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ЗМІСТ

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Effect of cerium dioxide nanoparticles on metabolic processes in the body of broiler chickens

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The scope of application of cerium dioxide and its special physical and chemical properties are considered in the work. The effect of the size factor on the properties of nanodispersed cerium dioxide determines the biological activity of the material, low toxicity and high oxygen non-stoichiometry. Specific properties of CeO_2 include the ability to regenerate oxygen non-stoichiometry, which is expressed in the ability of cerium dioxide nanoparticles to return to their initial state after participating in the redox process in a relatively short period of time, which enables their repeated use.

Nanoparticles, due to their small size, easily penetrate into the body through the respiratory, digestive, and skin organs and exhibit more pronounced biological activity due to the large surface area per unit mass. The change in the physical and chemical mechanisms of action of nanoparticles is due to the fact that most of the atoms are on the surface. Such an arrangement changes the physical, chemical, biological, toxicological properties of the substance and facilitates the interaction of nanoparticles with a living organism. Once inside a biological system, nanoparticles come into contact with a number of physical and chemical features of the organism, which affect their properties and can change the response. These features are largely due to the ability to pass through the redox cycle between two natural oxidation states (Ce^{3+} and Ce^{4+}).

The influence of cerium dioxide nanoparticles on metabolic processes in the body of broiler chickens has been established. Their introduction contributed to an increase in the content of total lipids in the blood by 24.6–31.3 %, albumins – by 16–22 %, and a decrease in the content of uric acid to the level of 63–67 % of the control. Non-toxicity of poultry meat treated with nano-cerium for consumers was established.

The high degree of biocompatibility, low toxicity and catalytic activity of nanodispersed cerium dioxide make it possible to consider it as a promising nanobiomaterial for use in biology, medicine and agriculture.

Key words: nanobiotechnologies, nanoparticles, ceriumdioxide, layinghens, lipids.

Problem statement and analysis of recent research. In the list of ten priority nanomaterials, experts of the interdepartmental program on the correct management of chemical preparations (IOMC) included nanodispersed cerium dioxide [13]. Cerium is the most common rare earth metal and is an element of industrial importance. Cerium dioxide (CeO2), which is also called cerium, is the most famous compound of the element, due to its redox properties and ability to guarantee excellent oxygen mobility [22]. It is a powerful oxidizing agent used in catalysis, biology, and medicine. Nanodispersed cerium dioxide is a promising material that is widely used in modern high-tech industries [1].

Considerable interest in the study of cerium dioxide is due to the fact that upon transitioning to the nanocrystalline state, this compound significantly changes its physicochemical properties in a rather unusual way [1]. In particular, as the particle size decreases, the unit cell parameter of CeO_2 increases. At the same time, there is a change in the oxygen non-stoichiometry of cerium dioxide due to an increase in the proportion of atoms located on the surface of the particles, which causes a change in its electronic and electrophysical properties.

The pronounced influence of the size factor on the physical and chemical properties of nanodispersed cerium dioxide determines the unique biological activity of the material, low toxicity and high oxygen non-stoichiometry. The first factor ensures the comparative safety of using cerium dioxide nanoparticles *in vivo*. The second determines the activity of nanodispersed CeO_2 in redox processes in a living cell, especially in the case of inactivation of active forms of oxygen [19, 27]. Specific properties of CeO_2 include the ability to regenerate oxygen non-stoichiometry, which is expressed in the ability of cerium dioxide nanoparticles to return to their initial state after participating in the redox process in a relatively short period of time, which provides the possibility of their multiple use [12, 18].

Nanoparticles, due to their small size, easily penetrate into the body through the respiratory, digestive, and skin organs and exhibit more pronounced biological activity due to the large surface area per unit mass. The change in the physical and chemical mechanisms of action of nanoparticles is due to the fact that most of the atoms are on the surface. This arrangement changes the physical, chemical, biological, toxicological properties of the substance and facilitates the interaction of nanoparticles with a living organism [11, 19].

Engineered nanoparticles (from 1 to 100 nm) may have different physical and chemical properties than those found in nature, their impact on human health should be evaluated depending on their size and shape [16]. The modern strategy for obtaining nanoparticles of cerium dioxide involves the use of the principles and approaches of "green chemistry" [3, 29].

Once inside the biological system, nanoparticles come into contact with a number of physical and chemical features of the body, which affect their properties and can change the response [2, 21, 23]. These features are largely due to the ability to pass through the redox cycle between two natural oxidation states (Ce^{3+} and Ce^{4+}). However, CeO_2 nanoparticles were previously thought to be stable and sparingly soluble [15] or insoluble in environmental conditions, depending on the carrier, pH, and particle size [16]. The dissolution of nanoparticles depends on the ratio between Ce^{3+} and Ce^{4+} on their surface layer [2]. As the size of the nanoparticles decreases, oxygen vacancies in the lattice are increasingly freed, which leads to a local decrease in the amount of Ce^{4+}.

In recent years, there have been reports in the literature about the use of metal nanoparticles, in particular cerium, in animal husbandry, since the use of antibiotics as growth promoters is prohibited in the European Union. It is reported that rare earth elements (REEs) can be successfully used as new natural feed additives to increase animal productivity [5, 7].

REE can activate the metabolism of proteins and other nutrients by stimulating the activity of hormones (growth hormone and T_3), induce the synthesis of metallothioneins and increase the content of glutathione in the liver. The antimicrobial and antioxidant effects of REE for animals have been established. In the case of their use in the diet of pigs (100 mg/kg), a positive effect on the feed conversion ratio and growth indicators was found.

Changes in the pro-oxidant-oxidant status of the blood of cows with hypogonadism and after their treatment when using the drug caplaestrol, which contains CeO_2 (cerium dioxide) nanoparticles, were established. The efficiency of ovarian repair and restoration of reproductive capacity of cows was also determined. The use of cerium nanoparticles in combination with drugs makes it possible to normalize the structure and function of the mammary gland and increase the level of colostralimmunoglobulins [25].

The use of REE had positive results for poultry [5, 13, 28], contributing to a significant increase in egg production, egg weight and fertilization rate of hatching eggs of 6-month-old laying hens and significantly improving egg production rate and egg weight.

Addition of REE citrate to the diet helps to increase the productivity of broilers [5]. One of the mechanisms of influence is increased secretion of digestive juices. The addition of REE to the diet contributed to a significant increase in SOD activity in the blood of fish and chickens.

Addition of different amounts of cerium dioxide to laying hens did not have a significant effect on feed consumption and egg weight, however, the feed conversion ratio improved and egg production increased ($p<0.05$). Egg quality criteria, with the exception of shell breaking strength, did not change. In particular, the addition of 200 and 300 mg/kg of cerium dioxide to the feed of laying hens led to a significant ($p<0.01$) increase in the

breaking strength of the eggshell. The concentration of Calcium and Phosphorus in blood serum increased significantly ($p<0.05$) after the administration of 100 mg/kg of cerium oxide [5]. It was also noted that the superoxide dismutase activity and concentration of malondialdehyde (MDA) in blood serum significantly decreased with the addition of cerium dioxide. Different doses of cerium dioxide addition had no significant effect on the activity of aminotransferases, the content of glucose, triglycerides, total cholesterol, high and low density lipoproteins in blood serum [7]. When cerium dioxide was included in the diet of chickens, a significant decrease in the content of TBC-AP in egg yolk was observed. At the same time, the addition of cerium dioxide improves the oxidative stability of eggs, and this may have a favorable effect on their shelf life. In the applied dose, nanocrystalline cerium dioxide does not accumulate in eggs and parenchymal organs of poultry [29].

Drinking nanocrystalline cerium dioxide to quail has a positive effect on egg productivity. When using nanocerium in a dose of 1 mM/l of drinking water, the laying capacity of quails increased by 7.8 %, the mass of eggs – by 16.9 %, and the intensity of laying – by 6.7 %. In doses of 0.1–10 mM/l of drinking water, nanocerium does not accumulate in eggs and parenchymal organs of poultry [26, 20]. The influence on the intensity of growth and feed consumption of young quails was revealed [29, 30].

The effect of nanocrystalline cerium dioxide was studied and lethal and semi-lethal doses of the drug were determined. The Ld_{50} of nanocrystalline cerium dioxide is greater than 2000 mg/kg, which confirms that this compound belongs to the V class of toxicity and indicates very low toxicity [29]. The positive antibacterial potential of CeO_2 nanoparticles against poultry pathogens, namely *Klebsiella sp., E. coli*, *Staphylococcus sp*. and *Salmonella sp*.was revealed. [9].

The high degree of biocompatibility, low toxicity and catalytic activity of nanodispersed cerium dioxide makes it possible to consider it as a promising nanobiomaterial for use in biology, medicine and agriculture. However, currently all possible mechanisms of its biological activity are poorly studied and require further research [4].

Target organs and reaction-response development mechanisms differ for different metal nanoparticles. They are able to induce reactive oxygen species, disrupt membrane structures, penetrate tissue barriers, enter cells and interact with intracellular components [6, 8, 16]. The issue of researching the positive effect and toxicity of metal nanoparticles is ambiguous and multifaceted, requiring a complex approach. This especially applies to nanoparticles that are used in pharmacology, medicine and agriculture, which contributes to their direct entry into the human body.

The aim of the research – to study of the effect of cerium dioxide nanoparticles on metabolic processes in the body of broiler chickens and the non-toxicity of the obtained poultry meat.

Material and methods of research. The synthesis of nanodispersed cerium dioxide was performed at the Department of Interferon and Immunomodulators of the D.K. Zabolotny Institute of Microbiology and Virology National Academy of Sciences of Ukraine. In the case of studying the effect on the body of nanodispersed cerium dioxide in the conditions of the educational research center of the Ukrainian National Academy of Sciences, 3 groups of broiler chickens of the Ross 308 cross were formed – control and 2 experimental groups of 100 heads each. Broilers were kept in BKN-3 cage batteries with free access to feed and water. The main parameters of the microclimate corresponded to zootechnical standards. Determination of live weight of broilers was carried out weekly by individual weighing. For 58 days, the research groups were given the preparation of nanodispersed cerium dioxide orally with drinking water at a dose of 8.6 mg/dm3 during the first 14 days, after the 7th (2ndNanocerium group) and 14th (3rdNanocerium group) day break, the course was repeated.

Blood stabilized with EDTA was used for hematological studies, and blood serum was used for biochemical studies. In blood serum, the following were determined: total protein, activity of enzymes (AlAT, AsAT, LDH), urea, total lipids, total cholesterol, calcium and phosphorus content with the help of a semi-automatic biochemical analyzer-HumaLyzer 3000 using standard sets of the company Human.

The biological value and toxicity of poultry meat was evaluated in the research laboratory "Veterinary and sanitary examination of livestock products" by the express method on the test culture *Tetraximena Piriformis*, laboratory strain WH-14.This test culture reacts to the action of chemical and biological factors adequately to higher animals, which makes it possible to quickly obtain reliable information. Toxicity was assessed by the express method, by detecting dead ciliates or altered forms, by motility and growth inhibition of *Tetrachimena Piriformis* ciliates [30]. The criterion of relative biological value was the number of ciliate cells, expressed as a percentage, which grew in three days on the studied object in relation to the cells in the control product [30].

Results and discussion. The study of the positive effect and toxic effect of metal nanoparticles is ambiguous and multifaceted, requiring a complex approach [1, 14, 17, 24]. This especially applies to nanoparticles used in agriculture, which contributes to their direct entry into the human body [10].

The results of the study show that blood indicators of broilers, which characterize the main types of metabolism (protein, lipid, mineral) during the period of the experiment, were within the physiological norm. The morpho-functional development of animals and poultry and the adaptive capabilities of their organism at an early age depend significantly on the intensity of synthesis and the degree of use of structural lipids (phospholipids, cholesterol) and reserve lipids (triacylglycerols) in energetic tissue processes [10]. Therefore, the tendency to a higher level of these indicators of lipid metabolism in the blood serum of chickens under the influence of the studied additive characterizes the intensive growth of young broilers. During the study of the indicators, no significant differences between the groups were found, but throughout the experiment there was a tendency to increase the content of total lipids and cholesterol in the experimental groups of chickens (Fig. 1). Thus, in the case of the introduction of cerium dioxide nanoparticles for 14 days with a seven-day break, the content of total lipids increased by 24.6 %, and in the case of a 14-day break – by 31.3 %.

During the study of the total protein content, no significant differences between the groups were found, but a significant increase in the albumin content in the blood of the broilers of the experimental groups was observed – inthe first group by 16 %, in the second – by 22 %. Regarding the content of uric acid, this content was lower in the experimental groups and amounted to 63 and 67 % of the control, respectively (Fig. 2).

It can be stated that in the case of the introduction of nanoparticles of cerium dioxide into the bird's body, activation of protein metabolism was observed, which contributed to assimilation processes and a decrease in the number of catabolism products.

In clinical studies, the activity of transaminases, in particular, alanine and aspartate aminotransferases, which characterize the degree of liver damage, are determined as marker enzymes. It was found that the birds of the 2nd research group showed a tendency to decrease the activity of AsAT in blood serum (Fig. 3). Such a change in the activity of aminotransferases is due to a certain hepatoprotective effect of cerium dioxide nanoparticles.

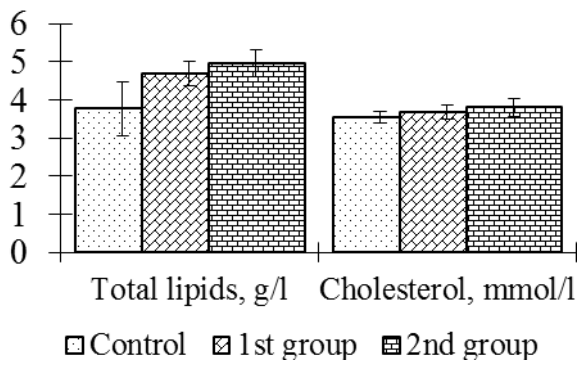

Fig. 1. **Content of total lipids and cholesterol in the blood serum of ROSS 308 broiler chickens after the addition of cerium dioxide nanoparticles.**

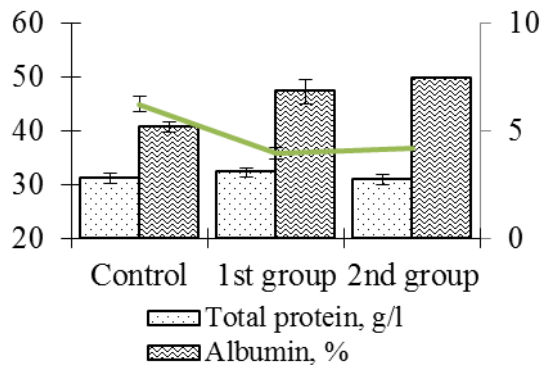

Fig. 2. **Content of total proteins, albumin and uric acid in the blood serum of ROSS 308 broiler chickens with the addition of cerium dioxide nanoparticles.**

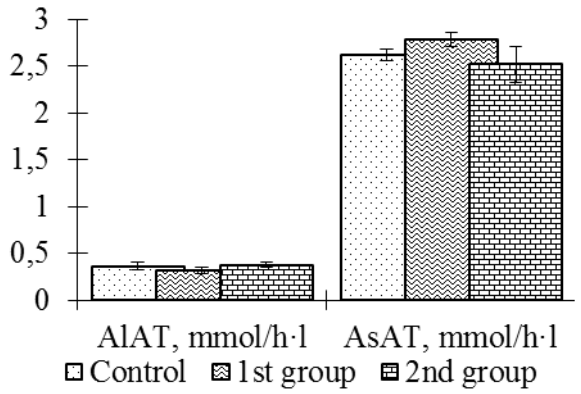

Fig. 3. **Activity of blood serum transaminases of ROSS 308 broiler chickens with the addition of cerium dioxide nanoparticles.**

It has been proven that a specific property of nanocerium is the ability to return to its original state after participating in the redox process in a relatively short period of time, which provides the possibility of its repeated use [6, 8, 13, 19]. Probably, such a unique property of nanocerium caused certain changes in the exchange of proteins and lipids.

As a result of the conducted research, an increase in the ratio of Calcium to Phosphorus was established, which makes it possible to assert the positive effect of cerium dioxide nanoparticles on mineral metabolism in the bird's body (Fig. 4).

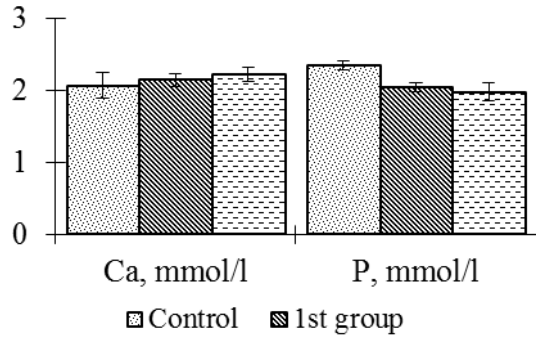

Fig. 4. **Calcium and Phosphorus content in the blood serum of ROSS 308 broiler chickens with the addition of cerium dioxide nanoparticles.**

The introduction of the drug into the diet of broilers from 1 day to 58 days of age with different intervals provides a tendency to a stable increase in their growth intensity, while the introduction of the supplement at an interval of 14 days probably ($p<0.05$) affected the increase in body weight.

Biochemical indicators of blood reflect metabolic processes, and also characterize the effect on the body of feed additives, antioxidants and other biologically active substances [19]. In further research, it is advisable to study the effect of the addition of cerium dioxide nanoparticles on the quality of poultry products.

The final stage of checking the use of any additive in animal husbandry and poultry farming is checking the product's quality. The main product of broiler chickens is meat. Studies were conducted to determine the toxicity and biological value of the meat of broiler chickens of different experimental groups. 0.56 % sea salt solution and glucose-peptone medium were used as controls.

Toxicity assessment was carried out by an express method, by detecting dead or changed forms, by mobility and inhibition of growth of *Tetraximena Piriformis* ciliates.

Tetraximena Piriformis ciliates in the 1st control group, which contained a 0.56 % sea salt solution, were active, but their growth was inhibited, which is explained by the neutrality of the medium. In the 2nd control group, the ability of ciliates to active movement and reproduction was noted. In all experimental groups, *Tetraximena Piriformis* cilia were mobile, reproduced well, pathological and dead cilia were absent. The obtained results indicate the non-toxicity of the meat of broiler chickens under the conditions of exposure to cerium dioxide nanoparticles.

The criterion of relative biological value was the number of ciliate cells, expressed as a percentage, which grew in three days at the studied object in relation to the cells in the control product (Table1).

Table 1 – **Results of the express method on the test culture of the ciliate *Tetraximena Piriformis* on the relative biological value of broiler chicken meat,** %; M±m; n=10

Indicators	Meat of broiler chickens		
	1st group	2ndgroup	3rdgroup
The number of cells, $\times 10^6$ in 1 cm^3 of medium	2,83±0,18	2,78±0,13	2,75±0,21
Relative biological value, % of control	100	98,2	97,2

The relative biological value of the meat of broiler chickens of the experimental groups was at the same level as that of intact birds. The noted decrease in biological value is insignificant (1.8–2.8 %) and is not probable.

Conclusion. The paper examines the effect of cerium dioxide nanoparticles on metabolic processes in the body of broiler chickens. Their introduction contributed to an increase in the content of total lipids in the blood by 24.6–31.3 %, albumins – by 16–22 %, and a decrease in the content of uric acid to the level of 63–67 % of the control, which indicates the activation of protein metabolism. Non-toxicity of poultry meat treated with nano-cerium for consumers was established. In the future, it is appropriate to study the effect of the drug in the diet of poultry on economic indicators.

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Вплив наночастинок діоксиду церію на метаболічні процеси в організмі курчат-бройлерів

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У роботі розглянуто сферу застосування діоксиду церію та його особливі фізико-хімічні властивості. Вплив розмірного фактору на властивості нанодисперсного діоксиду церію обумовлює біологічну активність матеріалу, низьку токсичність і високу кисневу нестехіометрію. Специфічними властивостями CeO_2 є і здатність до регенерації кисневої нестехіометрії, яка виражається у здатності наночастинок діоксиду церію після участі в окисно-відновному процесі за порівняно невеликий проміжок часу повертатися до вихідного стану, що забезпечує можливість їх багаторазового використання.

Наночастинки завдяки маленьким розмірам легко проникають у організм через органи дихання, травлення, шкіру та проявляють більш виражену біологічну активність внаслідок великої площі поверхні на одиницю маси. Зміна фізико-хімічних механізмів дії наночастинок зумовлена тим, що більшість атомів знаходиться на поверхні. Таке розташування змінює фізичні, хімічні, біологічні, токсикологічні властивості речовини та сприяє полегшенню взаємодії наночастинок з живим організмом. Потрапивши у біологічну систему, наночастинки стикаються з низкою фізичних і хімічних особливостей організму, які впливають на їх властивості та здатні змінити відповідь. Ці особливості значною мірою обумовлені здатністю до проходження у окисно-відновному циклі між двома природними станами окиснення (Ce^{3+} і Ce^{4+}).

Встановлений вплив наночастинок діоксиду церію на метаболічні процеси в організмі курчат-бройлерів. Їх введення сприяло підвищенню у крові вмісту загальних ліпідів на 24,6–31,3 %, альбумінів – на 16–22 % та зниженню вмісту сечової кислоти до рівня 63–67 % від контролю. Встановлена не токсичність м’яса птиці, що отримувала нано-церій, для споживачів.

Високий ступінь біосумісності, низька токсичність і каталітична активність нанодисперсного діоксиду церію дає змогу його розглядати як перспективний нанобіоматеріал для застосування у біології, медицині та сільському господарстві.

Ключові слова: нанобіотехнології, наночастинки, діоксид церію, кури-несучки, ліпіди.

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Features of herd formation based on beta- and kappa-casein of different dairy cattle breeds

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In dairy cattle breeding, much attention is paid to indicators of the milk quality and technological properties. These indicators are significantly influenced by milk proteins. Therefore, the goal of our research is to evaluate the effectiveness of creating dairy herds of domestic dairy cattle breeds that are characterized by the desired beta- and kappa-casein genotypes. To fulfill these tasks, the genotyping of cows of the Ukrainian Brown dairy, Sumy intrabreed type of the Ukrainian Black-and-White dairy, Simmental and Lebedyn breeds was carried out. The beta- and kappa-casein gene polymorphism was determined in the genetic laboratory of the Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine using real-time molecular biological analysis of allele recognition by polymerase chain reaction (PCR).

As a result of the conducted studies, it is found that cattle of Lebedyn and Ukrainian Brown dairy breeds have a higher frequency of the desired kappa-casein BB genotype. As a result, they are also characterized by a higher frequency of the B allele. More than 50 % of cattle of Simmental breed have a heterozygous AB genotype. Cattle of the Sumy intrabreed type of the Ukrainian Black-and-White dairy breed have a higher frequency of the homozygous AA genotype and the A allele. Cattle of the Ukrainian Brown dairy breed are characterized by the highest frequency of the A2A2 genotype and the A2 allele of beta-casein. Cattle of the Simmental breed, which are also characterized by the highest frequency of the A1A2 heterozygous genotype, have a slightly lower frequency. Homozygous A1A1genotype is more common in cattle of the Sumy intrabreed type of the Ukrainian Black-and-White dairy breed.

Using the principles of the model for creating a herd with the desired genotype, which is proposed by Mencarini IR et all, it is found that when testing the entire breeding stock, inseminating it with semen of homozygous stud bulls with the A2A2 genotype, random cow disposal at the level of 22 %, it takes 9–10 years to create herds with the desired genotypes of the Ukrainian Brown dairy, Simmental and Lebedyn breeds, respectively. To reduce the period for creating herds, it is essential to increase the percentage of cow disposal and simultaneously disposecattle with undesirable genotypes.

Key words: genotype, casein, stud bull, breeding, allele.

Problem statement and analysis of recent research. High-quality and technological indicators of milk are given great attention in dairy cattle breeding. The composition of milk proteins significantly affects the physical and chemical properties of milk and, as a result, its technological indicators.

In cheese manufacture, much attention is paid to the quality of dairy raw materials, namely the type of kappa-casein that is part of these raw materials. The kappa-casein gene polymorphism has been studied for more than 40 years. To date, thirteen genetic variants of bovine kappa-casein have been described. The most common genetic vari-

ants are A and B [6]. It is found that the B allele is associated with the production of milk that is most suitable for cheese manufacture in terms of technological properties. This explains the fact that cattle with the homozygous BB genotype have better cheese making of milk [4, 5, 20].

Different breeds of cattle and the same breed differ significantly in the frequency of kappa-casein genotypes and alleles. The Jersey cattle are characterized by a high frequency of the B allele (0.69) and BB genotype (0.45) [9, 13]. The Holstein breed is characterized by significant differentiation on this basis, depending on the country of origin (breeding). The frequency of the desired B allele, depending on the country of origin, was 41–49 % (Serbia, Iraq) and 14–17 % (Poland, China). Accordingly, the BB genotype was more common in the Serbian population – 23 %, the least – in the Canadian population (2.7 %) [1, 2, 8, 12, 15].

Recently, beta-casein polymorphism has attracted great interest from both the scientific community and the dairy sector due to its impact on milk quality. The two main subvariants of beta-casein (A1 and A2) are encoded by the CSN2 gene. It is found that the consumption of A1 variant, in comparison with A2 variant, potentially negatively affects human health after its digestion, but at the same time its availability improves the technological properties of milk [7, 16, 18].

The researchers have concluded that the amino acid content, fatty acid content, and milk color may be affected by the CSN2 A1A1, A1A2, and A2A2 genotypes. As a result of selective breeding of genotypes with the desired qualities, milk and dairy products can be improved [19].

The researchers state that the simplest process for creating a herd of cattle with the A2A2 genotype is to use bull semen with the A2A2 genotype by beta-casein. However, the transformation function is curved and asymptotic, and this strategy itself cannot achieve herd purity. The conversion rate can be significantly increased by genotyping calves and/or cows. This is required for completing the conversion process. The mating of heifers with semen with the A2A2 genotype by beta-casein can have a significant impact, especially in connection with an increase in the level of cow disposal. Combining these strategies can lead to pure herds with the A2A2 genotype within 5–8 years, depending on the initial structure of the herd. The use of sexed semen can further accelerate the conversion period [14].

The results of studies to estimate the frequency of beta-casein variants in the offspring of pre-genotyped cows inseminated with homozygous A2A2 semen indicate that the frequency of cattle with the A2A2 genotype has almost doubled – from 37 to 69 % [17].

Similar work may be carried out on the creation of dairy herds with the BB genotype by kappa-casein.

The goal of the research is to evaluate the prospect of creating dairy herds with the desired genotypes by beta- and kappa-casein of domestic dairy cattle breeds.

Material and methods of research. The genotyping of cows of the Ukrainian Brown Dairy breed (n=44), Sumy intra-breed type of the Ukrainian Black-and-White Dairy breed (n=26), Simmental (n=30), Lebedyn (n=59) breeds was carried out.

The kappa-casein gene polymorphism was determined in the genetic laboratory of the Bogomoletz Institute of Physiology of the National Academy of Sciences of Ukraine using real-time molecular biological analysis of allele recognition by polymerase chain reaction (PCR). Blood samples were taken to the 2.7 ml Monovette (Sarstedt, Germany), followed by freezing the samples and storing them at -20ºC. DNA for genotyping was obtained from samples using the Monarch® New England BioLabGenomic DNA Purification Kit (USA) in accordance with the manufacturer's protocol.

Allele frequencies were calculated taking into account the number of homozygotes and heterozygotes found for the corresponding allele using the formula:

$$P(A)=\frac{2N1+N2}{2n}$$

where N_1 and N_2 are the number of homozygotes and heterozygotes for the allele under study, respectively;
n is the sample number.

In order to assess the statistical reliability of the discrepancy between the distributions of the obtained results, the Pearson criterion was used as follows:

$$\chi^2=\frac{\sum(A-T)^2}{T}$$

where: A is the actual number of genotypes;
T is the theoretical number of genotypes.

Theactual (available) heterozygosity was determined by direct calculation using the formula:

$$H_O=\frac{N2}{n}$$

The expected heterozygosity was determined by the formula:

$$H_E=1-\sum_{i=1}^{n} p_i^2$$

where p_1, p_2,….p_n are allele frequencies.

The research results were processed using mathematical statistics using the Statistica-6.1Package in the Windows PC environment.

Results and discussion. According to the frequency of the desired genotype of BB kappa-casein, cattle of the Lebedyn and Ukrainian Brown dairy breeds have an advantage. They also predominate among representatives of other breeds in terms of the frequency of the B allele. The Simmental breed has a higher frequency of heterozygous (AB) genotypes, in which it is almost 50 %. Cattle of the Sumy intrabreed type of the Ukrainian Black-and-White Dairy breed have the highest frequency of the homozygous AA genotype and Aallele. In all the studied breeds, except Simmental, there is an advantage of the expected heterozygosity over the actual one (Table 1).

Studying the beta-casein polymorphism, it is found that the highest frequency of both the A2A2 genotype and the A2 allele is found in cattle of the Ukrainian Brown Dairy breed. Half of them have this genotype. Cattle of the Simmental breed have a slightly lower frequency of the A2A2 genotype (40 %). At the same time, cattle of this breed have the highest frequency of heterozygous A1A2 genotype (57 %). Cattle of the Sumy intrabreed type of the Ukrainian Black-and-White Dairy breed have the highest frequency of homozygous A1A1type (38 %). They are also characterized by the advantage of the expected heterozygosity over the actual one (Table 2).

The number of cattle with the desired homozygous A2A2 genotype by beta-casein and BB genotype by kappa-caseincan be increased by selecting parent pairs. Complete penetrance, i.e. 100 % of the frequency and probability of phenotypic gene expression, can be achieved if:

♂ ♀
A2A2 x A2A2 = 100 % penetrance;
A2A2 x A1A2 = 50–70 % penetrance;
A1A2x A2A2 = 50–70 % penetrance;
A2A2 x A1A1 = 50–70 % penetrance;
A1A2 x A1A2 = 25–50 % penetrance;
A1A1 x A1A2= 25–50 % penetrance;
A1A2 xA1A1= 25–50 % penetrance.

Table 1 – **Frequency of alleles and genotypes by kappa-casein genelocus**

Distribution	Genotypes, %			Allele,pcs.		χ^2
	AA	AB	BB	A	B	
Ukrainian Brown Dairy						
Actual	30	40	30	0,500	0,500	1,455
Theoretical	25	50	25			
Ukrainian Black-and-White Dairy						
Actual	58	27	15	0,712	0,288	3,079
Theoretical	51	41	8			
Simmental						
Actual	43	47	10	0,667	0,333	0,075
Theoretical	44	44	11			
Lebedyn						
Actual	15	36	49	0,328	0,672	2,014
Theoretical	11	44	45			

Table 2 – **Frequency of alleles and genotypes by beta-casein genelocus**

Distribution	Genotypes, %			Allele, pcs.		χ^2
	A1A1	A1A2	A2A2	A1	A2	
Ukrainian Brown Dairy						
Actual	7	43	50	0,284	0,716	0,167
Theoretical	8	41	51			
Ukrainian Black-and-White Dairy						
Actual	38	35	27	0,558	0,442	2,314
Theoretical	31	49	20			
Simmental						
Actual	3	57	40	0,317	0,683	2,871
Theoretical	10	43	47			
Lebedyn						
Actual	19	51	30	0,447	0,553	0,048
Theoretical	20	49	31			

An individual approach to the issues of selection, control of the processes of gene transfer from parents to offspring using DNA diagnostics methods will make it possible to replenish herds with carriers of the A2 or B gene in a relatively short time [10, 11].Taking into account the initial frequency of genotypes and alleles of the studied casein fractions in the studied breeds, using the principles of the herd creation model with the desired genotype [14], it is found that when using bull semen with the BB or A2A2 genotype on a number of cows and heifers without testing them, random cow disposal at the level of 22 % to create herds with the desired genotypes takes more than 15 years. Similar results will be obtained when using cow testing. It is possible to reduce the time required to create a herd by using genotype testing of heifers or cows and heifers. The best indicators regarding the timing of herd creation can be obtained by testing cows and heifers in combination with the use of sexed semen of stud bulls with the A2A2, BB genotypes. At the same time, over a 10-year period, such a herd can be created using genetic testing of heifers. In the case of using genetic testing of cows and heifers, such a herd can be created in nine years. Thus, in 9 years it is possible to create a herd of cattle of the Lebedyn breed with the BB genotype by kappa-casein and cattle of the Ukrainian Brown dairy and Simmental breeds with the A2A2 genotype by beta-casein. More time is needed to create similar herds of cattle of other studied breeds (Table 3).

It should be note that it is possible to reduce the time required to create such herds by increasing the percentage of accidental cow disposal and disposal of cattle with undesirable genotypes.

Conclusion. Results of the conducted studies indicate prospects for creating dairy herds with cattle of the desired genotypes by kappa-casein (BB) and beta-casein (A2A2). Given that the frequency of desired genotypes is higher in cattle of the Lebedyn and Ukrainian Brown Dairy breeds, this work is more promising with these breeds. In 9 years, the breeders can create a herd of cattle of the Lebedyn breed with the BB genotype by kappa-casein and cattle of the Ukrainian Brown Dairy and Simmental breeds with the A2A2 genotype by beta-casein. To reduce the time frame for creating herds, it is essential to increase the percentage of cow disposal and simultaneously dispose cattle with undesirable genotypes.

Table 3 – **Analysis of mechanisms for creating herds with the desired genotype**

Optionsforcarryingoutthework	Genotype fractionin 10 years in cattle of the mainherd		Required time, years	
	CSN2	CSN3	for creating a herd of cows with a genotype	
	A2A2	BB	CSN2 (A2A2)	CSN3 (BB)
Ukrainian Brown Dairy				
Lack of testing of cows and calves	0,78	0,66	>15	>15
Genetictestingofcows	0,85	0,71	>15	>15
Genetictestingofcalves	1,00	0,99	10	12
Genetic testing of cows and calves	1,00	1,00	9	10
Genetic testing of cows and calves and the use of sexed semen (A2A2) or (BB)	1,00	1,00	9	10
Ukrainian Black-and-White Dairy				
Lack of testing of cows and calves	0,66	0,54	>15	>15
Genetic testingofcows	0,71	0,57	>15	>15
Genetic testingofcalves	0,99	0,93	12	14
Genetic testing of cows and calves	1,00	0,95	10	11
Genetic testing of cows and calves and the use of sexed semen (A2A2) or (BB)	1,00	1,00	10	10
Simmental				
Lack of testing of cows and calves	0,78	0,54	>15	>15
Genetic testingofcows	0,85	0,57	>15	>15
Genetic testingofcalves	1,00	0,93	10	14
Genetic testing of cows and calves	1,00	0,95	9	11
Genetic testing of cows and calves and the use of sexed semen (A2A2) or (BB)	1,00	1,00	9	10
Lebedyn				
Lack of testing of cows and calves	0,71	0,78	>15	>15
Genetic testingofcows	0,99	0,85	>15	>15
Genetic testingofcalves	1,00	1,00	12	10
Genetic testing of cows and calves	1,00	1,00	10	9
Genetic testing of cows and calves and the use of sexed semen (A2A2) or (BB)	1,00	1,00	10	9

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Особливості формування стад за бета- та капа-казеїном молочної худоби різних порід

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У молочному скотарстві велику увагу приділяють показникам якості та технологічності молока. На ці показники істотний вплив мають білки молока. Тому метою цих досліджень є оцінка ефективності створення молочних стад вітчизняних порід молочної худоби, які характеризуються бажаними генотипами за бета- та капа-казеїном. Для виконання поставлених завдань проведено генотипування корів української бурої молочної, сумського внутрішньопородного типу української чорно-рябої молочної, симентальської та лебединської порід. Визначення поліморфізму гену капа-бета-казеїну проводили в генетичній лабораторії Інституту фізіології ім. Богомольця НАН за допомогою молекулярно-біологічного аналізу розпізнавання алелів методом полімеразної ланцюгової реакції (ПЛР) у реальному часі.

У результаті проведених досліджень встановлено, що тварини лебединської та української бурої молочної порід мають вищі значення частоти бажаного

генотипу ВВ капа-казеїну. Як наслідок, для них характерна і вища частота алеля В. Понад 50 % тварин симентальської породи мають гетерозиготний генотип АВ. Більшу частоту гомозиготного генотипу АА та алеля А мають тварини сумського внутрішньопородного типу української чорно-рябої молочної породи. Для тварин української бурої молочної породи характерна найбільша частота генотипу А2А2 та алеля А2 бета-казеїну. Дещо нижчу частоту мають тварини симентальської породи, для яких також характерна найбільша частота гетерозиготного генотипу А1А2. Гомозиготний генотип А1А1 частіше зустрічається у тварин сумського внутрішньопородного типу української чорно-рябої молочної породи.

Використовуючи принципи моделі створення стада з бажаним генотипом, яку запропоновано Mencarini IRetall, встановлено, що при тестуванні всього маточного поголів'я, осіменіння його спермою гомозиготних плідників з генотипом А2А2, випадковому вибракуванні корів на рівні 22 % для створення стад з бажаними генотипами української бурої молочної, симентальської та лебединської порід, відповідно необхідно 9–10 років. Для скорочення строків створення стад необхідно збільшити відсоток вибракування корів та паралельно вибраковувати тварин з небажаними генотипами.

Ключові слова: генотип, казеїн, плідник, селекція, алель.

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Significance of breeding value indicators for prediction of milk yield

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A comparative assessment of the significance of various indicators of the breeding value of breeding bulls for predicting milk yield in the highly productive herd of the farm economy "Alfa" was carried out. The milk yield of cows, whose bulls-fathers were evaluated in Ukraine and abroad, was studied. It was established that in the period from 2009 to 2014, the milk yield of first-calf heifers increased by 23.9 % and reached 5894.3 kg. Accordingly, during the specified period, most of the quantitative indicators of the breeding value and milk productivity of the bulls' daughters also increased: the breeding value of the parent bulls in terms of milk yield increased from +245.3 kg in 2009 to +540.4 kg in 2014; the breeding value of parent bulls in terms of the total amount of milk fat per lactation increased from +10.2 kg to +29.7 kg. A correlation analysis of the relationships between various indicators of breeding value of parent bulls and the milk yield of their daughters was carried out. It was established that the actual milk yield of first-calf heifers in FE "Alfa" most (correlation coefficients r higher than 0.8) and most significantly ($p<0.001$) depended on the average milk yield and amount of milk fat yield of bull's daughters in the herds where the breeding value of these bulls was evaluated. The breeding value of the sire bulls by the amount of milk fat yield was also characterized by high ($r=0.675$) and significant ($p<0.01$) relationship with the actual milk yield of the first-calf heifers (daughters of these sires). The variance analysis of the influence of the breeding value estimation method of breeder bulls (BV, ETA, ZW, DRV, RPC) on the actual milk yield of their daughters during the first lactation established that this influence was significant ($p=0.001$), and the power of influence was $\eta^2=0,59$. Significant differences were mostly observed between milk yields of first-calf heifers whose parent bulls were evaluated abroad and in Ukraine. The biggest difference in terms of actual milk yield was revealed between the daughters of bulls evaluated by the ETA method (Canada) and by the "daughter-of-the-same-age (DRV)" method (Ukraine). This difference was 2640 kg of milk ($p<0.01$) in favor of first-calf heifers from Canadian bulls.

Key words: breeding bulls, breeding value, milk productivity, daughters of bulls, milk yield, selection index, evaluation method.

Problem statement and analysis of recent research. Ukrainian catalogs of breeder bulls list more than a dozen different indicators of their breeding value in terms of milk productivity. Each of these indicators characterizes quantitative, qualitative or complex aspects of evaluation [1–4]. In particular, among the breeding value indicators there is a block of indicators that evaluate exactly breeding value by milk productivity, i.e. the potential advantage of the assessed bull's daughters compared to daughters of other bulls (for example, plus-minus according to the milk yield of daughters).

The second block of indicators is characteristics of average milk productivity of daughters, by which the sire was evaluated (for example, the average milk yield of his daughters per lactation).

Each block usually includes 5 indicators. Based on them, it is possible to additionally deter-

mine several more complex characteristics of milk productivity.

In addition, the catalogs have certain indexes and categories of breeding value, which characterize sires simultaneously by several breeding traits. They act as integral criteria for assessing the breeding value. In countries where breeding work is carried out at a high level, the calculation of the breeding value index is carried out simultaneously based on 4–6 economic and 17–18 breeding indicators. Usually such indicators in numerical form characterize the genetic potential of livestock from the point of view of its influence on the complex of economic and useful traits of offspring [4–11].

Breeding indexes mostly include the most significant indicators of productivity, reproductive capacity, technological, genomic and other characteristics of animals in a certain ratio in the way that it is convenient to achieve breeding goals with their help [12–18].

It should be noted that all indicators of breeding value, which are given in the catalogs, were determined on animals that are kept in certain conditions. And these conditions do not always correspond to the level that occurs in a specific domestic farm [19–21]. So, today most of the sperm of parent bulls used in Ukrainian farms have foreign origin. In particular, the cows on which the breeding value of sires was evaluated are characterized by milk yield of 7–12 thousand kg per lactation. And this implies the appropriate level and quality of feeding and comfort for animals. The vast majority of Ukrainian farms have not yet reached this level.

Thus, the comparative assessment of the significance of various indicators of breeding value for predicting milk yield of cows in low-yielding herds simultaneously faces several methodological obstacles regarding the correctness of such an assessment. First of all, we are talking about the additional impact of the non-compliance of farm conditions with those that took place during the evaluation of bulls.

In view of this, the **goal of the conducted research** is quite relevant. It consists in a comparative assessment of the significance of bull's breeding value indicators for predicting the milk yield of their daughters in such Ukrainian farms, the level of milk yield in which corresponds to foreign ones, and the quality of feeding and comfort of keeping can be compared with those that took place in the process of evaluating bulls abroad.

Material and methods of research. The research was conducted on the basis of data obtained at the farming economy "Alfa" (FE "Alfa") of Zolochiv District, Kharkiv Region. In this farm, the milk productivity of cows was increasing significantly for a long time. In 2010–2021, milk productivity of cows in FE "Alfa" reached the level that occurred when assessing the breeding value of most breeding bulls used in this farm.

FE "Alfa" is a dairy cattle breeding specializes farm. Ukrainian Black-And-White dairy cattle are bred here. They use tethered and untethered keeping of animals. The technologies of keeping different sex-age groups of animals, as well as keeping them in different seasons of the year, are somewhat different.

About 300 dairy cows were kept in the farm. The milk yield of dairy cows in the period 2007–2009 was 5545–6497 kg (an average of 6062 kg). In the period of 2012–2014, milk yield was 7503–8010 kg, that is, it increased on average to 7838 kg. Milk yield per cow during these controlled periods is significantly different ($p = 0.005$).

The average daily growth of heifer's mass during the considered years of research ranged from 616 to 872 g. This is a fairly high level. In this regard, and in view of the results of our previous studies [22], the level of daily growth of heifer's mass unlikely effects significantly on the results of evaluating of various breeding value characteristics of sires.

The material for the research was the data on the milk yield (MY) of the first-calf heifers, born from the use of sperm production of different breeder bulls in different periods. In addition, available indicators of breeding value of sires used in FE "Alfa" were selected from breeding bull's catalogs, 1-mol cards and other sources. In particular, the following indicators of breeding value of sires were monitored:

- Selection index of bull (SI), points;
- Pedigree index of bull (PI), points;
- Breeding value of bull by milk yield per lactation (BVMY), kg;
- Breeding value of bull by fat content in milk (BV%F), %;
- Breeding value of bull by amount of milk fat per lactation (BVFat), kg;
- Breeding value of bull by protein content in milk (BV%P), %;
- Breeding value of the bull by amount of milk protein per lactation (BVProt), kg;
- Average milk yield of bull's daughters per lactation in herds where the breeding value evaluation of the sire was carried out (ADMY), kg;
- Average fat content in milk of bull's daughters in the herds where the breeding value evaluation of the sire was carried out (AD%F), %;
- Average amount of milk fat per lactation of bull's daughters in the herds where the breeding value evaluation of the sire was carried out (ADMF), kg;

• Average protein content in the milk of bull's daughters in the herds where the breeding value evaluation of the sire was carried out (AD%P), %;

• Average amount of milk protein per lactation of bull's daughters in the herds where the breeding value evaluation of the sire was carried out (ADMP), kg.

In addition, two more complex indicators were calculated on the basis of these breeding value indicators:

• Breeding value of bulls by total amount of milk fat and milk protein per lactation (BVFatPr), kg;

• The average total amount of milk fat and milk protein per lactation of bull's daughters in the herds where the breeding value evaluation of the sire was carried out (ADMFP), kg.

The dynamics of indicators of breeding value and milk yield of first-calf heifers in FE "Alfa" at 2009 and 2014 were analyzed.

The relationships between the above parameters of breeding value of sires and the milk yield of first-calf heifers were investigated using correlation analysis. Statistical processing was carried out in three variants:

– using data for the entire period of research;

– with the involvement in the processing of only indicators determined as of 2009;

– with the involvement in the processing of only indicators determined as of 2014;

The package of applied computer statistical programs SPSS-22, the procedures "Correlation – Pairwise" and "General linear model – Univariate" were responsible for software support.

The correlation coefficients between various indicators of breeding value of sires and the actual milk yield of first-calf heifers (their daughters) in FE "Alfa" was compared. At the same time, the most significant and most important correlations were highlighted. This made it possible to determine the characteristics of breeding value of sires, which are most significant for predicting future milk yields.

In addition, trends of changing these correlation coefficients over time were monitored. At the same time, significant trends in the validity of the use of bulls' semen with certain indicators of breeding value were highlighted.

Results and discussion. It was established that the average milk yield of first-calf heifers in FE "Alfa" (table 1) during the entire period of research was M = 5306.7 kg. The mean square (standard) deviation of milk yields between daughters of different sires was σ = 1023.0 kg. At the same time, it should be noted that as of 2009, this average milk yield was significantly lower (M = 4759.1 kg) and less variable (σ = 694.0 kg). At 2014 it had increased by 23.9 % to 5894.3 kg. Milk yield variability between daughters of different bulls in 2014 is significantly higher compared to 2009: mean square (standard) deviation increased by 45.5 %.

Accordingly, most of the quantitative indicators of breeding value of sires and milk productivity of their daughters increased during the specified period. In particular, the breeding value of bulls by milk yield in 2014 reached +540.4 kg, by the total amount of milk fat per lactation of their daughters +29.7 kg, against +245.3 kg and +10.2 kg, respectively, in 2009.

The average milk yield of bulls' daughters per lactation in the herds, where the evaluation of breeding value of the sire was carried out, increased from 2009 to 2014 by 2582.3 kg, reaching 7487.6 kg. Accordingly, the average amount of milk fat per lactation of bulls' daughters in the herds where breeding value was evaluated increased by 59.9 % over the same period and reached 286.3 kg in 2014.

A correlation analysis of the relationships between the characteristics of the breeding value of bulls and the milk yield of first-calf heifers (their daughters in FE "Alfa") was carried out (table 2). It was established that the actual milk yield of first-calf heifers in FE "Alfa" most (correlation coefficients r higher than 0.8) and most significantly ($p<0.001$) depended on the average milk productivity (milk yield and amounts of milk fat) of their parent bull's daughters in the herds where the evaluation of the bulls' breeding value was carried out.

From the direct indicators of the breeding value of bulls, the breeding value of the sire bull by the amount of milk fat was characterized by a slightly lower ($r=0.675$), but also significant ($p<0.01$) relation with the actual milk yield of first-calf heifers (daughters of these sires) in FE "Alfa".

In addition, a considerable and significant relationship between the actual milk yield of first-calf heifers and the values of the selection indexes of parent bulls SI and PI was revealed.

All the given regularities are fully consistent with the conclusion we made earlier in the studies conducted on the database, which consisted of animals from many breeding farms of Ukraine [16].

If we analyze the correlations between different indicators of the breeding value, it is worth paying attention to the positive and significant relationships between the direct indicators of the breeding value of the sires and the average milk productivity of their daughters in the herds where the breeding value of these bulls was assessed. This indicates a parallel improvement over time in both the sires' breeding values of the themselves and the milk productivity of the dams on which these sires are tested.

Table 1 – **Characteristics of the variability of breeding value indicators of bulls-fathers in FE "Alfa"**

Indicators	Mean, M	Standard deviation, σ	Number of assessed bulls, heads
Breeding value of bull by milk yield per lactation (BVMY), kg	+436,2	428,5	17
Breeding value of bull by fat content in milk (BV%F), %	+0,071	0,211	17
Breeding value of bull by amount of milk fat per lactation (BVFat), kg	+22,8	23,5	17
Breeding value of bull by protein content in milk (BV%P), %	+0,099	0,127	7
Breeding value of the bull by amount of milk protein per lactation (BVProt), kg	+33,7	11,1	7
Breeding value of bulls by total amount of milk fat and milk protein per lactation (BVFatPr), kg	+77,0	24,0	7
Average milk yield of bull's daughters per lactation in herds where the breeding value of the sire evaluation was carried out (ADMY), kg	6576,2	2329,0	17
Average fat content in milk of bull's daughters in the herds where the breeding value evaluation of the sire was carried out (AD%F), %	3,771	0,246	17
Average amount of milk fat per lactation of bull's daughters in the herds where the breeding value evaluation of the sire was carried out (ADMF), kg	248,4	95,2	17
Average protein content in the milk of bull's daughters in the herds where the breeding value evaluation of the sire was carried out (AD%P), %	3,341	0,220	7
Average amount of milk protein per lactation of bull's daughters in the herds where the breeding value evaluation of the sire was carried out (ADMP), kg	300,9	28,9	7
The average total amount of milk fat and milk protein per lactation of bull's daughters in the herds where the breeding value evaluation of the sire was carried out (ADMFP), kg	652,7	66,6	7
Selection (SI) or pedigree (PI) index of bull, points;	639,4	479,0	15

A dispersion analysis of the effect of the breeding value estimation method of breeder bulls (BV, ETA, ZW, DRV, RPC) on the actual milk yield of their daughters during the first lactation in FE "Alfa" was carried out. It was found that this effect was significant ($p=0.001$), and the power of influence was $\eta^2 = 0.59$. Significant differences were mostly observed between milk yields of first-calf heifers whose parent bulls were evaluated abroad and in Ukraine.

Thus, the biggest difference by milk yields was recorded between the daughters of bulls evaluated by the ETA method (Canada) and by the "daughter-of-the-same-age (DRV)" method (Ukraine). This difference was 2640 kg of milk ($p<0.01$) in favor of first-calf heifers from Canadian bulls. In FE "Alfa" the average milk yield of first-calf heifers from Canadian sires evaluated by the ETA method was 7,503 kg against the milk yield of 4,863 kg in first-calf heifers that were daughters of bull sires evaluated in Ukraine by the DRV method.

In the rest of the researched options, first-calf heifers obtained from sires who were evaluated abroad prevailed in terms of milk yield of first-calf heifers, whose parents were evaluated in Ukraine, by 1173–2245 kg. All but one of these differences at milk yields were significant.

A comparison of the milk yield of the first-calf heifers of FE "Alfa" obtained from bulls of different breeding value categories was carried out. A significant ($p<0.05$) difference was established between the milk yields of first-calf heifers whose parent bulls had "P5" and "H+" breeding value categories. This difference was 1571 kg in favor of the "P5" category.

Table 2 – **Correlation between the milk yields of first-calf heifers and indicators of breeding value of their parent bulls for the entire monitored period**

Indicators		BVMY	BV%F	BVFat	BV%P	BVProt	BVFatPr	ADMY	AD%F	ADMF	AD%P	ADMP	ADMFP	SI/PI	MY
BV	r	1,00	–0,27	0,47	–0,60	0,46	–0,37	0,49*	–0,03	0,45	–0,52	–0,35	–0,52	0,26	0,38
MY	p		0,29	0,06	0,15	0,29	0,41	0,05	0,91	0,07	0,23	0,45	0,23	0,34	0,13
BV	r	–0,27	1,00	0,71**	0,67	–0,26	0,67	0,38	0,66**	0,50*	0,28	0,56	0,73	0,50	0,43
%F	p	0,29		0,00	0,10	0,57	0,10	0,14	0,00	0,04	0,54	0,20	0,06	0,06	0,09
BV	r	0,47	0,71**	1,00	0,65	0,03	0,89**	0,70**	0,56*	0,78**	0,00	0,61	0,78*	0,67**	0,68**
Fat	p	0,06	0,00		0,12	0,95	0,01	0,00	0,02	0,00	1,00	0,15	0,04	0,01	0,00
BV	r	–0,60	0,67	0,65	1,00	0,42	0,76*	–0,15	0,75	0,43	0,67	0,26	0,37	0,77*	0,60
%P	p	0,15	0,10	0,12		0,34	0,05	0,75	0,05	0,34	0,10	0,58	0,42	0,05	0,16
BV	r	0,46	–0,26	0,03	0,42	1,00	0,49	–0,07	–0,10	–0,15	0,08	–0,03	–0,10	0,24	0,43
Prot	p	0,29	0,57	0,95	0,34		0,27	0,88	0,83	0,76	0,87	0,96	0,84	0,60	0,34
BV	r	–0,37	0,67	0,89**	0,76*	0,49	1,00	0,39	0,28	0,69	0,03	0,52	0,63	0,50	0,58
FatPr	p	0,41	0,10	0,01	0,05	0,27		0,39	0,55	0,09	0,94	0,23	0,13	0,25	0,18
AD	r	0,49*	0,38	0,70**	–0,15	–0,07	0,39	1,00	0,33	0,98**	–0,66	0,86*	0,82*	0,61*	0,81**
MY	p	0,05	0,14	0,00	0,75	0,88	0,39		0,20	0,00	0,11	0,01	0,02	0,02	0,00
AD	r	–0,03	0,66**	0,56*	0,75	–0,10	0,28	0,33	1,00	0,52*	0,85*	–0,15	0,02	0,67**	0,43
%F	p	0,91	0,00	0,02	0,05	0,83	0,55	0,20		0,03	0,02	0,74	0,97	0,01	0,09
AD	r	0,45	0,50*	0,78**	0,43	–0,15	0,69	0,98**	0,52*	1,00	–0,11	0,90**	0,98**	0,71**	0,83**
MF	p	0,07	0,04	0,00	0,34	0,76	0,09	0,00	0,03		0,82	0,01	0,00	0,00	0,00
AD	r	–0,52	0,28	0,00	0,67	0,08	0,03	–0,66	0,85*	–0,11	1,00	–0,19	–0,15	0,61	0,26
%P	p	0,23	0,54	1,00	0,10	0,87	0,94	0,11	0,02	0,82		0,69	0,75	0,15	0,57
AD	r	–0,35	0,56	0,61	0,26	–0,03	0,52	0,86*	–0,15	0,90**	–0,19	1,00	0,97**	–0,15	0,77*
MP	p	0,45	0,20	0,15	0,58	0,96	0,23	0,01	0,74	0,01	0,69		0,00	0,76	0,04
AD	r	–0,52	0,73	0,78*	0,37	–0,10	0,63	0,82*	0,02	0,98**	–0,15	0,97**	1,00	–0,03	0,71
MFP	p	0,23	0,06	0,04	0,42	0,84	0,13	0,02	0,97	0,00	0,75	0,00		0,96	0,07
SI /	r	0,26	0,50	0,67**	0,77*	0,24	0,50	0,61*	0,67**	0,71**	0,61	–0,15	–0,03	1,00	0,62*
PI	p	0,34	0,06	0,01	0,05	0,60	0,25	0,02	0,01	0,00	0,15	0,76	0,96		0,02
MY	r	0,38	0,43	0,68**	0,60	0,43	0,58	0,81**	0,43	0,83**	0,26	0,77*	0,71	0,62*	1,00
	p	0,13	0,09	0,00	0,16	0,34	0,18	0,00	0,09	0,00	0,57	0,04	0,07	0,02	

* – p≤0,05; ** – p≤0,01.

Conclusions. 1. It was established that in the period from 2009 to 2014, in FE "Alfa" the average milk yield of first-calf heifers increased by 23.9 % and reached 5894.3 kg. Accordingly, during the specified period, mean square (standard) deviation increased by 45.5 %.

2. Most of the quantitative indicators of the breeding value and milk productivity of the bulls' daughters from 2009 to 2014 also increased: the breeding value of the parent bulls in terms of milk yield increased from +245.3 kg in 2009 to +540.4 kg in 2014; the breeding value of parent bulls in terms of the total amount of milk fat per lactation increased from +10.2 kg to +29.7 kg.

3. The actual milk yield of first-calf heifers in FE "Alfa" most (correlation coefficients r higher than 0.8) and most significantly ($p<0.001$) depended on the average milk yield and amount of milk fat yield of bull's daughters in the herds where the breeding value of these bulls was evaluated.

4. The breeding value of the sire bulls by the amount of milk fat yield was characterized by high ($r=0.675$) and significant ($p<0.01$) relationship with the actual milk yield of the first-calf heifers (daughters of these sires).

5. The variance analysis of the influence of the breeding value estimation method of breeder bulls (BV, ETA, ZW, DRV, RPC) on the actual milk yield of their daughters during the first lactation established that this influence was significant ($p=0.001$), and the power of influence was $\eta^2 = 0,59$. Significant differences were mostly observed between milk yields of first-calf heifers whose parent bulls were evaluated abroad and in Ukraine.

6. The biggest difference in terms of actual milk yield was revealed between the daughters of bulls evaluated by the ETA method (Canada) and by the "daughter-of-the-same-age (DRV)" method (Ukraine). This difference was 2640 kg of milk ($p<0.01$) in favor of first-calf heifers from Canadian bulls.

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Значущість показників племінної цінності для прогнозування надоїв

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Проведено порівняльне оцінювання значущості різних показників племінної цінності бугаїв-плідників для прогнозування надоїв у високопродуктивному вітчизняному стаді фермерського господарства «Альфа». Досліджено надої корів, батьками яких були бугаї, оцінені в Україні та за кордоном. Встановлено, що за період з 2009 до 2014 рік надої первісток збільшилися на 23,9 % і сягнули 5894,3 кг. Зросла також більшість кількісних показників племінної цінності та молочної продуктивності дочок бугаїв: племінна цінність бугаїв-батьків за надоєм зросла зі +245,3 кг у 2009 році до +540,4 кг у 2014 році; племінна цінність бугаїв-батьків за сумарною кількістю молочного жиру за лактацію – збільшилася з +10,2 кг до +29,7 кг. Здійснено кореляційний аналіз зв'язків між різними показниками племінної цінності бугаїв-батьків і надоями їхніх дочок. Встановлено, що фактичні надої первісток у ФГ «Альфа» найбільше (коефіцієнти кореляції r вищі за 0,8) і найвірогідніше ($p<0,001$) залежали від середніх надоїв і кількості молочного жиру дочок бугаїв у стадах, на яких проводили оцінювання племінної цінності цих бугаїв. Суттєвим ($r=0,675$) і вірогідним ($p<0,01$) зв'язком із фактичним надоєм первісток (дочок цих плідників) у ФГ «Альфа» характеризувалася також племінна цінність бугая-батька за кількістю молочного жиру. Дисперсійним аналізом впливу методу оцінювання племінної цінності бугаїв-плідників (BV, ETA, ZW, ДРВ, РПЦ) на фактичні надої їхніх дочок за першу лактацію встановлено, що цей вплив був вірогідним ($p=0,001$), а ступінь впливу становив $\eta^2 = 0,59$. Статистично значущі різниці здебільшого спостерігали між надоями первісток, батьки яких оцінені за кордоном і в Україні. Найбільшу різницю за фактичними надоями зафіксовано між дочками бугаїв, оцінених за передавальною здатністю методом ETA (Канада) і методом «дочки-ровесниці (ДРВ)» в Україні. Ця різниця становила 2640 кг молока ($p<0,01$) на користь первісток від канадських бугаїв.

Ключові слова: бугаї-плідники, племінна цінність, молочна продуктивність, дочки бугаїв, надій, селекційний індекс, метод оцінювання.

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Effect of mineral-vitamin premix on milk productivity and hematological blood parameters of cows

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The application of specialized premixes containing all the necessary elements for effective ration enrichment is the main direction of normalizing the mineral and vitamin nutrition of cows. The introduction of biologically active substances into animal diets in the form of premixes helps to increase the efficiency and productivity of the feed application.

The purpose of our study was to investigate the effect of the mineral-vitamin premix Biomix on milk productivity and hematological blood parameters of cows. The experiment was carried out on Ukrainian Black-and-White Dairy cows. Two groups were formed for experiment; each group contains 10 heads. The animals of the experimental group were fed by premix Biomix at the rate of 50 g per 1 head per day as a part of the main diet. The control group was fed by the equivalent amount of monocalcium phosphate.

The introduction of the Biomix premix into the diet of the research group made it possible to eliminate the deficiency of phosphorus, copper, zinc, cobalt, and iodine, and to bring the level of vitamin D_3 to normal.

Optimizing of the cows' mineral and vitamin nutrition through the application of Biomix premix ensured the dairy productivity and the milk quality increase. The average daily milk yield of the experimental cows increased by 5.9 % for 90 experimental days. The fat and protein content increased by 0.05 and 0.07 %, respectively. Cows of the experimental group surpassed the control ones by 153.1 kg in gross milk yield, and by 1.71 kg in average daily yield in terms of 4 percent milk; it is 7.4 % relative to the control ($P<0.05$). The level of dry matter, calcium, phosphorus, and lactose have increased in milk; its total energy value has also increased.

Feed consumption per 1 kg of milk decreased by 5.1 % and 5.4 % in terms of energy and digestible protein.

Feeding Biomix premix did not have a negative effect on hematological changes in the body. Morphological and biochemical blood parameters of cows corresponded to physiological norms. An increase in total protein ($P\leq0.95$), hemoglobin ($P\leq0.95$), and creatinine ($P\leq0.95$) was noted. There was a tendency to increase the level of erythrocytes, glucose, urea, and calcium in the blood.

Key words: cows, premix Biomix, vitamins, minerals, milk, fat, protein, blood, erythrocytes, hemoglobin.

Problem statement and analysis of recent research. The practical experience of cattle breeding shows the relationship between biologically complete feeding of cows, metabolic processes in their bodies, milk productivity and quality, and preservation of reproductive functions, health and viability of newborn calves. Realization of the genetic potential of milk productivity can be achieved only by providing cows with the necessary amount of energy, nutrients and biologically active substances in accordance with physiological needs [1, 2].

The key to achieving the feed maximum efficiency is the creation of a feeding system that

would provide the most optimal conditions for the intensification of anabolism processes. The vitamin-mineral factor occupies one of the leading places because it affects energy, protein, carbohydrate, and lipid metabolism and participates in almost all links of decay and synthesis, redox reactions as catalysts [3, 4].

The level of providing livestock with mineral elements and vitamins at the farms of the forest-steppe zone of Ukraine is characterized by its shortage in water, soil, and feed. Therefore, cattle rations without the inclusion of special feed additives do not provide full mineral and vitamin nutrition [5].

Due attention should be paid to the mineral nutrition of cowsbecause trace elements take an active part in the metabolism and affect the conversion of feed nutrients into livestock products [6]. According to scientific publications [7–9], a lack, excess or violation of the ratio of trace elements in the body of cows, metabolic processes are first disrupted, then various diseases develop, the productivity of animals decreases and their life expectancy decreases, too. The optimal content and ratio of trace elements in the tissues lead to a stable metabolism ensuring a normal state of health and high productivity.

The application of specialized premixes is an effective option for normalizing the mineral nutrition of cows, because premixes contain all the necessary elements for effective ration enrichment [10, 11]. Premixes are dosed to coarse and juicy feeds, enriching the diet with minerals and vitamins [12].

It has been proven that the complex introduction of biologically active substances into animal diets in the form of premixes balances and contributes to increasing the efficiency of feed application. Therefore, the productivity of animals, their state of health and reproductive capacity increases [13, 14].

The action of premixes is due to the presence of vitamins (A, D_3, E, K, C, group B), trace elements (iron, manganese, copper, cobalt, iodine, and selenium), macroelements (magnesium, and sulfur), amino acids, enzymes, antioxidants, antimicrobial drugs in optimal amounts and ratios [15, 16].

Mineral compounds can be introduced into premixes and compound feeds in inorganic and organic form. Inorganic forms such as oxides or sulfates are cheap and available. However, they have low bioavailability due to the antagonism of some metals in the intestine because they limit their assimilation. In organic forms, the trace element is chemically linked to amino acids or to small peptides. These minerals are much more available and bioactive. They interact less than inorganic ones in the digestion processeasily reaching the intestinal wall where they are absorbed into the blood. Organic minerals were created to maximize biological availability, the basis of their production technology is the process of chelates formation [17, 18]. Trace elements, in particular zinc, copper, manganese, associated with amino acids, have the same properties as natural organic trace elements found in plants.

Lots of studies have proven better assimilation of trace elements from their chelated compounds than from inorganic forms. Milk productivity increased by 14.9 % when using a premix with chelated forms of such trace elements as zinc, manganese, cobalt in the diet of dairy cows for the first 100 days of lactation. The increase in average daily milk yield, compared to the control, was 9.82 % due to the use of zinc, manganese, cobalt and copper sulfates in the premix [19].

The use of premixes increased feed digestibility, the balance of nitrogen, manganese, and cobalt [20, 21, 22, 23]. The experimental cows gave birth better and there were no cases of placental retention [24].

In the summer, the introduction of mineral premix Intermix into the diet of dairy cows had a positive effect on the processes of the intensity of ruminal metabolism, as evidenced by the increase in the concentration of raw biomass of microflora and volatile fatty acids [25].

The range of premixes, mineral and vitamin supplements is increasing every year due to the introduction of new biologically active additives into their composition, i.e., prebiotics, probiotics, and phytobiotics. Phytogenic supplements include extracts and essential oils obtained from herbs or spices. The presence of carotenoids, flavonoids, and steroid glycosides has a nutritional effect on the digestion and assimilation of feed nutrients.

This type of mineral and vitamin concentrates includes premix Biomix. It contains oregano extract; its biological effect is to inhibit pathogenic microflora and stimulate the development of lactobacilli.

The purpose of our study was to investigate the effect of the mineral-vitamin premix Biomix on milk productivity and hematological blood parameters of cows.

Material and methods of research. The scientific experiment was conducted to study the effectiveness of the mineral-vitamin premix Biomix-application in feeding dairy cows in the conditions of the Zolotyy Kolos farm in the Vinnytsia district. Two groups of Ukrainian Black-and-White Dairy cows according were formed, each group had 10 cows (Table 1). Cows of 2–3 lactations were se-

lected for the experiment in the third-fourth week after calving with a weight of about 600 kg, the average daily yield of milk of natural fat content was 25 kg with a fat content of 3.67 %.

The animals were fed the main ration fodder twice a day, in particular, bulk fodder was fed in the form of a feed mixture, concentrated fodder was fed twice a day during morning and evening milking. The control group received monocalcium phosphate (50 g/head per day) as part of the main diet.The monocalcium phosphate was replaced by an equivalent amount of the premix Biomix Standard in the diet of the experimental group.

The studied mineral-vitamin complex included such fat-soluble vitamins as 830 thousand IU/1kg of retinol (A), 220 thousand IU/1kg of calciferol (D_3), 11,600 mg of tocopherol; macroelements (18 % of calcium and 37 % of phosphorus) and such trace elements as 9,000 mg of zinc, 6,000 mg of manganese, 2,000 mg of copper, 120 g of cobalt, 300 g of iodine, 60 mg of selenium, antioxidants, andorigano extract. Wheat bran, saponite flour, chalk were used as fillers. The total energy content of 1 kg of premix was 7.2 MJ of exchangeable energy and 79 g of crude protein.

The calculation of milk productivity was carried out with the help of the Ekomilk ultrasonic analyzer based on the results of the decennial control milking.

Blood samples were taken from 5 animals from each group to analyze hematological indicators. Blood was collected from the jugular vein in the morning in 0.5 hours before feeding and 2 hours after feeding. Blood parameters (morphological and biochemical) were studied according to the methods of the Institute of Animal Biology of the National Academy of Sciences (Vlizlo et al. 2012).

Results and discussion. The introduction of Biomix premix in the amount of 50 g to the diet of the experimental groupmade it possible to balance the mineral and vitamin composition of the diet. The nutritional value of the diets of the control and experimental groups are shown in Table 2.

Table 1 – **Feeding pattens**

Group	Number of animals, heads	Cows feeding	
		Egalitarian, 15 days	Main, 90 days
1 – control	10	BD	BD
2 – experimental	10	BD	BD + premix Biomix (50g/head per day)

Table 2 – **Nutritional value of the control and experimental groups diets**

Indicator	Animal group		± control	According to the norm
	control	experimental		
Dry matter, g	21.8	21.84	0.04	20.5
Exchange energy, MJ	200.14	200.51	0.37	200
EFU	20.02	20.05	0.03	20.0
Crude protein, g	2668.2	2672.8	4.6	2810
Digestive protein, g	1830.3	1834.2	3.9	1825
Crude fiber, g	4542.8	4546.3	3.5	4510
Crude fat, g	640.5	641.9	1.4	625
Starch, g	2674.6	2674.6	–	2740
Sugar, g	1412.4	1214. 3	3.9	1825
Calcium, g	121.86	125.75	0.49	126
Phosphorus, g	73.22	84.62	7.4	85
Magnesium, g	36.17	36.38	0.31	32
Potassium, g	284.18	284.30	0.12	132
Sulfur, g	34.01	34.10	0.09	42
Ferum, mg	4318.5	4327.0	17	1390
Cuprum, mg	100.3	200.8	100.5	175
Zinc, mg	562.7	1122.7	460	1130
Cobalt, mg	10.22	16.22	6.0	13.9
Manganese, mg	847.83	1247.57	399.7	1130
Iodine, mg	6.05	21.05	15.0	15.7
Carotene, mg	837.49	837.49	–	785
Vitamin E, mg	2075.2	2655.2	680	695
Vitamin D, thousand IU	4.67	15.67	11.0	17.4

The introduction of the Biomix premix into the diet of the research group made it possible to eliminate the deficiency of phosphorus, copper, zinc, cobalt, and iodine, and to bring the level of vitamin D_3 to normal (15.67 thousand IU against 17.4). The deficiency of phosphorus was 13.9 %, copper was 42.8 %, zinc was 50.2 %, cobalt was 26.5 %, manganese was 25 %, iodine was 61.5 %, vitamin D3 was 73.2 % in the diet of the control group. All indicators are within the normal range, the deviation does not exceed 1 % except for vitamin D_3, its deficiency was at the level of 4.5 % in the experimental group.

It has been established that optimizing the mineral and vitamin nutrition of cows by Biomix premix ensures an increase in milk productivity of cows. Data on the productivity of cows and indicators of milk quality premix are shown in Table 3.

The milk yield (natural fat content) of the experimental cows was 2412.5 kg for 90 days of the main period. It is by 135 kg (5.9 %) more than the productivity of the control cows of the group. The average daily yield of the experimental cows fed the Biomix premix increased by 1.5 kg (P<0.05).

The share of fat and protein in milk increased by 0.05 % and 0.07 %, but the difference is unlikely.

In terms of 4.0 % milk, the cows of the experimental group exceeded their counterparts from the control group by 153.1 kg in gross milk yield, and by 1.71 kg in average daily milk yield, it is 7.4 % relative to the control (P<0,05).

The amount of milk fat was higher in the experimental group by 6.12 kg or 7.38 %. The cows of the experimental group surpassed the control animals by 5.9 kg and 8.3 % in terms of milk protein obtained.

The chemical composition of the experimental cows' milk is presented in Table 4.

According to Table 4, the higher energy value of milk was noted in the experimental group. Its indicator exceeds the control group by 0.7 %. The density of milk depends on the temperature and the content of its components. In the experimental group this indicator did not differ significantly and was in the range of 29.15-29.40 A.

Table 3 – **Milk productivity of cows,** M±m, n=10

Indicator	Group	
	control	experimental
Gross yield for the accounting period (90 days) of the experiment, kg	2277.4±45.49	2412.5±34.65*
Average daily yield of milk with natural fat content, kg	25.3±0.43	26.8±0.36*
Mass fraction of fat, %	3.64±0.04	3.69±0.03
Amount of milk fat, kg	82.90±1.08	89.02±1.44*
Mass fraction of protein, %	3.12±0.05	3.19±0.06
Amount of milk protein, kg	71.06±1.52	76.96±2.31
Milk yield for 90 days of the accounting period in terms of 4 % fat milk, kg	2072.4 ± 43.18	2225.5 ±13.42*
Average daily yield of 4 % fat milk, kg	23.02 ±0.41	24.73 ±0.35*

* – P<005.

Table 4 – **Chemical composition and quality indicators of milk,** M±m, n=10

Indicator	Group	
	control	experimental
Energy value, MJ	2.85±0.07	2.87±0.06
Density, A	29.15±0.37	29.40±0.35
Acidity, T	17.4±0.02	17.6±0.03
MSNF, %	8.85±0.10	8.91±0.10
Dry matter, %	12.67±0.19	12.78±0.18
Lactose, %	4.60±0.05	4.64±0.05
Ash, %	0.69±0.03	0.71 ±0.03
Calcium, g	1.25±0.01	1.26±0.02
Phosphorus, g	1.03±0.03	1.04±0.02

Milk solids include all components determining its nutritional and technological properties. The content of dry matter in the milk of the experimental cows was by 0,9 % higher than that of the control animals. The share of dry skimmed milk residue is also higher by 0.7 % in the experimental group than in the control one.

The level of lactose was 4.64 % in the milk of the experimental group cows, it is by 0.90 % more than in the control group. Milk sugar is part of enzymes-coenzymes involved in the synthesis of proteins, fats, vitamins, and it is necessary for normal intracellular metabolism in the body.

The calcium content was higher by 0.8 % and phosphorus content was higher by 1.0 % in the milk of the experimental cows than the control ones. According to the indicators of titrated acidity and density, the milk of cows of both groups meets the requirements of the first class, no deviations between the groups were recorded.

The consumption of nutrients for milk production is an important indicator for evaluating the effectiveness of feed nutrients utilization (Table 5).

Thus, the experimental cows had a higher feedconsumption. They spent 0.75 EFU per 1 kg of milk, it is by 5.1 % lower than in the control group, and it is by 3.9 g or 5.4 % lower than in the control group in terms of digestible protein.

According to the research results on the morphological and biochemical composition of the blood of the experimental and control cows conducted on the 30th day of the experiment, the hematological changes in the animals' bodies after the application of the mineral-vitamin premix had no pathological changes and were within physiological limits (Table 6).

Table 5 – **Nutrient consumption per** 1 kg of milk

Indicator	Group	
	control	experimental
Milk obtained during the main period of the experiment, kg	2277.4±45.49	2412.5±34.65*
Feed consumed for milk production:		
energy feed units (EFU)	1801.8	1804.5
digestible protein, kg	164.73	165.08
Feed consumption per 1 kg of milk:		
EFU	0.79	0.75
digestible protein, g	72.3	68.4

Table 6 – **Hematological parameters of the cow blood** (n=3)

Indicator	Group	
	1 – control	2 – experimental
Erythrocytes, T/l	5.76±0.11	6.15±0.26
Leukocytes, g/l	8.35±1.12	9.17±0.53
Basophils	0.63±0.16	0.85±0.23
Eosinophils	6.89±0.11	6.22±0.23*
Neutrophils:		
stab	7.24±0.52	7.84±0.19
segmented	24.09±0.31	23.67±0.21
Lymphocytes	60.08±0.09	60.29±0.08*
Monocytes	3.71±0.26	4.23±0.31
Hemoglobin, g/l	111.74±2.17	122.46±3.75*
Total protein, g/l	72.56±1.73	78.49±2.31*
Immunoglobulins, mg/ml	23.76±0.59	23.44±1.85
Glucose, mmol/l	2.42±0.1	2.64±0.27
Cholesterol, g/l	2.14±0.25	2.35±0.33
Urea, mmol/l	3.14±0.25	3.52±0.33
Creatinine, μmol/l	107.0±6.76	123.9±4.08*
Calcium, mmol/l	1.17±0.09	1.37±0.18
Carotene, mg %	0.51±0.01	0.58±0.05

Erythropoiesis is one of the important indicators of the hematopoietic organs functional state- and the trace elements exchange. The number of erythrocytes in the blood of the experimental group increased by 5.76 % relative to the control, and the hemoglobin level was by 9.6 % higher (P≤0.95). The leukocyte formula of both groups' animals corresponded to the limits of homeostasis. A slight increase in basophils in the blood of the cows of the experimental group was accompanied by a decrease in eosinophils. The difference in the number of leukocytes was improbable. An increase in total protein and creatinine (P≤0.95) was also noted, it may indicate an increase in protein metabolism in the body.

The results ofour research are in line with the data obtained by Gading (2020) and Cavallini (2020), they confirm that the use of mineral premixes with phytobiotics increased the growth indicators of calves and milk productivity of cows. No significant changes in the hematological parameters were noted.

Conclusion. The introduction of Biomix premix into the diet of dairy cows at the rate of 50 g per head per day made it possible to optimize the mineral and vitamin nutrition of animals and increase the productive feed effect.

The average daily yield of milk of the experimental cows fed the Biomix premix increased by 5.9 %, and the fat and protein content of the milk increased by 0.05 % and 0.07 %. The energy value of milk, the level of lactose, calcium and phosphorus increased. Feed consumption in terms of EFU per 1 kg of milk decreased by 5.1 %.

The system of control group cow homeostasis was in a state of equilibrium, i.e., hematological indicators remained at the level of the physiological norm. An increase in total protein (P≤0.95), hemoglobin (P≤0.95), creatinine (P≤0.95) was noted. There was a tendency to increase the level of erythrocytes, leukocytes, glucose, urea, and calcium in the blood.

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Вплив мінерально-вітамінного преміксу на молочну продуктивність та гематологічні показники корів

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Основним напрямом нормалізації мінерального та вітамінного живлення корів є застосування спеціалізованих преміксів, які у своєму складі містять всі необхідні елементи для ефективного збагачення раціону. Введення до раціонів тварин біологічно активних речовин у вигляді преміксів сприяє підвищенню ефективності використання кормів, у результаті чого зростає продуктивність.

Метою досліджень було вивчити вплив згодовування мінерально-вітамінного преміксу «Біомікс» на молочну продуктивність та гематологічні показники корів. Дослід проведено на двох групах корів української чорно-рябої молочної породи по 10 голів в кожній. Тварини дослідної групи у складі основного раціону отримували премікс «Біомікс» із розрахунку 50г на 1 голову за добу, а контрольної – рівноцінну кількість монокальційфосфату.

Введення до складу раціону дослідної групи преміксу «Біомікс» дало змогу ліквідувати дефіцит фосфору, купруму, цинку, кобальту, йоду, майже до норми довести рівень вітаміну $Д_3$.

Оптимізація мінерального та вітамінного живлення корів, шляхом використання преміксу Біомікс, забезпечила підвищення молочної продуктивності корів та якості молока. Середньодобовий надій корів дослідної групи протягом 90 днів основного періоду досліду зріс на 5,9 %. Вміст жиру та білку в молоці збільшились на 0,05 та 0,07 % відповідно. У перерахунку на 4-відсоткове молоко корови дослідної групи перевершували своїх аналогів з контрольної групи за валовим надоєм на 153,1 кг, та за середньодобовим – на 1,71 кг, що становить 7,4 % відносно контролю (P<0,05).

У молоці підвищився рівень сухої речовини, кальцію, фосфору, лактози, зросла його загальна енергетична цінність.

Витрати кормів на 1 кг молока за рівнем енергії та перетравного протеїну знизились на 5,1 та 5,4 %.

Згодовування преміксу «Біомікс» не мало негативного впливу на гематологічні зміни в організмі. Морфологічні та біохімічні показники крові корів відповідали фізіологічним нормам. Відмічено збільшення загального білка (P≤0,95), гемоглобіну (P≤0,95), креатиніну (P≤0,95). Спостерігалась тенденція до збільшення в крові рівня еритроцитів, глюкози, сечовини, кальцію.

Ключові слова: корови, премікс «Біомікс», вітаміни, мінеральні речовини, молоко, жир, білок, кров, еритроцити, гемоглобін.

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Nettle hay meal feeding and development of replacement pig stock

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The effect of different amount of nettle hay meal replacing concentrated feed on the development of replacement pig stock in terms of live weight and external measurements are studied. Indicators of growth patterns, nutrient consumption per 1 kg of pigs under test are cinsidered as well. Hematological indicators were researched. The main group of pigs was fed with the basic diet in the comparative and main periods of the experiment. (barley, wheat, pea and corn grits and sunflower meal). Nettle hay meal was added to the diet of the second group under test. It replaced 12.5 % of the protein of the above-mentioned diet. The third diet of pigs under test included 25 % nettle hay meal to replace the protein in concentrated feed. A mixture of micro- and macroelements was used as a mineral additive. The meal was given as dry form twice a day. Nettle hay meal was dried when nettle grass in the budding phase. Nettle hay was ground with a mill of 2 mm grid diameter. Long-term feeding with nettle hay meal in the amount between 12.5 % and 25 % of protein in the concentrated feed diet does not reduce the growth index of the pigs under test and does not increase the consumption of feed units, metabolism and protein per 1 kg of their growth compared to stock fed with concentrated feed rations. The growth indicator analysis demonstrates that the assessing stabable growth of piglets under test is higher than in the rest of groups under test. A similar tendency is observed in the growth tension index assessment. When growing the proportions of piglets' body structure change, it depends not only on changes in their live weight. So at 8 months of age the pigs that comsumped 12.5 and 25 % nettle hay meal had the highest linear growth rates compared to the other group under test. The results of hematological studies proved the replacement of concentrated fodder protein with nettle hay meal contributed to the increase in the erythrocyte amount, hemoglobin and total protein in the blood.

Key words: pigs, young stock, nettle, hay meal, concentrated feed, average daily, absolute, relative growth, mineral elements, feed consumption, formation intensity, stress index, hematological indicators.

Problem statement and analysis of recent research. Pigs are a kind of dangerous for people as they consumpt grain and its products. The grain on the food market is constantly increasing. Therefore, the researches on the maximum using of cheap non-grain feed for pigs is of great importance [6, 11, 14]. Scientists: P.S. Avramenko, I.G. Brushinin, V.G. Golubev, V.I. Yaremenko have made a significant contribution to the study of issues of feeding pigs with minimal grain consumption [1, 5, 7, 18]. A valuable non-cereal component in pig diets can be nettle, which takes the leading place among green fodder because of its nutritional qualities and beneficial effects on the body.

There are 10 types of nettles growing in Ukraine. It grows everywhere in darkened places or ravines, around houses, on the banks of reservoirs, on wastelands. Urticadioica, a perennial root plant with a four-sided hollow stem 50–150 cm high, densely covered with a kind stinging fur, has the greatest fodder value. The leaves of dicotyledonous nettle are egg-shaped, heart-shaped,

and coarsely toothed. Its leaves and shoots contain 140–300 mcg of carotene, 1000–2000 mcg of carotene, 1000–2000 mcg of vitamin C, 20–20 mcg of vitamin B2, 24–25 mcg of vitamin K per 1 kg of dry matter. Nettle significantly exceeded many other and wild plants in terms of content mineral substances. So, there is three times more iron and manganese and five times more copper and zinc in nettle than in alfalfa. In addition, it contains tannins, organic acids and a number of other biologically active substances. However, a restraining factor for pig feeding is the high rate of fiber and some biologically active substances [17]. Dried nettle is a good fodder and is eaten with appetite by every farm animal. If mowed before flowering and thoroughly dried, nettles turn into good hay, containing as much protein as alfalfa or clover. The plant accumulates iron, calcium, magnesium, zinc, iodine, selenium, cobalt, water and fat-soluble vitamins thanks to its medicinal properties [29]. Research by Nepali scientists has proved the beneficial effect of annual productivity in pig farming, grain crops are less and grow in excess, which would otherwise be lost throughout the hilly region of the country. In spite of the fact that there is no proves in gemativ and antiparasitic effects, the nettle obtains non-specific to the mammals. So the pigs under test appeared to be very healthy despite of the low energy diet and low dry matter content compared to other groups [16, 19, 20, 22]. Nettle is a good source of vitamins and minerals. It regulates the digestive system and stimulates appetite, it makes a positive effect on immune system and has some antibacterial activity. Nettle improves biochemical, hematological and immunological indicators of rabbits [19]. Nettle powder can replace the antibiotic in pig feed, as its amount is 3.0–7.5 % of the total weight of the feed [27].

Good results obtained when feeding rabbit with nettle hay meal provokes some experiments to test its effect on pigs [10, 16, 20]. The efficiency decrease in pig farming in many complexes has been caused by the low pig productivity, the slow growth of young stock and a death significant rate. This has been caused by poor-quality feed with insufficient content of vital vitamins, mineral elements, other biologically active substances and their absence. Mineral elements are the structural material of the animal body, they participate in the digestion of feed nutrients, absorption, synthesis, decay and excretion of metabolic products from the body. They create conditions for normal function of vitamins, enzymes, hormones, stabilize acid-alkaline balance and osmotic pressure [2, 4, 8, 15]. Grain feed satisfies only 50–85 % of mineral element need. Their lack is compensated by mineral additives, hay meal as part of compound feed, feed mixtures. The use of hay meal is one of the increasing productivity elements for pigs. Its composition includes more than 40 different micro-ingredients, namely: trace elements, vitamins, amino acids and other substances. Recently many countries there the pig farming is developed have been conducting research on revising and clarifying the rules for introducing hay meal out of legumes, cereals, and other types of plants (nettle). Their inpact as thesources of energy, protein, macro- and microelements, sorbents of toxins, substances that contribute to the removal of heavy metals from the body, and their therapeutic effect on diarrhea in pigs have not yet been fully studies [9, 12, 13, 19].

The lack of nutrients in pig's diet effects negative not only on their productivity, but on the process of maturation of cells responsible for immune reactions as well. Deficiency of protein, amino acids, vitamins, macro- and microelements in rations adversely affects the productivity and reproductive functions of pigs. Their sources are grain, animal feed, waste from various industries, hay meal [13, 21, 24]. Scientists [23, 25, 26, 28] believe that one of the reasons for the unsatisfactory state of pig farming is the lack of high-quality fodder, a deficiency in the rations of protein and biologically active substances, including vitamin and mineral supplements. This stimulates the search for additional feed additives.

The aim of the research is to analyse the effect of feeding different doses of nettle hay meal replacing concentrated feed on the growth and development of young pig stock in terms of live weight and external measurements, parameters for evaluating the patterns of growth of piglets, consumption of nutrients per 1 kg of experimental animals, hematological indicators.

Material and methods of research. The scientific and economic experiment has been conducted on of the Large White pigs. For the experiment, according to the principle of analogues, 54 piglets of 5–6 months of age were selected, one main and two groups under test (10 sows and 8 piglets in each group) were formed. The experiment lasted 146 days. During this period, piglets of the main group were kept on diets containing barley, wheat, pea and corn grits and sunflower meal. In the diet of the second group under test, hay mealcontained nettle, replacing 12.5 % of the protein of the above-mentioned feeds. The diet of the pigs of the third group included 25 % nettle hay meal protein instead of concentrated feed. A mixture of micro- and macroelements was used as a mineral supplement. Feed was given in the form of dry feed twice a day. Nettle hay meal was pre-

pared from dried nettle grass in the budding phase. Nettle hay was ground with a special mill of grid diameter of 2 mm.

Intensive growth and development of the pigs under test was calculated in absolute values of weight gain and linear indicators. For this, the average daily increase in live weight was determined according to the generally accepted formula.

The absolute increase in live weight was calculated according to the formula:

$$A = W_2 - W_1$$

A is the absolute increase in the live weight, kg;
W1, W2 - live weight, at the beginning and at the end of the period, kg.

Average daily growth was determined by the formula:

$$СП = \frac{A}{t}$$

SP is the average daily increase, g
t – period between two weighings, days.

To select the criteria for evaluating the patterns of growth of pigs in early ontogeny, the following indicators were determined:

– relative growth according to the formula:

$$ВП = \frac{(W_2 - W_1)}{W_1} \times 100$$

W1 is the live weight of animals at the beginning of the period;
W2 – live weight of animals at the end of the period.

– the intensity of the formation of animals (Δt) was determined by the formula:

$$\Delta t = \frac{W_6 - W_4}{0{,}5 \times (W_6 + W_4)} - \frac{W_8 - W_6}{0{,}5 + (W_8 + W_6)}$$

W4, W6, W8 are the live weight of pigs at 4, 6, and 8 months of age.

Indices of stress (In) and uniformity of growth according to V.P. Kovalenko's method:

$$In = \frac{At}{ВП} \times СП$$

where СП is the average daily increase, g
ВП – relative growth, %

$$Ip = \frac{1}{1 + \Delta t} \times СП$$

Hematological studies were carrieed out with 5 animals from each group. Blood from pigs under test was taken from the lateral branch of the auricular veins by puncturing it. The following blood parameters were studied: the number of erythrocytes according to the generally accepted method, by counting in the Goryaev chamber; hemoglobin content – according to the generally accepted method with the help of Sali'shemometer; the content of total protein in blood serum – by the refractometric method.

Calculation of feed costs per 1 kg of live weight gain (3k) in feed units according to the formula:

$$З_к = \frac{К_к}{П}$$

Зк – feed consumption per 1 kg of live weight gain, feed units;
$К_к$ – the amount of fodder fed during the accounting period, fodder units;
П – gross increase in live weight, kg.

Biometric processing of the results was carried out by generally accepted methods of variational statistics using the program MS Excel 2010

Results and discussion. Feeds that were available on the farm were included in the rations: barley, wheat, pea, corn grits, sunflower meal, nettle hay meal in accordance with the experiment scheme, and mineral supplements. The replacement of concentrated feed protein with nettle hay meal protein in the diets of the experimental groups was excluded. of them protein-rich feed: sunflower meal and pea grits, and also changed the ratio of other components.

The rations for the groups under test were calculated to obtain average daily gains of 450–600 g. During the main period of the experiment, the pigs of the first and second experiments used four doses of feed, composed, respectively, of live weight (40, 60, 80 and 100–120 kg).

The nettle hay meal in the diets of the pigs of the second experimental group, depending on their age and weight, was 0.120–0.240 kg. The share of nettle protein from the total amount of it in the diet was 12.5 %. From 0.240 to 0.410 kg of nettle hay meal (25 % protein) was added to the rations of the pigs of the third group. The introduction of nettle hay meal into the rations of pigs reduced the need for additional doses of mineral fertilizers, especially calcium and manganese.

For 1 kg of gain, pigs of the main group of the first spent 6.46 feed units, 4.72 dry matter, 69.2 MJ of exchangeable energy, 0.60 kg of digestible protein. The indicated parameters of the pigs of the groups under test differed little from the indicated parameters of the pigs of the control group (Table 1).

He–pigs of the main group increased their weight by 72.9 kg in 146 days, and pigs by 72.8 kg, their average daily weight gain was 499 and 497 g (Table 1). The introduction of 12.5 % nettle hay

meal protein into the diet of experimental pigs (the second research group) did not negatively affect their weight gain. The total weight gain of piglets was 72.1 kg, and gilts – 72.5 kg, the average daily weight gain was 493 and 496 grams, in parallel. When the amount of nettle hay meal in the rations of pigs (the third group) was increased to 25 % by protein, a decrease in growth indicators was not observed.

During the experiment, it was established that he-pigs and she-pigs had different growth rates depending on the period of the experiment (Table 2).

At the beginning of the experiment, at the age of 5 months, the animals of all groups had almost the same live weight, but at the age of 6 months he-pigs and she-pigs of the main group exceeded the counterparts of the main group by 1.3 kg according to this indicator (P>0.99). Subsequently, the process of equalizing the live weight of young stock was stated, and at the age of 9 months, the pigs of the 2nd group under test weighed 131.4 kg, which is 2.3 kg more (P>0.99) compared to the main group (129.1 kg) (Table 3).

Table 1 – **Nutrient Consumption per 1 kg of growth of the pigs under test**

Measures	Groups under test		
	main	1 tested group	2 tested group
Fodder unit	6,46	6,53	6,68
Dry matter, kg	4,72	4,81	5,14
Matabiloc energy, MJ	69,2	71,3	73,8
Protein, kg	0,78	0,79	0,82
Soddenprotein, kg	0,60	0,61	0,63

Table 2 – **Weight increase** $\overline{X} \pm S_{\overline{X}}$ (n=18)

Measures	Groups under test		
	main	1 tested group	2 tested group
he-pig, n=9			
Starting life weight, kg	57,7±0,84	57,8±0,93	57,7±0,81
Result life weight, kg	130,6±3,20	129,9±3,21	129,1±3,23
Body weight increase, kg	72,9±2,16	72,1±2,78	71,4±2,42
Daily avarege body weight increase, g	499±12,4	493±13,3	489±17,5
she-pig, n=9			
Starting life weight, kg	56,4±1,29	56,2±1,21	56,6±1,18
Result life weight, kg	129,0±4,84	128,7±4,42	130,1±7,03
Body weight increase, kg	72,6±3,13	72,5±3,13	73,5±2,92
Daily avarege body weight increase, g	497±24,7	496±21,4	503±17,3

Table 3 – **Life weight of he- and she- pigs,kg** $\overline{X} \pm S_{\overline{X}}$ (n=18)

Age (months)	Groupd under test		
	main	1 tested group	2 tested group
he-pigs			
5	57,7±0,64	57,8±0,73	57,7±0,71
6	69,9±0,73	72,2**±0,74	71,4±0,64
7	84,1±0,64	85,6±0,44	85,1±0,63
8	109,3±0,88	110,4±0,77	109,1±0,58
9	130,6±3,10	129,9±3,31	129,1±3,49
she-pigs			
5	56,4±1,39	56,2±1,31	56,6±1,28
6	71,2±2,13	72,5**±1,25	71,6±1,64
7	84,9±3,14	85,8±2,21	85,9±3,18
8	106,1±1,68	107,3±2,17	106,8±1,69
9	129,1±4,64	128,7±4,24	131,4**±6,05

Note: **(P<0,01).

The of growth pattern assessment of pigs based on average daily growth, relative growth, formation intensity, growth stress index, growth uniformity is presented in Table 4. The index of the formation of animals was greater in main group by 0.142 than for pigs of group 2 and by 0.088 in parallel with main group. The growth stress index of young stock in the main and 1–2 groups under test was: 1.018; 0.697; 0.499. The growth uniformity index of gilts for group 2 was 0.427, and the main group was 0.368. There was almost no difference in the growth of group 1 gilts (0.369) compared to the main group.

The index of the pigs' formation was greater in main group by 0.137 than in piglets of group 2 and by 0.089 in parallel of experimental group 1. The growth stress index of young stock in the main and 1–2 groups under test, respectively, was: 1.028; 0.699; 0.497. The growth index of piglets in group 2 was 0.423, and the main group was 0.367. There was almost no difference in the uniformity of growth of piglets of experimental group 1 (0.368) compared to the main group.

While growing the proportions of pigs' body structure change depending not only on changes in live weight. Characteristics of the growth and development of young animals based on linear body measurements are more complete and accurate. Linear growth is determined by measuring animals. In the process of rearing repair pigs with different doses of nettle feeding, some difference in changes in the linear diameters of the animals was established (Table 5).

It was proved 6 month old animals of groups 1 and 2 were characterized by higher indicators of body length. At this age, it was 111.6 and 111.4 cm in parallel. This is 1.9–1.7 cm more than that of the piglets of the main group. By breast girth 6 month old pigs of the main group prevailed over peers of the 2 other group by 11.4 cm (P>0.999). Some difference in height at the shoulder was established. According to these indicators, the animals of the main group were inferior to the analogues of the 1st experimental group by 0.9 cm and the 2nd by 1.3 cm. When 8–month old the piglets with 12.5 % and 25 % nettles hay meal had the highest indicators of linear growth, in comparison with the main group.

Productivity of pigs depends on the amount of total protein in blood serum: with an increase in the level of productivity, the amount of protein also increases. The hematological parameters of the pigs under test are shown in the Table 6.

The results of hematological studies have proved that replacing the protein of concentrated feeds with nettle hay meal (12.5 % and 25 % by protein) contributed to the increasing rate in the blood: the content of erythrocytes by 1.30 and 1.42 mg % (P>0.999) and the hemoglobin content, respectively, by 1.37 and 1.49 g % (P>0.999), total protein content by 0.25 and 0.47 g % (P>0.999).

Table 4 – **Indicators of evaluation of patterns of growth of piglets**

Measures	Groups under test		
	main	1 tested group	2 tested group
Avarige per day, g	497	496	503
Relative growth, %	129,4	129,1	132,4
Intensivity (Δt)	0,283	0,194	0,146
Growth power index (In)	1,028	0,699	0,497
Growth Constancy (Ip)	0,367	0,368	0,423

Table 5 – **Age-related changes in linear diameters of pigs under test**,см $X \pm S_x$ (n=9)

Group	Body length	Chest girth	Height at the shoulder
6 month old pigs			
Main	109,8±0,09	110,3±0,014***	59,9±0,07
1 group under test	111,7±0,06	112,1±0,08***	60,4±0,06
2 group under test	111,5±0,07	98,9±0,08***	60,3±0,05
8 month old pigs			
Main	117,9±0,06	119,4±0,07***	65,5±0,03
1 group under test	119,4±0,07	124,9±0,08***	66,4±0,06
2 group under test	118,3±0,08	121,8±0,07***	66,7±0,04

Note: ***P>0,999.

Table 6 – **Hematological rates of pigs under test** $X \pm S_x$(n=5)

Measures	Groups under test		
	main	1 tested group	2 tested group
Erythrocytes, mg %	4,05±0,03	5,35±0,009***	5.47±0,008***
Hemoglobin, г %	10,47±0,03	11,84±0,08***	11,96±0,06***
Notal protein, г %	10,58±0,04	10,93±0,03***	10,95±0,06***

Note:***P>0,999.

Wide use of nettle hay meal in pig diet is recommended by Ukrainian and foreign scientists [1, 5, 7, 18, 23, 27, 28]. Nettle is a fairly high-energy feed. The nutritional value of nettle herbage ranges from 0.18 to 0.22 k.o., and the digestible protein content is from 24 to 29 g per 1 kg and depends on the vegetation phase and dry matter content. In nettle hay meal, the concentration of the indicated indicators increases 3–4 times [17, 22, 26]. As a result of the conducted research, it is possible to speak with high reliability about the expediency of using nettle in feeding pigs as a valuable non-cereal component, which due to its nutritional qualities and favorable influence on the body occupies one of the first places among plants. age of nettle hay meal in a scientific and economic experiment that lasted 146 days, the live weight of animals at the beginning of the experiment was: 57.7–57.8 kg in boars and 56.2–56.6 in gilts. At the end of the research, the increase in the live weight of he-pigs was 130.6 kg in the main group and in the 2nd group under test was 129.9, where the level of protein replacement of concentrated feed with hay meal increased to 12.5 %, and in the 3rd group to 129.1 kg, where the level of protein replacement of concentrated feed with hay meal was 25 %. At the same time, the dose of mineral feeding was reduced to balance the diet. Live weight of pigs in the main group at the beginning of the research was 56.4 kg, at the end – 129.1 kg, and in the second group – 56.2 kg. After replacing protein concentrate feed with nettle protein, their live weight increased to 128.7 kg, and when replacing 25 % of protein in the 3rd group from 56.6 kg to 131.4 kg, which exceeded the results of the second group and the main group. At the same time, the consumption of nutrients was as follows: c.o.d. – 6.46 main group, 6.53 – 2 group and 6.68 – 3 group. Dry matter: 4.72 kg – main group, 4.81 kg – 2 group under test and 5.14 – 3 group. Protein 0.78 kg – main group, 0.79 – 2 group and 0.82 kg – 3 group. The following indicators were obtained for digestible protein: in the main group, the consumption was 0.60 kg, in the 2nd group – 0.61 kg, and in the 3rd group – 0.63 kg. Therefore, the introduction of nettle hay meal into the diets of repair pigs in the amount of 12.5 up to 25 % protein, replacing it with cereal protein, does not affect the indicators of feed payment and the consumption of basic nutrients. According to our data, the average daily gain of piglets of the main group was 6g less than that of the analogues of the second group and 1g more compared to animals the main group. The relative increase in young animals of the second group reached 132.4 %, and in the main and first groups: 129.4 and 129.1 %, respectively. The index of animal formation was greater in the sows of the main group than in the counterparts of the first and second groups under test. The uniformity of the growth of piglets of the main group was almost not different compared to the other groups. The characteristics of the growth and development of young stock according to the data of linear body diameters are more complete and accurate. In the process of raising pigs with different doses of nettle feeding, some difference in the linear measurements of the animals was established. We found that at the age of 6 months, the animals of the first and second experimental groups were characterized by higher body length indicators, and in terms of breast girth, at the specified age, the animals of the main group exceeded the peers of the second experimental group by 11.4 cm (P>0.999). A certain difference in height at the shoulders was established: according to this indicator, the animals of the main group were inferior to the analogues of the group 1 to 0.9 cm and the group 2 to 1.3 cm. By the end of 8 months, piglets that received 12.5 % and 25 % nettle hay meal by protein, had the highest linear growth rates compared to the control group. Thus, the obtained data testify to the positive effect of protein replacement of concentrated feeds with nettle protein on age-related changes in the exterior of pigs, hematological indicators and coincide with the results of research by Ukrainian and foreign scientists [11, 13, 15, 18, 19, 25, 27, 29].

Conclusion. Nettle hay can be a highly nutritious feed not only for poultry, but also for pigs. In terms of protein and essential amino acids in the dry matter, nettle is not worse to such widely used forages in pig feeding as leguminous meal and

sunflower meal, it is rich in minerals and vitamins. Long-term feeding of hay meal to pigs with nettles in the amount of 12.5 % and 25 % of the protein in the diet instead of concentrated feed does not reduce the intensity of their growth. Raising pigs on rations with nettle hay meal does not lead to an increase in the consumption of feed units, exchangeable energy and protein per 1 kg of their growth compared to animals kept on concentrated rations. The analysis of indicators of of the regular growth assessment of piglets shows that the index of formation of animals is higher amoung the young stock of the main group than in parallel of the experimental groups. A similar terndency is observed in the assessment of the growth tension index. During the growth of pigs, the proportions of their body structure change, which depend not only on the change in live weight, so at 8 month old pigs receiving 12.5 % and 25 % nettle hay meal had the highest linear growth rates compared to the main group. The results of hematological studies showed that the replacement of concentrated feed protein with nettle hay meal increased the erythrocytes, hemoglobin and total protein rates in the blood.

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Вплив згодовування сінного борошна кропиви на ріст і розвиток ремонтного молодняку свиней

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Досліджено вплив згодовування різних доз сінного борошна кропиви при заміні ним концентрованих кормів на ріст та розвиток молодняку свиней за живою масою та екстер'єрними промірами, показниками оцінки закономірностей росту свинок, витратами поживних речовин на 1кг піддослідних тварин, гематологічними показниками. Контрольна група свиней у зрівняльний і основний періоди досліду отримувала основний раціон (ОР: ячмінну, пшеничну, горохову та кукурудзяну дерть і соняшниковий шрот). До складу раціону другої дослідної групи вводили сінне борошно кропиви шляхом заміни 12,5 % протеїну зазначених вище кормів. До раціону піддослідних свиней третьої (дослідної) групи включали 25 % за протеїном сінного борошна кропиви замість концентрованих кормів. Як мінеральну добавку використовували суміш мікро-і макроелементів. Корми задавалися у вигляді сухого корму два рази на добу. Сінне борошно кропиви готували із висушеної трави кропиви, скошеної у фазі бутонізації. Сіно із кропиви подрібнювали за допомогою спеціального млина з діаметром решіток 2 мм. Тривале згодовування свиням сінного борошна кропиви в кількості 12,5 та 25 % протеїну раціону замість концентрованих кормів не знижує інтенсивності їх росту, не призводить до підвищення витрат кормових одиниць, обмінної енергії та протеїну на 1 кг їх приросту порівняно з тваринами, що утримувались на концентрованих раціонах. Аналіз показників закономірності росту свинок свідчить, що індекс інтенсивності формування тварин є вищим у молодняку контрольної групи, ніж у аналогів дослідних груп. Аналогічна тенденція спостерігається за оцінкою індексу напруги росту. У процесі росту свиней змінюються пропорції будови їх тіла, які залежать не тільки від зміни живої маси. Так, у 8-місячному віці свинки, що отримували 12,5 та 25 % сінного борошна кропиви мали найвищі показники лінійного росту у порівнянні з контрольною групою. Результати гематологічних досліджень показали, що заміна протеїну концентрованих кормів сінним борошном кропиви сприяла збільшенню в крові вмісту еритроцитів, гемоглобіну та загального білка.

Ключові слова: свині, молодняк, кропива, сінне борошно, концентровані корми, середньодобовий, абсолютний, відносний приріст, мінеральні елементи, витрати кормів, інтенсивність формування, індекс напруги, гематологічні показники.

БІОТЕХНОЛОГІЇ ТА БІОІНЖЕНЕРІЯ

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Use of agricultural production waste in relation to bio nano technology for the synthesis of functionalized selenium nanoparticles

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Бітюцький В.С., Цехмістренко С.І., Демченко О.А., Цехмістренко О.С., Мельниченко О.М., Олешко О.А., Мельниченко Ю.О. Використання відходів сільськогосподарського виробництва у біонанотехнології синтезу функціоналізованих наночастинок селену. Збірник наукових праць «Технологія виробництва і переробки продукції тваринництва», 2022. № 2. С. 42–50.

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The application of nanotechnological innovations to increase the effectiveness of the bioflavonoid quercetin is considered. The ability to functionalize with specific ligands that target specific organs or cells is very important because it is possible to increase the concentration of quercetin at the desired target level while reducing side effects. Quercetin, as a flavonoid antioxidant, is widely used to reduce oxidative stress and activate important signalling pathways in cells. However, poor solubility in water, intensive first-pass metabolism limits its use. A strategy for the development of nanocomposites is presented, which involves combining quercetin obtained from agricultural waste with selenium nanoparticles. In vitro results demonstrated that quercetin-nanoselenium has high water solubility compared to individual flavonoids. It has been proven that quercetin-nano-selenium nanoparticles are capable of reducing 1,1-dipheny l-2-picrylhydrazyl (DPPH) radicals and are characterized by high antioxidant activity. The ability to functionalize nanoparticles with specific ligands that target the modulation of specific signaling pathways (Keap1/Nrf2/ARE, Nf-kB, mTOR) in individual cells is very important, as it allows increasing the concentration of quercetin to the desired level while reducing side effects.

Considering the beneficial effects of quercetin, a strategy has been developed that involves the use of selenium nanoparticles to deliver quercetin in a specific and controlled manner. Research in this direction is promising, as it will contribute to clarifying the expediency of using such nanosystems, which have not yet been widely used. The use of onion waste and its extracts in biotechnology, biomedicine, pharmaceuticals and the agro-industrial sector can be an optimal solution for reducing environmental damage. In addition, it will provide an economically determined alternative for the production of feed additives for animals and poultry. Compounds of quercetin with nanoselenium should take a worthy place in clinical medical practice, biology and agriculture. The beneficial effects of quercetin can be further enhanced with the help of nanotechnology, which will contribute to the effective use of this compound with such great biological potential.

Key words: nanobiotechnologies, waste, onion peel, 1,1-dipheny l-2-picrylhydrazyl, antiradical activity.

Introduction. Bionanotechnology emerged as an integration of biotechnology and nanotechnology for the development of biosynthetic and environmentally safe technologies for the synthesis of nanomaterials, and "green" synthesis and surface modification of nanoparticles are crucial in this field [34]. Nanoparticles (NPs), synthesized using plant extracts or phytocomponents, are of great importance in the development of various therapeutic and diagnostic tools [30]. Biogenic, "green" synthesis of metal nanoparticles, metal oxides and metalloids with the participation of plants and microorganisms combines biological principles with biochemical approaches to obtain nano-sized functional particles with the necessary properties. Environmental cleanliness of the production of nanostructures, the use of which is growing at a significant rate, is an urgent problem today. The advantages of further synthesis by biological means, i.e. using microorganisms (yeast, fungi, bacteria) and plant parts (stem, root, leaves, bark, flowers, etc) include non-toxicity, easy reproducibility and economic efficiency [5, 40, 41].

Various strategies and biomodifiers are used for surface modifications/functionalization of these nanomaterials, in particular with the participation of flavonoids. Flavonoids are one of the most widespread classes of compounds found in vegetables and fruits. Flavonoids are divided into classes, and although structurally unique, they share a common core structure formed by three rings known as the flavan core. The position of the hydroxyl group (–OH) in one of the rings determines the mechanisms of action of flavonoids and reveals complex multifunctional activity. Flavonoids are widely used as antioxidants, analgesics, and anti-inflammatory agents, showing safe preclinical and clinical profiles [16]. Flavonoids are divided into classes: flavones (flavone, apigenin, and luteolin), flavonols (quercetin, kaempferol, myricetin, and fisetin), and flavanones (flavanone, hesperetin, and naringenin).

Quercetin (Q) (3,3',4',5,7-pentahydroxyflavone) is a flavonol found in various types of fruits, vegetables, and plants, including: berries, apples, tomatoes, cocoa, onions, and certain medicinal plants [17, 27].

The properties of quercetin as a dual-acting antioxidant are due to its ability to act as a direct antioxidant, removing active forms of oxygen and nitrogen. These highly reactive ions are formed during metabolic processes or come from exogenous sources such as pollution, radiation, etc In addition, quercetin may act as an indirect antioxidant by activating the Nrf2 signaling pathway and the transcription of antioxidant enzymes.

Quercetin is an antioxidant flavonoid that exhibits anti-inflammatory activity due to the inhibition of pro-inflammatory cytokines [18], oxidative stress [6, 7, 9, 42], NLRP3 inflammasome activity in macrophages [13], activation of p65 NF-κB [19, 26], MAP kinase signaling in macrophages [26], p50 NF-κB activation in primary human keratinocytes [44], and TNF-α, IL-1β, and IL-6 production in LPS-stimulated cells [10]. In addition to the inhibition of the above-mentioned pro-inflammatory signaling pathways, part of this effect is carried out due to the activation of the Nrf2/ HO-1 pathway [7, 19]. Quercetin can activate the AMPK pathway to modulate mTOR in a dose-dependent manner and contribute to the reduction of nuclear translocation of NF-κB to inhibit pro-inflammatory processes [8].

However, poor water solubility and intensive first-pass metabolism limit the clinical use of quercetin. It was found that selenium nanoparticles conjugated with quercetin inhibit the aggregation of amyloid-β structures, which are a sign of Alzheimer's disease, and exhibit antioxidant properties. Such nanocomposite carriers significantly increase water solubility and bioavailability of quercetin (QNPSe) [37].

Biological synthesis is one of the most environmentally friendly methods of synthesis of nanostructures. Moreover, if the synthesis is carried out with the help of waste, in particular plants, then the synthesis process not only becomes green, but also environmentally friendly. Onion husks are one of these vegetable wastes. In the case of onion processing at the domestic or industrial level, a large amount of waste is generated, including unfit for food: the upper and lower parts, the outer skin and two outer layers [12]. It is reported that among all producers, only European countries can generate almost 0.6 million tons of onion waste annually [23]. In addition, these wastes can have a harmful effect on the environment if not properly disposed of, as they are not suitable for making feed, nor can they be used as fertilizer [2]. Therefore, onion waste remains underutilized even after establishing the fact that it is a rich source of bioactive compounds such as phenols, flavonoids, and flavanols. Thus, increasing the value of onion waste and its extracts in the field of biotechnology, biomedicine, pharmaceuticals and the agro-industrial sector can be an optimal solution to reduce environmental damage and provide an economical, inexpensive alternative for the production of medical supplements or drugs [24].

Onion husks are considered to be waste, which is detected 20 times more quercetin and glycosides of quercetin than the edible part [25]. It is believed that onions have numerous phytochemicals, flavo-

noids and enzymes that help in the synthesis of nanoparticles [24].

Despite the differences in varieties or extraction methods, the main phenolic compound identified in onions is quercetin. The identified compounds included quercetin 4'-o-glucoside and quercetin, as well as other quercetin glycosides, cyanidine 3-O-glucoside and a number of degradation products and oxidation of quercetin [24].

Quercetin has a protective effect on cancer and cardiovascular diseases, chronic inflammation, oxidative stress and neurodegenerative pathology due to its anti-radical and anti-inflammatory properties, however, its poor bioavailability inhibits the potential beneficial effects of this flavonoid. In this sense, different types of nanosis have been developed to improve quercetin solubility, as well as to develop fabric-specific delivery systems. These studies allow you to improve the bioavailability of quercetin and increase its concentration in the right places. Thus, quercetin can become a promising compound if you use nanotechnology as a tool for improving its therapeutic efficacy [33].

Instead of using commercially affordable but expensive quercetin, as a stopping agent for the synthesis of gold nanoparticles, researchers [38] used onion husks as a cheap quercetin source, which can act as a strong reducing agent, as well as a calculating agent, and its synergistic. In this work, giving priority to the rational use of natural resources, all the crude onion extract was cleaned up to four fractions, namely: ethyl acetate (EA), butanol, methanol and water [31]. Unlike the use of organic extractants, studies [1] used aqueous onion husk extract in case of green synthesis of silver nanoparticles. Among the various nanostructures of selenium nanoparticles are characterized by unique properties, such as high catalytic and biological activity, and biogenic selenium nanoparticles obtained by "green" chemistry methods, affect the redox-sensitive Keap1/NRF2/are different stressful effects by activating transcription and synthesis of a number of antioxidant and detoximizing enzymes [4]. Quercetin has been proven to counteract neuro-steaming by activating NRF2/HO-1 and inhibiting the transmission of NF-KB signals [39]. Thus, the functionalization of selenium nanoparticles can contribute to the effects of nanocomposition on transcription factors NRF2 and NF-KB, key pathways that regulate the subtle balance of cellular oxidative status and response to stress and inflammation. Our studies are aimed at synthesis of nanobioconjugates selenium with quercetin for the use of water extract of onion husk, which is a waste of agro-industrial production.

The aim of the research is studying the process of synthesis of functionalized flavonoids of selenium nanoparticles for the use of water extract of onion husk, research of radical-absorbent activity of nanobioconjiangists of selenium.

Material and methods of research. Onion (Allium cepa L), widely known as common onion, belongs to the Amaryllidaceae family. Onion husks were used as a source of flavonoids for the preparation of functionalized selenium nanoparticles. The onion husks were washed several times with distilled water to remove dust and dirt on it, and dried in the air. The dried vegetable raw material was crushed and sifted through a sieve and placed in the extractor. The extraction was carried out in the ratio of raw materials: extractant - 1:25 at extraction temperatures 90 °C for 15 minutes. Water was purified as extractants. In the event of extraction, the extracts were filtered through filter paper. For the preparation of selenium nanoparticles as a stabilizing factor and the reducing agent used fresh extract made from onion husk. 50 ml of filtrate was taken for synthesis of nanoparticles (NPs). Selenium nanoparticles (SeNPs) were performed as follows: 200 ml of 10 mm of sodium selenite (Na_2SeO_3) were dissolved by the precursor in distilled water and added drops of onion husk extract with constant mixing to change the color of the reaction mixture. The solution was left in mixing conditions for 3 hours after the incubation time confirmed the synthesis of the SeNPs. UV- and visible spectra of functionalized selenium nanoparticles were recorded in various chronological intervals (1 hour, 2, 3 hours). Antioxidant (radical-absorbent) activity of synthesized nanoselenium compounds was determined by the method based on the ability of antioxidant molecules of the test substance to restore free radical radicals 2.2-dipheny l-1-1-pyxilhydrazil (DPPH). DPPH is a stable free radical, the solution of which is purple. The DPPH solution (0.4 mm in 80 vol. The absorption was measured at 517 nm using a spectrophotometer [46].

Results and discussion. Selenium nanoparticles obtained from onion husks, which were discarded as biowaste in landfills, were used as the object for this study.

The reduction of sodium selenite to zero-valent selenium was monitored using a UV-visible spectrophotometer, and the presence of a strong surface plasmon resonance, a characteristic phenomenon exhibited by selenium nanoparticles, confirmed the presence of nanoselenium in the medium.

Visual coloring is the first stage of research into the process of selenium nanoparticle formation. At the first stage of the reaction, the color of

the mixture was yellow, which gradually changed to red-brown over time (Fig.1). After 3 hours of incubation, no further color changes were observed. The appearance of a red-brown color is caused by the excitation of plasma resonance surface oscillations by selenium nanoparticles, which provides spectroscopic evidence of their formation. Analysis of the spectrum showed an absorption peak at λ=370 nm, which confirms the formation of selenium nanoparticles.

The acquired color can also reveal the morphology of the nanoparticles, as red solutions tend to be monocyclic or amorphous (spherical), while dark and black solutions tend to be triangular, linear, or rod shaped [14, 22]. The height of the absorption peak increased with time, but no further increase in its intensity was observed after 3 hours of reaction, which indicates the maximum transformation of SeO_3^{2-} into Se^0. (Fig. 2). As a result of the conducted research, a pronounced radical-absorbing effect of nanoselenium conjugates with phenolic compounds from aqueous extracts of the husk was established, compared to the standard – a quercetin solution. Quercetin showed its properties as a reducing agent by 72,4 %, and nanoselenium conjugates reduced DPPH radicals by DPPH on 86,7 %.

DPPH is a compound that contains a nitrogen radical in the centre and has a deep purple colour in ethanol solution. It was established that in the presence of conjugated selenium nanoparticles with flavonoids (QSeNPs) from onion peel, the color of the DPPH solution gradually changes from intense purple to light yellow, which corresponds to the transition of DPPH into a non-radical form (Fig. 3). The evolution of the reaction was observed by analysing the UV-visible spectroscopy at the absorption peak at λ=517 nm (characteristic UV-visible peak of DPPH) in the presence of the QSeNPs catalyst.

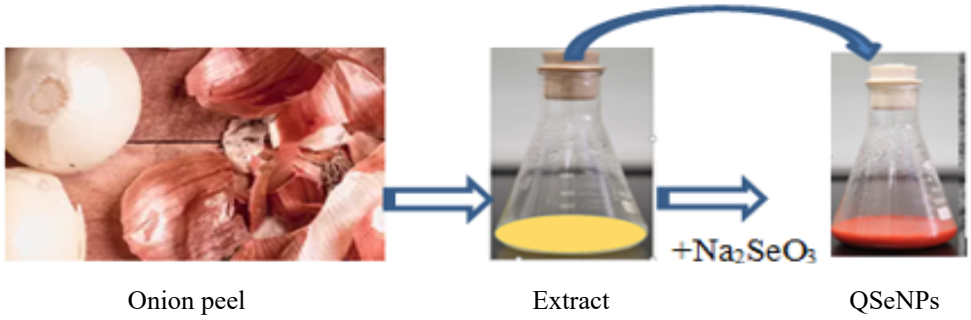


Fig.1. **Synthesis of nanobioconjugates of selenium and quercetin using aqueous onion peel extract.**

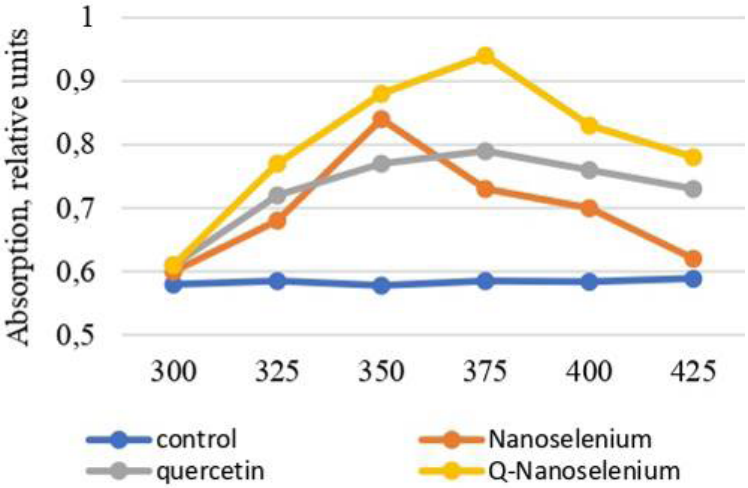
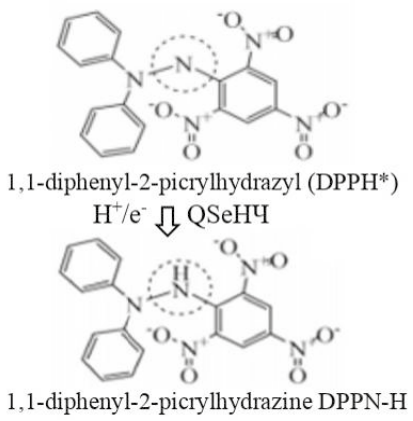


Fig. 2. **Absorption spectra of selenium nanoparticles and conjugates of nanoselenium with quercetin (QSeNPs).**

Fig. 3. **Conversion of 1,1-diphenyl-2-picrylhydrazyl (DPPH*) to 1,1-diphenyl-2-picrylhydrazine DPPN-H by quercetin-nanoselenium (QSeNP) conjugates and color change from purple to light yellow.**

The colour change occurs due to the conversion of 1,1-diphenyl-2-picrylhydrazyl (DPPH*) into 1,1-diphenyl-2-picrylhydrazine DPPN-H by quercetin-nanoselenium conjugates (QSeNPs). The reaction proceeds according to the scheme:



Thus, the obtained selenium nanoconjugates show high anti-radical activity against DPPH* due to their hydrogen/electron transfer ability.

Numerous studies, conducted mainly in vitro, show that flavonoids can be classified as non-enzymatic antioxidants capable of directly or indirectly attenuating or preventing cell damage caused by free radicals [36].The antioxidant properties of many flavonoids are much stronger than those of vitamins C and E [35].

Flavonoids can prevent cell damage caused by free radicals by various mechanisms (Fig. 4).

The mechanism of free radical binding by flavonoids was proposed and supported by a number of researchers for the first time in the form of a hypothesis [32, 36]. The proposed hypothesis includes three main targets (Fig. 5).

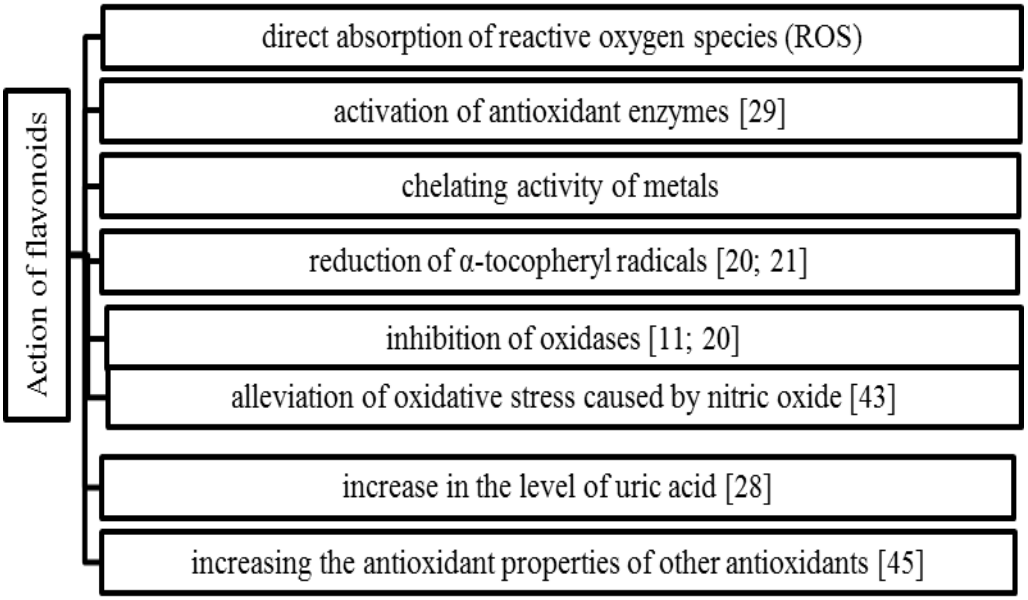


Fig. 4. **Main metabolic pathways of flavonoids action.**

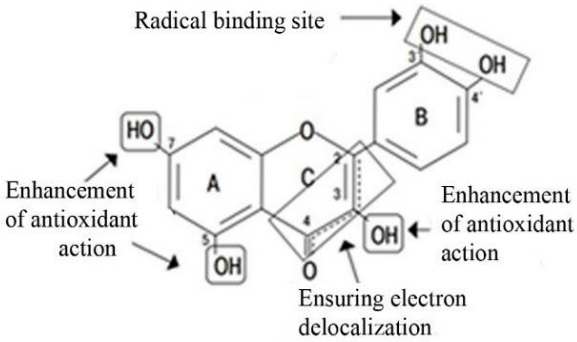


Fig. 5. **Main targets in the flavonoid molecule (using the chemical structure of quercetin as an example) that ensure the binding of free radicals (adapted from [6]).**

1) Hydroxyl groups 3´and 4´ associated with ring B (catechal structure) are the main characteristic of flavonoids, necessary for "quenching" free radicals. At the same time, the hydroxyl groups in the B-ring probably play the most important role in the sequencing of reactive oxygen species (ROS), while similar substituents in the A rings have a much lower antioxidant effect.

2) The double bond 2, 3 in conjugation with the 4-oxo-(ketone)-group in ring C provides electron delocalization from ring B. Electron delocalization of aromatic rings is known to stabilize the formed radicals (probably due to resonance) when flavonoid interacts with RFK.

3) Hydroxyl groups connected to rings A and C in the 3rd, 5th and 7th positions, together with the 4-oxo group, also increase the antioxidant activity of flavonoids, probably ensuring hydrogen bonding with the oxo group.

In vitro experiments have established that flavonoids, which are characterized by all the noted features of the chemical structure, have the greatest ability to inactivate free radicals. Such polyphenols include the flavonols quercetin and myricetin, as well as flavan-3-oleepicatechinagalate, epigallocatechin and, especially, epigallocatechin gallate. At the same time, a significant role in increasing antiradical activity is played by the hydroxyl group in position 3, which brings additional activity to flavonols and flavan-3-ols [36].

Conclusion. The paper presents an innovative "green" synthesis of quercetin-functionalized selenium nanoparticles using onion peel extract, which allows the use of biological waste for the preparation of environmentally friendly nanoconjugates. The presented bionanotechnology is effective, environmentally safe, economically attractive and allows obtaining stable composite selenium nanoparticles. Selenium nanoparticles, functionalized with quercetin, synthesized from agricultural waste (onion husks), are biocompatible, have antioxidant / antiradical properties and can be included in feed additives for animals and poultry.

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Використання відходів сільськогосподарського виробництва у біонанотехнології синтезу функціоналізованих наночастинок селену

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Розглянуто застосування нанотехнологічних інновацій щодо підвищення ефективності діїбіофлавоноїда кверцетину. Здатність до функціоналізації за допомогою специфічних лігандів, які націлені на певні органи або клітини, дуже важлива, оскільки можна збільшити концентрацію кверцетину на бажаному цільовому рівні, одночасно зменшуючи побічні ефекти.Кверцетин, як флавоноїдний антиоксидант, широко використовується для зменшення оксидативного стресу та активації важливих сигнальних шляхів у клітинах. Однак погана розчинність у воді, інтенсивний метаболізм першого проходження обмежують його застосування. Представлена стратегія розробки нанокомпозитів, яка передбачає поєднання кверцетину, одержаного з відходів сільськогосподарського виробництва, з наночастинками селену. Результати invitro продемонстрували, що кверцетин-наноселен має високу розчинність у воді порівняно з окремими флавоноїдами. Доведено, що наночастинки кверцетин-наноселену здатні довідновлення радикалів 1,1-дифеніл-2-пікрилгідразилу (DPPH) та характеризуються високою антиоксидантною активністю. Здатність функціоналізації наночастинок за допомогою специфічних лігандів, які націлені на модуляцію специфічних сигнальних шляхів (Keap1/Nrf2/ARE, Nf-kB, mTOR) у окремих клітинах, дуже важлива, оскільки дозволяє збільшити концентрацію кверцетину на бажаному рівні, одночасно зменшуючи побічні ефекти.

Зважаючи на сприятливий вплив кверцетину, розроблена стратегія, яка передбачає використан-

ня наночастинок селену для доставки кверцетину специфічним і контрольованим способом. Перспективним є дослідження у цьому напрямку, так як сприятимуть з'ясуванню доцільності використання таких наносистем, які не одержали ще широкого застосування. Використання відходів цибулі та її екстрактів у біотехнології, біомедицині, фармацевтиці та агропромисловому секторі може стати оптимальним рішенням для зменшення шкоди навколишньому середовищу. Окрім того це забезпечить економічно обумовлену альтернативу для виробництва кормових добавок для тварин та птиці. Сполуки кверцетину із наноселеном повинні зайняти достойне місце у клінічній медичній практиці, біології та сільському господарстві. Корисні ефекти кверцетину можуть бути додатково посилені за допомогою нанотехнологій, що сприятиме ефективному застосуванню цієї сполуки з таким великим біологічним потенціалом.

Ключові слова: нанобіотехнології, Селен, відходи,лушпиння цибулі, 1,1-дифеніл-2-пікрилгідразил, антирадикальна активність.

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БІОТЕХНОЛОГІЇ ТА БІОІНЖЕНЕРІЯ

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Cultivation of worms on a substrate containing poultry droppings fermented with addition of biodestructors

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Significant accumulations of poultry droppings near large poultry enterprises are a problem both in Ukraine and abroad. An urgent issue is the search for rational methods of disposal of these wastes with the involvement of natural, ecologically safe methods such as composting with the use of microorganisms and vermiculture. The use of microorganisms during the fermentation of broiler chicken droppings with litter (in the form of sawdust of non-coniferous trees) leads to a reduction of time for preparation of organic biomass for vermiculture. However, it is of scientific and practical interest to establish the effectiveness of growing worms on a substrate containing broiler chicken droppings fermented with various doses of microorganism preparations compared to the option where the droppings were fermented in the traditional way during 18 months.

During the research, 4 groups were formed with 4 micro-beds in each. The weight of the compost and the volume of the micro-beds were identical. In the control version, the compost contained 92.0 % (by weight) of broiler chicken manure fermented during 18 months and 8.0 % of shredded wheat straw. In the experimental micro-beds, the ratio of manure to straw was identical by mass, however, the manure was fermented during 180 days using 143, 1430 and 2860 mg of bio-destructors per ton of biomass. The influence of broiler chicken droppings on the number of sexually mature and immature worms, their mass and the number of laid cocoons was studied for 100 days. The content of protein, biotic metals, and toxic metals in vermiculture biomass was studied.

It was proven experimentally that the inclusion of broiler chicken droppings with a litter of fermented droppings in the amount of 2860 mg/t of bio-destructors leads to an increase in the number of sexually mature and immature worms in the micro-bed and their mass by 45.5 and 29.3 % respectively, and 63.9 and 56.7 % relatively to the control where the litter was fermented during 18 months. Cultivation of vermiculture on compost with manure fermented for 180 days (III experimental group) makes it possible to obtain 41.4 % more cocoons and 17.1 % more cocoons compared to the control group. The biomass of worms grown on compost containing manure fermented with the highest dose of bio-destructors probably did not differ by the content of biotic metals and toxic metals.

Key words: worm cocoons, protein, biotic metals, toxic metals, bio-destructors, vermiculture, compost.

Problem statement and analysis of recent researche. The dynamic development of poultry farming in most countries of the world leads to the accumulation of significant volumes of litter with and without bedding in limited areas [1, 2]. In Ukraine, the issue of the accumulation of poultry droppings in limited areas is also quite relevant. Poultry droppings, especially of broiler chickens, are a source of soil, air and water pollution, while at the same time it contains a significant amount of nutrients for microorganisms, invertebrates, insects and plants. Among the nutrients of poultry droppings there is a certain concentration of proteins, carbohydrates, lipids, macroelements

and microelements [3, 4]. Promising methods of rational disposal of broiler chicken droppings are its active composting under the action of a conglomerate of microorganisms and vermiculture [5–7]. Therefore, conducting research aimed at accelerating biotechnological methods of disposal of broiler chicken droppings with the use of bio-destructors and vermiculture is of important scientific and practical importance.

For the implementation of vermiculture, dung worms or a hybrid of the red California worm are used. Worms (vermiculture) belong to the oligochaete group and are hermaphrodites. The alimentary canal of worms runs throughout the body. Worms breathe takes place through the skin, which has a large number of blood capillaries. The mass of a sexually mature individual is from 0.35 to 1.4 g. Sexual maturity of worms occurs in 90–95 days. After mating, each individual forms a cocoon with eggs in the middle (up to 15–22 eggs), and after 15–24 days a new generation appears. Under optimal conditions, worms mate every 8–9 days. The substrate for vermiculture is pre-prepared organic mass [4, 7, 8].

Various prepared (composted) organic wastes are used for the cultivation of vermiculture: crop production (straw, chaff, spoiled hay, spoiled hay, silage, etc), livestock production (poultry droppings, animal manure, and waste from slaughterhouses), industrial and food waste [7, 9, 10, 11].

Vermicomposting involves mesophilic biooxidation of organic waste [12]. The use of worms contributes to the active improvement of soil fertility, increasing the content of humus in them, and increasing the yield of agricultural crops. This is achieved due to the ingress of worm coprolites (biohumus) into the soil. Vermiculture coprolites are an effective organic fertilizer. With coprolites, the soil is enriched with humic, fulvic acids, useful microorganisms, polysaccharides, proteins and nitrogenous substances. The greater the number of earthworms per unit area of the soil, the higher its aeration and fertility is. Water penetration into compacted soils increases. As a result of the action of worms, the mineralization of Nitrogen, Phosphorus and other chemical elements is accelerated, thereby increasing bioavailability for plants [13–16].

It is forbidden to use fresh manure of cattle, horses, pigs, and especially poultry droppings for vermiculture. Before using manure and droppings for worms, it is necessary to ferment them. Traditional fermentation of poultry droppings lasts up to 12–18 months. The use of bio-destructors accelerates the processes of manure fermentation by 2 times [17].

During vermicomposting, worms and microorganisms simultaneously act on organic waste [12, 18]. Studying the possibilities of reducing the production time of organic fertilizer from poultry droppings and increasing the biomass of worms by combining vermicomposting with composting under the action of a conglomerate of microorganisms has economic, ecological and practical significance.

The aim of the study is to establish the effectiveness of growing vermiculture on compost containing broiler chicken droppings fermented with different doses of bio-destructors.

Material and methods of research. The study of the influence of the substrate containing broiler chicken droppings with litter (sawdust of non-coniferous trees) fermented under different conditions was carried out in the vivarium of the Biological and Technological Faculty of Bila Tserkva National Agrarian University. The air temperature in the room was maintained at the level of 22–23 ˚C. Both in the control and in the experimental groups, 4 micro-beds were formed. For the experiment, sexually mature worms (hybrid red California worms) were used, the average weight of which was 0.72±0.01 g. After mixing the litter of broiler chickens with straw, the humidity of the substrate in all groups was adjusted to 67±0.5 %. Once every two days, aeration of the substrate was carried out in micro-beds. The duration of the experiment was 100 days. At the end of the experiment, sexually mature worms, young and cocoons were counted in each micro-bed (Table 1).

Before determining the content of protein and trace elements in the biomass of vermiculture, worms were cleaned of compost, washed with distilled water and placed in desiccators with crushed, moistened filter paper for 36 hours in order to clean the gastrointestinal tract from humus and coprolites.

The protein content in the biomass of worms was determined by the method of O. Lowry. For this, a homogenate was made from worms [19]. The content of biotic metals (Copper, Zinc, and Ferrum) and toxic metals (Plumbum and Cadmium) in vermiculture biomass was determined using atomic absorption spectroscopy [20]. The mass of worms and their cocoons was determined using techno-chemical and analytical scales.

The obtained research data were processed using standard methods of variation statistics using the Statistica program.

Results and discussion. On the hundredth day of the experiment, it was established that there were an average of 132 sexually mature worms in the control micro-beds. This means that in the first 10 days after settlement, only 3–4 cocoons were formed. Worms hardly mated during this peri-

od. When using the litter of broiler chickens fermented with the use of 143 mg/t of bio-destructors for 6 months (I experimental group-micro-beds), the number of sexually mature individuals was 13.6 % higher than in the control one. The fastest adaptation of worms was found in the III experimental group. During the first 10 days, the most cocoons were formed there. Therefore, the number of sexually mature worms was 45.5 % higher than in the control one (Table 2).

The average weight of one sexually mature worm in the control group is 0.71 g. In the first experimental group, this indicator was 5.6 % higher than the control value. The weight of one individual in the II and III experimental groups was the same and amounted to 0.8 g each, which is 12.7 % more than in the control group.

Analyzing the number of worms that had not reached sexual maturity, it was found that the lowest rate was recorded in the control group. Under the cultivation of vermiculture on a substrate fermented with the use of a bio-destructors in the amount of 1430 mg/t (II experimental group), the number of worms was greater than in the control group by 25.4 %. In the III experimental group, the growth of the population was established. The number of immature individuals was 29.3 % higher compared to the data in the control group.

The average weight of worms in the control group that had not reached sexual maturity was 0.033 g. When growing worms on compost fermented with the use of a bio-destructors (I experimental group), the average weight of one individual increases by 12.1 %. The highest average weight of one immature worm was recorded in the III experimental group. The difference with the control one constituted 21.2 %.

Based on the obtained indicators of the number of sexually mature and immature individuals for 100 days of the experiment, it was calculated that for the processing of 1 ton of compost in the control group, it is possible to obtain 41.5 kg of worm biomass. It was calculated that the use of compost obtained by the accelerated method (III experimental group) makes it possible to obtain 28.6 % more of vermiculture biomass.

Table 1 – **Study scheme**

Group of micro-beds	The number of introduced worms in one micro-bed, units	The mass of the substrate in one micro-bed, kg	Characteristic of the substrate
Control group	100	15,0	Fermented litter of broiler chickens for 18 months without the addition of bio-destructors (92.0 % by weight) + crushed wheat straw (8.0 % by weight)
I experimental group	100	15,0	Fermented litter of broiler chickens for 180 days with the addition of bio-destructors – 143 mg/t (92.0 % by weight) + crushed wheat straw (8.0 % by weight)
II experimental group	100	15,0	Fermented litter of broiler chickens for 180 days with the addition of bio-destructors – 1430 mg/t (92.0 % by weight) + crushed wheat straw (8.0 % by weight)
III experimental group	100	15,0	Fermented litter of broiler chickens for 180 days with the addition of bio-destructors – 2860 mg/t (92.0 % by weight) + crushed wheat straw (8.0 % by weight)

Table 2 – **Indicators of growth and development of worms on the 100th day of the experiment,** M±m, n=6

Group-micro-beds	Mature worms in the micro-bed		Immature worms in one micro-bed		Mass of worms, which is grown under processing a ton of the studied substrate, kg
	quantity, units.	mass, g	quantity, units.	mass, g	
Control group	132±2,3	93,7±0,86	3866±56,3	127,6±3,77	41,5
I experimental group	150±3,5	112,5±0,69	4799±74,2	177,6±5,18	45,5
II experimental group	185±2,8	148,0±0,74	4850±65,5	194,0±7,33	50,2
III experimental group	192±4,5	153,6±1,33	5000±70,7	200,0±4,11	53,4

It is possible to judge the intensity of mating of sexually mature worms by the number of cocoons. The lowest number of mating was observed in the control group, where worms were grown on compost containing fermented broiler chicken droppings, for 18 months, on the 100th day of the experiment, the number of cocoons constituted 145 units. The established pattern was that the higher the concentration of the bio-destructors in the litter of broiler chickens, the greater number of cocoons in the micro-bed was. In the III experimental group, the number of cocoons was greater by 41.4 % compared to the control indicators (Fig. 1).

The mass of cocoons correlates with the number of individuals that develop in them. The average mass of cocoons in the 1st experimental group was 7.8 % greater than in the control one. The largest mass of one cocoon was in the III experimental group. The difference with the control one constituted 17.1 % (Fig. 2).

In addition to the influence of different compost on the technological parameters of vermiculture, the chemical composition of worm biomass was also studied. In vermiculture biomass from the control group, the protein content constituted 683 g/kg of dry matter (Fig. 3). Cultivation of worms on compost containing broiler chicken droppings probably did not affect the increase of protein in their biomass. The difference with the control group was within 0.4–1.0 %.

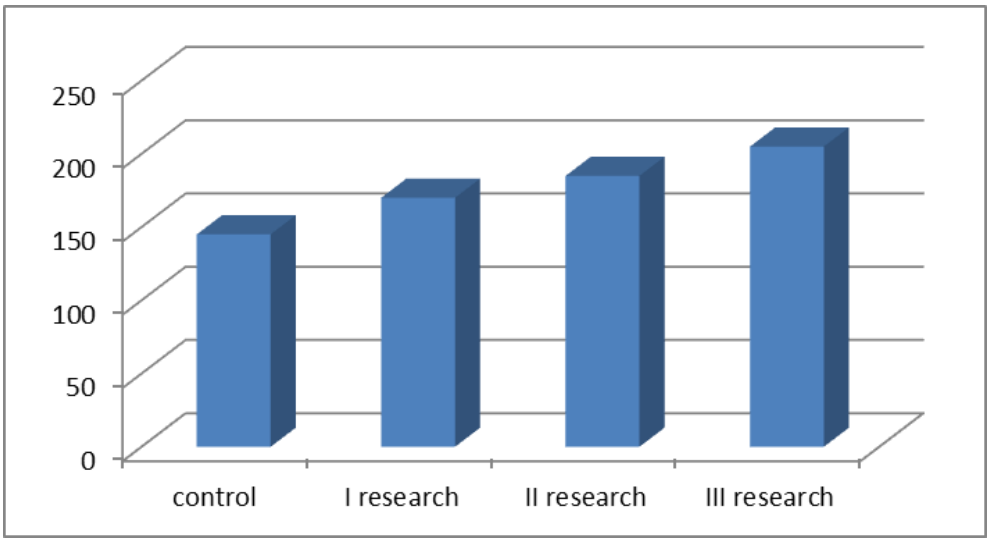

Fig. 1. **Number of cocoons, units.**

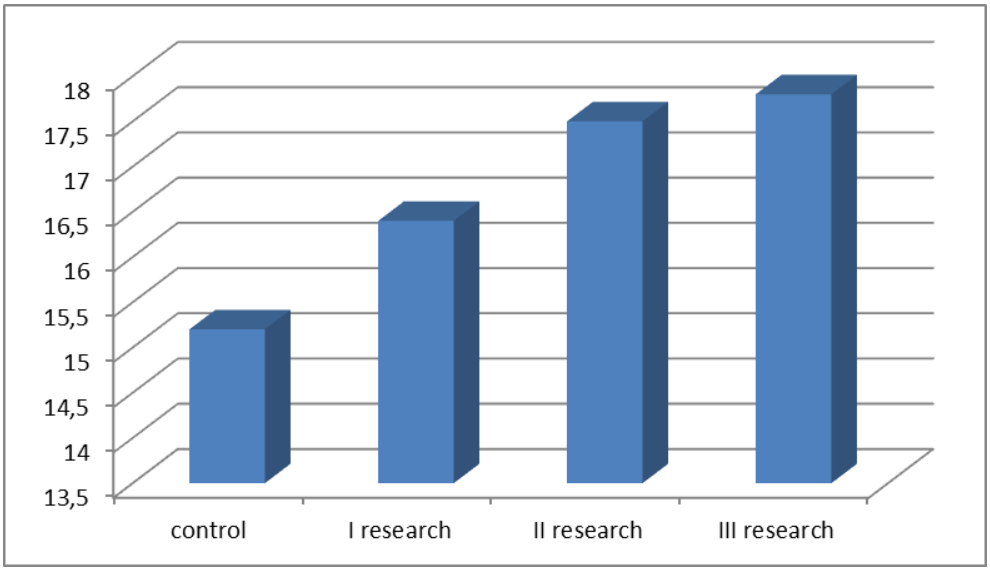

Fig. 2. **Mass of cocoons, mg.**

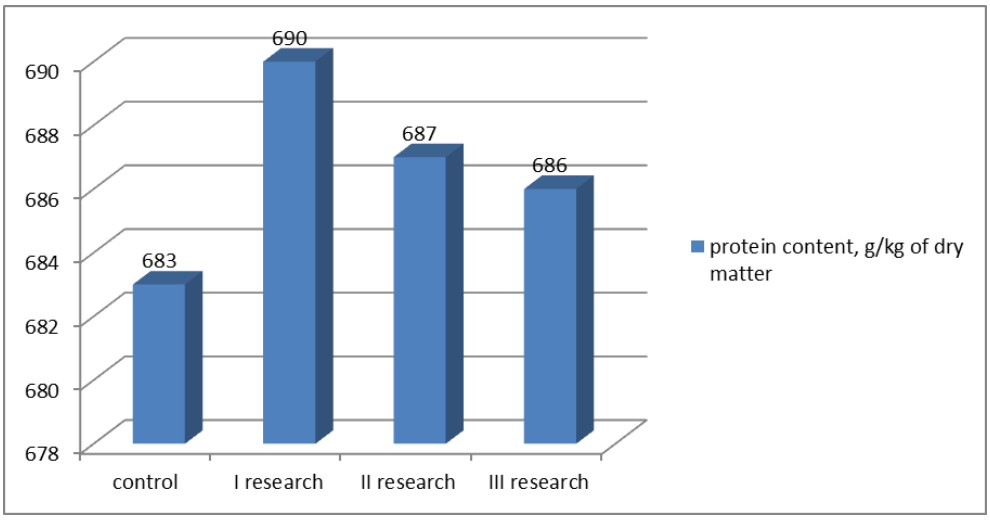

Fig. 3. **Protein content in vermiculture biomass, g/kg of dry matter.**

Among biotic metals, the content of Ferrum, Copper and Zinc in the biomass of worms was determined. The content of copper in worms from the control group was 7.9 mg/kg of dry matter. The lowest metal content was found in vermiculture biomass from the 1st experimental group. The indicator was 12.6 % lower than in the control group, but it was not significantly different from the control. In worms from the II and III experimental groups, the copper content was lower than in the control one by 5.1–7.6 %. The difference had the nature of a trend (Table 3).

Investigating the content of Zinc in the biomass of worms, it was established that the highest rate was recorded in the control group. It has been observed a pattern in the research groups that with an increase in the content of the bio-destructors in the litter of broiler chickens, the content of zinc in worms increases.

The content of Ferrum in vermiculture biomass from the control group constituted 875.5 mg/kg of dry matter. When growing worms on compost with broiler chicken droppings, which was fermented with the highest dose of bio-destructors (III experimental group), the content of Ferrum was lower than in the control group by 1.63 %. The difference was within the margin of error.

Studying the content of Lead it was found that the highest concentration of this toxic metal was in vermiculture biomass from the control group. The lowest content of Lead was recorded in worms from the 1st experimental group. The difference with the control group was 11.6 % and was not probable. It was revealed that with the increase of the destructor during the fermentation of broiler chicken droppings, which is the part of the compost, the metal content increases within the margin of error (Fig. 4).

Cadmium content in the biomass of worms from the control group was at the level of 0.15 mg/kg. In the worms from the experimental groups, the metal content was lower by 6.7–13.3 % compared to the control group. The difference was with in the margin of error.

Table 3 – **The content of biotic metals in the biomass of worms**, mg/kg of dry matter

Group-micro-beds	Metals		
	Copper	Zinc	Ferrum
Control group	7,9±0,48	75,1±2,55	875,5±18,12
I experimental group	6,9±0,52	73,4±3,45	845,2±22,45
II experimental group	7,3±0,39	74,2±1,99	857,4±35,53
III experimental group	7,5±0,37	74,7±4,15	861,2±17,69

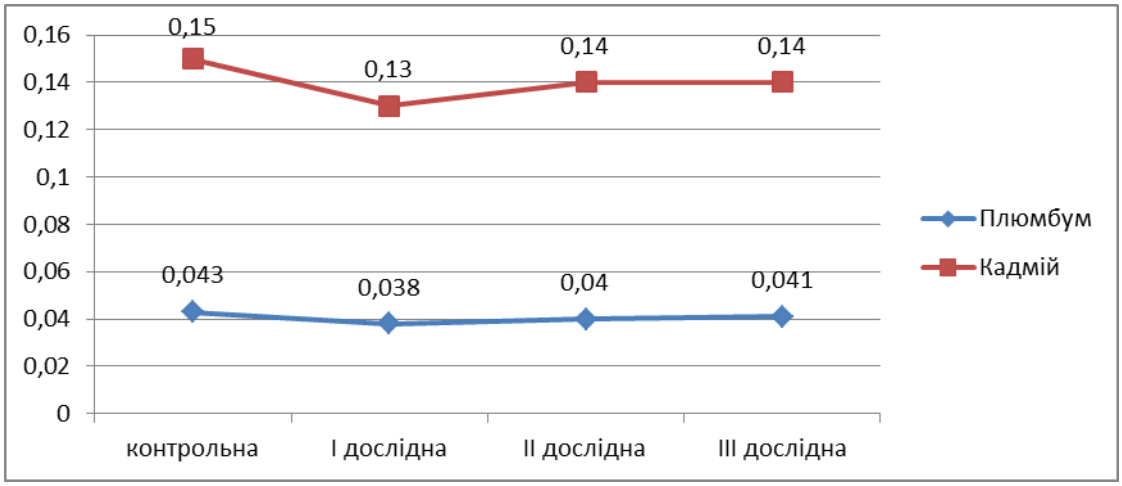

Fig. 4. **Content of toxic metals, mg/kg of dry matter.**

Conclusion. 1. The use of 92.0 % by mass of broiler chicken droppings fermented for 180 days with the use of bio-destructors at the rate of 2860 mg/t as a substrate makes it possible to increase the productivity of vermiculture. This regularity is confirmed by an increase in the number of sexually mature worms and worms that have not reached sexual maturity and their mass, respectively, by 45.5 and 29.3 % and 63.9 and 56.7 % relatively to the technology where poultry droppings, which was fermented for 18 months, were used.

2. Broiler chicken droppings fermented with the highest dose of bio-destructors in the composition of compost contributes to an increase in the number of formed cocoons of worms and their weight, respectively, by 41.4 and 17.1 % compared to the control group.

3. The use of broiler chicken droppings fermented with the use ofbio-destructors in the composition of compost revealed an increase within the margin of error of protein and a decrease in biotic metals and toxic metals in vermiculture biomass.

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Вирощування черв'яків на субстраті з вмістом посліду птиці ферментованого за участі біодеструкторів

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Значні накопичення посліду птиці біля великих птахопідприємств є проблемою як в Україні, так і за кордоном. Актуальним питанням є пошук раціональних методів утилізації цих відходів із залученням природних, екологічно безпечних методів, таких як компостування за участі мікроорганізмів та вермикультури. Застосування під час ферментації посліду курчат-бройлерів із підстилкою (у вигляді тирси нехвойних дерев) мікроорганізмів призводить до скорочення часу підготовки органічної біомаси для вермикультивування. Науково-практичний інтерес представляє встановлення ефективності вирощування черв'яків на субстраті з умістом посліду курчат-бройлерів ферментованого різними дозами препарату мікроорганізмів у порівняні з варіантом, де послід ферментували традиційним способом впродовж 18 місяців.

Під час проведення досліджень формували 4 групи по 4 мікроложів кожній. Маса компосту і об'єм мікролож були ідентичними. В контрольному варіанті компост містив 92,0 % (за масою) посліду курчат-бройлерів, ферментованого 18 місяців, і 8,0 % подрібненої соломи пшениці. У дослідних мікроложах співвідношення посліду до соломи було ідентичне за масою, проте послід ферментували 180 діб, застосовуючи 143, 1430 та 2860 мг біодеструктора на тонну біомаси. Досліджували вплив упродовж 100 діб посліду курчат-бройлерів на кількість статевозрілих, нестатевозрілих черв'яків, їх масу і кількість відкладених коконів. Вивчали вміст у біомасі вермикультури білка, металів-біотиків та металів-токсикантів.

Було доведено, що включення у компост посліду курчат-бройлерів із підстилкою ферментованого посліду за участі біодеструктора в кількості 2860 мг/т призводить до підвищення кількості статевозрілих та нестатевозрілих черв'яків у мікроложі та їх маси відповідно на 45,5 і 29,3 % та 63,9 і 56,7 % відносно контролю, де послід ферментувався 18 місяців. Вирощування вермикультури на компості з послідом, ферментованим впродовж 180 діб (III дослідна група), дає можливість отримати на, 41,4 % більшу кількість коконів та на 17,1 % їх масу у порівняні з контролем. Біомаса черв'яків, вирощених на компості, з умістом посліду ферментованого найбільшою дозою біодеструктора вірогідно не відрізнялась за вмістом металів-біотиків та металів-токсикантів.

Ключові слова: кокони черв'яків, білок, метали-біотики, метали-токсиканти, біодеструктор, вермикультура, компост.

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Application of mineral carriers for immobilization of *Trichoderma viride*

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There is no doubt in the fact that preparations containing *Trichoderma* as the main component are currently a real alternative to synthetic agrochemicals as antagonists of soil-borne plant diseases and growth stimulators. The use of this kind of drugs does not always give sufficiently stable results. In addition, technological problems can often arise, which are associated with the short shelf life of liquid preparations based on *Trichoderma*. The use of the solid form of the drug often causes the problem of self-inhibition of conidia germination. And therefore a more careful calculation of the optimal dose for each specific strain is required. The development of multifunctional bio-preparations that are more stable in their effectiveness, especially preparations that include *Trichoderma*, is one of the most important tasks in the system of biotechnology development, which determined the relevance of the research.

The aim of the work is to establish the optimal carrier for the immobilization of *Trichoderma viride* cells and the effect of immobilization on the stability of drugs. It has been experimentally established that the growth and development of the immobilized fungus *Trichoderma viride* is influenced by the nature and method of modification of the carrier. It was established that when the fungus immobilized on native and modified carriers (particle size 0.5 μm – 2.5 mm) was seeded on a sterile nutrient medium, a decrease in the growth rate of *Trichoderma viride* was found, compared to the control one where the native fungus was used. It has been proven that the optimal amount of grinding of both native and modified media is – 150 μm – 1.5 mm. It has been found that saponite modified with starch is the optimal carrier for *Trichoderma viride* immobilization.

It has been proven that upon immobilization of the fungus *Trichoderma viride*, the latter becomes more resistant to the negative factors of the growth environment (antagonistic action of the natural conglomerate of microorganisms residing on spoiled alfalfa hay).

Key words: fungi, cell immobilization, carriers, modified starch, humic acids, saponite, zeolite, zeolite-containing basalt tuff.

Problem statement and analysis of recent research. Native culture or preparations of *Trichoderma viride* are widely used in agriculture. The fungus as the main component of the drug is used to struggle against a number of soil diseases, increase the yield of grain and leguminous crops. Preparations containing fungi are used for the disposal of crop production waste, unfit for use as feed of plant origin, etc [3, 21]. The use of *Trichoderma viride* together with arbuscular mycorrhizal fungi makes it possible to replace chemical fertilizers and stimulate an increase in crop yields [16]. Stimulation of plant growth can be manifested taking into account a number of limiting factors

such as seeding rate, type of formulation, growing conditions [4, 17]. Fungi are used in the form of preparations in the form of liquid and powder. However, with different technologies that use preparations containing Trichoderma, various difficulties arise, under which the effectiveness of the fungi decreases. The use of native preparations of *Trichoderma* in technologies of crop production or utilization of organic waste does not always give stable results [21]. These phenomena are due to the fact that the liquid form of preparations containing fungi has a short shelf life. When using loose form of fungi preparations, the feature of autoinhibition of the process of conidia germination is manifested. Therefore, this phenomenon requires careful calculations of the necessary dose of the drug for each particular strain of fungi [21]. In addition, there is a problem of the lack of local fungal delivery systems to reduce diseases caused by soil bacterial pathogens [19].

The multi-vector use of *Trichoderma viride* in agriculture requires the development of technologies for the production of fungi preparations that are stable in their effectiveness. The issue of the effectiveness of immobilization (stabilization) of the fungi on domestic natural minerals – saponins, zeolites and zeolite-containing basalt tuffs is unexplored.

Trichoderma refers to filamentous spore fungi. These fungi produce ascomycetes and belong to the large class Deuteromycetes. These fungi are one of the types of mold that is found in almost all types of soil. *Trichoderma viride* is the most common and cultivated fungus. These fungi are widely used in the national economy [7, 20].

In world practice, a number of methods of their immobilization have been developed for more effective use of microorganisms, including fungi. Immobilization of cells of microorganisms, their parts and enzymes is a physical limitation or localization in a certain spatial location. The latest involves restricting their free movement to demonstrate hydrodynamic characteristics that differ from those of the external environment, while preserving their biological and catalytic properties. Immobilization allows repeated and continuous use of cells of microorganisms. Depending on the method and method of immobilization of microbial cells, it is possible to prolong their viability and improve their growth in adverse conditions [5, 8, 18].

The selection of a suitable carrier (matrix) for the immobilization of cells of microorganisms is an important process for obtaining a finished product with the desired biological properties. The carriers had to be accessible, have a large surface area, which would later provide an opportunity for the growth of microorganisms (satisfactory interstitial space). Under certain technological conditions, the matrix should be easily regenerated and reusable, not exhibit toxic effects in relation to the cells of microorganisms, and promote the manifestation of catalytic activity. Carriers should have mechanical and thermal resistance, biological and chemical stability [9, 11, 12, 14].

Immobilization of cells of microorganisms is carried out using physical and chemical methods, including the use of adsorption, encapsulation, and encapsulation in a gel [15]

To maintain the stability of *Trichoderma* in the soil during the warm season, materials (as a matrix) with sorption properties and are a source of nutrition for the culture are introduced into the soil together with fungi preparations: peat, cereal bran, tree bark, compost obtained from the manure of farm animals, substrates after seafood cultivation [10, 13].

Biotechnologies of fungi spore encapsulation with starch borate, starch xanthan or carrageenan gel have been developed to stabilize drugs.

Technologies for manufacturing alginate granules with microorganisms and alginate granules with pyrophyllite clay filler are used [13].

Immobilization of *Trichoderma* conidia and chlamydiospores was carried out on wheat bran and kaolin clay in alginate gel [13]. Both organic and inorganic carriers are also used for the immobilization of microorganisms. Among organic carriers, starch, pectin, cellulose, gelatin and their forms modified by physical and chemical methods are used [1, 2, 6]. As a matrix, calcium alginate, modified coal from animal bones is used [5, 12, 18].

The aim of the study is to establish the optimal carrier and the size of its grinding for the immobilization of *Trichoderma viride* fungi cells and its growth stability in non-sterile conditions.

Materials and methods of research. The growth of *Trichoderma viride* fungi was checked using the PDA medium – potato dextrose agar with the content of antibacterial drugs (sterile benzylpenicillin sodium salt (1000000 units) 0.5 g/dm^3 and streptomycin sulfate 0.4 g/dm^3). Before using the medium, it was thermally treated – autoclaved (25 minutes at a temperature of 121–123 °C).

The native culture of *Trichoderma viride* (control) and its immobilized forms were sown on PDA containing antibiotics. In addition, spoiled alfalfa hay was treated with native and immobilized drugs. Hay with a moisture content of 64.5 % was not subjected to heat treatment and treatment with antimicrobial drugs (non-sterile conditions). After 14 days of cultivation of hay treated with native and immobilized preparations of *Trichoderma viride* at a temperature of 25–26 °C, the investigat-

ed fungus was isolated from its samples on potato-dextrose agar.

The prepared PDA nutrient medium was poured into Petri dishes of 15 cm3 each. Each Petri dish was numbered, taking into account the experimental group.

Incubation of cultures of native and immobilized *Trichoderma viride* on the medium and cultures of alfalfa silage treated with these fungi was carried out in a thermostat at a temperature of 26±0.5 ˚C.

For the immobilization of *Trichoderma viride*, such natural minerals were used as carriers: saponite of the Tashkiv region, gray zeolite, and zeolite-containing basalt tuff with different levels of grinding (Table 1). The same grinding was used for modified saponite, modified gray zeolite and modified zeolite-containing basalt tuff. Natural carriers were modified with starch, humic acids using calcium chloride.

Table 1 – **Carrier characteristics**

Carrier	The amount of grinding of the native and modified form
Saponite	From 0,5 up to 150 µm
	From 150 µmup to 1,5 mm
	From 1,5 mm up to 2,5 mm
Gray zeolite	From 0,5 up to 150 µm
	From 150 µm up to 1,5 mm
	From 1,5 mm up to 2,5 mm
Zeolite-containing basalt tuff	From 0,5 up to 150 µm
	From150 µm up to 1,5 mm
	From 1,5 mm up to 2,5 mm

Immobilization of spores and mycelium of *Trichoderma viride* on native and modified carriers was carried out by a physical method. Viridin preparations were the source of fungi biomaterial. A carrier was added to the solution of *Trichoderma viride* in the presence of 0.05 M calcium chloride and incubated for 20–22 hours at a temperature of 4.0–4.5 °C, periodically subjecting the mixture to thorough mixing every 1.5–2.0 hours.

Results and discussion. It was established that the CFU index in the control (native form of the fungus) was 3.1x109. Investigating the growth and development of immobilized *Trichoderma viride* fungi, it has been established that these processes are influenced by the nature and method of carrier modification.

After sowing the fungi immobilized on native saponite (particle size 0.5–150 µm), the growth rate of *Trichoderma viride* on the nutrient medium the decrease was found by 238 times compared to the control. Compared with the options where saponite modified with starch and humic acids was used, the growth of the fungus was also lower, respectively, by 8.4 and 4.2 times compared to the control (Table 2).

The lowest rate of growth of the immobilized fungus on native saponite was recorded in the variant where the size of the carrier was crushed – 1.5–2.5 mm compared to the control and variants where the modified carrier was used.

Under using modified saponite (particle size 1.5–2.5 mm) with starch and humic acids, the CFU indicator of *Trichoderma viride* decreases compared to the option where saponite with a particle size of 150 µm – 1.5 mm was used, respectively, in 8.8 and 23, 7 times.

Table 2 – **The Growth of *Trichoderma viride* on PDA nutrient medium**

Indicator	Native carrier	Carrier modified with starch	Carrier modified with humic acids
Control	$3,2x10^9$	$3,1x10^9$	$3,3x10^9$
The drug immobilized on saponite (0,5–150 µm)	$1,3\ x10^7$	$1,1\ x10^8$	$5,5\ x10^7$
The drug immobilized on saponite (150 µm – 1,5 mm)	$5,3\ x10^7$	$4,7\ x10^8$	$9,5\ x10^7$
The drug immobilized on saponite (1,5–2,5 mm)	$6,9\ x10^6$	$5,3\ x10^7$	$0,4\ x10^7$
The drug immobilized on zeolite (0,5–150 µm)	$0,9\ x10^6$	$0,5\ x10^7$	$6,2\ x10^6$
The drug immobilized on zeolite (150 µm – 1,5 mm)	$8,5\ x10^6$	$7,3\ x10^7$	$3,8\ x10^7$
The drug immobilized on zeolite (1,5–2,5 mm)	$7,7\ x10^5$	$6,1\ x10^6$	$2,0\ x10^6$
The drug immobilized on zeolite-containing basalt tuff (0,5–150 µm)	$1,7\ x10^6$	$1,0\ x10^7$	$6,0\ x10^6$
The drug immobilized on zeolite-containing basalt tuff (150 µm – 1,5 mm)	$9,3\ x10^6$	$8,2\ x10^7$	$4,3\ x10^7$
The drug immobilized on zeolite-containing basalt tuff (1,5–2,5 mm)	$4,5\ x10^6$	$5,2\ x10^7$	$1,5\ x10^7$

Comparing the effects of saponite modification methods, it was proven that upon immobilization of *Trichoderma viride* on starch-modified saponite with a particle size of 150 µm – 1.5 mm, the CFU index of the fungus was higher than when using native saponite and modified with humic acids, respectively, by 8.8 and 4 ,9 times. It was found that the optimal grinding value of both native and modified saponite is – 150 microns – 1.5 mm. Thus, when using starch-modified saponite with a particle size of 150 µm to 1.5 mm, the growth of fungi on the medium was 4.2 times higher than when using such a medium with a particle size of 0.5–150 µm.

Sowing of *Trichoderma viride* immobilized on native zeolite (particle size 0.5–150 µm) showed that the number of CFU of the fungus was 3444 times less compared to the control. Relative to variants with a similar particle size of zeolite modified with starch and humic acids, it was found that the growth of *Trichoderma viride* was lower, respectively, by 55.5 and 65.9 times. The greatest growth and development of the fungus was recorded in the variant where the culture immobilized on starch-modified zeolite (150 µm – 1.5 mm) was used, compared to other experimental samples. The indicator was higher in comparison with the option where the immobilization of the fungus was carried out on the native carrier and the carrier modified with humic acids, respectively, by 8.6 times and by 92.1 %.

Studying the growth intensity of *Trichoderma viride* immobilized on zeolite-containing basalt tuff revealed a decrease in CFU indicators compared to the control. Comparing between the immobilized fungi, it was established that the greatest culture growth was on the medium where zeolite-containing basalt tuff with a particle size of 150 µm – 1.5 mm modified with starch was used.

Comparing the effect of carriers on the growth of the fungus, it has been found that immobilization on saponite, zeolite, and zeolite-containing basalt tuff leads to a significant decrease in the CFU index of *Trichoderma viride* compared to the control. Saponite modified with starch was found to be the most effective carrier for immobilization of the fungus. The most optimal particle size of natural minerals, both native and modified, was 150 µm – 1.5 mm.

During the treatment of spoiled hay with the native preparation (control) *Trichoderma viride*, it was established that during 14 days of cultivation, the CFU indicator was $4.4x10^3$. It was found that the growth indicators of the immobilized fungi on the hay were influenced by the carrier and the size of the particles of the carrier. During the treatment of spoiled alfalfa silage with a fungus immobilized on saponite, it was found that the growth of fungi was 75 times greater than in the control when using preparations with native saponite with a particle size of 0.5–150 µm. When using *Trichoderma viride* immobilized on saponite modified with starch with a similar size of particles, the growth of the fungus was 3.9 times greater compared to the version where native saponite was used (Table 3).

Table 3 – **The Growth of *Trichoderma viride* on spoiled hay**

Indicator	Native carrier	Carrier modified with starch	Carrier modified with humic acids
Control	$4,4x10^3$	$4,0x10^3$	$3,9x10^3$
The drug immobilized on saponite (0,5–150 µm)	$3,3\ x10^5$	$1,3\ x10^6$	$4,4\ x10^5$
The drug immobilized on saponite (150 µm – 1,5 mm)	$9,7\ x10^5$	$4,3\ x10^6$	$0,8\ x10^6$
The drug immobilized on saponite (1,5–2,5 mm)	$7,8\ x10^4$	$6,7\ x10^5$	$9,9\ x10^4$
The drug immobilized on zeolite (0,5–150 µm)	$2,8\ x10^4$	$5,8\ x10^5$	$3,9\ x10^4$
The drug immobilized on zeolite (150 µm – 1,5 mm)	$7,9\ x10^4$	$9,9\ x10^5$	$8,9\ x10^4$
The drug immobilized on zeolite (1,5–2,5 mm)	$9,7\ x10^3$	$8,9\ x10^4$	$0,7\ x10^4$
The drug immobilized on zeolite-containing basalt tuff (0,5–150 µm)	$0,8\ x10^4$	$2,9\ x10^5$	$2,8\ x10^4$
The drug immobilized on zeolite-containing basalt tuff (150 µm – 1,5 mm)	$7,5\ x10^4$	$9,6\ x10^5$	$8,8\ x10^4$
The drug immobilized on zeolite-containing basalt tuff (1,5–2,5 mm)	$5,5\ x10^4$	$5,9\ x10^5$	$6,2\ x10^4$

Comparing the growth of the fungus immobilized on saponite with different sizes of particles and different methods of modification, it was found that the highest growth of *Trichoderma viride* on spoiled alfalfa hay was recorded in the variant where the fungus was immobilized on saponite modified with starch with a particle size of 150 μm – 1.5 mm.

When hay was treated with preparations of a fungus immobilized on native zeolite with a particle size of 0.5–150 μm, the growth of the latter was 6.3 times greater than in the control. When using a fungus immobilized on both native and modified zeolite, the whitest growth of microorganisms was recorded when using carriers with a particle size of 150 μm – 1.5 mm. Comparing the effect of immobilization of *Trichoderma viride* on different media on the growth of the fungus, it was established that the highest CFU indicators were recorded on treated hay in the variant where drugs immobilized on zeolite modified with starch were used.

The growth of the fungus immobilized on native zeolite-containing basalt tuff with a particle size of 0.5–150 μm on the hay was more intense than in the control. The difference according to the CFU indicator was 1.8 times. Immobilization of the fungus on zeolite-containing basalt tuff (particle size 150 μm – 1.5 mm) modified with starch and humic acids allows to obtain preparations with better growth on alfalfa hay compared to preparations immobilized on the native mineral, respectively, by 12.8 times and by 17.3 %. Comparing the growth indicators of *Trichoderma viride* immobilized on native and modified zeolite-containing basalt tuff on alfalfa hay, it was established that the most intense growth was obtained with the use of the fungus immobilized on a natural mineral modified with starch.

Analyzing the growth of native and immobilized preparations of *Trichoderma viride* on alfalfa hay it has been revealed that the highest rates of CFU of the fungus were obtained using preparations immobilized on modified saponite (particle size 150 μm – 1.5 mm).

Thus, we have proved that with the help of immobilization, the fungus *Trichoderma viride* becomes more resistant to negative factors of the external environment (the antagonistic effect of the natural conglomerate of microorganisms of spoiled alfalfa hay), which confirms the data of researchers [5, 13].

Conclusion. 1. When *Trichoderma viride* is immobilized on modified saponite with a particle size of 150 μm – 1.5 mm, the growth intensity of the fungus on a sterile nutrient medium PDA decreases by 238 times compared to the native preparation.

2. Among natural minerals (saponite, gray zeolite and zeolite-containing basalt tuff) and their modified forms, saponite modified with starch is the optimal carrier for immobilization of Trichoderma viride fungus.

3. Immobilization of *Trichoderma viride* on starch-modified saponite with the participation of calcium ions allows to maintain 977 times greater growth of the fungus in an environment with unfavorable external conditions compared to its native form.

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Застосування мінеральних носіїв для іммобілізації *Trichoderma viride*

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Препарати, що містять *Trichoderma* як основний компонент, нині є реальною альтернативою синтетичним агрохімікатам як антагоністи ґрунтових хвороб рослин й стимулятори росту. Застосування таких препаратів не завжди дають стабільні результати. Окрім того, часто можуть виникати технологічні проблеми, які пов'язані із коротким терміном придатності рідкої форми препаратів на основі *Trichoderma*. Застосування твердої форми препарату часто викликає проблему самогальмування проростання конідій. І тому потрібний більш ретельний розрахунок оптимальної дози для кожного конкретного штаму. Розроблення більш стабільних за своєю ефективністю поліфункціональних біопрепаратів, особливо препаратів, до складу яких входить *Trichoderma*, є одним із найважливіших завдань біотехнології, що зумовило актуальність дослідження.

Метою роботи є встановлення оптимального носія для іммобілізації клітин *Trichoderma viride* та впливу іммобілізації на стабільність препаратів. Експериментально встановлено, що на ріст і розвиток іммобілізованого гриба *Trichoderma viride* впливає природа і спосіб модифікації носія. За посіву на стерильному поживному середовищі іммобілізованого на нативних і модифікованих носіях гриба (величина часточок 0,5 мкм-2,5 мм) виявлено зниження показника росту *Trichoderma viride* порівняно з контролем, де використовували нативний гриб. Доведено, що оптимальною величиною подрібнення як нативних так і модифікованих носіїв є 150 мкм – 1,5 мм. Виявлено, що оптимальним носієм для іммобілізації *Trichoderma viride* є модифікований крохмалем сапоніт.

Доведено, що за іммобілізації гриба *Trichoderma viride* останій стає стійкішим до негативних факторів середовища росту (антагоністична дія природнього конглометару мікроорганізмів, що перебуває на зіпсованому сінажі люцерни).

Ключові слова: гриби, іммобілізація клітин, носії, модифікований крохмаль, гумінові кислоти, сапоніт, цеоліт, цеолітовмісний базальтовий туф.

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ХАРЧОВІ ТЕХНОЛОГІЇ

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Quality Evaluation Research of Low-Calorie Milk Ice Cream

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A modern approach to nutrition requires the creation and further development of new types of ice cream that are more beneficial for health in comparison with the traditional ones. The solution to this issue lies in the creation of the low-calorie ice cream. It gets its distinctive properties and features due to the reduction of fat and sugar content along with the mixing of various functional components.

The aim of the research was to study the effects of milk processing products and plant ingredients on the quality of low-calorie milk ice cream.

The organoleptic, physical, chemical and microbiological indicators of low-calorie milk ice cream were examined. It was established that according to taste profiles, experimental samples of ice cream had good indicators of smell and color in the absence of extraneous odors. According to physical, chemical and microbiological indicators, samples of low-calorie milk ice cream met the requirements of the State Standard of Ukraine (DSTU) 4733:2007.Studies of the microstructure of low-calorie milk ice cream have shown that there is a tendency to increase the dispersion of the air phase in its samples. It is proven that the new low-calorie ice cream components possess properties of the moisture-binding components. The amount of sugar was reduced by a third comparing to the traditional composition of ice cream precisely due to the utilization of the components.

The composition of low-calorie milk ice cream in which the protein and vegetable components was substantiated, and the organoleptic, physical, chemical and microbiological parameters were studied. The expediency of the protein and plant components usage in low-calorie milk ice cream recipes has been proven.

Key words: low-calorie milk ice cream, quality, organoleptic indicators, physical and chemical indicators, microbiological indicators, microstructure, whey processing products.

Problem statement and analysis of recent research. Ice cream is the most popular dessert and is in steady demand among the population, especially children. A variety of raw materials are used in mixtures to produce ice cream. The main raw material used for the production of ice cream is milk and dairy products. Sucrose and other sweeteners, stabilizers, etc are also used in the recipes, while fruits, berries, vegetables, etc diversify the overall assortment of ice cream [1]. The composition of ice cream includes fats, proteins, carbohydrates, minerals, etc, which come in the form of true and colloidal solutions and emulsions. True solutions are formed by salts, lactose and sucrose, whilst emulsions are formed by fats. Milk proteins, stabilizers and a certain amount of calcium phosphate are in ice cream structure in the form of colloidal solutions [2]. The ice cream composition after freezing predominantly consists of ice crystals, in some cases it includes small amount of lactose crystals, agglomerated particles of fat, protein, stabilizer, which are all distributed in the plasma [3, 4].

In Ukraine, ice cream has been produced on an industrial scale for a long time [5]. To withstand the competition, manufacturers try to constantly improve and expand their range of varieties, however, the quality of ice cream itself is not always the center of the attention. The range of domestic ice cream variants consists mainly of products with a high fat content, but the demand for low-calorie ice cream, sour milk ice cream and ice cream enriched with functional ingredients is growing every year in the world [5, 6].

In recent years, the dairy products and nutrition department of the Institute of Food Resources of NAAS of Ukraine has been studying whey processing products obtained using membrane technologies [7, 8]. Nevertheless, the test usage of such ingredients as a component of ice cream recipes was not carried out, although there are already data available on the improvement of organoleptic characteristics as well as structural and mechanical properties of food products. [9, 10]. In addition, various structuring agents with fillers are used to secure a certain structure of the dessert. The final consumer properties of the finished product are formed due to the fillers and their features. Such structure formers include inulin and rice flour, which have a high moisture-binding capacity. They can be used as natural thickeners during the production of dairy products, in particular, ice cream [11, 12].

Therefore, it is relevant to carry out research on the substantiation of the composition and development of the technology of low-calorie milk ice cream, which has functional ingredients in its composition, in particular from milk processing products.

The aim of the research was to study the influence of milk processing products and plant ingredients on the quality of low-calorie milk ice cream.

Material and methods of research. The subjects of research were: Dairy raw materials (whole and skimmed milk, cream, skimmed milk powder, butter), vegetable ingredients (inulin, rice and sesame flour, apple powder), milk processing products (dry concentrate of whey proteins obtained by ultrafiltration, with a mass fraction of protein 80 % and whey dry demineralized with a demineralization level of 90 %, and a mass fraction of protein of 27 %), the finished product (low-calorie milk ice cream).

The selection of organoleptic indicators was carried out on the basis of literature data and a list of parameters was selected that play an important role when evaluating the quality of ice cream, namely: *color, taste, smell, consistency, aftertaste.* Each of the listed indicators was evaluated based on the maximum score of 5 points [13]. To compare organoleptic indicators of low-calorie milk ice cream, a quantitative descriptive (profile) test was used, which allows you to visually compare taste characteristics [14].

Physical, chemical and microbiological indicators of ice cream samples were determined according to DSTU 4733:2007 [15].

The microstructure of ice cream samples was determined using a Motic (Fischer Bioblock) light microscope with a video camera. A thin layer of the ice cream sample was applied to a glass slide where it was dried. Observations were carried out at a 400-fold magnification [16, 17].

Results and discussion. We developed the technology of low-calorie ice cream production, in which whey protein concentrate (WPC) and demineralized whey powder (DWP) were used for protein enrichment and as an emulsifier; inulin as a natural polysaccharide, stabilizer and prebiotic; apple powder, rice and sesame flour as a thickener.

As a check control, milk ice cream was used, its content as follows: fat is 4.0 % and dry matter is 29.1 %, including 15.5 % of sugar. The following was added to the experimental samples of low-calorie dairy ice cream: 1 – apple powder and DWP; 2 – sesame flour and DWP; 3 – rice flour and WPC.

The generalized results of the ice cream organoleptic evaluation are depicted in Figure 1.

According to the analysis of organoleptic data by taste profiles (Figure 1) all ice cream samples had good smell and color indicators following in the absence of extraneous odors. According to all organoleptic indicators the best sample was the ice cream with rice flour and WPC, which differed from the others in its vivid creamy flavor, dense consistency, absence of protein lumps and ice crystals (Figure 1).

It should be noted that the control checks and samples of ice cream with sesame flour or apple powder and DWP showed an intermediate value of the taste profile (Figure1). At the same time, the consistency of the control ice cream sample was rated at 4 points and was characterized as excessively "fluffy" with palpable ice crystals.

The results of low-calorie ice cream samples research according to physical, chemical and microbiological indicators are presented in table 1.

Analysis of the data obtained along the research (Table 1) shows that the value of the a_w indicator in all ice cream samples lies in a narrow range – from 0.957 to 0.962, as well as the cryoscopic temperature (negative (4.00–4.42 °C), which is associated with quantitative composition of carbohydrates."Water activity" (a_w) is an important parameter that must be taken into account during the development of dairy products with new composition and properties, to ensure

the production of high-quality products [18]. In the case of its decrease, the possibility of using moisture for the metabolism of microorganisms decreases whilst the energy of the connection between the moisture and the material increases. At the same time, as a rule, it becomes more difficult for microorganisms to use the available moisture for their biological needs [19].

As a result of studies of physical, chemical and microbiological indicators of low-calorie milk ice cream, it was established that the mass fraction of dry substances, titrated acidity, the number of mesophilic aerobic and facultatively anaerobic microorganisms and bacteria of coliform group in the experimental samples were within the acceptable range according to DSTU 4733:2007 (Table 1).

Freezing is as important during the production of ice cream, as the parameters which affect the microstructure of the finished product [20]. Ice cream is frozen in two stages: the first defines a rapid but partial freezing in the freezer to a temperature of negative 4–5 °C with simultaneous air saturation of the mixture with intensive stirring, and the second is hardening, during which the partially frozen product is hardened without stirring in low-temperature chambers. During hardening and subsequent storage, already formed ice crystals and air bubbles increase in size, but new structural elements are no longer formed [20, 21]. The dispersion of structural elements of ice cream is determined mainly by their shape and size: the smaller and more evenly distributed they are in the total mass of ice cream, the better is the quality [21]. The shape and size of ice crystals and air bubbles largely depend on the speed of freezing and the degree of mechanical impact on the product. Therefore, the microstructure of the control and experimental ice cream samples during hardening was investigated (Figure 2).

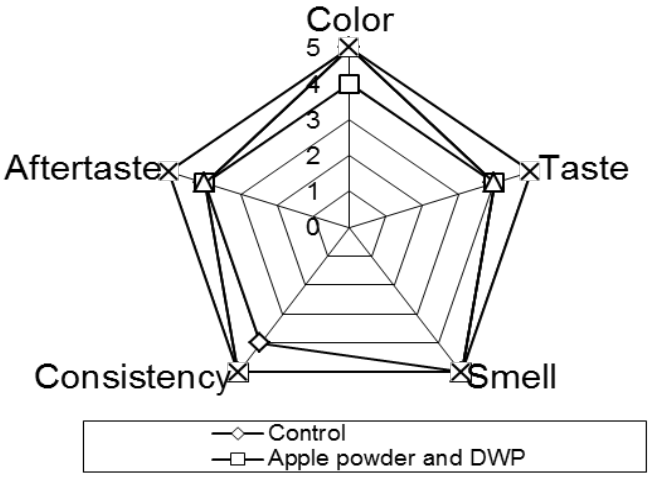

Fig. 1. **Organoleptic indicators of low-calorie milk ice cream.**

Table 1 – **Physical, chemical and microbiological indicators of low-calorie milk ice cream**

Indicator	DSTU 4733:2007	Ice cream with		
		apple powder and DWP	sesame flour and DWP	rice flour and WPC
Titrated acidity, °T	22	23±1	21±1	22±1
Active acidity, units of pH	–	6,622±0,043	6,982±0,026	7,012±0,013
Mass fraction of dry substances, %, including	29,0	29,5±0,3	30,9±0,5	29,8±0,2
Fat, %	4,0	4,3	4,3	4,3
DSMR, %	–	15,4	15,1	15,8
Sugar mass fraction, %	15,5	8,4	8,4	8,4
Water activity (a_w)	–	0,962±0,003	0,958±0,002	0,957±0,001
Cryoscopic temperature ($t_{кр}$), °C	–	–4,00±0,33	–4,42±0,16	–4,21±0,11
The amount of mesophilic aerobic and facultative anaerobic microorganisms, CFU in 1 g of ice cream	$1*10^5$	$4,5*10^4$	$5,0*10^4$	$4,0*10^4$
Bacteria of the coliform group in 0.1 g of ice cream	Not allowed	Not detected		
Yeasts and mold fungi	Not normalized	Not detected		

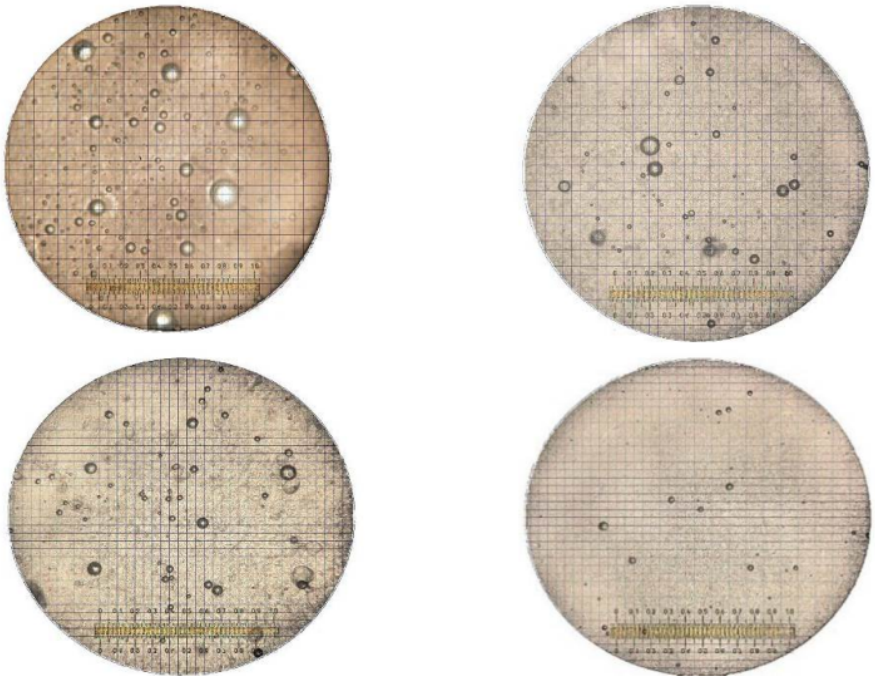

Fig. 2. **Microstructure of ice cream samples:** 1 – control; 2 – with apple powder and DWP; 3 – with sesame flour and DWP; 4 – with rice flour andWPC.

Study of the microstructure of control and experimental ice cream samples (Fig. 2) made it possible to conclude that the test samples are more saturated with air, and, therefore, their knockdown is higher compared to the control. The membranes of the air cells are not disturbed. The outer air bubbles are covered with a fat emulsion. Air bubbles in the test samples are homogeneous and evenly distributed over the entire surface of the ice cream. It should be noted that the weak stabilization of the fat globules of the control sample of ice cream due to the lower mass fraction of dry substances in the serum phase allows the liquid fat phase to penetrate into the space between them, forming clusters of milk fat (Fig. 2).

It is known that the size of air bubbles affects the consistency of ice cream [22]. It should be noted that the dimensions of the structural elements of ice cream are not directly regulated in regulatory documents. However, the size of air bubbles, in particular, is indirectly taken into account when evaluating the state of the structure and consistency of ice cream [20, 22]. However, the size of air bubbles, in particular, is indirectly taken into account when evaluating the state of the structure and consistency of ice cream [20, 22]. Therefore, the distribution of the air phase in the control and experimental ice cream samples was analyzed (Fig. 3).

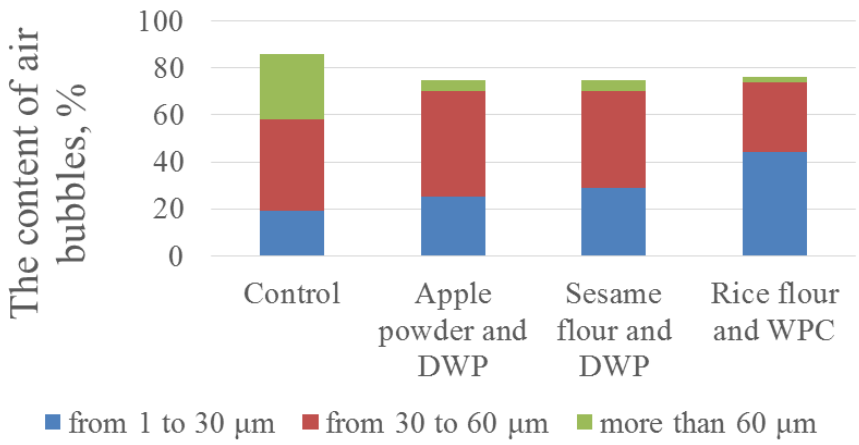

Fig. 3. **Air phase distribution in ice cream.**

Figure 3 shows that the content of finely dispersed air bubbles in samples of ice cream with rice flour and WPC, with sizes from 1 to 30 μm reaches 60 %, and bubbles with sizes larger than 60 μm are absent in the field of the studied microphotographs. Moreover, in experimental samples of ice cream with DWP and vegetable components (apple powder or sesame flour), the content of air bubbles with sizes from 30 to 60 μmis within 50–55 %, from 1 to 30 μm – within 40–45 %, At the same time, in the control sample of ice cream, the share of coarsely dispersed bubbles (D>60 μm) is about 30 %, finely dispersed bubbles (up to 30 μm) is only 25 %.

Therefore, the replacement of traditional stabilizers with WPC, DWP, rice flour and inulin in low-calorie milk ice cream does not increase the value of the water activity indicator, but, on the contrary, lowers this indicator. Moreover, the new components of low-calorie ice cream are good moisture-binding components, due to the use of which the amount of sugar has been reduced by a third in comparison with the traditional composition of ice cream. Based on the results of the microstructural analysis of low-calorie milk ice cream using milk processing products and vegetable ingredients, it can be stated that there is a tendency to increase the dispersion of the air phase in it.

Conclusion. The composition of low-calorie milk ice cream with the usage of protein and vegetable components was substantiated, and the organoleptic, physical, chemical and microbiological parameters were examined throughout the research. The expediency of using protein and plant components in recipes of low-calorie milk ice cream has been proven. It was established that the indicators of flavor, color and consistency of low-calorie milk ice cream, as well as mass fractions of dry substances and dry defatted substances, titrated and active acidity, the number of mesophilic aerobic and facultative-anaerobic microorganisms and the coliform bacteria were within the acceptable range according to DSTU 4733:2007. Studies of the low-calorie milk ice cream microstructure showed that its samples are more saturated with air, the membranes of air cells are not disturbed, the air bubbles are homogeneous and evenly distributed over the entire surface of the ice cream, and they are covered with a fat emulsion on the outside.

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Дослідження якості низькокалорійного молочного морозива

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Сучасний підхід до харчування передбачає необхідність створення нових видів морозива, корисніших для здоров'я, ніж традиційні. Вирішенням цього питання є створення морозива, що має низьку калорійність внаслідок зниження вмісту жиру та цукру і додавання різноманітних функціональних компонентів.

Метою роботи було дослідження впливу продуктів переробки молока та рослинних інгредієнтів на якість низькокалорійного молочного морозива.

Досліджено органолептичні, фізико-хімічні і мікробіологічні показники низькокалорійного молочного морозива. Встановлено, що за смаковими профілями дослідні зразки морозива мали гарні показники запаху і кольору за відсутності сторонніх запахів. За фізико-хімічними і мікробіологічними показниками зразки низькокалорійного молочного морозива відповідали вимогам ДСТУ 4733:2007. Дослідження мікроструктури низькокалорійного молочного морозива показали, що існує тенденція до підвищення дисперсності повітряної фази в його зразках. Показано, що нові складники низькокалорійного морозива є гарними вологозв'язувальними компонентами, за рахунок використання яких, зменшено на третину кількість цукру у порівнянні із традиційним складом морозива.

Обґрунтовано склад низькокалорійного молочного морозива з використанням білкових і рослинних компонентів та досліджено органолептичні, фізико-хімічні і мікробіологічні показники. Доведено доцільність використання білкових та рослинних компонентів у рецептурах низькокалорійного молочного морозива.

Ключові слова: низькокалорійне молочне морозиво, якість, органолептичні показники, фізико-хімічні показники, мікробіологічні показники, мікроструктура, продукти перероблення сироватки.

ЕКОЛОГІЯ

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Comparative analysis of the diversity of bees in agroecosystem habitats

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Biodiversity has a great importance on agroecosystems, since it determines their actual and potential productivity. Bees provide crucial ecological service in the agricultural landscape in most geographical regions because they are considered to be predominant and most economically important group of pollinators. The objective of the study was the assessment of bees (domestic, wild, bumblebees) diversity in different types of habitats in agroecosystems of Central Forest-Steppe zone of Ukraine. Bee communities were investigated in agrocenosis, semi-natural habitats and ecotones between on territories of 6 farms. In total were sampled 1131 individuals of bees that were presented by 60 species. Species composition, density and richness of bees were investigated. Indexes of Shannon, Simpson and Sorensen were used for biodiversity analysis. The results indicated that the species richness of bees grows by gradient: agrocenosis – semi-natural habitat – ecotone. The most common and spread species were *Apis mellifera* L., *Bombus lapidarius* L., *B. terrestris* L., *Halictus simplex* Blüthgen, *Systropha curvicornis* Scopoli, *Lasioglossum leucozonium* Schrank. Density of *Apidae* increases in agrocenosis and falls in semi-natural habitats. Forming of bees' fauna in agrocenosis depends on bees fauna of semi-natural habitats. Availability of ecotones promotes increasing of bee diversity in agroecosystems because it performs preservation function for biota and improves the spreading of bees and other species. The presented results could be used to predict changes in the formation of bee entomocomplexes in order to preserve their biodiversity.

Key words: agroecosystems, habitats, bees diversity, species richness, species density.

Introduction. The development of agriculture takes on a variety of forms, among which extensive land use tends to land transformation through the destruction and fragmentation of habitats. Such an approach can lead to a reduction of the biodiversity in agroecosystems. Over the past 50 years, intensification of agriculture has led to the disappearance of many wild plant and animal species both at the regional and national levels and has led to profound changes in the functioning of agroecosystems [8, 12].

Reducing the species diversity due to the intensification of agriculture can affect the sensitivity of agroecosystems to exogenous changes in the environment. The consequences of these processes are currently poorly investigated, but biodiversity is known to be of great importance for agroecosystems, since it determines their actual and potential productivity [6]. Unfortunately, the definition of an optimal level of biodiversity is very complicated, because the removal of pests, competitor species and pathogens can positively affect the productivity of agriculture, but at the same time reduce the resistance of agroecosystem to the impact of external environmental factors.

Preservation of biodiversity is one of the priorities of the state ecological policy in many countries, among which the relevance of implementation of continuous monitoring of quantitative and qualitative indicators of natural resources, as well as the creation of a system of scientifically based assessment of biodiversity objects using the ecosystem approach are indicated.

Considering the importance of invertebrate for continental ecosystems in the schemes of biomonitoring special attention is paid to insects. According to various scientific estimates, the percentage of insects in biota is from 53 to 75 %, and their total biomass exceeds the biomass of all other animals. Therefore, insects provide a significant portion of the biotic cycle of matter, energy and information in the biosphere, which determines the maintenance of environmental equilibrium. It is insects, such as cicadas [24], ants [17], butterflies [10], earthen bugs [16], locust [1], spiders [5], etc, are the most commonly used bioindicators of a satisfactory state of biota in general and of its zonal peculiarities in particular.

Bees (*Apidae, Sphecidae, Eumenidae, Pompilidae*) occupy a special place among entomoindicators. They are characterized by complex life histories and have specific requirements for foraging and nesting recourses [15]. They need habitats rich in flowering plants [3], as a large proportion of the species only collect pollen from certain plants. In addition, bees have specific nesting sites, such as dead wood, bare soil, plant stems or small rock cavities which should be close to feeding sites.

Bees provide crucial ecological service in the agricultural landscape in most geographical regions because they are considered to be predominant and most economically important group of pollinators [14]. A decline in bee diversity will affect the pollination of many insect-pollinated crops and wild plant species. Although the honeybee (*Apis mellifera L.*) is generally regarded as the most important bee pollinator [25], wild bees are also relevant [22]. There has been growing concern about suspected declines in wild bee populations and the implications for agricultural and natural ecosystems [11]. The role of the landscape context and of the land-use change on pollination has been comprehensively synthesized by Kremen et al. [13]. There is also a greater likely hood of toxicological effects of insecticides in agriculturally dominated landscapes [21].

With respect to farming systems, Holzschuh et al. (2010) demonstrated that organic farming increases bee diversity by enhancing flower availability. In addition, bee diversity was influenced by the landscape context and the interaction of both, organic farming being more effective in homogeneous landscapes [9].

Since 1962, the bee has increasingly been employed to monitor environmental pollution by heavy metals in territorial and urban surveys, pesticides in rural areas and radionuclide presence in the environment [4].

As a result of a number of studies, several features associated with agriculture management make farm poor habitat for bees and other pollinators. Intensification of agriculture has led to a more homogenous landscape, characterized by large crop fields and fewer non cultivated habitats. Loss of complex landscape elements between farmland and adjacent ecosystems, as well as the increased use of agrochemicals, has been linked to the reducing in richness of bee speciesin agroecosystems.

Locally, species richness and abundance depend on plant species richness and cover as well as on the habitat composition and diversity in the surrounding landscape [9, 22]. Furthermore, Schweiger et al. [19] showed in an extensive sampling across Europe that wild bee communities are first influenced by the land use intensity in a region, then by the landscape structure, i.e., the proportion of semi-natural elements in the landscape. Other investigations demonstrated a response of bees to field margins and boundaries, which suggests that they may be good indicators of agri-environmental schemes [6, 23]. By observed the decline in species richness and crop visitation rate for pollination in response to the distance to natural habitats for several crops worldwide, Ricketts et al. [18] emphasized the importance of conserving and managing sufficient resources for wild pollinators within the agricultural landscape to maintain the pollination services.

The bee thus enables us to throw light on a situation of environmental change and risk that otherwise would have remained hidden in shadow.

The aim of the study was the assessment of bees (domestic, wild, bumblebees) diversity in different types of habitats in agroecosystems of Central Forest-Steppe zone of Ukraine.

Materials and methods of recearch. Our study sites were located in Dniester-Dnipro province of Central Forest-Step of Podilska and Pridneprovska hills. The study sites were situated in Kiev region (villages Yablunivka, Bloschintsi, Terezine, Matyushi, Bugayivka, Karapishi) (Figure 1).

The habitat mapping method is based on generic system of habitat definitions “General Habitat Categories” [7]. We applied QGIS tool (GNU General Public License, http://qgis.org) for creating digital maps of surveyed habitats. Data validation was carried out in a field conditions.

At each farm, studied habitats were divided into 3 groups: agrocenoses – fields of winter wheat, soybeans, corn, barley, buckwheat; ecotones–ecotone between agrocenosis and single-row wind-protection trees, ecotone between agrocenosis and forest band, a grass band on a field road between agrocenoses; semi-natural territories – grasslands (Figures 2, 3).

Fig. 1. **Location of the case study area** (scale: 1:288980 m): 1 – Karapishi, 2 – Matyushi, 3 – Terezine, 4 – Bloschintsi,5 – Yablunivka, 6 – Bugayivka.

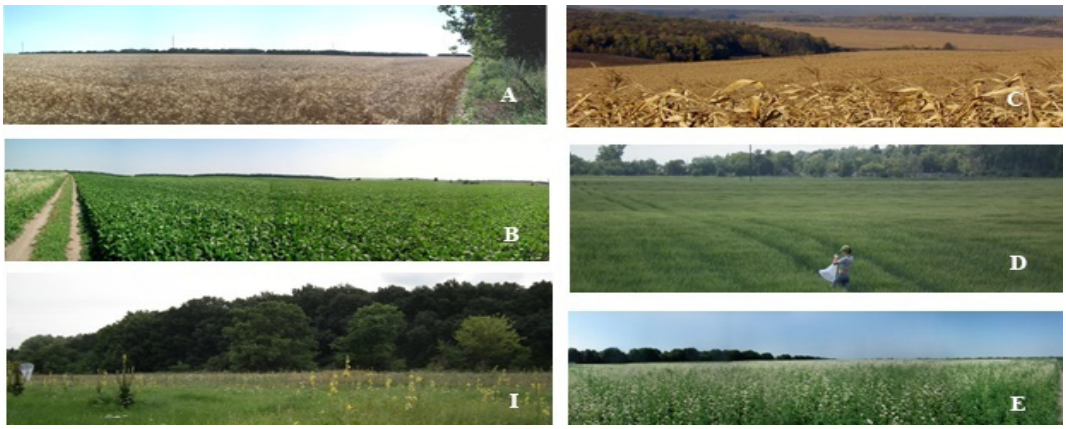

Fig. 2. **Agrocenosis and semi-natural habitats:** A – winter wheat, B – soybean, C – corn, D – barley, E – buckwheat, I – grassland (pasture).

Fig. 3. **Ecotones: F – between agrocenosis and single-row wind-protection trees, G –between agrocenosis and forest band, H – grassband on a field road between agrocenoses.**

The management was rather similar at all farms. Agro-chemicals are not applied on the grasslands, stocking rates are very low (0.15–1.75 LU/ha grassland). Zero or low inputs of fertilizers (15–50 t/4 year solid cattle manure or 20–30 kg N/ha/year inorganic fertilizer) and one or two pesticide applications are usual on the arable fields.

Bees were captured with an insect net. The aerial net method along transect ("belt") walks has been used for years in ecological studies [2]. Slow walks along 100 meters long surveyed each habitat/field plot and 2-meter-wide transect crossing the middle of the location of the vegetation. In case of shorter plots than 100 m, 2 x 50 m transects were surveyed. The transect walk lasted 15 minutes (the speed of walking was of about 6-7 m per minute). While walking, the collector caught all individual bees seen within the 2 m wide "belt" with a standard entomological aerial net.

Captured specimens were transferred into a vial with ether. Then samples of bees were pierced with an entomologic needle, brought to the laboratory and then accumulated before dispatch to a taxonomist for identification. Each sample was labeled with date, habitat name, conditions and place of sampling. When bees could be identified in the field (for example, domestic bees), they were registered and released. Particular attention was put on bee species of Anthophoridae and to a less extent Megachilidae because they are wasp-like in appearance.

Sampling was carried out only between 10.00 and 19.00 hours on days that are sunny, not too windy and a temperature higher than 15° C.

During the agriculture season, each plot of the farms was surveyed three times – in May, July and September. Transect walks were carried out in habitats when vegetation was present. One habitat was surveyed at different times of the day for each of three sampling dates (the start point of the route was changed for each survey). In agrocenosis transect walks were made during the growing season of the cultivated plant, in natural and semi-natural habitats – when vegetation height was >15 cm.

Species composition, density and richness of bees were investigated. Indexes of Shannon, Simpson and Sorensen were used for biodiversity analysis [20].

Results and discussion. Bee communities were investigated in agrocenosis, semi-natural habitats and ecotones between. In total were sampled 1131 individuals of bees that were presented by 60 species (Table 1).

The species richness of bees grows by gradient: agrocenosis – semi-natural habitat – ecotone. Increasing of species' richness was established in grass stripes on an agrocenosis edge close to forest bands, one-row wind-protection trees and meadows. In total 40 species were sampled in ecotones. The lowest number of bee species was found in agrocenosis (18 species), and medium species number – in semi-natural habitats (28).

The dominant speciesin agrocenosis, semi-natural habitats and ecotones was *Apis mellifera* L. with the highest density in agrocenosis (7.9 samples per 100 m^2) during blooming period. Other species were rare in fields. Some of them could be observed on specific plant species only. *Andrena pilipes*, *Megachile centuncularis* were found in soya cenosis only, likewise *Evylaeus leucopus*, *Lasioglossum sexnotatum*, *L. xanthopus* – could be seen in barley, and *Osmia cerinthidis*, *Sphecodes sp*. – in cenosis of buckwheat and alfalfa. At the same time *Osmia cerinthidis* is typical for South regions, it is not often found in north regions of Ukraine.

Ecotones on the edge of agrocenosis close to forest bands and meadows were presented by 40 species of bees. The most spread and common species were *Apis mellifera* (4.5 samples/100 m^2), *Bombus lapidarius* (0.8 samples/100 m^2), *B. terrestris* (0.8 samples/100 m^2), *Halictus simplex* (0.7 samples/100 m^2). We have found *Bombus argillaceus* (0.03 samples/100 m^2) from the Red List of Ukraine in grass stripes between agrocenosis and wind-protection trees.

Ecotones between agrocenosis were not rich for bee species and were presented by 5 species only. The most spread were *Systropha curvicornis* (0.5 samples/100 m^2), *Lasioglossum leucozonium* (0.3 samples/100 m^2). The same species were common for other types of ecotones.

Density of *Apidae* increases in agrocenosis and decreases in semi-natural habitats. The average density of *Apidae* was 1.0±0.21 samples/100 m^2in agrosenosis, 0.9±0.20 – in ecotones and 2.4±0.38 – in semi-natural habitats.

The comparative analysis showed the strong correlation between abundance of bees species and habitat affiliation to semi-naturalterritories (biotopes) (r=0.59). We have found the highest average numbers of bee species diversity in meadows and pastures (0.8 species per 100 m^2) that could be explained by the diversity of flowering plants (Fig. 4).

The lower level of average bee species diversity (0.2 species/100 m^2) and density (1.0 individual/100 m^2) were noticed in agrocenosis that linked to monoculture and agromanagement treats (mineral fertilizing, pesticides applying etc). At the same time, we have found the strong correlation between numbers of bee species and sizes of agrocenosis plots.

Table 1 – **Species composition of bees and bumblebees**

№	Species	Samples
1	*Ammobatoides abdominalis* (Eversmann, 1852)	2
2	*Andrena dorsata* (Kirby, 1802)	3
3	*Andrena flavipes* Panzer, 1799	19
4	*Andrena gelriae* van der Vecht, 1927	1
5	*Andrena haemorrhoa* (Fabricius, 1781)	1
6	*Andrena nanaeformis* Noskiewicz, 1925	1
7	*Andrena ovatula* (Kirby, 1802)	2
8	*Andrena pilipes* Fabricius 1781	2
9	*Andrena polita* Smith, 1847	5
10	*Andrena subopaca* Nylander, 1848	1
11	*Apis mellifera* L.	766
12	*Bombus muscorum* (Linnaeus, 1758)	1
13	*Bombus (Psithyrus) rupestris* (Fabricius, 1793)	1
14	*Bombus argillaceus* (Scopoli, 1763)	1
15	*Bombus lapidarius* (Linnaeus, 1758)	28
16	*Bombus pascuorum* (Scopoli, 1763)	9
17	*Bombus sylvarum* (Linnaeus, 1761)	5
18	*Bombus terrestris* (Linnaeus, 1758)	56
19	*Dasypoda altercator* (Harris, 1780)	1
20	*Eucera chrysopyga* Pérez, 1879	1
21	*Evylaeus calceatus* (Scopoli, 1763)	5
22	*Evylaeus laticeps* (Schenck, 1869)	8
23	*Evylaeus leucopus* (Kirby, 1802)	1
24	*Evylaeus malachurus* (Kirby, 1802)	1
25	*Evylaeus politus* (Schenck, 1853)	4
26	*Evylaeus sexstrigatus* (Schenck, 1870[", 1869"])	2
27	*Halictus maculatus* Smith, 1848	3
28	*Halictus quadricinctus* (Fabricius, 1776)	15
29	*Halictus sajoi* Blüthgen, 1923	10
30	*Halictus sexcinctus* (Fabricius, 1775)	3
31	*Halictus simplex* Blüthgen, 1923	43
32	*Halictus tetrazonius* (Klug, 1817)	1
33	*Hoplosmia spinulosa* (Kirby, 1802)	1
34	*Lasioglossum discum* (Smith, 1853)	14
35	*Lasioglossum laterale* (Brullé, 1832)	1
36	*Lasioglossum leucozonium* (Schrank, 1781)	8
37	*Lasioglossum majus* (Nylander, 1852)	2
38	*Lasioglossum sexnotatum* (Kirby, 1802)	1
39	*Lasioglossum sp.*	1
40	*Lasioglossum xanthopus* (Kirby, 1802)	1
41	*Lasioglossum zonulum* (Smith, 1848)	5
42	*Lithurgus cornutus* (Fabricius, 1787)	2
43	*Megachile centuncularis* (Linnaeus, 1758)	1
44	*Megachile melanopyga* Costa, 1863	1
45	*Melitta tricincta* Kirby, 1802	6
46	*Osmia cerinthidis* F.Morawitz, 1876	4
47	*Panurgus calcaratus* (Scopoli, 1763)	2
48	*Pseudoanthidium nanum* (Mocsáry, 1879)	2
49	*Rophites algirus* Pérez, 1895	5
50	*Rophites hartmanni* Friese, 1902	11
51	*Seladonia confusa* (Smith, 1853)	2
52	*Seladonia kessleri* (Bramson, 1879)	1
53	*Seladonia semitecta* (Morawitz, 1874)	2
54	*Seladonia subaurata* (Rossi, 1792)	3
55	*Seladonia tumulorum* (Linnaeus, 1758)	3
56	*Sphecodes albilabris* (Fabricius, 1793)	1
57	*Sphecodes sp.*	2
58	*Systropha curvicornis* (Scopoli, 1770)	33
59	*Systropha planidens* Giraud, 1861	13
60	*Tetralonia malvae* (Rossi, 1790)	1
	Total	1131

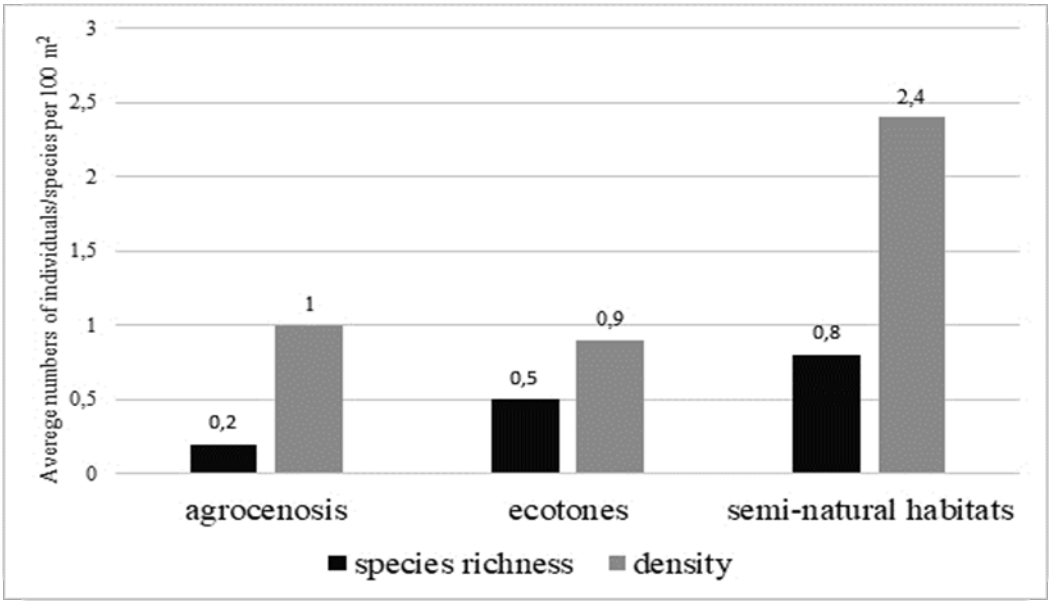

Fig. 4. **Species diversity and density of bees in different types of habitats.**

The average numbers of bee species diversity and density were about 0.5 species/100 m^2 and 0.9 individuals/100 m^2 in ecotones. Giving this, availabilityof ecotones promote increasing of bee diversity in agroecosystems because it performs preservation function for biota and improves the spreading of bees and other species.

In total 28 species of *Apidae* were found in semi-natural biotopes (habitats). The dominant species were *Apis mellifera* L. (12.1 individuals/100 m^2), *Bombus terrestris* Linnaeus (2.1 individuals/100 m^2), *Systropha curvicornis* Scopoli (1.3 individuals/100 m^2), *Halictus quadricinctus* Fabricius (0.9 individuals/100 m^2), *H. simplex* Blüthgen (0.8 individuals/100 m^2). Most of them we have also found in ecotones on the edge of fields close to forest bands, meadows and in gross stripes between fields.

The highest numbers of Shannon index for bees diversity were established in grass stripes between agrocenosis and forest bands, meadows (H=2.12). The species evenness in ecotones was J=0.76. Lower level of species diversity and higher level of evenness were found in agrocenosis (H=1.73, J=0.97). The lowest numbers of species diversity and evenness were noticed in semi-natural habitats (H=1.45, J=0.75).

The highest similarity of bees species were found in both agrocenosis and semi-natural habitats (Sorensen similarity index – 0.50).That points toward dependents of bees fauna forming in agrocenosis from fauna of semi-natural habitats. The decreasing of Sorensen index was established for semi-natural habitats and ecotones between them and agrocenosis (down to 0.3). The lowest similarities were observed in ecotones and agrocenosis.

The results obtained make it possible to assume that bees respond to changes in their environment and in particular to increased intensiveness of agriculture management. That makes them a reliable indicator and allows their use in biomonitoring of the environment.

Conclusion. The species richness of bees grows by gradient: agrocenosis – semi-natural habitat – ecotone and presented by 60 species in the observed farm territory of Kiev region (Dniester-Dnipro province of Central Forest-Step of Podilska and Pridneprovska hills). The most common and spread species are *Apis mellifera* L., *Bombus lapidarius* L., *B. terrestris* L., *Halictus simplex* Blüthgen, *Systropha curvicornis* Scopoli, *Lasioglossum leucozonium* Schrank.

Density of *Apidae* increases in agrocenosis and falls in semi-natural habitats. Forming of bees fauna in agrocenosis depends from fauna of semi-natural habitats. Availability of ecotones promotes increasing of bee diversity in agroecosystems because it performs preservation function for biota and improves the spreading of bees and other species.

The presented results could be usedto predict changes in the formation of bee entomocomplexes in order to preserve their biodiversity.

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Порівняльний аналіз різноманіття бджіл в оселищах антропогенного типу

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Відомо, що біорізноманіття має велике значення для агроекосистем, оскільки визначає їх реальну та потенційну продуктивність. Важливий екологічний сервіс у сільськогосподарських ландшафтах більшості географічних регіонів забезпечують бджоли, оскільки їх вважають переважною та найбільш економічно значущою групою запилювачів. Метою дослідження було оцінювання різноманіття бджіл (домашньої, диких, джмелів) у різних оселищах антропогенного типу Центрального Лісостепу України. Бджолині угруповання досліджували в

агроценозах, напівприродних біотопах та екотонах між ними на територіях 6 господарств. Всього було відібрано 1131 особин бджіл, які було представлено 60 видами. Досліджено видовий склад, щільність і видове багатство бджіл. Для аналізу біорізноманіття використовували індекси Шеннона, Сімпсона та Соренсена. Результати показали, що видове багатство бджіл зростає за градієнтом: агроценоз – напівприродне оселище – екотон. Найбільш поширеними видами були *Apis mellifera L., Bombus lapidarius L., B. terrestris L., Halictus simplex Blüthgen, Systropha curvicornis Scopoli, Lasioglossum leucozonium Schrank*. Щільність *Apidae* зростає в агроценозах і зменшується в напівприродних оселищах. Формування фауни бджіл в агроценозах залежить від їх різноманіття в напівприродних оселищах. Наявність екотонів сприяє збільшенню різноманіття бджіл в агроекосистемах, оскільки виконує функцію збереження біоти та покращує розповсюдження бджіл та інших видів. Представлені результати можуть бути використані для прогнозування змін у формуванні ентомокомплексів бджіл з метою збереження їх біорізноманіття.

Ключові слова: агроекосистеми, оселища, різноманіття бджіл, видове багатство, щільність особин.

ВОДНІ БІОРЕСУРСИ ТА АКВАКУЛЬТУРА

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Role of microorganisms of the aquatic environment in the formation of the ecological and sanitary state of water bodies

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Due recent years, humanity has faced the problem of lack of clean water for its needs, so the main goals of science are to increase the efficiency of natural resources that would not impair the quality of water. Chemical and biological methods have traditionally been used to assess water quality, but biological methods are more efficient because they are less costly and more informative. Biological control of water quality has a number of advantages over chemical and physical methods, since the grouping of living organisms mirrors all changes in the ecological state of the aquatic environment, while responding to a complex of various factors and pollutants. The method makes it possible to assess the consequences of both permanent and volley contamination. One of the most important biological methods for assessing the ecological state of reservoirs is the assessment of water quality by microbiological indicators, which is the first and most informative component of biota that responds to the allochthonous introduction of organic substances. Microorganisms are involved in optimizing the conditions of the aquatic environment, namely in the building of hydrological and gas regimes and in the self-cleaning of reservoirs, which ultimately determines the quality of water. Therefore, the assessment of the mechanisms of microbiocenosis formation makes it possible to determine the sanitary state of reservoirs by ensuring the functioning of the ecosystem with a bacterial link. The introduction of new technologies should not bring or form any threats to the environment, health and safety of human life, which is why an important task today is to study the impact of microorganisms on the formation of the ecological and sanitary state of aquatic ecosystems.

Key words: microorganisms, aquatic environment, water classes, bioindication, sapidity zones, heterotrophic bacteria.

Problem statement and analysis of recent research. It is known that the disposal and transformation of organic matter formed in the process of photosynthesis are carried out by microorganisms. They adapt quickly to the environment, and their short life cycle allows to trace the change in the structure of the population and its dynamics and the deviation of some metabolic processes under the influence of organic matter. One of the most important biological methods for assessing the ecological state of reservoirs [24, 26–30] is the assessment of water quality by microbiological indicators, since they are the first to respond to the alochtone of the introduction of organic substances [1].

As we know, water pollution is the cause of various diseases, so today the main goal is to determine the sanitary and epidemiological threats and the risks associated with them. The main source of pathogenic microorganisms of the aquatic environment is the inflow of wastewater [25]. The factors of changes in the aquatic environment can be anthropogenic or natural. Today, it is difficult to find a reservoir that does not suffer pollution as a result of human activity. The deterioration of the water quality of natural water bodies is an extremely serious problem for Ukraine. In the most of rivers and lakes receive insufficiently treated effluents from industrial enterprises, domestic effluents from cities and villages, effluents from

livestock farms, etc And here's the result: not only can we not drink water from most of our reservoirs without prior multi-stage water preparation, but swimming in them is sometimes dangerous to health. This is necessary not only to state the fact that water is clean or polluted, but also to develop a set of measures by local authorities and communities to improve the environmental situation on water bodies [2, 23].

For today, it is extremely important to increase the efficiency of natural resources without deteriorating the quality of the aquatic environment as a complex of abiotic and biotic factors necessary to obtain a high-quality food resource, namely, the microbial population is involved in optimizing the conditions of the aquatic environment, namely in the formation of hydrological, gas regimes and in the self-cleaning of reservoirs, which ultimately determines the quality of water [3, 21, 25].

The aim of the study is to analyze the role of microorganisms of the aquatic environment in the formation of the ecological and sanitary state of reservoirs.

Material and methods of research. Chemical and biological methods are used to assess water quality, the latter being more widely used because they are less costly and more informative.

Sanitary and microbiological assessment of water is carried out according to the following indicators: oxidability – the amount of oxygen dissolved in water; the total number of microorganisms is the number of colonies that grew by MPA with 1 ml of water at a temperature of 37 °C for 24 hours; coli-titer is the smallest volume of water (in milliliters), in which one E. coli is found; coli-index is the number of E. coli per 1 liter of water. Normal values for drinking water:Coli index ≤3 (in 1 liter); Coli titer ≥300; Microbial number ≤100 [4].

When determining water quality by microbiological indicators, the coli-titre and coli-index are calculated. The coli-titer of water is measured by the minimum amount of water (ml), in which Escherichia coli bacteria are found, and the coli-index by the number of these bacteria contained in 1 liter of the test water. These indicators are determined by the titration (fermentation) method or by the method of membrane filters. With the titration method, the isolation and identification of bacteria on nutrient media is carried out. When using the membrane filter method, water is first filtered using a Bunsen flask with a Seitz funnel with a membrane filter mounted in it and water is run through filters using a vacuum pump, which are then placed on the surface of the nutrient medium in Petri dishes and, after incubation in a thermostat, the number of colonies that have grown, typical of E. coli bacteria, is counted, and then identified using biochemical tests. Sanitary and microbiological control of drinking water quality. Sanitary and microbiological study of drinking water consists of determination of total microbial count (TMC), the number of Enterobacteria, spores of sulfite-reducing Clostridia and Coliphages [5, 6].

The presence of Coliphages (bacteriophages that parasite on *E.coli*) is determined in the water of surface sources and drinking water prepared from it, as well as in wastewater. They are indicators of the effectiveness of groundwater protection and drinking water treatment. The study is carried out using the Grazia agar layers method. To titrate the Coliphages, the test water is mixed with molten and cooled feed agar, where the E.coli indicator strain is also introduced. The resulting mixture is poured with a second layer onto a nutrient agar in a Petri dish and after solidification of the medium, the dish is incubated at 37 °C for 24 h. As a result, the indicator culture forms a uniform solid growth, and in the presence of Coliphages, transparent colonies are formed. The result of the study is expressed in colony-forming units (CFU/ml). Standards for drinking water: the number of microorganisms in 1 ml of water – no more than 50, coli-index – no more than 3 bacteria in 1 liter of water. Enterobacteriaceae and thermophilic bacteria should be absent in 300 ml of drinking water, spores of sulfite-reducing clostridia should be absent in 20 ml of water, and coliphages – in 100 ml of water.

Total microbial count (TMC) is the number of mesophilic aerobic and facultative anaerobic bacteria in 1 cc (1 ml) of water, which is determined in all types of water.

1 ml of the test water is introduced into two sterile Petri dishes and poured with molten and cooled feed agar (depth inoculation method). Incubate at 37 °C for 24 h, then at room temperature for another 24 h. Calculate the number of colonies on 2 plates (on the surface and in the depth of nutrient agar) and calculate the arithmetic mean value [8].

To detect mold and yeast fungi, the test water is seeded with 0.5 ml per Sabouraud medium and incubated at room temperature for 3–4 days. The number of colonies is calculated and the arithmetic mean is also calculated. The result of the total microbial number is calculated by summing the average arithmetic number of bacteria, yeasts and molds and expressed in colony-forming units (CFU/ml).

The total microbial count allows to estimate the level of microbial contamination of water, complementing the indicator of faecal contamination, and at the same time to detect pollution from

other sources, for example, industrial emissions. A sharp increase in TMC, even within the norm, detected repeatedly, is a signal for finding the cause of pollution. Also, this indicator is indispensable for the urgent detection of massive microbial contamination of unknown origin in drinking water.

When assessing the quality of water, the number of Enterobacteriais determined. The presence of *Enterobacteria* (coliform bacteria) is also determined in all types of water. The term "coliform bacteria" includes bacteria of the *Enterobacteriaceae* family and thermotolerant coliform bacteria.

Enterobacteriaceae bacteria include gram-negative asporogenic sticks that do not have oxidase activity and ferment lactose to form acid and gas at 37 °C for 24–48 hours (or ferment glucose to form acid and gas at 37 °C for 24 hours). Detection of *Enterobacteriaceae* in drinking water indicates a potential epidemic hazard of water use. The presence of bacteria of the genus *Escherichia* in food, water, soil, on the equipment indicates fresh faecal contamination of great sanitary and epidemiological importance. *Citrobacter and Enterobacter* are believed to be indicators of older (several weeks) faecal contamination and therefore have a lower sanitary and indicative value compared to *Escherichia*.

Thermotolerant coliform bacteria have the same characteristics, but additionally ferment the lactose to form acid and gas at 44.5 °C after 24 h. Thermotolerant coliform bacteria quickly die in the external environment, so their detection indicates fresh faecal contamination of water [9].

To determine the presence of Enterobacteriain drinking water, the method of membrane filters is used. The test water (3 samples of 100 ml) is passed through 3 bacterial filters of nitrocellulose, which are then placed on Endo medium and incubated at 37 °C for 24 h. Count the number of lactose-positive colonies identified as coliform bacteria grown on the filters. From 2–3 colonies of red color, swabs are prepared, stained according to Gram and oxidase activity is determined. For this purpose, the filter with the bacterial colonies grown on it is transferred with tweezers, without overturning, onto a patch of filter paper moistened with dimethyl-phenyldiamine. When oxidase is present, the indicator stains the colony blue. 2–3 colonies that did not change the original coloration are seeded in a semi-liquid medium with 0.5 % glucose solution. The crops are incubated for 24 hours at 37 °C. In the presence of gas formation, the number of red colonies on the filter is counted and the circle-index is determined.

Enterococcus bacteria are normal intestinal residents, but are excreted in the external environment in a smaller amount than *E. coli.* Enterococci are more likely to die in water and soil. As a rule, they do not reproduce in these objects, which allows them to be considered as an indicator of fresh faecal contamination. The presence of enterococci is considered an additional indicator of faecal contamination of water and other objects. However, their isolation requires more complex media in preparation and they grow more slowly.

Proteus bacteria live in both human and animal gut (*P.mirabilis*) and rotting residues (*P.vulgaris*). The presence of proteins in environmental objects indicates their contamination with decomposing substrates and extremely unfavorable sanitary conditions [10].

Also, in the environmental assessment of water, studies are carried out to identify spores of sulfite-reducing clostridia. Spores of sulfite-reducing clostridia are more resistant to decontamination and adverse environmental factors than other indicator bacteria. This feature is of particular importance in the evaluation of primary chlorination, since it inactivates almost all indicator bacteria. Detection of clostridia in water before entering the distribution network indicates insufficient purification and that decontamination-resistant pathogenic microorganisms are not likely to have died during purification. To determine the clostridium, the water under study is introduced into the molten and cooled medium of Wilson-Blair. The medium contains thiosulfate (hyposulfite) and a colourless iron salt. As a result of the germination of spores, the reproduction of clostridia and their reduction of sulfite, iron sulfide is formed, which gives the environment a black color [11].

Results and discussion. Traditionally, qualityof water is determined by chemical and bacteriological methods. Biological control of water quality has a number of advantages over chemical and physical methods, since the groups of living organisms mirror all changes in the ecological state of the aquatic environment, while responding to a complex of various factors and pollutants [12]. The method allows to assess the consequences of both permanent and salvo contamination, since it averages the "contamination effect" in time. The most common is the system of assessment of the ecological state of the reservoir and water quality, which is based on the study of the qualitative and quantitative composition of the types of indicators, that is, bioindication of fresh water. During biomonitoring, information about the state of the ecosystem ofthe water body is accumulated, changes occurring in it are detected, and measures are developed to improve its ecological state. To bioindicate the quality of the environment, those types of bioindicators living in a rather small range of environmental conditions are

selected. In the case of organizing and conducting environmental monitoring of the state of the reservoir, the use of bioindicators usually provides more valuable information than the assessment of pollution by chemical methods or special devices that determine only individual factors of pollution. Instead, bioindicator species respond to a complex of pollutants or general changes in external conditions. Various hydrobionts are used for biotesting – algae, microorganisms, invertebrates, fish. The most popular objects are juvenile *forms of* planktonic crustacean filters *Daphnia magna, Ceriodaphnia affinis*. For example, a seven-day test on daily young ceriodaphnia *Ceriodaphnia affinis* allows for a shorter period of time (7 days) than on *Daphnia magna* (21 days) to give a conclusion about chronic water toxicity [13, 14].

An important condition for the correct conduct of biotesting is the use of genetically homogeneous laboratory cultures, because they undergo sensitivity testing, are held in special laboratory conditions provided by the standards, which ensure the necessary reproducibility of research results, as well as maximum sensitivity to toxic substances.

Each group of organisms can be used as bioindicators of the state of the environment, but it is very important that the method is relatively cheap and fast. Therefore, the most extensive use is made of microbiological studies, since the earliest and most effective indicator that responds to the introduction of organic matter into the aquatic environment is the number of heterotrophic microorganisms Natural reservoirs are very different in terms of the quality of water, which is conventionally divided into several classes. I class of water quality – very clean, water of similar quality is mainly noted in mountain rivers and lakes, where the impact of humans on nature is still extremely small. II class of water quality – pure, the amount of nutrients in the water increases, but the oxygen regime remains quite favorable. There is a high species diversity of algae, clams, crustaceans, larvae and insects. The overgrowth of immersed plants is predominant, which are spread over significant areas of the water area. III class of water quality – contaminated. In such waters, the content of biogenic elements, organic matter, is significantly increased, as a result of which the bioproductivity of the reservoir increases dramatically. The consequence of this is the emergence of such a phenomenon as the "flowering" of water due to the mass development of microscopic algae, first of all blue-green. Class IV water quality – dirty, this class includes very silt water with poor oxygen regime, frequent phenomena of suffocation and low transparency of water. V class of water quality – very dirty. Determined in reservoirs where the concentration of dissolved oxygen is extremely low (less than 10 %), and the bottom sediments contain hydrogen sulfide. Aquatic plants and bottom macroinvertebrates are usually absent or very rare [15].

Water is not an environment conducive to the reproduction of pathogenic microbes, for which natural biotopes are human and animal organisms. The viability of pathogenic bacteria is affected by concomitant, competitive flora (antagonist microbes, phages, the simplest, algae), as well as temperature, insolation, various chemicals, etc [16, 17].

However quantitative and qualitative ratios in biocenoses are unstable and change under the influence of various factors, that is, they change in sapidity. The term "saprosity" (from Greek *Sapros* – rotten) denote a set of features of the reservoir, including the composition and amount of microorganisms in the water, which contains organic and inorganic substances in certain concentrations. As you know, sapidity is the ability of aquatic organisms to live in water containing different amounts of organic substances. The processes of self-purification of water in reservoirs occur sequentially and continuously, with a gradual change in biocenoses. Polysaprobic, mesosaprobic and oligosaprobic zones are distinguished. The probability zones are also determined by microbiological indicators:

Polysaprobic zones (heavily polluted areas) contain large amounts of organic substances that are easily decomposed, and are almost completely devoid of oxygen. Microbial biocenosis of similar zones is especially rich, but the species composition is limited by anaerobic bacteria, fungi, actinomycetes. The number of bacteria in 1 ml of water in the polysaprobic zone reaches a million or more. Very many saprophytic microflora, well-developed heterotrophic organisms: filamentous bacteria (Sphaerotilus); sulfurous bacteria (Beggiatoa, Thiothris); bacterial zoogles (Zoogloearamig era); the simplest − infusorias (Paramecium putrinum, *Vorticella putrina*); colorless hookworms; oligochaetes (Tubifex *tubifex, Polytomauvella water)*.

Mesosaprobic zones (zones of moderate pollution) are characterized by the dominance of oxidative and nitrification processes. The quality composition is varied. These are mainly nitrifying, obligate aerobic bacteria, as well as species *Clostridium, Pseudomonas, Mycobacterium, Streptomyces, Candida,* etc The total number of microorganisms is hundreds of thousands in 1 ml. Alpha mesosaprobe zone – aerobic decomposition of organic substances begins, ammonia, carbon dioxide is formed, oxygen is scarce, hydrogen sul-

fide and methane are absent, the number of saprophytic bacteria is determined by tens and hundreds of 1 ml, individual organisms develop in the mass such as bacterial zooglions; filamentous bacteria; fungi; from algae – oscillatorium, stigeoclonium, chlamydomonas, euglena. Beta mesosaprobe zone – there are no unstable organic substances, there is complete mineralization, saprophytes are thousands of cells in 1 ml, and their number sharply increases during the period of plant death, the content of oxygen and carbon dioxide fluctuates depending on the time of day, during the day excess of oxygen, carbon dioxide deficiency and at night vice versa.

Oligosaprobic zones (clean water zones) are characterized by a finished self-cleaning process, a small content of organic compounds and the end of the mineralization process. Water is distinguished by a high degree of purity. The number of bacteria is from 10 to 1000 in 1 ml of water [88]. These are practically pure water bodies. There is no flowering, the content of oxygen and carbon dioxide does not fluctuate.

Pathogenic microorganisms entering reservoirs are quite abundant in polysaprobic zones, gradually die in mesosaprobic zones and are practically not detected in oligosaprobic zones [18].

Our previous studies have shown that when assessing the water quality of fish ponds according to the ecological and sanitary classification when applying fertilizers, the water quality ranged from the class "clean", the discharge "sufficiently clean" to the class "contaminated", the discharge "conditionally contaminated – very contaminated", which corresponds to the β-oligosaprobic – λ-mesosaprobic zone. A comparative analysis of the average seasonal numbers of heterotrophic bacteria when fertilizing showed an increase of 42 % and 37 % compared to the control. Significant fluctuations in environmental and sanitary indicators of water quality when applying fertilizers indicate that microflora is a highly informative biological component of the aquatic ecosystem, which quickly responds to the application of both traditional and new fertilizers in fish farming. The assessment of the water quality of fish ponds according to the ecological and sanitary classification showed that fertilizers lead to the intensification of the development of bacterioflora and heterotrophic microorganisms, but does not lead to a deterioration in water quality [19].

Therefore, the sanitary and microbiological assessment of water is conditional and mandatory. Basic sanitary and microbiological methods are aimed at determining the total microbial contamination (total microbial count), detection of pathogenic microorganisms and their metabolites in the studied objects, determining the degree of water pollution. Practical sanitary microbiology uses two main methods of assessing the sanitary and epidemic state of the external environment: direct detection of pathogenic microorganisms and detection of indirect signs of pathogens in the external environment. According to the current regulatory documents, the following are subject to sanitary and microbiological supervision: drinking water (centralized and local water supply, i.e. from water supply, wells, springs and wells); pool water; open water (rivers, reservoirs); wastewater (domestic and fecal, industrial, melt and runoff); purified water for the preparation of medicines; medical and domestic ice.

The microflora of reservoirs are formed by two groups of microorganisms: autochthonous (constantly live and multiply in the reservoir) and allochthonous (get in from outside when contaminated with various sources). Microorganisms that have adapted to living conditions in the water and are regularly found in it can be considered water-specific flora. These include aerobic coca: micrococa, sarcins, *Serratiamarcescens, Bacillus cereus, Bacillus mycoides,* bacteria of the genera *Pseudomonas, Proteus, Leptospira*. The number of microorganisms in open water bodies varies widely: from several tens, hundreds to millions in 1 ml, which depends on the type of water body, the degree of its pollution, the seasons, etc The microflora of reservoirs depends on the substances contained in them and on biocenosis, that is, the species composition and abundance of other living creatures. Yes, phages and the simplest ones in the water destroy bacteria. Microorganisms capable of forming antibiotics cause the death of other bacteria sensitive to these substances.

Water microorganisms play a significant role in the circulation of substances in nature. They break down organic matter to form substrates that use other aqueous microorganisms in food. Biological activity in reservoirs is maximum in the summer-autumn period.

The number of microorganisms in the water of surface runoff in the spring flood period increases to 2.8–3 million in 1 ml. During the flood period, secondary contamination of the water supply network is possible. The microflora of economic and faecal wastewater consists of microorganisms isolated from the intestines of humans and animals, among which there are representatives of normal and opportunistic flora (Escherichia, enterococci, klebsiels, clostridia, fungi of the *genus Candida*, the simplest, etc), but can also be pathogenic – pathogens of intestinal infections (salmonella, shigel, vibrio, yersinia, leptospira, polio, hepatitis A, E viruses, etc). The danger of infection with

the latter is particularly great if insufficiently decontaminated wastewater from infectious diseases hospitals enters the reservoirs [20].

Conclusion. To assess the state of water bodies, it is the biological analysis that can be considered the most accurate and convenient. One of the most important biological methods for assessing the ecological state of reservoirs is to assess the quality of water precisely by microbiological indicators, since microorganisms are the first and most informative component of biota that responds to pollution. Maintaining this vector of studying and assessing the sanitary status of water bodies and solving problems regarding the purification of water bodies is the main task of the present.

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Роль мікроорганізмів водного середовища у формуванні еколого-санітарного стану водойм

Савенко Н.М., Присяжнюк Н. М.

Протягом останніх років людство стикнулося з проблемою браку чистої води для своїх потреб, тому основними завданнями науки є підвищення ефективності природних ресурсів, які б не погіршували якість води. Для оцінювання якості води традиційно використовуються хімічні та біологічні методи, проте біологічні методи є ефективнішими, оскільки вони менш затратні та більш інформативні. Біологічний контроль якості води має ряд переваг перед хімічними і фізичними методами, оскільки угрупування живих організмів віддзеркалює усі зміни екологічного стану водного середовища, одночасно реагуючи на комплекс різноманітних чинників і забруднювачів. Метод дає змогу оцінити наслідки як постійного, так і залпового забруднення. Одним із найважливіших біологічних методів оцінювання екологічного стану водойм є оцінювання якості води за мікробіологічними показниками, що є найпершим і найінформативнішим компонентом біоти, який реагує на алохтонне внесення органічних речовин. Мікроорганізми беруть участь у оптимізації умов водного середовища, зокрема у формуванні гідрологічного та газового режимів та в самоочищенні водойм, що в кінцевому результаті і визначає якість води. Тому оцінювання механізмів формування мікробіоценозу дає можливість визначати санітарний стан водойм забезпечення бактеріальною ланкою функціонування екосистеми. Впровадження нових технологій не повинно приносити чи формувати будь яких загроз довкіллю, здоров'ю та безпеці життєдіяльності людини, саме тому важливим завданням сьогодення є вивчення впливу мікроорганізмів на становлення еколого-санітарного стану водних екосистем.

Ключові слова: мікроорганізми, водне середовище, класи води, біоіндекація, зони сапробності, гетеротрофні бактерії.

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