено низку препаратів на основі пробіотиків для використання у ветеринарії та медицині. Вони широко використовуються як допоміжні лікарські засоби у профілактиці захворювань, що супроводжуються порушенням нормальної мікрофлори в організмі тварин і людей [2, 19]. Втрати популяції медоносних бджіл за останні десятиліття загрожують як екосистемі, так і продовольчій безпеці країни. Дослідники і пасічники за цих умов особливу увагу приділяють факторам, які погіршують резистентність організму, функціональний стан його окремих систем та продуктивність бджіл. Застосування у бджільництві препаратів природного походження [13, 18] дозволяє уникнути багатьох побічних ефектів, оскільки механізми дії цих препаратів істотно відрізняються від хімічносинтезованих і ґрунтуються на фізіологічній активації захисних реакцій організму.

Тому науковий супровід сучасного бджільництва спрямований на отримання безпечної екологічної продукції, розроблення засобів і методів стимуляції розмноження і підвищення стійкості бджіл до різних збудників хвороб, а також захист від несприятливих умов навколишнього середовища [13]. Показано, що вплив чинників абіотичного, біотичного походження та антропогенної діяльності порушують фізіологічні процеси в організмі *Apis mellifera*, пригнічуючи антиоксидантну та імунну системи, що призводить до загибелі цілих колоній [1, 14, 17].

Вважається, що мікроорганізми та їхні метаболіти, які входять до складу пробіотиків, позитивно впливають на людей і тварин, оздоровлюють їх організм. На сьогодні дослідження мікробіоти кишківника медоносної бджоли показують, що вона впливає на харчування господаря, збільшення ваги, ендокринну передачу сигналів, імунну функцію та стійкість до патогенів [18, 23].

Погіршення кормової бази або її різка зміна — один із чинників, що негативно впливає на здоров'я бджіл і розвиток колоній та може призвести до їхньої загибелі [6, 21]. Дефіцит корму або незначне порушення компонентного складу може ослаблювати АОС, детоксикаційну та імунну системи бджіл, внаслідок чого їхній організм стає вразливішим до застосування хімічних препаратів захисту рослин і захворювань різної етіології.

Дія вказаних чинників сприяє надмірній генерації АФК в організмі бджіл, що призводить до розвитку оксидативного стресу [9]. АФК можуть реагувати з поліненасиченими жирними кислотами ліпідних мембран та індукувати перекисне окиснення ліпідів (ПОЛ), що впливає на фізіологічну функцію клітинних мембран. Кінцевим продуктом цих реакцій є малоновий диальдегід (МДА), маркер ПОЛ, як наслідок окисного стресу. Тому необхідно вивчати нові ефективні засоби натурального походження для боротьби з хворобами та покращення здоров'я медоносних бджіл,

використання яких допоможе уникнути багатьох побічних ефектів. Доведено, що механізми їхньої дії відрізняються від синтетичних речовин і препаратів за рахунок активації захисних реакцій організму на фізіологічному рівні [20].

Особливої уваги у системі профілактики захворюваності бджіл заслуговують дослідження щодо фізіологічного обґрунтування застосування пробіотиків, антибактеріальні й антифунгальні властивості яких обумовлені високою антагоністичною активністю до широкого спектру патогенних та умовно патогенних мікроорганізмів, можливості їх синергічного поєднання з мікроелементами [13, 15].

Нормалізація кишкової бактеріальної мікрофлори медоносних бджіл — основа для їх фізіологічного росту, розвитку і розмноження, підсилення імунної відповіді та резистентності до дії патогенів [5, 13]. Після надходження в шлунково-кишковий тракт пробіотики чинять як пряму дію на патогенні та умовно-патогенні мікроорганізми, так і непряму — активуючи специфічні та неспецифічні захисні системи організму.

За різних експериментальних інфекційно-запальних моделей пробіотик *Lactobacillus casei* IMV B-7280 проявляв ефективну терапевтичну дію. Вплив цього пробіотика пов'язаний із фізіологічною нормалізацією кишкової мікрофлори та участю в модуляції запальних реакцій. У шлунково-кишковому тракті пробіотики чинять як пряму дію на патогенні та умовно патогенні мікроорганізми, так і непряму — активуючи специфічні та неспецифічні захисні системи організму [5, 13].

У зв'язку з вищевикладеним, метою досліджень було визначення впливу за різної періодичності застосування пробіотичного препарату класу *L. casei* IMV B-7280 на вміст загального білка, вміст продуктів перекисного окиснення ліпідів та активність каталази у гомогенатах тканинах організму бджіл.

Матеріали і методи

Дослідження проведені в Інституті біології тварин НААН на медоносних бджолах карпатської породи, відібраних для досліду з лабораторної пасіки. У дослідженнях використано ліофілізований пробіотичний штам *L. casei* IMV B-7280, виділений у відділі проблем інтерферону і імуномодуляторів з асоційованої культури біологічного матеріалу та депонований в Українській колекції мікроорганізмів Інституту мікробіології та вірусології імені Д. К. Заболотного НАН України.

Дослідження були проведені в умовах лабораторного термостату на п'ятьох групах по 60–90 бджіл у кожній, аналогів за масою, силою сім'ї, віком матки. Бджоли контрольної (С) групи отримували підгодівлю з 60% цукрового сиропу в кількості 1 см³/групу/добу. Дослідна група 1 бджіл (Е1) додатково до 1 см³ цукрового сиропу отримувала розчин пробіотика *L. casei* B-7280 у концентрації 10⁶ КУО/см³ щодобово. Дослідна група 2 бджіл (Е2) аналогічно отримувала 1 см³ ЦС

щодобово і розчин пробіотика *L. casei* B-7280 у концентрації 10^6 КУО/см³ через добу. Дослідна група 3 (E3) отримувала 1 см³ ЦС щодобово і розчин пробіотика *L. casei* B-7280 через кожні 3 доби. Дослідна група 4 (E4) отримувала 1 см³ ЦС щодобово і розчин пробіотика *L. casei* B-7280 один раз на тиждень.

Бджіл контрольної та дослідних груп утримували в садках-контейнерах об'ємом 4 дм³ в аналогічних умовах лабораторного термостата ТС-80М-3 з мікро-вентиляцією за температури 30°C, вологості 74–76% протягом чотирьох тижнів дослідження.

У підготовчий період, а також на 28-у добу дослідного періоду з контрольної та дослідних груп відбирали бджіл для проведення фізіолого-біохімічних досліджень.

Для приготування гомогенату тканин всього організму групу бджіл масою 0,5 г гомогенізували з фізіологічним розчином у співвідношенні 1:10 за допомогою гомогенізатора (*Homogenizer Type 302*, Польща) на льоду. Проби центрифугували за 3000 g протягом 5 хв. Супернатант використовували для подальшого вимірювання ферментативної активності.

Вміст загального білка в організмі бджіл проводили за методом Кельдаля [10].

Активність каталази (1.11.1.6) визначали за допомогою здатності гідрогенпероксиду утворювати із солями молібдену стійкий кольоровий комплекс, інтенсивність якого визначали на спектрофотометрі (*Unico*, США) за довжини хвилі 410 нм проти води [12]. Активність каталази визначали в мкгМоль/хв·мг протеїну.

У гомогенаті тканин організму бджіл визначали вміст гідроперекисів [18] та ТБК-активні продукти за цією методою [11].

Усі отримані цифрові дані статистично опрацьовані за допомогою комп'ютерної програми *Statistica* з використанням методу варіаційної статистики, а також

програми *Excel* з пакету послуг *Microsoft Office 2007* та 2010. Відмінності між групами вважали статистично вірогідними $P < 0.05$.

Результати й обговорення

Відомо, що білкові структури відображають стан організму, а також зміни, які відбуваються в ньому під впливом внутрішніх або зовнішніх чинників.

Аналіз результатів досліджень показує збільшення вмісту загального протеїну у тканинах цілого організму бджіл в групах E1–E3, але ці різниці невірогідні, що може вказувати на відсутність суттєвого впливу пробіотика *L. casei* B-7280 на концентрацію протеїнів у тканинах бджіл.

Дослідженнями вмісту загального протеїну у гомогенатах тканин організму бджіл показали збільшення його рівня в групі E4 на 17.19% і 24.60% ($P < 0.05$) відповідно щодо контролю та між підготовчим і дослідним періодами (рис. 1). Ці дані вказують на те, що, незалежно від терміну згодовування *L. casei* B-7280, вони не мають суттєвого впливу на синтез білків, окрім одноразового на тиждень внесення пробіотика.

Відомо, що активність каталази є одним з основних індикаторів загального стану антиоксидантної системи, яка бере участь у захисті організму від надмірної дії активних форм кисню, що призводять до розвитку оксидативного стресу.

Разом з тим, за результатами дослідження встановлено збільшення каталазної активності тканин організму бджіл на 79.80% і 38.07% в групах E1, E2 ($P < 0.05$) щодо контролю, а для бджіл групи E1 — на 52.75% ($P < 0.05$) порівняно з підготовчим періодом (рис. 2).

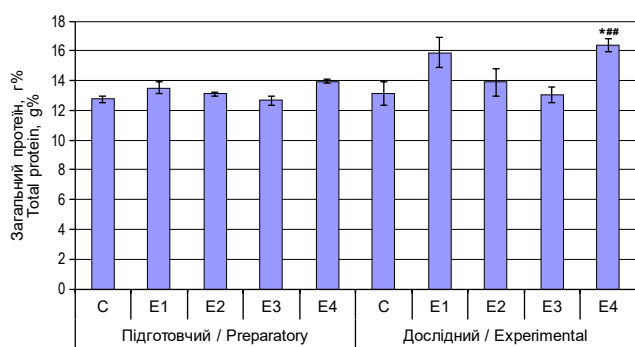


Рис. 1. Вміст загального протеїну у гомогенатах тканин організму бджіл ($M \pm m$, $n=3$)
Fig. 1. Total protein content in bee body tissues homogenates ($M \pm m$, $n=3$)

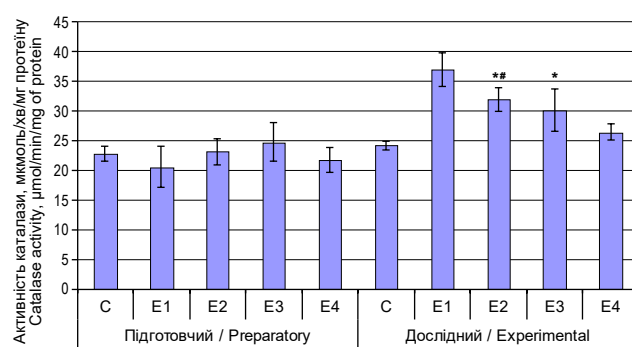


Рис. 2. Активність каталази у гомогенатах тканин організму бджіл, мкмоль/хв/мг протеїну ($M \pm m$, $n=3$)
Fig. 2. Catalase activity in bee body tissues homogenates, μmol/min/mg of protein ($M \pm m$, $n=3$)

Примітка. На цьому та наступному рисунках: * — $P < 0.05$ — вірогідні різниці між контрольною та дослідними групами; # — $P < 0.05$, ## — $P < 0.01$ — вірогідні різниці в групі між періодами дослідження. C — контрольна група, E1 — перша дослідна група, E2 — друга дослідна група, E3 — третя дослідна група, E4 — четверта дослідна група.

Note. In this and the next figures: * — $P < 0.05$ — significant differences between the control and experimental periods by groups; # — $P < 0.05$, ## — $P < 0.01$ — significant differences in a group between study periods. C — control group, E1 — first experimental group, E2 — second experimental group, E3 — third experimental group, E4 — fourth experimental group.

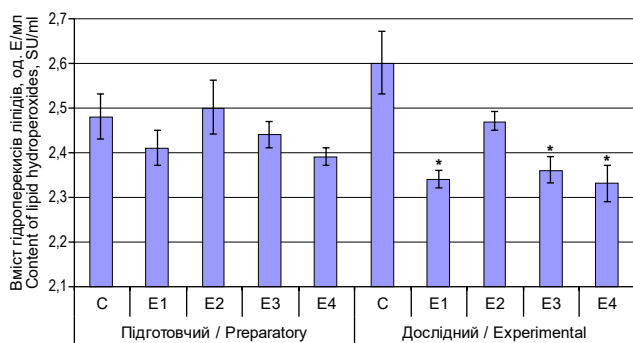


Рис. 3. Вміст гідроперекисів ліпідів в гомогенатах

тканин організму бджіл ($M \pm m$, $n=3$)

Fig. 3. Lipid hydroperoxides content in bee body tissues homogenates ($M \pm m$, $n=3$)

Таким чином, пробіотики можуть надавати стимулюючу дію на опірність і життєздатність організму медоносних бджіл. Водночас у контрольній групі бджіл, які отримували розчин цукрового сиропу впродовж усього досліджу, активність каталази залишалася на сталому рівні [8, 22].

Надмірна активація процесів ПОЛ за зниженої активності антиоксидантної системи організму може призвести до значних патологічних змін. Це супроводжується насамперед пошкодженням субклітинних та клітинних мембран. Продукти ПОЛ призводять до порушення білок-ліпідних зв'язків в біомембранах, що провокує зміни еластичності волокон, ініціює фібропластичні процеси та старіння колагену [3, 7].

Вміст ГПЛ знижувався на 10.00%, 9.23% та 10.38% ($P < 0.05$) у групах E1, E2 і E3 порівняно з контрольною групою (рис. 3). Вміст ТБК-активних продуктів у дослідних групах E1, E2, E3 мав тенденцію до зменшення на тлі вірогідно нижчого рівня в групі E4 на 14.98% ($P < 0.01$) порівняно з підготовчим періодом (рис. 4).

Ці результати вказують, що додавання *L. casei* B-7280 впливало на антиоксидантну дію в організмі бджіл за різної тривалості підгодівлі; це супроводжувалося зниженням вмісту ТБК-активних продуктів і гідроперекисів ліпідів в гомогенатах тканин організму бджіл усіх дослідних груп, окрім E3, та впливало на життєздатність, що узгоджується з даними інших авторів [14].

Загалом наші дослідження показали, що згодовування бджолам до цукрового сиропу *L. casei* B-7280 за різної тривалості підгодівлі мало позитивний вплив на антиоксидантну систему організму бджіл.

Отже, за підгодівлі бджіл цукровим сиропом та розчином пробіотику *L. casei* B-7280 спостерігали зміни активності каталази, яка є маркером загального стану антиоксидантної системи бджіл. Підгодівля бджіл *L. casei* 10^6 КУО/мл характеризувалась відмінностями вмісту загального протеїну у тканинах цілого організму бджіл дослідних груп (E4 — $P < 0.05$) та зниженням вмісту ТБК-активних продуктів і гідроперекисів ліпідів, окрім дослідної групи E3. Отже, пробіотичні культури можуть мати стимулюючу дію на життєздатність бджіл та впливати на стан антиоксидантної системи організму бджіл.

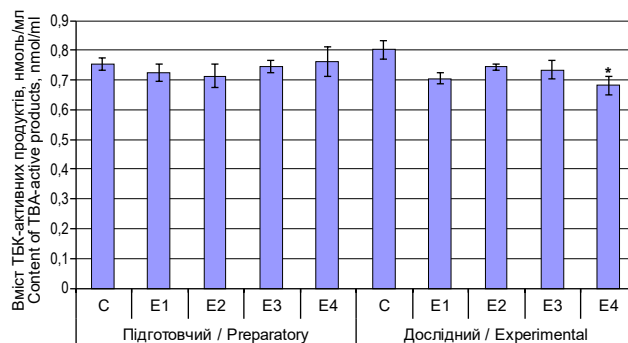


Рис. 4. Вміст ТБК-активних продуктів в гомогенатах

тканин організму бджіл ($M \pm m$, $n=3$)

Fig. 4. Content of TBA-active products in bee body tissues homogenates ($M \pm m$, $n=3$)

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Study of the probiotic *Lactobacillus casei* B 7280 action for different durations of use on the resistance of the bee organism

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The scientific support of modern beekeeping is aimed at obtaining safe ecofriendly products, developing tools and methods for stimulating reproduction and increasing the bees' resistance to various pathogens, as well as protection from adverse environmental conditions. There is a trend to use new effective means of natural origin, whose mechanisms of action differ from synthetic substances and preparations due to the activation of the body's protective reactions at the physiological level. Probiotic *Lactobacillus casei* IMV B-7280 has an effective stimulating effect on physiological processes under various environmental and experimental conditions of bees' life. The physiological effect of this probiotic is associated with the intestinal bacterial microflora normalization and taking part in the body's protective reactions modulation. Therefore, the purpose of the research was to determine the effect of the probiotic preparation *L. casei* B-7280 on the content of protein, peroxidation products, and catalase activity in homogenates of bee body tissues for different durations of use. Bees of the control (C) group were fed with 60% sugar syrup in the amount of 1 ml/group/day. Experimental groups additionally received a probiotic *L. casei* B-7280 solution at a concentration of 10⁶ CFU/ml: experimental group 1 (E1) — daily; experimental group 2 (E2) — once every two days; experimental group 3 (E3) — once every four days; experimental group 4 (E4) — once a week. The total duration of drinking syrup and probiotics was 4 weeks. An increase in the content of total protein in the tissues of the entire bees' body was established in E1–E3 groups, but these differences are not significant, which may indicate the absence of an appreciable effect of the probiotic *L. casei* B-7280 on the protein concentration in bee tissues. An increase in the catalase activity of bee body tissues was observed by 79.80% and 38.07% in E1, E2 groups ($P < 0.05$) compared to the control, and for bees of E1 group by 52.75% ($P < 0.05$) compared to the preparatory period. The content of LHP decreased by 10.00%; 9.23% and 10.38% ($P < 0.05$) in E1, E2 and E3 groups, respectively, compared to the control. The content of TBC-active products tended to decrease in experimental groups E1, E2, E3, and in E4 by 14.98% ($P < 0.01$) compared to the preparatory period. The research results showed that the addition of *L. casei* B-7280 affected the antioxidant activity in the bees' body at different times of its feeding, which was accompanied by a decrease in the content of TBA-active products (MDA) and lipid hydroperoxides in the homogenates of the bees' body tissues in all experimental groups, except E3 group, and affected the viability of bees.

Key words: bees, probiotic, protein, peroxidation products, catalase activity



The exposure of lead nitrate on germination and vigor processes of *Helianthus* (sunflower) and *Lathyrus* (pea) seeds

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It is hereby studied the exposure of lead ions on germination and growth viability of helianthus and lathyrus seeds in laboratory conditions. Certain tendencies have been revealed showing the impact of different doses of the lead ions on germination of helianthus and lathyrus as well as on the length of the root and sprout in germinating the seed. Furthermore, a particular decrease ($P < 0.99$) in the helianthus seed germination was observed under the lead exposure at the concentration of 5×10^{-3} mol/L. Whereas, at the lead ions concentration in their amount from 5×10^{-6} mol/L to 5×10^{-4} mol/L, no considerable influence on helianthus and lathyrus seed germination was noticed. However, the helianthus and lathyrus root length has been exposed by the lead ions more significantly. It was noticed that the helianthus root had significantly decreased in length at the lead ions concentration of 5×10^{-3} mol/L, 5×10^{-4} mol/L, 5×10^{-5} mol/L and 5×10^{-6} mol/L ($P < 0.999$, $P < 0.999$, $P < 0.99$ and $P < 0.999$, respectively). The difference in terms of the lathyrus root length at 5×10^{-3} mol/L, 5×10^{-4} mol/L, 5×10^{-5} mol/L and 5×10^{-6} mol/L concentration of lead ions was found significant ($P < 0.955$, $P < 0.99$, $P < 0.95$ and $P < 0.99$, respectively). The study results with regard to the exposure of different doses of the lead nitrate on the average length of the helianthus sprout showed an significant difference rate ($P < 0.999$) in these values at the lead ions concentration in the solution of 5×10^{-3} mol/L, 5×10^{-5} mol/L and 5×10^{-6} mol/L. There was no significant difference between the lathyrus sprout length under the lead ions exposure between 5×10^{-6} mol/L and 5×10^{-3} mol/L. Thus, as far as the research results are concerned, there has been highlighted a significant lead nitrate exposure on economically useful indicators of the helianthus and lathyrus growing. A clear tendency of the lead nitrate impact on the helianthus and lathyrus seed germination and on their root and sprout length was revealed.

Key words: lead ions, the helianthus (sunflower) seed, the lathyrus (pea) seed, germination, root, sprout

Analysing Primary Sources

The activity of a technogenic agent, i.e. the people, has led to the accumulation of toxic contaminants in the soil, among which heavy metals are the leading ones.

The analysis of the harshness of accumulation of heavy metals in soil shows that this process increases

on the year-over-year growth basis. Heavy metals enter the soil mainly as insoluble compounds and over the time a part of them becomes soluble. Heavy metals may accumulate in the soil environment in large quantities and move both horizontally and vertically.

Part of the heavy metals, being in their exchangeable form, is absorbed by plants and their products.

The major part of heavy metals is concentrated in the root system of the plant, owing to the plant's buffering system, and only some of these toxicants pass into the vegetative mass: the stem, leaves and inflorescence, including the seed. [3, 5].

It has been found that heavy metals in large quantities, the lead in particular, negatively affect the plant's physiological and biochemical processes. It is commonly known that the lead-induced toxicity for most plants is in the range of 1000–2000 mg/kg. However, some plants are highly resistant to lead exposure even up to 10 g/kg. The intensity of lead accumulation in plants vary depending on many factors, and first of all, on their botanical origin. A high level of heavy metals accumulation is observed in plants of the *Brassicaceae* family, i.e. the cabbage, mustard, etc. [2, 7].

Adaptive responses of plants to lead ions are shown in their ability to tolerate high concentrations of lead ions without any significant metabolic disorders. The level of tolerance to heavy metals is determined by the ratio between their accumulation in plants and their effective detoxification. Research has revealed a difference in the distribution of these pollutants and their particles between the roots and sprouts of the *helianthus* plants. The roots, immersed into such ion's environment, accumulated much larger amounts of pollutants and the toxic exposure of cadmium ions was much stronger than the exposure of lead ions. Obviously, the sensitivity of plants heavy metals exposure, and, accordingly, the tolerance index depends primarily on their effective binding and deposition into the vacuoles. Plants showed adaptation only under conditions of stress that caused no irreversible damage, while exposure to extreme stress did not allow adaptive mechanisms to manifest, and the tolerance index under the mentioned conditions was decreasing. [1, 4].

One of the effects of heavy metals on plants is the production of hydrogen peroxide, which in its excess negatively affects proteins and lipids of membranes and leads to DNA damage. This may indicate that one of the adaptive responses of plants to the heavy metal's exposure is the accumulation of hydrogen peroxide, which likely acts as a signalling molecule for further triggering of protective mechanisms in the plant cell. However, there is a need for further research of the adaptation response involving hydrogen peroxide, since there is a lack of knowledge with regard to accurate concentration of peroxide necessary to stimulate the antioxidant response and the adequate conditions for its regulation.

The exposure of lead ions on the activity of glutathione reductase means that these pollutants produce the stimulating effect at low concentrations, which leads to an increase in the activity of the enzyme, while high concentrations, with some exceptions, suppress activity of the enzyme.

The studies of oats in the conditions of anthropogenic contamination of the turf and podzolic soil revealed that when the concentration of lead in the soil was increased to the level of 5 and 10 TLV, the growth and development

of plants was suppressed, the absorbent hairs and the root itself undergone deformation and were underdeveloped, there was observed an inhibitory effect on plant development, and, in some cases, a plant death. [6, 8].

Materials and Methods

The *helianthus* and *lathyrus* seeds were germinated at different concentrations in the distilled water with the addition of lead nitrate as follows: control stage — distilled water without the lead nitrate, experiment no. 1 — lead nitrate at concentration of 5×10^{-3} mol/L, experiment no. 2 — lead nitrate at concentration of 5×10^{-4} mol/L, experiment no. 3 — lead nitrate at concentration of 5×10^{-5} mol/L, experiment no. 4 — lead nitrate at concentration of 5×10^{-6} mol/L.

The exposure of lead nitrate on *helianthus* and *lathyrus* seeds was evaluated by the following parameters: germination, root and sprout length.

Statistical processing of the research results was carried out using the Student's *t*-test.

Results and Discussion

Our research results proved that there is a certain exposure of different concentrations of lead nitrate in the aqueous solution on the germination of *helianthus* seeds, the length of root shoots and stem (see table 1).

Moreover, at the concentration of lead nitrate in the aqueous solution of 5×10^{-3} mol/L, 5×10^{-4} mol/L and 5×10^{-6} mol/L, the germination of *helianthus* seeds decreased by 7%, 3%, and 3%, respectively, as compared to the experiment where the lead nitrate was not added to the aqueous solution.

The root length on the third day of its germination was lower by 33.4%, 42.2%, 38.2% and 34.8% upon adding into an aqueous solution the lead nitrate in the concentration of 5×10^{-3} mol/L, 5×10^{-4} mol/L, 5×10^{-5} mol/L and 5×10^{-6} mol/L respectively, as compared to *helianthus* seeds at their control stage, where the lead nitrate was not added to the aqueous solution. A similar tendency was displayed on the seventh day of the *helianthus* root ontogeny. Thus, upon adding into an aqueous solution the lead nitrate in the amount of 5×10^{-3} mol/L, 5×10^{-4} mol/L, 5×10^{-5} mol/L and 5×10^{-6} mol/L, there was noticed a decrease in *helianthus* seed root length by 25.5%, 19.0%, 20.0% and 25.2%, respectively, as compared to the stage where the *helianthus* seeds were germinated without adding the lead nitrate into an aqueous solution.

The analysis of the lead nitrate exposure on the growth vitality of the sprout of *helianthus* seed vegetative mass showed that at 5×10^{-3} mol/L, 5×10^{-5} mol/L and 5×10^{-6} mol/L concentrations of the named toxicant in the aqueous solution the sprout decreased in length by 20.6%, 0.3% and 13.1%, respectively, as compared to the control stage values.

Table 1. The exposure of lead nitrate on germination and vigor processes of helianthus seeds

Experiment stages	Seed germination, %	Root length, mm		Sprout length on the 7 th day, mm
		3 rd day	7 th day	
Control stage	100	64.1±26.2	177.8±70.4*	143.2±31.9
5×10 ⁻³ mol/L	93**	42.7±15.9*	136.0±27.8*	113.7±36.1*
5×10 ⁻⁴ mol/L	97	37.0±13.3*	143.9±46.3*	142±46.9
5×10 ⁻⁵ mol/L	100	39.6±16.4**	142.1±57.3*	108.6±27.2*
5×10 ⁻⁶ mol/L	97	41.8±15.2*	132.9±59.7*	124.4±27.0*

There is also a proved exposure of the lead nitrate on germination of lathyrus seeds, their root and sprout length (see table 2). Thus, at the concentration of lead nitrate in the aqueous solution of 5×10⁻³ mol/L, 5×10⁻⁵ mol/L and 5×10⁻⁶ mol/L, the germination of lathyrus seeds increased by 1%, 1%, and 2%, respectively, as compared to the control stage values. At the lead nitrate concentration of 5×10⁻⁴ mol/L in the aqueous solution, no difference between the experimental and control stage values was found. Analysing the lead nitrate exposure on the lathyrus seed root length, it should be highlighted that at 5×10⁻³ mol/L, 5×10⁻⁴ mol/L and 5×10⁻⁵ mol/L concentrations of the named toxicant in the aqueous solution, the lathyrus root length was found lower on the third day by 87%, 13.3% and 12.4%, respectively, as compared to the control stage values. At the lead nitrate concentration of 5×10⁻⁶ mol/L in the aqueous solution, no difference between the experimental and control stage values was found. The lathyrus root length on the seventh day at 5×10⁻³ mol/L, 5×10⁻⁴ mol/L, 5×10⁻⁵ mol/L and 5×10⁻⁶ mol/L lead nitrate concentrations in the aqueous solution, was lower by 27.1%, 13.2%, 20% and 10.0%, respectively, as compared to the control stage values.

There is also proved that on the seventh day of the experiment the lathyrus seed sprout length at the lead nitrate concentration of 5×10⁻³ mol/L, 5×10⁻⁴ mol/L, 5×10⁻⁵ mol/L and 5×10⁻⁶ mol/L in the aqueous solution, was lower by 1.5%, 3.4%, 4.2% and 1.0% as compared to the control stage value.

Thus, the results of the research show a particular impact of the lead nitrate on economically useful indicators of the helianthus and lathyrus growing. A clear tendency of the lead nitrate exposure on the helianthus and lathyrus seed germination and on their root and sprout length was revealed.

A particular decrease (at P<0.99) in the helianthus seed germination was revealed at the lead nitrate concentration of less than 5×10⁻³ mol/L in the aqueous solution.

As far as a particular decrease in the helianthus and lathyrus seeds root length at the lead nitrate concentration between 5×10⁻⁶ mol/L and 5×10⁻³ mol/L is concerned, i.e. in all experiments regarding the lead nitrate

Table 2. The exposure of lead nitrate on germination and vigor processes of lathyrus seeds

Experiment stages	Seed germination, %	Root length, mm		Sprout length on the 7 th day, mm
		3 rd day	7 th day	
Control stage	98	48.1±17.5	204.±137.5	92.3±23.7
5×10 ⁻³ mol/L	99	43.9±20.3***	148.7±27.6*	90.9±30.7
5×10 ⁻⁴ mol/L	98	41.7±21.4**	177.1±34.8*	89.1±23.8
5×10 ⁻⁵ mol/L	99	42.1±19.9**	163.2±44.5*	88.4±24.9
5×10 ⁻⁶ mol/L	100	48.1±17.5***	183.6±41.0*	91.4±21.2

exposure on the length of helianthus seed sprout, it should be specified that a particular difference likely occurs at all experiment stages, except for the one where the concentration of the named toxicant amounted to 5×10⁻⁴ mol/L.

As far as the experiment on the lathyrus seed is concerned regarding the exposure of the lead nitrate on the sprout length, no particular difference was found.

As suggested by the results of the research, it was found that the germination of the helianthus and lathyrus seeds in the aqueous solution (distilled water) with the addition of the lead nitrate in the amount between 5×10⁻⁶ mol/L and 5×10⁻³ mol/L contributed to a slight increase in seed germination, a particular decrease in root length on the third and seventh day of experiment and the length of the sprout.

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Вплив нітрату свинцю на схожість та ростові процеси насіння соняшнику та гороху

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Вивчено вплив іонів свинцю на схожість та ростові процеси насіння соняшнику та гороху за їх пророщування в лабораторних умовах. Виявлено певні тенденції щодо впливу різних доз іонів свинцю на схожість соняшнику і гороху та довжину кореня і паростка за пророщування насіння. Зокрема, вірогідне зниження ($P < 0.99$) схожості насіння соняшнику спостерігали за впливу свинцю, концентрація якого складала 5×10^{-3} моль/л, тоді як за концентрації іонів свинцю від 5×10^{-6} моль/л до 5×10^{-4} моль/л вірогідного впливу на схожість насіння соняшнику та гороху не спостерігали. Помітніший вплив іонів свинцю виявлено на довжину кореня соняшнику та гороху. Спостерігали вірогідне зниження довжини кореня соняшнику за концентрації іонів свинцю 5×10^{-3} моль/л, 5×10^{-4} моль/л, 5×10^{-5} моль/л та 5×10^{-6} моль/л ($P < 0.999$, $P < 0.999$, $P < 0.99$ та $P < 0.999$ відповідно). Різниця в довжині кореня гороху за концентрації іонів свинцю 5×10^{-3} моль/л, 5×10^{-4} моль/л, 5×10^{-5} моль/л та 5×10^{-6} моль/л виявилась вірогідною ($P < 0.955$, $P < 0.99$, $P < 0.95$ та $P < 0.99$ відповідно). Результати досліджень з вивчення впливу різних доз нітрату свинцю на середню довжину паростка соняшнику засвідчили вірогідну різницю ($P < 0.999$) у цих показниках за концентрації іонів свинцю у розчині 5×10^{-3} моль/л, 5×10^{-5} моль/л та 5×10^{-6} моль/л. Вірогідної різниці між довжиною паростка гороху за впливу іонів свинцю від 5×10^{-6} моль/л до 5×10^{-3} моль/л не виявлено. Отже, за результатом проведених досліджень виявлено суттєвий вплив нітрату свинцю на господарсько корисні показники вирощування соняшнику та гороху. Встановлено певну тенденцію впливу нітрату свинцю на схожість насіння соняшнику і гороху та довжину кореня і паростка.

Ключові слова: іони свинцю, зерно соняшнику, зерно гороху, схожість, корені, паростки



Comparative analysis of different approaches for determining microbiological criteria in feed samples for animals and poultry

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KNV: Conceptualization; Methodology; Investigation; Data curation; Visualization.
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The article presents research results on microbiological criteria of animal and poultry feed conducted under the requirements of the State Monitoring of Animal and Poultry Feed in accordance with the Order of the State Service of Ukraine on Food Safety and Consumer Protection for routine studies of feed samples and for in-depth studies to detect the entire species composition of microorganisms in feed samples coming from feed production enterprises in Ukraine. Isolation and identification of isolates were carried out according to current documentation. Feed is one of the main components of the food chain within the "One Health" concept to which Ukraine is committed. In-depth microbiological studies isolated from animal and poultry feed samples isolates of *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria monocytogenes*, and *Listeria innocua*, confirming high levels of contamination by pathogens indicating potential risks of their spread and danger due to possible antimicrobial resistance (AMR) and potential transmission of acquired resistance to the normal microbiota of animals and humans through feed consumption. Furthermore, animal and poultry feed are not included in the list of objects of the National Strategy of Ukraine for Containing the Development of Antimicrobial Resistance.

Key words: animal and poultry feed, premixes, mixed fodder, bran, meal, fish meal, animal-origin meal, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria monocytogenes*, *Listeria innocua*

Introduction

The intensification of livestock production involves the introduction of biologically complete regulated feeding, which ensures a high level of growth, development, and productivity of animals and prevents diseases associated with metabolic disorders and poor feed quality. Additionally, the contamination of animal feed with conditionally pathogenic and pathogenic microorganisms, including zoonotic ones, which can cause bacterial diseases in animals and poultry, presents a significant problem [4]. The greater danger lies in the detection of antibiotic-resistant pathogens, especially those with acquired resis-

tance. Animal feed is a component of the food chain, thus directly relating to the "One Health" and "Global Health Security" strategies developed by leading global organizations such as the Food and Agriculture Organization of the United Nations (FAO), World Organization for Animal Health (WOAH, founded as OIE), and World Health Organization (WHO). The main priorities of these strategies are to preserve human and animal health and ensure the production of quality and safe products.

Ukraine is also involved in these strategies and conducts monitoring of raw materials and products throughout the agricultural sector, including the quality and safety of agricultural products. Specifically, within these monitoring

studies, information is collected, analyzed, and systematized regarding the contamination of animal and poultry feed with biotic contaminants — bacterial pathogens, including zoonotic ones [2, 11, 19].

However, the feed monitoring system in Ukraine does not cover all risks from feed production, identify critical points in technological processes, nor develop a prediction system for possible bacterial contamination of certain components of animal and plant origin and raw materials from other industries used for feed production. This situation is dangerous as feeding contaminated feed can pose risks of infectious diseases among animals or poultry and have epidemiological consequences. It is known that in EU countries, the USA, Japan, and other highly developed countries, the number of bacterial diseases associated with the food chain, where animal and poultry feed is a major component, is increasing [17, 18, 21]. Therefore, food safety is a high-priority issue in all countries, including Ukraine [8, 12, 19, 20].

Moreover, the most urgent problem of our time is the antibiotic resistance of pathogenic microorganisms, particularly those isolated during microbiological control of cattle, pig, and poultry feed materials, dry feed, and canned feed, as there remains a risk of transmission of such resistance to other animals, poultry, and humans. Although Ukraine has implemented a National Action Plan to combat antimicrobial resistance in line with the World Health Organization's (WHO) Global Strategy to Contain Antimicrobial Resistance, the plan does not cover Ukrainian feed production facilities.

Therefore, it was of interest to establish the entire species spectrum of microorganisms in animal and poultry feed and further test their sensitivity to antimicrobials and check for acquired resistance enzymes.

Materials and Methods

The research was conducted by scientists from the Research Microbiology Department of the State Scientific Research Institute for Laboratory Diagnostics and Veterinary-Sanitary Examination (Kyiv) and the Institute of Animal Biology NAAS (Lviv).

Microbiological monitoring studies were conducted on samples of various types of animal and poultry feed for non-compliance according to the Order of the State Service of Ukraine on Food Safety and Consumer Protection no. 56 dated 21.01.2023 and Order no. 898 dated 27.12.2023 on the approval of the State Monitoring Plan for Feed for 2023 and 2024, respectively.

The determination of microbiological criteria by routine methods for compliance with QMAFAnM, Coliform bacteria, *Staphylococcus aureus*, *Salmonella pathogenes*, *Listeria monocytogenes*, and sulfite-reducing clostridia was carried out according to current regulatory documentation, as well as on the demand of manufacturers according to their regulatory documentation.

Additionally, in-depth own studies were conducted to determine the complete species composition of bacterial microorganisms that are feed contaminants. These in-depth studies involved appropriate cultures from enrichment media, previously inoculated with feed samples and incubated at 37°C for 24 hours. Further inoculations were made on various differential-diagnostic media for the detection of *Escherichia coli*, bacteria of the genus *Staphylococcus*, and *Listeria* [3, 5, 6, 13–15].

For the isolation and identification of *E. coli* isolates from the accumulation medium, the cultures were made on special media: Endo, XLD, Ramback, Simons, and trisugar agar (TSA), media with phenol red and carbohydrates glucose, lactose, sucrose, maltose, arabinose, rhamnose, xylose, dulcitol, indole was tested and incubated in a thermostat at 37±1.0°C for 24 hours.

To isolate and identify isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis* from the accumulation medium, the samples were streaked on milk-salt agar, egg yolk salt agar, glucose-blood agar, and special Baird-Parker medium to identify the specific growth pattern, plasma-coagulation reaction with sterile rabbit blood plasma was performed, the presence of enzymes for the fermentation of carbohydrates lactose, glucose, mannitol, sucrose, maltose, xylose, arabinose, mannitol was studied, and tests for the detection of catalase and oxidase were performed.

For the isolation and identification of *Listeria monocytogenes* and *Listeria innocua* isolates from the accumulation medium, half Fraser's broth, full Fraser's broth, L-mono medium, selective PALCAM agar, and the CAMP test were performed.

Results and Discussion

During the study period from 01.07.2023 to 01.04.2024, 382 samples of various types of feed were examined for microbiological indicators, including premixes — 36; compound feed and bran — 47; meal and cake — 127; feed for non-productive animals — 66; fish meal — 9; animal-origin meal — 9; other types of feed — 88.

Analysis of the research results for the detection of *E. coli* isolated 21 isolates and identified them. When inoculated on Endo medium, *E. coli* had a characteristic growth in the form of red colonies with a metallic sheen and reddening of the medium beneath them. On XLD medium, *Escherichia* colonies were yellow with an opalescence zone around the colonies. On Rambak medium, green colonies were grown. On Simons medium, the test culture did not grow, and the medium did not change color, which is typical for *Escherichia*.

The growth characteristics of *E. coli* on the slanted column of triple sugar agar (TSA) were characterized by a color change from red to yellow in the slanted part and in the agar thickness due to the fermentation of sugars to acid by *Escherichia* and the change in medium pH. This indicated the characteristic biochemical properties of the studied *E. coli*.

The results of biochemical studies of *Escherichia* isolates to detect enzymes for fermenting glucose, lactose, sucrose, maltose, arabinose, rhamnose, xylose confirmed their presence. The isolates did not ferment dulcine. Biochemical properties confirmed the typical characteristics of *E. coli*. The production of indole, formed during the complete breakdown of proteins and manifested as reddening of the strip impregnated with Kovac's reagent, was confirmed in all *E. coli* isolates.

Thus, the analysis of microbiological research results established that 21 cultures were identified as *E. coli* because they possessed all the typical properties characteristic of this pathogen.

The analysis of the research results for the detection of staphylococcal infection pathogens isolated 38 isolates and identified them. Inoculations from enrichment media on milk-salt agar after cultivation in a thermostat showed the growth of opaque round colonies with even edges, convex, pigmented in yellowish, yellow, and white colors, of small and medium sizes. On yolk-salt agar, staphylococcal colonies grew light-colored, of medium size, with a zone of cloudiness around the colonies with an iridescent fringe, indicating lecithinase production. On Baird-Parker medium, characteristic growth of staphylococcus in the form of characteristic black colonies with a metallic sheen and a clear opalescence zone around them, typical for *S. aureus*, was observed.

Smears were made from individual characteristic colonies on Baird Parker medium to check the purity of the *S. aureus* isolate. The preparations were fixed and stained using the Gram's method. The microscopy of the preparations showed homogeneous Gram-positive cocci located separately, in pairs, in clusters and in packets, which confirmed the purity of the test culture of *S. aureus*. The results of the plasma coagulation test showed complete coagulation of rabbit blood plasma in 16 experimental isolates of staphylococci isolated from samples of pre-mixes, feed and bran and meal. Plasma coagulation was observed after different time periods from 2 h 30 min to 6 h and confirmed one of the characteristic typical properties of *S. aureus*. The plasma coagulation test revealed 22 cultures that did not coagulate blood plasma. During the study of biochemical properties of the experimental isolates, 16 experimental isolates were found to have sacrolytic enzymes for the fermentation of lactose, glucose, mannitol, maltose, which confirm the enzymatic properties typical of *S. aureus*. In 22 isolates, fermentation of mannose, maltose, sucrose and the absence of mannitol fermentation, which are characteristic of *S. epidermidis*, were detected. According to the results of catalase and oxidase tests, 16 test isolates showed catalase production and the absence of oxidase, which confirmed their belonging to the pathogen *S. aureus*.

Taking into account the results of studies on the haemolytic properties of the experimental isolates, complete haemolysis of sheep erythrocytes (β -haemolysis) with a transparent zone around the colonies was detected, which confirms one of the typical properties of *S. aureus*.

Based on the analysis of the results of tests for the detection of listeriosis pathogens, 8 isolates of *L. monocitogenes* and 4 isolates of *L. innocua* were identified. The growth of *Listeria* in Fraser broth one and two was characterised by darkening of the medium, which confirmed the presence of the pathogen. On selective PALCAM agar, the growth of small, dark colonies with a sunken centre and blackening of the medium beneath them was observed. On L-mono medium, *Listeria* bacteria grew in the form of greenish colonies, but a clear zone of opalescence was formed around the *L. monocitogenes* colonies (fig. 2), while such a zone was absent around the *L. innocua* colonies.

The analysis of the CAMP test results showed the presence of the pathogen *L. monocitogenes* by the presence of a zone of hemolysis and a zone of expansion and clearance of hemolysis around the *S. aureus* streak with a narrow zone of hemolysis near the *Rhodococcus equi* streak (fig. 3).

Based on the results of the CAMP test for the identification of *L. innocua*, the absence of haemolysis was detected in relation to the *S. aureus* and *Rh. equi* streaks.

A comparative analysis of the data obtained after the State Monitoring of Animal and Poultry Feed, as well as the data of microbiological tests conducted by routine methods and the results of in-depth studies of animal and poultry feed, revealed that in the first and second cases, the results of the studies do not provide a true picture of the epizootic situation regarding the level of contamination and species circulation of various types of microorganisms at feed production enterprises in Ukraine. In-depth studies have shown a rather problematic picture, since in addition to conditionally pathogenic microorganisms, zoonotic pathogens *E. coli*, *S. aureus*, and *L. monocitogenes* were identified (table).

Analysing the data obtained, it is evident that certain genera and species of pathogens are detected in samples of animal and poultry feed and their number in in-depth microbiological studies is higher than in other data obtained.

The research results showed that monitoring and routine studies do not detect or take into account a significant number of different bacterial contaminants of animal and poultry feed, which creates real risks of infection of animals with opportunistic and pathogenic microorganisms, including zoonotic ones. Our research results are confirmed by scientific data from other researchers [11, 12, 20].

Within the framework of the "One Health" concept, scientists emphasise the need to create a strong feed base, which includes a system and structure of feed production and is one of the main conditions for the economic modernisation of livestock production and ensuring food security in Ukraine [7, 9, 10].

The health, productivity and reproductive capacity of animals largely depend on the quality and suitability of feed for feeding. To determine these characteristics, laboratory and on-farm feed evaluation is mandatory [4, 16].

Table. Results of research on animal and poultry feed samples according to microbiological criteria using different research approaches ($n_1=1$; $n_2=3$; $n_3=81$; piece)

Microbiological non-compliance (type of pathogen)	Total tested/ isolated isolates of positive samples	Premixes	Grain	Compound feed, bran meals	Meals, cake	Feed for unproductive animals	Canned feed for animals	Fish meal	Meal of animal origin	Other types of feed
Results of monitoring studies										
Sulphite-producing clostridia	1	—	—	—	—	—	—	—	—	1 (cattle feed)
Total:	1	—	—	—	—	—	—	—	—	1
Results of routine studies										
<i>Salmonella</i> spp.	2	—	—	—	2	—	—	—	—	—
<i>Enterobacter</i> spp.	1	—	—	—	—	1	—	—	—	—
Bacterial contamination	3	—	—	1	—	—	—	1	—	—
Total:	3	—	—	1	2	—	—	1	1	—
Results of our own in-depth research										
<i>Escherichia coli</i>	21	2	6	7	6	—	—	—	—	—
<i>Staphylococcus aureus</i>	16	2	—	5	7	—	—	—	—	—
<i>Staphylococcus epidermidis</i>	22	2	2	9	8	—	—	—	2	1
<i>Listeria monocitogenes</i>	8	—	—	3	4	—	—	—	—	—
<i>Listeria innocua</i>	4	—	—	2	3	—	—	—	—	—
Total:	81	7	8	26	37	—	—	—	2	1



Fig. 1. Growth characteristics of *Staphylococcus aureus* on Baird-Parker medium. 1 — growth of *S. aureus* black colonies with a zone of opalescence

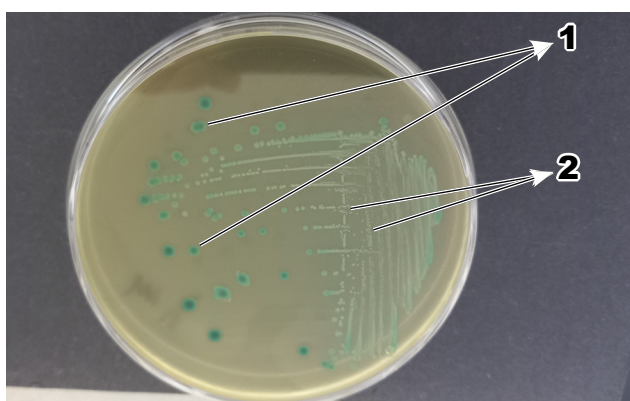


Fig. 2. Typical growth of *Listeria monocitogenes* and *Listeria innocua* colonies on L-mono medium. 1 — growth of greenish colonies with a zone of opalescence for *L. monocitogenes*; 2 — growth of *L. innocua* colonies of light colour and no opalescence zone

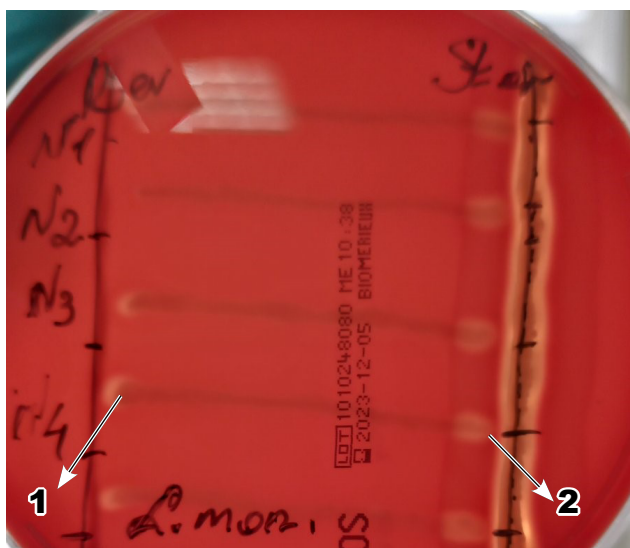


Fig. 3. Features of *Listeria monocitogenes* haemolysis manifestation in the CAMP test. 1 — a zone of expansion and clearance of haemolysis around the *Staphylococcus aureus* streak during the growth of *L. monocitogenes*; 2 — a narrow zone of haemolysis around the *Rhodococcus equi* streak during the growth of *L. monocitogenes*

Even greater risks lie in the fact that microorganisms isolated from animal and poultry feed are not currently monitored for antibiotic-resistant pathogens and acquired resistance to antimicrobials. As both animals and humans are part of the food chain in the “One Health” concept, the problem is exacerbated by the transmission of such acquired resistance to the normal microbiota of the intestinal tract of animals, poultry and humans.

It was found that among the 382 samples of premixes, grain, feed and bran, meal and cake, animal flour and cattle feed examined in depth, 81 samples were contaminated with opportunistic and pathogenic microorganisms that were not identified during monitoring and routine studies.

It was established that in-depth studies of microbiological criteria in animal and poultry feeds revealed a significant proportion of opportunistic pathogens and zoonotic pathogens of bacterial origin, including 21 isolates of *E. coli*, 16 isolates of *S. aureus*, 22 isolates of *S. epidermidis*, 8 isolates of *L. monocitogenes*, 4 isolates of *L. innocua*, which confirms the high level of contamination of animal and poultry feed with pathogens, indicates potential risks of their spread and danger due to the possible presence of antimicrobial resistance and acquired resistance mechanisms.

Prospects for further research are to test the isolated pathogen strains for antibiotic susceptibility, screening of enterobacteria and *Staphylococcus* spp. strains to identify and confirm the production of acquired enzymes, as animals, poultry and humans are integral parts of the food chain.

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Порівняльний аналіз різних підходів з визначення мікробіологічних критеріїв у зразках кормів для тварин і птиці

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В статті представлені результати досліджень мікробіологічних критеріїв кормів для тварин і птиці, виконаних за вимогами Державного моніторингу кормів для тварин і птиці згідно з наказом Державної служби України з питань безпеки та захисту прав споживачів, за проведення рутинних досліджень зразків кормів і за проведення поглиблених досліджень на виявлення всього видового складу мікроорганізмів у зразках кормів, які надходять із кормовиробничих підприємств України. Виділення і ідентифікація ізолятів проведено згідно з чинною документацією. Корми є однією із головних складових частин харчового ланцюга у концепції «Єдине здоров'я», до виконання якої долучена Україна. За поглиблених мікробіологічних досліджень виділені зі зразків кормів для тварин і птиці ізоляти *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria monocitogenes*, *Listeria innocua* підтверджують високий рівень їх контамінації патогенами, вказують на потенціальні ризики щодо їх розповсюдження, на небезпеку через ймовірну наявність у них стійкості до антибактеріальних препаратів (АБП) та ймовірну можливість передачі набутої резистентності нормальній мікробіоті організму тварин і птиці через споживання таких кормів та людині внаслідок споживання сировини і продукції тваринництва. До того ж корми для тварин і птиці не входять до переліку об'єктів моніторингу Державної стратегії України щодо стримування розвитку стійкості до протимікробних препаратів.

Ключові слова: корми для тварин і птиці, премікси, комбікорм, висівки, шрот, рибна мука, мука тваринного походження, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Listeria monocitogenes*, *Listeria innocua*



Features of *in vitro* cultivation of the field strain of canine parvovirus

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ROV: Investigation; Methodology; Formal analysis.

Declaration of Conflict of Interests:

None to declare.

Ethical approval:

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Intestinal infections remain one of the most important health care problems. According to WHO, viral diarrhea is the most common infectious disease in the world. A similar situation is registered among the diseases of dogs, where enteritis of infectious etiology occupies a leading place, and the leader is parvovirus enteritis from which dog owners have been suffering for the past forty years. Despite such a long period of time, unfortunately, proper means of treatment and prevention have not been created, and taking into account the persistence of the virus in the external environment and the ability to mutate, the correlation of diagnostic methods is always necessary, which, accordingly, indicates the relevance of studying parvovirus infection in dogs. The use of cell cultures as test objects for the cultivation and accumulation of viral biomass makes it possible to obtain an antigen suitable for use in the manufacture of diagnostic, prophylactic and therapeutic biological preparations. The publication contains research materials on the peculiarities of the cultivation of canine parvovirus isolated on the territory of Ukraine from a 2-month-old purebred puppy that died of parvovirus enteritis. In the biological material isolated from the dead animal by means of laboratory studies, mono-infection and the possibility of its cultivation *in vitro* were established. The possibility of using a transplantable line of rabbit kidney cell culture (RK-13) has been established. Freshly reseeded, after 24 hours of incubation, with at least 70% and no more than 80% cell monolayer and seeding concentration from 1.0×10^5 to 2.0×10^5 cells/ml, as a biological test object for cultivation and accumulation parvovirus biomass. Cultivation was carried out in a thermostat at $t = +37.5^\circ\text{C}$ for no more than 10 days, and a mixture of medium 199 and DMEM was used as a supporting medium. The effectiveness of cultivation was determined by the increase in the titer of the infectious activity of the virus, which by the fifth passage was $3.8 \pm 0.08 \lg \text{TCD}_{50/\text{cm}^3}$.

Key words: canine parvovirus, field isolate, cultural properties, cytopathogenic effect, RK-13



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Introduction

Recently, there has been a tendency in the world to increase the population of companion animals, among

which dogs occupy a special place. In connection with the increased interest in breeding and keeping small domestic animals and the growth in the number of the latter's population in personal and official use, led to an increase

in the registration of cases of various infectious diseases [11, 17]. But today, knowledge about the diagnosis, pathology of most infectious diseases, the use of effective methods of therapy is not enough. Therefore, the fight against infectious diseases of animals is the most urgent problem for modern veterinary medicine, among which a special place belongs to viral diseases [12, 22], which are characterized by a wide spectrum of distribution with a long-lasting effect on the health of animals, namely, extremely high lability of clinical signs, which complicate diagnosis for a practical veterinarian [2, 10].

The most widespread, highly contagious and key enteropathogen among dogs is parvovirus, which affects the dog population worldwide, causing parvovirus enteritis in the latter, which is one of the main causes of morbidity and mortality in puppies [19, 20]. The lethality of dogs from parvovirus enteritis ranges from 10 to 70%, depending on the form of the disease. The manifestation of parvovirus infection has several pronounced peaks of activity, namely: the beginning, which reached the scale of the pandemic in the eighties and remained at a significant level almost until the nineties; the next, less pronounced but characteristic peak of activity was recorded precisely in the nineties, and the third, the least pronounced, was established at the beginning of the two thousandths [1, 18]. Taking into account the high contagiousness against the background of not always effective vaccination, we get a constant circulation of pathogens, especially in places of significant crowding due to the keeping of dogs. The problem lies in the insufficiently deep study of the biological properties of canine parvovirus, in particular the cultural properties and the possibility of obtaining and accumulating a cultural antigen suitable for improving the diagnosis of this disease in dogs.

The detection of the virus in natural and clinical samples is a complex problem of research and diagnostics, therefore, the reproduction of the virus in laboratory conditions is an important experimental tool in fundamental virological research, which allows studying the peculiarities of the reproduction of the virus, its interaction with the host cell, and knowledge of the pathogenesis of the disease, the etiological factor of which there is a virus. In addition, the study of the features of virus reproduction provides a basis for the diagnosis of viral diseases and the production of vaccines, as well as the creation of recombinant viruses for potential use in gene therapy [5, 17].

Summarizing the above, the aim of our work was to study the *in vitro* cultural properties of a field isolate of canine parvovirus on transplantable lines of cell cultures, with the possibility of obtaining a biological object suitable for the accumulation of viral biomass.

Research Material and Methodology

During the research, the basic rules of good laboratory practice GLP were followed (*Good laboratory practice*), provisions of the "General Ethical Principles of Animal Ex-

periments", adopted by the First National Congress of Bioethics (Kyiv, 2001). The experimental part of the research was carried out in accordance with the requirements of international principles "European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Purposes" (Strasbourg, 1986), "Rules for conducting work with the use of experimental animals", approved by the order of the Ministry of Health no. 281 of November 1, 2000 "On measures to further improve organizational forms of work with the use of experimental animals" and the corresponding Law of Ukraine "On protection of animals from cruel treatment" (no. 3447-IV dated February 21, 2006, Kyiv). Euthanasia was performed using drugs that ensure rapid, painless death, in accordance with the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes" (conclusion on the procedure for carrying out scientific research in accordance with bioethical norms, approved at the meeting of the Department of Epizootology, Microbiology and Virology of the National University of Life and Environmental Sciences of Ukraine protocol no. 4 from 16.04.2024) [3, 4, 6, 13].

Diagnostic studies for the confirmation of parvovirus were carried out using *VetExpert* express test systems in dogs (CPV/CCV Ag, solid-phase immunochromatographic analysis for qualitative detection of Canine Parvovirus/Coronavirus antigen), manufactured in Poland. Diagnostic studies on the presence of mono-infection with only parvovirus antigen in dogs were carried out on the basis of the private veterinary laboratory of LLC "Bald" (Kyiv) using enzyme-linked immunosorbent assay and polymerase chain reaction. For isolation of parvovirus antigen from pathological material and accumulation of viral material, transplantable lines of cell cultures SPEV (pig embryo kidneys), BHK-21 (Syrian hamster kidney fibroblasts), RK-13 (rabbit kidneys) were used. Virological studies, namely the isolation and identification of viruses, were carried out using cell cultures, by infecting laboratory animals. Cells were grown in glass and plastic mattresses with a volume of 25, 50 and 100 ml. stationary method, as well as in the wells of polystyrene plates with a seed concentration from 1.0×10^5 to 2.0×10^5 cells/ml. Reproduction of field isolates of parvovirus was carried out on cell culture with the formation of a monolayer in the range of 70–80%. At the end of the incubation period, the infected culture was frozen at a temperature -30°C and subjected to thawing in order to destroy the structure of cell integrity due to temperature "stress" and ensure the complete release of the virus. Microbiological studies to determine bacterial contamination and antibiotic sensitivity of detected microorganisms were carried out by sowing pathological material on nutrient media. Calculation of the infectious titer was calculated according to the method of Reed and Mench. The cultured virus-containing liquid was used to perform RGA to detect the agglutinating properties of parvovirus and coronavirus to erythrocytes of animals of various species. A suspension of erythrocytes and RGA was obtained according to generally accepted methods [16, 21].

Statistical processing of data was carried out using the *Statistica* program, all experiments were performed in 3 repetitions. average values were considered significant at $P < 0.05$ [15].

Research Results and Their Discussion

The work was carried out at the department of epizootology, microbiology and virology in the conditions of the problematic research laboratory "Microbiology, virology and immunobiotechnology" of the Ukrainian National Institute of Biotechnology. A fragment of the dog's intestine was used to isolate the virus after it died from parvovirus enteritis. The intestine with its contents was removed during the post-mortem examination, placed in a sterile dish with physiological saline and frozen. After thawing, the material was crushed by grinding in a mortar with sterile quartz sand, diluting with Hanks' solution (1:10). Large particles were removed by centrifugation for 20–30 min at 2500 rpm./min ($g = 800$). Next, the supernatant liquid was collected, antibiotics were added to the sediment (1000 units of penicillin and streptomycin per 1 ml), and placed in a refrigerator at 4°C for 60 min. Control of the effectiveness of such treatment was carried out by means of sowing on nutrient media for aerobes, anaerobes and fungi. After receiving a negative result of bacteriological control, the virus-containing material was used to infect cell cultures.

For isolation, adaptation and accumulation of parvovirus, primary trypsinized and transplanted cell cultures are used. Successful cultivation of viruses is carried out in cell cultures obtained from animals sensitive to this virus. The most effective reproduction of canine parvovirus occurs in cells obtained from the organs of dogs and cats, including: in cultures of cat or dog kidney cells, mink lungs, without manifestation of a cytopathogenic effect. But, according to other researchers, canine parvovirus is able to detect the cytopathogenic effect of the virus in the cat kidney cell line [7, 8]. In order to expand the list of the use of biological objects suitable for the cultivation and accumulation of parvovirus, we conducted an experimental study on the suitability such transplantable lines of cell cultures as hamster kidney (BHK-21), rabbit kidney (RK-13) and pig kidney (SPEV) (fig. 1).

In the body of animals, most viruses show a tropism towards young, rapidly dividing cells, so we tested the infection of cell culture at different time periods after reseeding and cell population of cell cultures. It was established that newly transplanted cells with at least 70% and no more than 80% monolayer of cells after 24 hours of incubation were the most sensitive to the incubation of the virus-containing isolate. Control of the number of introduced cells during reseeding was carried out using a camera with a Goryaev grid. The most optimal seeding concentration was from 1.0×10^5 to 2.0×10^5 cells/ml. For the cultivation of transplantable lines of cell cultures, a nutrient medium was used, which included media 199 and DMEM — 45% each, fetal bovine serum — 10% and antibiotics.

Incubation of cell cultures was carried out in a thermostat at $t = +37.5^\circ\text{C}$ for no more than 10 days. For intensive accumulation of culture material that can be used for infection and accumulation of virus-containing material, cell cultures were reseeded every 72 h, this also stimulated cell growth at a more intensive rate, which is necessary for better contact of the virus with the cell. A solution of trypsin and versene in a ratio of 1:6 to 1:3 and an antibiotic was used for transplanting the culture. The concentration was adjusted depending on the state of "seeding" of the mattress with cells.

Infection was carried out as follows: the growth medium was drained from the mattress and 2.5–3 cm³ of viral material was introduced into it. The mattress was placed in a thermostat for an hour at a temperature of $+37.5^\circ\text{C}$. At the same time, every 15 min, the monolayer of cells was slowly washed with viral material 4–5 times, after which the latter was drained and a supporting medium was introduced into the mattress, which for the infected cell culture had the same composition as the nutrient medium, only without serum. Cultivation was carried out according to generally accepted methods, evaluating the cytopathogenic effect (CPD) according to the four-plus system, under an inverted microscope at low magnification, relative to the control.

The simplest signs that indicated the multiplication of the virus were degenerative changes in the cells, that is, the manifestation of the virus's CDP — the formation of visible morphological changes in the cell is called a cytopathogenic effect or action. Cytopathogenic changes in infected cell cultures depend on the biological properties and dose of the virus under study (fig. 2).

The cytopathogenic effect of parvovirus was manifested in the following sequence: inhibition of cell division processes, partial shrinkage of cells, partial darkening in the form of cell agglutination. Subsequently, the development of classical changes, namely the destruction of cells by their stretching and acquiring the shape of a "star", which was followed by the rupture of the cell wall. Incubation of viruses on cell cultures was carried out for no more than 10 days at $t = +37.5^\circ\text{C}$, monitoring the state of the cell monolayer every 24 h under a microscope at low magnification ($\times 56$) (fig. 2).

As a result of our experiment, it was established that the most sensitive cell culture line for cultivating a field isolate of parvovirus was rabbit kidney and slightly less sensitive hamster kidney after the third passage, and pig kidney cell culture was more sensitive in the first three passages. The reproduction of parvovirus in cell culture was controlled by the time of manifestation of CPD, which directly depended on the density of the monolayer of cells: after 70% — after 48 h, 80% — after 72 h, and if more than 90% of the monolayer — the destruction of cells occurred rather slowly, it was necessary to incubate the culture up to 10 days. At the end of the incubation period, the infected culture was frozen at a temperature -30°C and subjected to thawing in order to destroy the structure of cell integrity due to temperature "stress" and ensure the complete release of the virus.

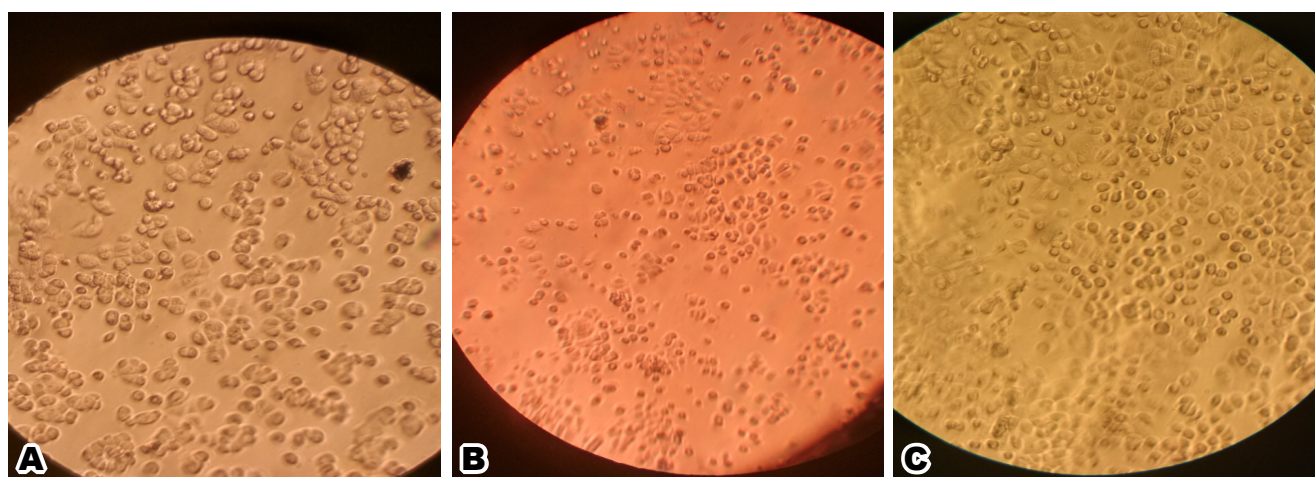


Fig. 1. Cell cultures, 12 hours after transplanting: A — RK-13; B — BHK-21; C — SPEV. $\times 56$

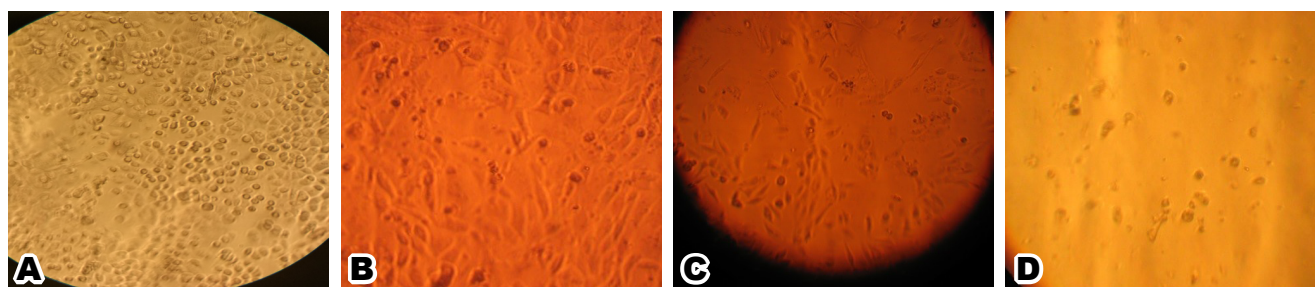


Fig. 2. Effect of parvovirus on RK-13 cell culture ($\times 56$). A — before infection; B — state of cell culture 48–72 hours after infection; B — state of cell culture 4–5 days after infection; D — state of cell culture 7–8 days after infection

The sensitivity of the cell lines was determined not only by the expression of the CPD of the virus on the cells, but also by the level of accumulation of the virus in the cell suspension. The titer of infectious activity of the material was determined by titration of viral material in cell culture on sterile 96-well microplates. Calculation of the infectious titer was performed according to the method of Reed and Mench. In the course of the experiment, the following results were obtained: at the beginning of the experiment, the titer of infectious activity on the culture of rabbit kidney cells averaged $1.5 \lg \text{TCD}_{50/\text{cm}^3}$, after the third passage — up to $2.7 \pm 0.06 \lg \text{TCD}_{50/\text{cm}^3}$, and after 1st — increased to $3.8 \pm 0.08 \lg \text{TCD}_{50/\text{cm}^3}$.

Therefore, analyzing literary sources, it was established that today most parvoviruses isolated from dogs are cultivated *in vitro* on cell cultures obtained from the organs of dogs and cats [9, 14]. We have established the suitability for cultivation and accumulation of a field isolate of parvovirus on the culture of rabbit kidney cells (RK-13), where after adaptation of the virus, the cytopathogenic effect was noted after 72 h. incubation, and on the 5th–7th day — 90–100% cytopathogenic effect, while the titer of infectious activity increased with each new passage of viral material and by the fifth was $3.8 \pm 0.08 \lg \text{TCD}_{50/\text{cm}^3}$.

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Особливості культивування *in vitro* польового штаму парвовірусу собак

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Кишкові інфекції залишаються однією із найважливіших проблем охорони здоров'я. За даними ВООЗ, найпоширенішими у світі серед інфекційних хвороб є вірусні діареї. Подібна ситуація реєструється і серед хвороб собак, де ентерити заразної етіології посідають провідне місце, а лідером є парвовірусний ентерит, від якого власники собак потерпають останні сорок років. Незважаючи на такий тривалий термін часу, на жаль, не створено належних засобів лікування і профілактики, а враховуючи стійкість вірусу у зовнішньому середовищі і здатність до мутації, завжди необхідною є кореляція діагностичних методів, що, відповідно, свідчить про актуальність вивчення парвовірусної інфекції у собак. Використання культур клітин як тест-об'єктів для культивування і накопичення вірусної біомаси дає можливість отримання антигена, придатного для використання у виготовленні діагностичних, профілактичних і лікувальних біопрепаратів. У публікації наведено матеріали досліджень особливостей культивування парвовірусу собак, виділеного на території України від безпородного цуценяти віком 2 місяці, який загинув від парвовірусного ентериту. В отриманому від загиблої тварини біологічному матеріалі лабораторними дослідженнями встановлено моноінфікування і можливість його культивування *in vitro*. Встановлено можливість використання перещеплювальної лінії культури клітин нирки кроля (RK-13), щойно пересіяної, після 24 годинної інкубації, з не менше 70% і не більше 80% моношаром клітин і посівною концентрацією від $1,0 \times 10^5$ до $2,0 \times 10^5$ кл./мл як біологічного тест об'єкта для культивування і накопичення парвовірусної біомаси. Культивування проводили в умовах термостату за $t = +37,5^\circ\text{C}$ не більше 10 діб., а як підтримуюче середовище використовували суміш середовища 199 і ДМЕМ. Ефективність культивування визначали за зростанням титру інфекційної активності вірусу, який до п'ятого пасажу становив $3,8 \pm 0,08 \lg \text{ТЦД}_{50/\text{гсм}}^3$.

Ключові слова: парвовірус собак, польовий ізолят, культуральні властивості, цитопатогенна дія, RK-13



Influence of Zinc, Selenium and Germanium citrates nanoparticles on hematological and biochemical parameters of rabbits under moderate heat stress

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Authors' Contributions:

YM: Conceptualization; Project management; Methodology; Formal analysis; Research; Resources; Data curation; Writing — original draft, review & editing; Visualization.

Declaration of Conflict of Interests:

None to declare.

Ethical approval:

A permission to conduct the study was obtained from the Bioethics Committee of the Institute of Animal Biology NAAS of Ukraine (Protocol no. 152 from 10.04.2024, Lviv, Ukraine).

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Climate change leads to increased environmental temperatures, which negatively affects the bodies of animals. Rabbits do not have sweat glands and are not able to regulate their body temperature, which, under conditions of heat stress, disrupts the physiological processes of the body: body thermoregulation, respiration, immune function, endocrine regulation, blood parameters, reproductive capacity, which are critical factors in ensuring the functioning of the body. In recent years, in order to mitigate heat stress, considerable attention has been focused on compounds produced by nanotechnology, which have a positive effect on the animal body compared to organic and inorganic substances and have a wide range of biological effects. However, the effect of macro- and microelement nanoparticles on the mammalian body depends on the amount used. Therefore, the purpose of the experiment was to study the hematological and biochemical parameters of rabbits after weaning under moderate heat stress by feeding Zinc, Selenium and Germanium citrates produced by nanotechnology.

Key words: rabbits, blood, moderate heat stress, nanoparticles, Zinc citrate, Selenium citrate, Germanium citrate, hematological and biochemical parameters

Introduction

In the context of global warming, an increase in ambient temperature becomes a negative factor for the development of rabbit breeding [10, 17]. Heat stress has negative consequences for the health of rabbits, leading to a decrease in daily weight gain by 20–25%, a decrease in feed intake by 8–15%, an increase in mortality by 9–12%, and a decrease in reproductive capacity by 6–10% [19]. Under heat stress conditions, rabbits cannot maintain a balance between heat production and heat release [26]. The optimal physiological values of the rabbit organism's temperature and humidity range are 18 to 21°C and humidity 55–65% [21, 22]. To reduce the adverse effects of heat stress in animal feeding, organic compounds of trace elements of nanotechnological origin are used. Trace element nanoparticles, in combination with organic

acids, are characterized by high bioavailability, surface activity, catalytic and adsorption properties, and low toxicity [9].

Zinc is a component of more than 300 enzymes. It is essential physiologically for the functioning of alcohol dehydrogenase, alkaline phosphatase, aldolase, lactate dehydrogenase, RNA and DNA polymerase, transcriptase, carboxypeptidase A, B, G, and superoxide dismutase [32]. It is involved in the biosynthesis of nucleic acids, cell division processes, and metabolism of proteins, lipids, and carbohydrates [4]. Zinc plays a vital role in the antioxidant defense system and inhibits the oxidation of DNA and protein macromolecules [5, 28]. It regulates proliferation, differentiation, apoptosis, and metallothionein gene expression [6]. The addition of zinc oxide nanoparticles to the diet of rabbits at doses of 20, 40, 60, and 80 mg/kg body weight reduces the level of alanine aminotransferase

and aspartate aminotransferase activity, creatinine, and urea, which may indicate an improvement in liver and kidney function [1]. A study by F. Hasan et al. found that rabbits treated with zinc oxide nanoparticles at 30 and 60 mg/kg body weight had better growth and feed intake than the control group [12]. Studies by D. A. Kamel et al. found that the addition of 50 mg of ZnO/kg or 30 mg of nano-Zn/kg in the diet of rabbits increased the levels of glutathione, glutathione-S-transferase, superoxide dismutase, IgG and IgM immunoglobulins, and high-density lipoprotein while reducing the concentration of triacylglycerols and TBA-active products in the blood serum of rabbits under heat stress [14].

Selenium is a critical element of the composition of selenoproteins, which regulate thyroid hormones, act as inhibitors of the nonspecific immune response, and neutralize inflammatory and phagocytic processes [11]. Adding 0.3 mg/kg of organic Selenium to the diet reduces rectal temperature, respiratory rate, and concentration of TBA-active products in the sperm plasma, increases the total number of spermatozoa, the number and body weight of the offspring of rabbits exposed to heat stress [13]. Studies by M. S. Ayyat et al. found that feeding organic Selenium at 0.03 mg/kg of rabbit diet mitigated the adverse effects of heat stress, which was reflected in a decrease in rectal temperature, respiratory rate, and heart rate in the summer [3]. Adding Selenium citrate to the diet at 25 and 50 mg/kg to rabbit feed increases the average daily weight gain, reduced glutathione, and catalase activity. It reduces the level of TBA-active products in the blood serum of rabbits under heat stress [30]. Toxicological studies have shown that selenium nanoparticles of 20–60 nm and Se-methionine in amounts of 30 and 70 µg Se/kg in the diet of mice improve Se accumulation in the blood, liver, and kidneys compared to the control [35].

It is known from the literature that organic germanium can adsorb free radicals and increase the body's antioxidant activity, especially during times of stress [15]. Once in the body, germanium interacts with hemoglobin and ensures cellular metabolism [18]. Germanium compounds improve the immunological characteristics of lymphocytes, including T-helper cells and cytotoxic T-suppressors, and stimulate the production of various cytokines, which can help strengthen the immune system in the fight against various diseases [31]. A significant interferon activity in the blood serum was found after oral administration of the organic germanium compound Ge-132 300 mg/kg body weight to mice [2]. Studies have shown that Ge-132 increases the cellular stimulatory factor IL-3 level in stem cells, which helps regulate the processes of their differentiation and proliferation and ensures regulation and balance in the processes of blood formation [18].

Given the above, the aim of the study was to determine the effect of Zinc, Selenium and Germanium Citrates obtained by nanotechnology on the morphological and biochemical parameters of rabbit blood after weaning under moderate heat stress.

Materials and Methods

The study was conducted in the vivarium of the Institute of Animal Biology, National Academy of Sciences (Lviv, Ukraine). Animals were kept in a room with a regulated microclimate in mesh cages measuring 50×120×30 cm. The study was conducted on young rabbits-analogues from 35 to 78 days of age, the Thermon White breed. For the study, groups of 6 animals were formed, 24 animals in total. During the experimental period, the room temperature was increased for 12–16 hours using electric adjustable heaters for 43 days. The temperature range during the study was from 27.8 to 28.9°C.

The temperature and humidity were monitored using a *Trotec BL30* thermo-hygrometer with a data logger. An electronic air analyzer measured humidity and temperature (patent No. 127047) [23]. The temperature-humidity index controlled the room temperature. Animals for the study were selected in the control and I, II, and III experimental groups, with an average body weight of 980±50 g. Rabbits of the control group were exposed to heat stress and kept on an essential diet of standard balanced granular feed and water without restriction.

Rabbits of I, II, and III experimental groups consumed pelleted feed as in the control but received citrates of trace elements with water for 24 hours daily for 43 days. Using individual drinkers for each animal and placing the animals in separate cages allowed us to control the amount of water each rabbit received. I experimental group received Zinc citrate in amount of 60 mg Zn/l or 12 mg Zn/kg body weight; II group — Selenium citrate, 300 µg Se/l or 60 µg Se/kg body weight; III group — Germanium citrate, 62.5 µg Ge/l or 12.5 µg Ge/kg body weight. "Nanomaterials and Nanotechnologies" LLC (Kyiv, Ukraine) manufactured the solutions for the study (patent no. 38391) [16]. The appearance of metal particles was studied using a *JEM 100CX II* transmission electron microscope. Permission to conduct the research was obtained from the Bioethics Committee of the Institute of Animal Biology of the National Academy of Sciences of Ukraine, Lviv (protocol No. 152 of 10.04.2024). All manipulations with experimental animals were carried out following the provisions of "The General Ethical Principles for Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001) and the rules of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986). The blood parameters of rabbits were studied on the 14th day of the preparatory period and the 14th and 29th days of supplementation in the experimental period under moderate heat stress conditions.

For hematological studies, blood was taken from the marginal ear vein of rabbits into tubes with the anticoagulant ethylenediaminetetraacetate (*EDTA-K2+*), and the total number of red blood cells and red blood cell indices were determined: absolute red blood cell count (RBC),

mean erythrocyte volume (HGB), hematocrit (HCT), mean erythrocyte corpuscular volume (MCV), mean erythrocyte hemoglobin content (MCH), mean erythrocyte hemoglobin concentration (MCHC), erythrocyte distribution width (RDW); the number of white blood cells (WBC) and their forms — lymphocytes (LYM), monocytes (MON), granulocytes (GRA); platelet count and platelet indices: absolute platelet count (PLT), mean platelet volume (MPV), thrombocrit (PCT), relative platelet distribution width (PDW) using an automatic hematology analyzer *Orphee Mythic 18* (Switzerland) [33]. Samples of whole blood were taken from the marginal ear vein of 6 animals from the group into tubes with 1% heparin for biochemical studies. The content of total protein, albumin, alanine aminotransferase (ALT) activity aspartate aminotransferase (AST), and alkaline phosphatase (ALP), triacylglycerols, cholesterol, total calcium, and inorganic phosphorus was determined using a *Hymalyzer 2000* biochemical analyzer [33].

The study results were analyzed using the *Statistica 7.0* software package (*Statsoft*, USA). The experimental data are presented as mean (M) ± standard deviation (SD). The study's quantitative data were tested for homogeneity of variances using the Levene test. Multiple comparisons were made using a two-factor analysis of variance (Two-way ANOVA), where factor A is time, factor B is trace element citrates, and AB is the interaction of time and trace element citrates. To detect statistical differences between the control and experimental groups, the *a posteriori* Tukey HSD method was used, and differences were considered significant at $P \leq 0.05$ [27].

Results and Discussion

Rabbits have the most negligible lung weight concerning the body weight of all farm animals, so they need a constant supply of clean air to ensure the body's functioning [4]. Studies have shown that feeding rabbits Zinc citrate and Selenium citrate in the blood, respectively, increased the number of red blood cells by 22.2 ($P < 0.01$) and 20.2% ($P < 0.05$) compared to the control at the final stage of the study, which may indicate a stimulating effect on erythropoiesis (table 1). The analysis of the results of hemoglobin concentration in animals of I, II, and III groups were respectively higher by 16.3% ($P < 0.01$), 28.5% ($P < 0.001$), and 21.9% ($P < 0.001$) on the 29th day of the study. The percentage of hematocrit value in the blood of rabbits of the I and II experimental groups increased by 27.3% ($P < 0.01$) and 20.7% ($P < 0.05$), respectively, on the 29th day of the experiment. The increase in the concentration of hemoglobin and the percentage of hematocrit value in the blood of rabbits directly correlates with the number of red blood cells.

The effect of time factor A ($P = 0.020794$) and the factor of trace element citrates B ($P = 0.019834$) was reflected in statistically significant changes in the absolute red blood cell content. The analysis of the combination of AB factors did not show a statistically significant result ($P = 0.147556$).

Table 1. Number of red blood cells, hemoglobin, and hematocrit in the blood of rabbits after feeding Zinc, Selenium, and Germanium citrates compounds under moderate heat stress (M±SD, n=6)

Indicators	Group	Period of research, age/day of supplementation		
		Preparatory period	Study period	
		49/1	63/14	78/29
RBC, $10^{12}/l$	C	5.76±0.51	5.81±0.70	5.04±0.62
	I E	6.08±0.69	5.84±0.53	6.16±0.55**
	II E	6.28±0.55	6.02±0.56	6.06±0.63*
	III E	6.45±0.42	5.93±0.69	5.41±0.26
HGB, g/l	C	132.67±5.75	133.00±10.21	109.17±6.11
	C	135.17±8.47	134.50±12.56	127.00±5.25**
	I E	149.66±16.57	145.66±13.54	140.33±9.77***
	II E	150.00±10.82	134.67±8.01	133.16±5.63***
HCT, L/l	C	0.413±0.07	0.387±0.06	0.362±0.02
	C	0.467±0.06	0.433±0.04	0.461±0.03**
	I E	0.449±0.05	0.416±0.07	0.437±0.07*
	II E	0.502±0.06	0.467±0.04	0.442±0.01

Note. In this and the following tables, the statistically significant differences compared to the control group are: * — $P < 0.05$; ** — $P < 0.01$; *** — $P < 0.001$.

Table 2. Erythrocyte indices in the blood of rabbits after feeding Zinc, Selenium, and Germanium citrates compounds under moderate heat stress (M±SD, n=6)

Indicators	Group	Period of research, age/day of supplementation		
		Preparatory period	Study period	
		49/1	63/14	78/29
MCU, fl	C	97.43±2.30	98.23±2.31	97.23±3.46
	I E	96.31±2.53	101.10±2.74	98.53±2.28
	II E	98.00±2.52	102.57±2.57	99.46±2.45
	III E	95.03±1.36	100.15±4.38	100.37±3.14
MCH, pg	C	23.56±1.11	23.53±0.95	23.83±0.84
	I E	23.83±0.43	23.90±1.23	24.61±0.90
	II E	24.38±0.77	25.03±0.88	24.40±0.94
	III E	24.68±0.57	24.71±1.23	25.10±0.15
MCHC, g/l	C	241.67±2.87	225.83±6.49	231.17±5.98
	I E	247.17±3.54	228.17±3.18	243.00±2.96**
	II E	243.67±5.27	235.83±3.65*	245.50±3.53***
	III E	244.67±1.75	232.17±6.01	234.00±6.35
RDW, %	C	10.23±0.42	10.38±0.60	10.43±0.58
	I E	10.68±0.36	10.81±0.34	10.98±0.53
	II E	10.36±0.20	10.46±0.62	10.60±0.43
	III E	10.75±0.59	10.96±0.52	10.50±0.63

The analysis of the time factor A ($P = 0.000021$) and the factor of trace element citrates B ($P = 0.000001$) resulted in statistically significant changes in hemoglobin content, where the expressed effect was observed for factors A and B. The results of the analysis of the combination of AB factors did not show a statistically significant result ($P = 0.079910$).

The effect of time factor A ($P = 0.051486$) had no statistically significant effect on the hematocrit value, but

the effect of trace element citrates B ($P=0.000400$) had a significant effect on the studied index. In the combined interaction of the two factors, there were no statistically significant changes in AB ($P=0.588368$).

In the blood of rabbits, an increase in the average concentration of hemoglobin in the erythrocyte was found after feeding Zinc citrate by 5.11% ($P<0.01$) on the 29th day of the study and Selenium citrate by 4.42 and 6.19% ($P<0.05-0.001$), respectively, on the 14th and 29th days of the experiment (table 2). Zinc is a necessary coenzyme for many enzymes and participates in the synthesis of DNA, RNA, and proteins. In particular, it is essential for the activity of ribonucleic polymerase, which is necessary for the transcription of genes encoding hemoglobin proteins and other essential components of red blood cells [1]. Selenium is a component of enzymes such as glutathione peroxidase and thioredoxin reductase, which protect cells from oxidative stress, reducing the level of damage to erythropoietic cells in the bone marrow, allowing them to multiply more efficiently and differentiate into mature red blood cells [6].

In our opinion, due to their antioxidant properties and essential function in the processes of enzyme activation, Selenium and Zinc indirectly affect the number of red blood cells and red blood cell indices, which is an essential indicator of the body's hematopoietic function under moderate heat stress. The analysis of the influence of factor A ($P=0.000000$), factor B ($P=0.000003$), and the interaction of time factors and citrates of trace elements AB ($P=0.001152$) was marked by statistically significant changes in the effect on the average concentration of hemoglobin in the erythrocyte.

The influence of time factor A ($P=0.000059$) was reflected in statistical changes in the effect on the average erythrocyte volume, but the factor of trace element citrates B ($P=0.089660$) did not affect the studied indicator. In the combined interaction of the two factors, there were no statistically significant changes in AB ($P=0.182130$). The determination of factor A ($P=0.468084$) was not marked by statistical changes in the average hemoglobin content in a single erythrocyte. The factor of action of trace element citrates B ($P=0.000730$) had a significant effect on the studied indicator. In the combined interaction of the two factors, no statistically significant changes in AB were observed ($P=0.778313$). The results of the analysis of factor A ($P=0.5578669$) did not show statistically significant changes in the effect on the width of red blood cell distribution. The influence of the factor of trace element citrates B ($P=0.021730$) had a statistically significant effect on the studied variable. In the interaction of the two factors, the data obtained were unaffected by statistical changes in AB ($P=0.734692$), indicating the absence of influence in the combination of these factors.

Studies have shown that the number of leukocytes in the blood of rabbits of the I and II experimental groups decreased by 9.01 ($P<0.05$) and 7.95% ($P<0.05$), respectively, and lymphocytes by 11.3 ($P<0.05$) and 12.5% ($P<0.05$) at the final stage of the study (table 3).

Leukocytes are cells of the body's defense system. They are within physiological values, but their decrease in the experimental groups may indicate the absence of inflammation or a reduced risk of inflammation with supplementation [1]. A reduction in the number of lymphocytes during the study period may indicate a lower risk of acute inflammation and a reduced risk of heat stress diseases under the influence of trace element citrates [19].

The effect of time factor A ($P=0.829832$) did not reveal statistical changes in leukocyte counts, but trace element citrates B ($P=0.000245$) significantly affected the studied index. In the combined interaction of the two factors, no statistically significant changes in AB were observed ($P=0.597066$). Analysis of the time factor A ($P=0.000338$) and the factor of micronutrient citrates B ($P=0.000878$) showed statistically significant changes in the absolute lymphocyte count, where the expressed effect was observed for factors A and B. The analysis of the combination of AB factors did not show a statistically significant result ($P=0.634944$).

The use of Zinc citrate supplementation led to an increase in the content of monocytes in the blood of rabbits by 23.5% ($P<0.05$) on the 29th day of the experiment. Zinc is an essential trace element involved in regulating the immune system. It promotes the development and functioning of immune cells, including monocytes [1]. An increased content of monocytes may indicate the activation of the immune system, which responds to stressful conditions, including heat stress. Therefore, a higher content of monocytes is involved in the immune response by producing various cytokines that activate the response of the immunophysiological system during exposure to elevated environmental temperatures.

Feeding citrates of trace elements in the blood of rabbits in I and III experimental groups increased the absolute content of granulocytes by 30 ($P<0.01$) and 66.3% ($P<0.001$), respectively, and the relative content of granulocytes by 13.5 ($P<0.05$) and 41.1% ($P<0.001$) during 29 days of the experiment. The addition of Selenium citrate to the diet of rabbits caused significant changes in the relative content of granulocytes by 17.1% ($P<0.01$) in the final period of the study compared to the control group (table 3). Germanium can activate the immune system by stimulating the formation of various types of immune cells, including granulocytes [24]. The intake of physiologically reasonable amounts of organic zinc and selenium compounds in the diet stimulates the activation of enzymes responsible for the synthesis of DNA and RNA, which promotes the proliferation and differentiation of cells, including granulocytes, which is an essential component of the immune system in protecting the body from infections and inflammation [19].

The effect of the time factor A ($P=0.000000$) and the factor of micronutrient citrates B ($P=0.007262$) was marked by statistically significant changes in the content of monocytes, where the expressed effect was observed for factor A. The results of the analysis of the combination of AB factors did not show a statistically significant

result ($P=0.0442296$). The study of the effect of factor A ($P=0.000000$), factor B ($P=0.001213$), and the interaction of time factors and citrates of trace elements AB ($P=0.000180$) were characterized by statistically significant changes in the effect on the number of granulocytes. The effect of time factor A ($P=0.005293$) and the effect of trace element citrates B ($P=0.008958$) was characterized by statistically significant changes in the relative content of lymphocytes. The analysis of the combination of factors AB did not show a statistically significant result ($P=0.262943$). The analysis of the time factor A ($P=0.000000$) was marked by statistical changes in the relative content of monocytes, but the factor of trace element citrates B ($P=0.076088$) had no effect on the studied indicator. In the combined interaction of the two factors, no statistically significant changes in AB were observed ($P=0.100019$). The determination of factor A ($P=0.000000$), factor B ($P=0.002499$), and the interaction of time and trace element citrates of AB ($P=0.006297$) were characterized by statistically significant changes in the effect on the relative content of granulocytes, where time is the predominant factor of influence.

In the case of micronutrient citrate supplementation, there was a tendency to increase in platelet count, mean platelet volume, thrombocrit value, and relative width of platelet distribution by volume during the study (table 4).

The effect of time factor A ($P=0.000000$) and the factor of trace element citrate B ($P=0.001883$) was marked by statistically significant changes in platelet count, where the expressed effect was observed for factor A. The analysis of the combination of AB factors did not show a statistically significant result ($P=0.665514$). The effect of the time factor A ($P=0.000000$) and the factor of influence of citrates of trace elements B ($P=0.001883$) was marked by statistically significant changes in platelet count, where a pronounced effect was observed for factor A. The data analysis shows that the combination of AB factors did not show a statistically significant result ($P=0.665514$). The influence of the factor of action of citrates of trace elements B ($P=0.014762$) had a statistically significant effect on the studied variable. In the interaction of the two factors, the data obtained were unaffected by statistical changes in AB ($P=0.297532$), indicating the absence of influence in the combination of these factors.

The results of the study of the biochemical parameters of rabbit blood, which characterize the state of metabolism and functioning of the body, indicate a positive trend in the feeding of Zinc citrate, Selenium citrate, and Germanium citrate compared to the control group. Feeding Selenium citrate to rabbits of the second experimental group in their blood, respectively, decreased the level of creatinine by 7.5% ($P<0.05$) and urea by 5.61% ($P<0.01$) on the 14th day and by 7.3% ($P<0.05$) and 12.3% ($P<0.01$). Germanium citrate feeding under conditions of moderate heat stress in III experimental group resulted in a decrease in urea by 14.7 ($P<0.001$) and 15.1% ($P<0.01$) on the 14th and 29th days of feeding compared to the control (table 5). Creatinine is formed in muscle tissue during

Table 3. The number of leukocytes and their forms in the blood of rabbits after feeding Zinc, Selenium, and Germanium citrates compounds under moderate heat stress ($M\pm SD$, $n=6$)

Indicators	Group	Period of research, age/day of supplementation		
		Preparatory period	Study period	
		49/1	63/14	78/29
WBC, $10^9/l$	C	9.58±0.83	9.35±0.59	9.43±0.41
	I E	8.66±0.69	8.63±0.80	8.58±0.44*
	II E	8.73±0.35	9.11±0.59	8.68±0.37*
	III E	8.70±0.31	8.96±0.32	9.15±0.48
LYM, $10^9/l$	C	5.26±0.63	5.16±0.47	5.75±0.27
	I E	4.66±0.58	4.88±0.29	5.10±0.34*
	II E	4.41±0.18	4.98±0.19	5.03±0.33*
	III E	4.43±0.95	4.80±0.40	5.23±0.42
MON, $10^9/l$	C	1.43±0.10	1.33±0.12	1.06±0.05
	I E	1.55±0.25	1.51±0.11	1.31±0.19*
	II E	1.65±0.24	1.50±0.20	1.15±0.08
	III E	1.50±0.20	1.55±0.10	1.16±0.13
GRA, $10^9/l$	C	1.81±0.56	2.11±0.54	2.20±0.14
	I E	2.38±0.27	2.25±0.24	2.86±0.57*
	II E	1.66±0.33	2.20±0.33	2.68±0.35
	III E	1.76±0.50	2.16±0.54	3.66±0.40***
LYM, %	C	54.96±6.14	54.87±4.70	57.31±3.35
	I E	48.55±4.68	54.00±1.96	55.38±2.10
	II E	55.61±2.62	53.91±2.39	56.33±2.29
	III E	49.98±4.56	52.80±2.49	54.21±3.32
MON, %	C	26.83±5.35	22.04±3.84	12.41±1.29
	I E	23.56±6.15	22.85±1.95	13.65±0.71
	II E	23.01±2.32	23.86±1.90	12.56±1.14
	III E	28.73±3.64	25.46±1.61	12.70±1.39
GRA, %	C	18.50±4.81	19.38±1.63	25.91±1.47
	I E	27.73±6.45	20.78±2.08	29.43±0.89*
	II E	21.36±4.43	21.20±4.78	30.35±2.85**
	III E	21.25±6.88	20.36±3.40	36.56±2.25***

Table 4. Platelet count and platelet indices in the blood of rabbits after feeding Zinc, Selenium, and Germanium Citrates compounds under moderate heat stress ($M\pm SD$, $n=6$)

Indicators	Group	Period of research, age/day of supplementation		
		Preparatory period	Study period	
		49/1	63/14	78/29
PLT, $10^9/l$	C	303.00±35.18	368.00±13.88	376.50±14.87
	I E	263.33±46.24	373.00±25.28	385.67±25.81
	II E	349.83±57.70	390.66±23.43	390.33±12.78
	III E	331.33±34.82	386.67±25.19	397.83±28.83
MPV, fl	C	4.96±0.30	5.01±0.38	4.86±0.37
	I E	5.36±0.29	5.25±0.21	5.06±0.19
	II E	5.25±0.24	5.61±0.57	5.15±0.24
	III E	5.28±0.40	5.66±0.38	5.21±0.31
PCT, %	C	0.262±0.02	0.212±0.02	0.189±0.01
	I E	0.233±0.03	0.217±0.03	0.200±0.01
	II E	0.237±0.02	0.221±0.02	0.212±0.01
	III E	0.239±0.04	0.218±0.02	0.211±0.02
PDW, %	C	12.45±1.21	13.71±0.98	12.55±1.41
	I E	14.31±0.97	14.51±0.53	13.01±0.91
	II E	13.50±0.84	14.01±1.01	13.58±0.47
	III E	13.91±1.83	13.78±0.74	13.90±0.76

creatinine metabolism, which is used to supply energy to the muscles. The kidneys filter creatinine and eliminate it from the body through the urine. The level of urea in the blood is an indicator of the functional state of the kidneys, as well as protein metabolism in the body [29]. Zinc citrate, Selenium citrate, and Germanium citrate have antioxidant properties that help reduce heat stress and protect the kidneys from the adverse effects of heat stress [8, 24]. The lower levels of creatinine and urea compared to

Table 5. The content of total protein, albumin, creatinine, and urea in the blood of rabbits after feeding Zinc, Selenium, and Germanium Citrates under moderate heat stress (M±SD, n=6)

Indicators	Group	Period of research, age/day of supplementation		
		Preparatory period	Study period	
		49/1	63/14	78/29
Total protein, g/l	C	60.78±5.60	57.20±4.54	54.01±3.29
	I E	62.31±4.55	58.08±3.36	55.51±5.24
	II E	63.40±2.11	62.76±3.09	59.81±3.15
	III E	60.33±2.73	60.06±4.59	57.33±2.56
Albumin, g/l	C	29.40±1.64	33.56±2.09	31.50±2.28
	I E	32.00±1.52	30.80±4.21	27.23±1.61
	II E	31.23±2.20	29.68±3.53	28.25±3.42
	III E	31.41±2.96	28.56±1.98	29.10±3.98
Creatinine, μmol/l	C	115.10±4.33	118.03±5.44	117.62±7.91
	I E	112.03±5.64	111.57±4.64	115.28±4.63
	II E	108.53±4.24	109.10±4.94*	108.95±3.45*
	III E	111.97±4.30	112.70±6.06	116.05±3.93
Urea, mmol/l	C	7.03±0.61	6.26±0.38	6.03±0.63
	I E	6.33±0.36	5.88±0.31	5.66±0.61
	II E	6.60±0.63	5.55±0.35**	4.96±0.30**
	III E	6.23±0.49	5.01±0.11***	4.80±0.33**

Table 6. The level of aminotransferase and alkaline phosphatase activity in the blood of rabbits after feeding Zinc, Selenium, and Germanium Citrates under moderate heat stress (M±SD, n=6)

Indicators	Group	Period of research, age/day of supplementation		
		Preparatory period	Study period	
		49/1	63/14	78/29
AST, u/l	C	24.70±2.14	31.90±1.40	29.48±0.96
	I E	22.83±2.38	20.71±1.79***	22.96±1.99***
	II E	26.01±3.93	27.05±2.30**	25.45±1.30**
	III E	25.53±3.36	29.96±3.10	28.85±2.43
ALT, u/l	C	58.75±5.80	74.73±4.52	63.45±3.67
	I E	51.38±4.90	62.31±3.83***	55.58±2.87**
	II E	53.00±4.82	66.63±2.70**	56.73±3.99**
	III E	56.51±6.26	70.05±2.07	60.91±4.00
Alkaline phosphatase, u/l	C	277.45±25.02	324.50±31.81	293.25±37.13
	I E	309.07±21.98	316.08±18.28	278.83±28.11
	II E	303.73±22.66	315.03±28.99	273.73±29.55
	III E	308.18±13.35	310.37±22.72	268.55±34.87

the control group may indicate an improvement in renal function under moderate heat stress.

Determination of the time factor A (P=0.167163) revealed no statistical changes in creatinine content. The influence of the factor of trace element citrates B (P=0.000131) had a significant effect on the studied index. In the combined interaction of the two factors, no statistically significant changes in AB were observed (P=0.918005). The effect of time factor A (P=0.000000) and the effect of trace element citrates B (P=0.000000) was marked by statistically significant changes in urea content, where a pronounced effect is observed for two factors. The effect of the time factor A (P=0.000014) and the factor of trace element citrate (P=0.001055) was marked by statistically significant changes in total protein content, where the expressed effect was observed for factor A. The results of the analysis of the set of AB factors did not show a statistically significant result (P=0.627532).

The effect of the time factor A (P=0.000000) had a statistically significant effect on albumin content. The action factor of trace element citrates B (P=0.178466) did not show any significant changes. Interaction of time and trace element citrates is statistically significant (P=0.021085) and indicates a combined effect on mitigating the effects of moderate heat stress.

The addition of Zinc citrate and Selenium citrate to the diet of rabbits reduces AST activity by 35.0 (P<0.001) and 22.1% (P<0.001), respectively, and by 15.2 (P<0.05) and 13.6% (P<0.05) on the 14th and 29th days of the study compared to the control group. Significant values were also found in rabbits of the I and II experimental groups for ALT activity, where a decrease of 16.6 (P<0.001), 12.4% (P<0.01), and 10.8 (P<0.01) and 10.5% (P<0.01) was observed on the 14th and 29th days of supplementation (table 6). A decrease in the level of aspartate aminotransferase and alanine aminotransferase activity within physiological parameters may indicate an improvement in rabbit liver function, which was more pronounced with Zinc citrate and Selenium citrate under moderate heat.

The effect of time factor A (P=0.001088) and the effect of micronutrient citrate B (P=0.000000) had a statistically significant effect on the level of AST activity, where the effect of factor B was expressed. In particular, the combined effect of time and micronutrient citrates was statistically significant AB (P=0.000597), which may indicate a better result on the enzyme activity. The effect of the time factor A (P=0.000000) and the factor of trace element citrate B (P=0.000000) was marked by statistically significant changes in the level of ALT activity, where the expressed effect was observed for factors A and B. The analysis of the combination of factors AB did not show a statistically significant result (P=0.840587). The results of the analysis of the time factor A (P=0.000060) were marked by statistical changes in the level of alkaline phosphatase activity, but the factor of trace element citrates B (P=0.940835) had no effect on the studied indicator. In the combined interaction of the two factors during the experimental period, AB had no statistically significant effect (P=0.225183).

The study of cholesterol content in the blood of rabbits of I and II experimental groups showed a corresponding decrease of 27.7 ($P<0.01$), 22.2% ($P<0.01$), and 20.3 ($P<0.05$), and 16.6% ($P<0.05$) on the 14th and 29th days of the experiment. Cholesterol is the main component of cell membranes and is involved in forming structural integrity and cell permeability (table 7). Selenium and Zinc are components of glutathione peroxidase and thioredoxin reductase, which help protect cells from oxidative stress, including cholesterol oxidation [36]. Zinc is essential for functioning the HMG-CoA reductase enzyme, which ensures cholesterol synthesis in cells. By regulating the activity of this enzyme, Zinc can affect the synthesis and breakdown of cholesterol [7]. The influence of the time factor A ($P=0.000000$), the trace element citrates factor B ($P=0.000002$), and the combination of factors AB ($P=0.000176$) during the experimental period were characterized by statistically significant changes in cholesterol content during the experimental period.

Feeding Selenium citrate increased the content of inorganic phosphorus by 10.7% ($P<0.05$) on the 29th day of the study. Phosphorus is vital to many biochemical processes, including energy metabolism, DNA and RNA synthesis, and protein phosphorylation [20]. Heat stress causes an increase in free radicals in the body, which damages cell membranes and structures. As a part of glutathione peroxidase, Selenium reduces oxidative stress by neutralizing free radicals, which helps maintain the functionality of cell membranes and enzymes, phosphatase and phosphorylase, involved in the transport and metabolism of phosphorus. Under conditions of moderate heat stress, activation of phosphatases is essential to provide free phosphorus, which is necessary for ATP synthesis and repair of damaged cells. Phosphorylases add phosphate groups to organic molecules, which is essential for the regulation of metabolic pathways, in particular, the breakdown of glycogen to glucose-1-phosphate, which promotes the release of glucose into the blood and can be used for the energy needs of the cell, under conditions of heat stress on the body of rabbits [34]. The influence of the factor of action of citrates of trace elements B ($P=0.001718$) has a statistically significant effect on the studied variable. In the interaction of the two factors, the data obtained were unaffected by statistical changes in AB ($P=0.334127$), indicating the absence of influence in the combination of these factors.

Analysis of the effect of time factor A ($P=0.021905$) had a statistically significant effect on the content of triacylglycerols. The effect of trace element citrates B ($P=0.069257$) did not reveal any significant changes. The interaction of time and trace element citrates factors is statistically significant ($P=0.030044$) and indicates the combined effect of these two factors on the variable. The effect of time factor A ($P=0.000000$) and the effect of trace element citrates B ($P=0.000000$) was marked by statistically significant changes in urea content, where a pronounced effect is observed for two factors. The results of the analysis of the set of factors AB did not show statistically significant results ($P=0.168903$). The effect

Table 7. The content of cholesterol, triacylglycerols, total calcium, and inorganic phosphorus in the blood of rabbits after feeding Zinc, Selenium, and Germanium Citrates under moderate heat stress ($M\pm SD$, $n=6$)

Indicators	Group	Period of research, age/day of supplementation		
		Preparatory period	Study period	
		49/1	63/14	78/29
Triacylglycerols, mmol/l	C	0.63±0.07	0.79±0.07	0.71±0.06
	I E	0.69±0.14	0.67±0.10	0.62±0.05
	II E	0.78±0.06	0.76±0.06	0.66±0.09
	III E	0.76±0.06	0.72±0.09	0.67±0.05
Cholesterol, mmol/l	C	0.17±0.02	0.54±0.07	0.54±0.06
	I E	0.19±0.03	0.39±0.03**	0.42±0.04**
	II E	0.20±0.01	0.43±0.04*	0.45±0.02*
	III E	0.21±0.02	0.52±0.07	0.48±0.05
Total calcium, mmol/l	C	3.06±0.26	3.06±0.20	2.83±0.41
	I E	3.26±0.19	3.10±0.24	2.86±0.28
	II E	3.35±0.15	3.13±0.29	2.98±0.37
	III E	3.30±0.14	3.20±0.23	2.90±0.25
Inorganic phosphorus, mmol/l	C	1.61±0.07	1.93±0.15	1.78±0.17
	I E	2.08±0.23	1.96±0.16	1.95±0.22
	II E	2.01±0.24	2.11±0.07	2.16±0.12*
	III E	2.13±0.24	1.95±0.23	1.90±0.24

of the time factor A ($P=0.000112$) indicated statistical changes in total calcium content. However, the factor of trace element citrates B ($P=0.254447$) did not affect the studied indicator. In the combined interaction of the two factors, there were no statistically significant changes in AB ($P=0.951526$).

During the period of the study, it was found that feeding rabbits with Zinc citrate in the blood increased the number of red blood cells ($P<0.01$), hemoglobin concentration ($P<0.01$), percentage of hematocrit value ($P<0.01$), mean hemoglobin concentration in erythrocyte ($P<0.05$), monocyte count ($P<0.05$), absolute and relative granulocyte content ($P<0.05$) and reduced the number of leukocytes and lymphocytes ($P<0.05$) during 29 days of experiment. The addition of Selenium citrate to the diet of animals increased the number of red blood cells ($P<0.05$), hemoglobin concentration ($P<0.001$), percentage of hematocrit value ($P<0.05$), relative content of granulocytes ($P<0.01$), average hemoglobin concentration in erythrocyte ($P<0.05-0.001$) and a decrease in the number of leukocytes ($P<0.05$), the number of lymphocytes ($P<0.05$) was noted during the study. Germanium citrate feeding had less effect on the blood parameters of rabbits under heat stress, with an increase in hemoglobin concentration ($P<0.001$), absolute granulocyte content ($P<0.001$), and relative granulocyte content ($P<0.001$) during 29 days of the experiment.

The change in the biochemical parameters of rabbit blood was more influenced by the supplementation of

Zinc citrate and Selenium citrate, which were characterized by a decrease in AST activity ($P<0.001$ and $P<0.001$; $P<0.05$ and $P<0.05$), ALT ($P<0.001$ and $P<0.01$; $P<0.01$ and $P<0.01$), cholesterol content ($P<0.01$ and $P<0.01$; $P<0.05$ and $P<0.05$) on the 14th and 29th days of the experimental period, respectively. Selenium citrate decreased the level of creatinine ($P<0.05$) and urea ($P<0.01$) during the experiment and increased the content of inorganic phosphorus ($P<0.05$) on the 29th day of the study. The addition of Germanium citrate caused a decrease in urea content ($P<0.001$ and $P<0.05$) on the 14th and 29th days of the experiment, respectively.

Thus, feeding rabbits with Zinc citrate (12 mg Zn/kg body weight) and Selenium citrate (60 µg Se/kg body weight) under moderate heat stress caused pronounced positive changes in the hematological and biochemical parameters of rabbit blood during the study. The addition of Germanium citrate (12.5 µg Ge/kg) to the diet had less pronounced effect on the body of rabbits, but mitigated the negative effect of heat stress, with more pronounced positive changes in blood parameters on hematocrit value, absolute and relative content of granulocytes on the 29th day of the study compared to the control.

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Вплив наночастинок цитратів цинку, селену та германію на гематологічні та біохімічні показники організму кролів за умов помірного теплового стресу

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Зміни клімату призводять до підвищених температур довкілля, що негативно впливає на організм тварин. Кролі не мають потових залоз і не здатні регулювати температуру свого тіла, що за умов теплового стресу порушує фізіологічні процеси організму: терморегуляцію тіла, дихання, імунну функцію, ендокринну регуляцію, параметри крові, репродуктивну здатність, які є ключовими чинниками функціонування організму. В останні роки для пом'якшення теплового стресу значну увагу зосереджують на сполуках, виготовлених методами нанотехнології, які проявляють позитивний ефект на організм тварин порівняно з органічними і неорганічними речовинами та мають широкий спектр біологічної дії. Однак вплив наночастинок макро- та мікроелементів на організм ссавців залежить від застосованої кількості. Тому мета експерименту полягала у дослідженні гематологічних та біохімічних параметрів організму кролів після відлучення в умовах помірного теплового стресу за випоювання цитратів цинку, селену та германію, виготовлених методами нанотехнології.

Ключові слова: кролі, кров, помірний тепловий стрес, наночастинок, цинку цитрат, селену цитрат, германію цитрат, гематологічні та біохімічні показники

Ключем до всякої науки є знак питання.

ІНСТИТУТ БІОЛОГІЇ ТВАРИН НААН ОГОЛОШУЄ НАБІР у ДОКТОРАНТУРУ

очна (денна) форма навчання

та АСПІРАНТУРУ

очна (денна/вечірня)

і заочна форма навчання



за спеціальностями:

091 — Біологія та біохімія

204 — Технологія виробництва та переробки
продукції тваринництва

211 — Ветеринарна медицина

У 2024 р. для допуску до вступного іспиту з іноземної мови для вступу на навчання для здобуття ступеня доктора філософії потрібні результати єдиного вступного іспиту (ЄВІ). Реєстрацію осіб, які бажають взяти участь у єдиному вступному іспиті (ЄВІ), здійснюють приймальні комісії закладів вищої освіти з 7 до 29 травня (для участі в основній сесії) і з 17 до 21 червня (для участі в додатковій сесії, у цей період матимуть змогу зареєструватися ті, хто не зміг цього зробити під час основного періоду реєстрації) за умови особистої присутності вступника(-ці) та/або дистанційно.

Підготовка в аспірантурі здійснюється за рахунок:

- коштів державного бюджету України (за державним замовленням);
- коштів юридичних та фізичних осіб (на умовах контракту).

Підготовка в докторантурі здійснюється за рахунок:

- коштів юридичних та фізичних осіб (на умовах контракту).

Термін подання документів — з 01.07 до 31.08.2024 р.

Проведення вступних іспитів — з 01.09 до 10.09.2024 р.

Зарахування вступників за кошти державного бюджету — до 13.09.2024 р.

Зарахування вступників за кошти фізичних/юридичних осіб — до 31.10.2024 р.

Початок навчання в аспірантурі — 16.09.2024 р.

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Запрошуємо до участі
**у XXII всеукраїнській конференції
молодих вчених,**

присвяченій 75-річчю від дня народження доктора ветеринарних наук,
професора, члена-кореспондента НААН
Ростислава Степановича ФЕДОРУКА (11.08.1949–21.06.2023)



19–20 вересня 2024 р., м. Львів, Україна

Місце проведення:

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Робочі мови:

Українська, англійська.

Формат проведення:

Змішаний офлайн/онлайн (цифрова платформа ZOOM). Участь передбачає усну доповідь з презентацією тривалістю до 10 хв, або стендову доповідь із представленням постера.

Тематика і основні напрями:

- Актуальні проблеми сільського господарства та екології у воєнний час.
- Фізіологія і біохімія.
- Мікробіологія та біотехнологія.
- Екологія.
- Живлення тварин.
- Ветеринарна медицина.
- Розведення, селекція та генетика тварин.

Реєстраційні внески:

Участь онлайн — 75 грн.: організаційні витрати, матеріали конференції в електронному форматі, електронний сертифікат учасника. **Участь офлайн — 150 грн** (за умови реєстрації **до 1 липня**), **200 грн (до 1 серпня)**, **250 грн (до 23 серпня)**: організаційні витрати, матеріали конференції в електронному форматі, друкований та електронний сертифікат учасника; перерви на каву/чай.

Додатково можна придбати друковану версію журналу, варієтсь — 200 грн.

Реквізити для організаційного внеску: 5169 3600 2658 3462 (Котик Богдан Іванович).

Важливі дати і терміни:

Подання матеріалів учасниками конференції	до 23 серпня 2024 р.
Повідомлення про прийняття або відхилення матеріалів	до 26 серпня 2024 р.
Публікація програми конференції	до 12 вересня 2024 р.

Реєстрація і подача матеріалів:

Для участі у роботі конференції та подання матеріалів потрібно пройти реєстрацію. Тези конференції будуть опубліковані у науковому журналі «Біологія тварин». Учасники, які виголосять найкращі доповіді, отримають запрошення опублікувати їх у формі статей.



Вимоги до оформлення тез:

Мова — українська, англійська. Обсяг тез — до 5000 символів (з пробілами).

Оргкомітет залишає за собою право надсилати тези на доопрацювання, відхиляти їх за невідповідності тематиці конференції або зазначеним вимогам.

Відповідальними за наукову достовірність поданих у матеріалах даних є автори.



**Сертифікати учасника будуть надані лише тим, хто представить свої доповіді!
Публікація тез без участі у конференції неможлива!**

Контактна інформація:

Котик Богдан Іванович
тел. (+38 093) 192-18-73
ibtyoungscientists@gmail.com

Сторінка
конференції:



Подія
у Facebook:



Запрошуємо до участі
у Міжнародній науково-практичній конференції
**«Актуальні проблеми
сучасної біології, тваринництва
та ветеринарної медицини»,**



присвяченій 100-річчю від дня народження доктора ветеринарних наук,
професора, академка УААН, заслуженого діяча науки і техніки України,
директора Інституту біології тварин НААН (1972–1993)
Петра Захаровича ЛАГОДІЮКА (08.06.1924–17.02.1994)

3–4 жовтня 2024 р., м. Львів, Україна

Місце проведення:

Інститут біології тварин НААН, вул. Василя Стуса, 38, м. Львів, 79034, Україна.

Робочі мови:

Українська, англійська.

Формат проведення:

Змішаний офлайн/онлайн (цифрова платформа ZOOM). Участь передбачає усну доповідь з презентацією тривалістю до 10 хв, або стендову доповідь із представленням постера.

Тематика і основні напрями:

- Фізіологія та біохімія.
- Живлення та годівля тварин.
- Ветеринарна медицина.
- Мікробіологія та біотехнологія.
- Розведення, селекція та генетика тварин.
- Екологія.

Важливі дати і терміни:

Подання матеріалів учасниками конференції
Повідомлення про прийняття або відхилення матеріалів
Публікація програми конференції

до 31 серпня 2024 р.
до 9 вересня 2024 р.
до 22 вересня 2024 р.

Реєстрація і подача матеріалів:

Для участі у роботі конференції та подання матеріалів
потрібно пройти реєстрацію на сайті конференції.
Тези конференції будуть опубліковані у науковому журналі
«Біологія тварин».



Вимоги до оформлення тез:

Мова — українська, англійська. Обсяг тез — до 5000 символів (з пробілами).
Оргкомітет залишає за собою право надсилати тези на доопрацювання,
відхиляти їх за невідповідності тематиці конференції або зазначеним вимогам.
Відповідальними за наукову достовірність поданих у матеріалах даних є автори.



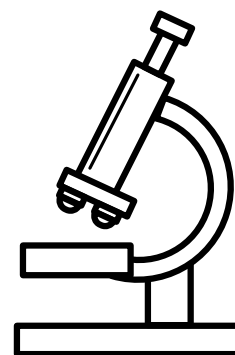
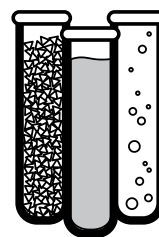
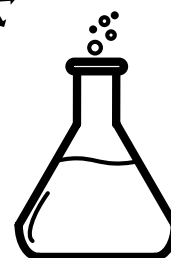
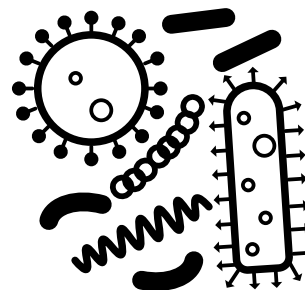
Контактна інформація:
Лучка Іван Васильович
тел. (+38 097) 384-21-77
inenbiol@gmail.com

Сторінка
конференції:



ІНСТИТУТ БІОЛОГІЇ ТВАРИН НААН ПРОВОДИТЬ:

- Дослідження біохімічних показників
(аналізатор *Humalyzer 2000*, Німеччина)
- Гематологічний аналіз
(аналізатор *Mythic-18Vet*, Швейцарія)
- Мікробіологічні дослідження
(посів на стерильність, антибіотикограма,
склад мікрофлори кишечника тварин,
мікробіологічний аналіз кормів, води, повітря)
- Імуноферментні дослідження
(аналізатор *Stat Fax 3000*, Німеччина)
- Оцінка репродуктивної здатності тварин,
штучне осіменіння, трансплантація ембріонів
- Селекційно-генетичні дослідження
- Дослідження кормів
- Дослідження молока
- Дослідження яєць
- Визначення показників якості меду
- Дослідження вовни і волосся
- Атомно-абсорбційний і атомно-емісійний аналіз
концентрації хімічних елементів
- Аналіз органічних добрив



Організовує проведення досліджень на лабораторних тваринах
і надає кваліфіковану інтерпретацію отриманих результатів.

* можливе проведення інших досліджень

** всі лабораторії Інституту акредитовані для проведення досліджень

Інститут біології тварин НААН
вул. В. Стуса 38, м. Львів, 79034
тел.: +38 (032) 270-23-89, +38 (96) 858-37-76
e-mail: markinfo@inenbiol.com.ua

Завжди раді співпраці з Вами!

Запрошуємо розмістити рекламу Вашої компанії на сторінках наукового журналу «Біологія тварин»!

Ми готові співпрацювати з Вами для створення ефективної рекламної стратегії, яка відповідає Вашим потребам і бюджету.

Пропонуємо різні варіанти розміщення реклами, зокрема банери, оголошення або статейно-рекламні матеріали.

Реклама в нашому журналі дозволить Вам:

- залучити увагу науковців і фахівців до Вашої компанії та продукції;
- підвищити впізнаваність бренду та позиціонування Вашої компанії на ринку;
- залучити нових клієнтів і розширити Вашу клієнтську базу.

Пропозиції до співпраці на 2024 рік:

- **рекламний блок на ½ сторінки** — 1000 грн в одному номері журналу, 2500 грн у трьох номерах журналу.
- **рекламний блок на 1 сторінку** — 2000 грн в одному номері журналу, 5000 грн у трьох номерах журналу.

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