ТЕХНОЛОГІЯ ВИРОБНИЦТВА І ПЕРЕРОБКИ ПРОДУКЦІЇ ТВАРИННИЦТВА

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Fatty acid composition of quail blood erythrocyte membranes under condition of feeding sodium selenite and nanoselenium

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Infectious diseases are a significant problem in poultry farming, because they significantly slow down the growth rate of the industry and lead to losses. For the productive development of the industry and the avoidance of danger to the health of consumers due to the uncontrolled use of these drugs, it is worth using alternative methods of protecting poultry and improving the quality of the obtained products, in particular drugs obtained by nanotechnological means. Nowadays, nanoparticles of various minerals are used in poultry farming, the mineral antagonism of which is reduced in the intestines, compared to preparations of the usual size, which contributes to the modulation of absorption mechanisms, optimization of the immune response, and increased efficiency of digestion.

Oxidative stress is a harmful factor for cellular integrity due to the constant release of reactive forms of oxygen. Se is widely used as a supplement to reduce oxidative stress. The element is found in organic and inorganic compounds, replaces Sulfur in protein molecules and is an important part of selenoproteins. Se is known for its antioxidant activity, plays a major role in optimizing redox potential, reproductive processes, hormone metabolism, muscle development, and anticarcinogenesis. Nano-Se results in higher Se retention activity due to smaller size and greater bioavailability.

Biogenic selenium nanoparticles (SeNPs), synthesized with the participation of bacteria, have unique physicochemical and biological properties compared to inorganic and organic compounds, and nanoselenium-enriched probiotic bacteria can be effectively used as an alternative to other forms of selenium as food and feed additives.

The work compared the composition of fatty acids of lipids extracted from erythrocytes of quail blood, which were injected with sodium selenite and pro-oxidant nano-Se. With the introduction of nanoselenium, a decrease in the level of NFAs was noted, as well as a significant increase in the level of the main PUFAs. The lack of compensatory accumulation of docosapolyene fatty acids in the cell membranes of erythrocytes of birds of the 2nd group can be considered as a factor of the beneficial effect of the administered drug, as well as a justification for the need to prescribe complex drugs to birds capable of modulating the fatty acid composition of cell membranes. The effectiveness of using a complex nanopreparation consists in normalizing the level of fatty acids and restoring their metabolism at the stage of eicosanoid formation.

Key words: fatty acids, quails, peroxide oxidation, selenium, sodium selenite, nanoselenium, nanoparticles, lipids.

Problem statement and analysis of recent research. Infectious diseases are a significant problem today in poultry farming, because they significantly slow down the growth rate of the industry and lead to losses. Vaccines and antibiotics, which are currently used to fight pathogenic microorganisms, can pose a danger to the health of consumers due to the uncontrolled use of these drugs. For the productive development of the poultry industry, it is worth using alternative methods of poultry protection and improving the quality of the obtained products, in particular drugs obtained by nanotechnology [5].

Currently, poultry farming uses nanoparticles of various minerals (Argentum, zinc oxide [5], cerium dioxide, copper [27], Ferrum, Selenium [5]), the potential of which has not yet been fully utilized due to insufficient knowledge. Mineral compounds are scarcely available from a biological point of view for animals, and in the nanoform, mineral antagonism in the intestines is reduced, which contributes to the modulation of absorption mechanisms, optimization of the immune response of the bird's body and increased digestion efficiency [25], reduction of cases of early embryonic mortality [16].

Nanomaterials, as biosensors, are used to obtain information about the course of various types of metabolism in tissues and cells, and thanks to the ultra-sensitive determination of the content of nutrients, their metabolites, and the activity of biologically active compounds, they increase the understanding of the nature of the interaction of these substances [16], their bioavailability, and food evaluation of the obtained products [25].

Nanoparticles (NPs) enter the body from food or water and through parenteral administration of nanopreparations [20]. Their bioavailability usually decreases when passing through the gastrointestinal barriers, intestinal mucosa and liver, and with direct introduction into the systemic circulation by parenteral injections, bioavailability is 100%. Depending on the size, nanoparticles can transit through the digestive tract without being absorbed by the body, or penetrate through the intestine and reach organs and tissues with the blood flow [23]. In monogastric animals, the mechanism of conversion of nanosized Se preparations into selenite assumes that the intestinal microbiota converts nano-Se into selenite, Se-phosphate or H_2Se, which ultimately leads to the synthesis of selenoproteins [20].

Addition of nanosilver and inorganic Se to the diet does not cause weight gain, feed consumption and changes in feed conversion ratio, but increases relative weight of liver and small intestine ($p<0.05$) of broilers [23]. Feeding Selenium nanoaquachelates with vitamin E had a positive effect on calcium-phosphorus metabolism in laying hens.

There are data on the effect of Selenium on enterocytes [14], in particular its effect on the reduction of intestinal tumorigenesis in multiple intestinal neoplastic mutations. Selenium supplementation can be hypothesized to have some effect on intestinal growth, including cellular differentiation of the intestinal mucosal epithelium. An important effect of Selenium on breeding chickens due to increased resistance to oxidative stress [12] and increased resistance and immunological status of the organism was revealed. There are reports that, in addition to having a positive effect on intestinal morphology, Selenium is able to improve the composition of the intestinal microbiota and act as an antioxidant.

Oxidative stress is a detrimental factor for cellular integrity due to the constant release of reactive oxygen species mediated by various biotic (bacteria, viruses, fungi, etc.) and abiotic stressors. Such a trace element as Selenium with a powerful antioxidant potential is widely used as a feed additive to reduce oxidative stress in living systems [3; 8; 11]. Selenium is widely found in organic and inorganic compounds [8]. It replaces Sulfur in protein molecules and is an important part of a number of enzymes (selenoproteins). Se is mainly known for its antioxidant activity and plays a major role in redox potential optimization, reproductive processes, thyroid hormone metabolism, muscle development, and anticarcinogenesis [18]. Nano-Se results in higher Se retention activity due to smaller size and greater bioavailability [15]. Glutathione peroxidase is the first selenoprotein discovered in biological systems with antioxidant activity [8].

Biogenic selenium nanoparticles (SeNPs), synthesized with the participation of bacteria, have unique physicochemical and biological properties compared to inorganic and organic compounds. Nanoselenium-enriched probiotic bacteria can be effectively used as an alternative to other forms of selenium as food and feed additives [28].

It has been established that biogenic selenium nanoparticles affect the redox-sensitive transcription factor Nrf2 (Keap1/Nrf2/ARE signaling), which activates gene expression and the synthesis of a number of antioxidant and cytoprotective proteins, including quinone oxidoreductase, glutathione peroxidase, heme oxygenase-1, glutathione-S-transferase, gamma-glutamylcysteine synthetase, glutathione reductase and superoxide dismutase [2; 24]. Biogenic nanoselenium particles activate the Nrf2-ARE system through p38, ERK1/2, and AKT-mediated phosphorylation of

Nrf2 to improve the antioxidant function of intestinal epithelial cells [24].

The addition of nano-Se is used in poultry diets to monitor the intensity of growth, redox and immune processes. The addition of nanoselenium improves the reproductive performance of poultry [28]. Nano-Se showed better results on body weight gain compared to sodium selenite in broiler diets [18]. Similar results were also observed when 0.3 mg/kg of Se was added to the basic diet in the form of nanoelemental Se, sodium selenite or selenium-containing yeast [1; 3; 15]. The combination of probiotics and Se nanoparticles also showed improvement in growth, skeletal muscle fatty acid profile, and serum α-tocopherol content in broilers. Nano-Se optimized the antioxidant status through the effect on the activity of antioxidant enzymes and increased the level of IgG and IgM compared to organic and inorganic Se compounds under conditions of oxidative stress [4] in chickens and thermal stress [11] in broilers, while improving growth and immunity indicators, activating the expression of cytokine genes.

Recent studies have focused on the use of Selenium to engage the antioxidant defense system [4; 6], however, there are quite a few reports on the use of nanoscale preparations of Selenium. Thus, feeding nano-Se to broilers significantly increases the activity of GSH-Px and superoxide dismutase (SOD) in blood serum and reduces the concentration of malondialdehyde [11]. Nano-Se increases the antioxidant capacity of the liver due to a decrease in the amount of oxidized GSH-Px in the liver. Selenium compounds normalize blood biochemical parameters in case of intoxication [7] and, according to current data, Selenium can modulate viral diseases, including COVID-19.

The aim of the research was to determine the composition of fatty acids of lipids, as the main substrate of peroxidation, extracted from erythrocytes of the blood of quails, which were injected with sodium selenite and nanoselenium.

Material and methods of research. The study used sodium selenite (a traditional mineral supplement for poultry diets) and a new nanopreparation of Selenium, obtained using strains of *L. plantarum* cultures provided from the collection of microorganisms of the Institute of Microbiology and Virologynamed after D.K. Zabolotny. In our previous studies, we evaluated the stability of nanoselenium, investigated acute and chronic toxicity in laboratory animals, and characterized the nanopreparation using transmission electron microscopy (TEM), which indicated the non-toxicity and stability of the synthesized nanopreparation.

In the future, comprehensive production studies were conducted on quails in order to study the lipid metabolism in the bird's body and compare the effects of traditional and new forms of selenium. During the research, the general principles of bioethics, legal norms and requirements were followed. In the case of studying the biological effect of various forms of Selenium, 120 quails of the Pharaoh breed were selected at the age of 1-day-old and 2 groups were formed using the method of analogues - a control group (received sodium selenite) and an experimental group (received nanoselenium) of 60 heads each. The conditions of keeping quails were the same and corresponded to zootechnical standards. The quails were kept in cages with a local heating system, fed compound feed according to recipes according to age. Poultry received complete compound feed with the addition of sodium selenite (group 1) and biogenic nanoselenium (strains of *L. Plantarum* cultures previously grown on a Selenium-enriched medium) (group 2) by multistage mixing. The dosage of Selenium drugs corresponds to the established effective amounts according to previous scientific studies [22] and was 0.3 mg/kg of feed.

For research, biological material was collected from 5 poultry heads at the same time to exclude daily fluctuations of physiological and biochemical parameters.

The extraction of lipids from plasma and blood cells was carried out with a chloroform-methanol mixture in a volume ratio of 2:1 [26]. Separation of lipids into fractions was carried out on thin-layer silica gel plates in the solvent system hexane : diethyl ether : glacial acetic acid (85 : 15 : 1). Detection of individual fractions of lipids on both plates was carried out in iodine vapor. The isolated lipid fractions were removed from the first plate and, after adding potassium dichromate solution to them, colorimetrically, and transesterified from the second plate. The identification of lipid fractions on the plates was carried out using standard lipids with the degree of purification of ChC. According to the results of thin-layer chromatography, the content of individual classes of lipids of the first plate was calculated according to the formula with correction coefficients for each studied fraction. These coefficients were calculated as the ratio of the extinction of the non-esterified form of cholesterol (internal standard) and the extinction of the investigated lipid fractions. Transesterification of lipids from the second plate was carried out by dissolving them in hexane. Later, a 5% solution of sodium methylate in methanol was added to the obtained hexane solution of lipids in a test tube and shaken for 3–4 minutes. After separating the contents of the test tube, the upper layer was removed with an automatic pipette, concentrated and introduced into the evaporator of a gas-liquid chromatographic apparatus, which has a column selective for long-chain fatty acids. A gas-liquid chromatograph (Chrom-5, Praha) with a steel column of 3700×3 mm was used for studies of methyl esters of fatty acids. The calculation of the content of individual fatty acids based on the results of gas chromatographic analysis was carried out according to the formula, which includes correction factors for each studied fatty acid [2626]. These coefficients were found as the ratio of the peak area of heptadecano (internal standard) and the studied fatty acid at a concentration of 1:1 in the isothermal mode of the chromatograph.

Variational and statistical processing of the data was carried out using the Microsoft Excel program according to the formulas created by us. Arithmetic mean value (M), standard error (m) and mean square deviation (σ) were determined. The reliability of changes was determined by Student's t-test.

Research results and discussion. The normal metabolism of the body depends on many factors, in particular, on the content of lipids and fatty substances. Being energy-intensive substances, lipids at the same time perform a number of other vital functions and can cause metabolic disorders if their amount or individual fractions ratio in the diet is incorrect. In particular, cholesterol is a substance from the group of sterols, which is found in large quantities in nervous and fatty tissues, the liver and is a precursor of steroid hormones, bile acids, lipoproteins and vitamin D. Its excess in the body leads to the formation of gallstones and cholesterol plaques in blood vessels. Triglycerols - esters of glycerol and VLC, are a natural reserve of fatty acids that participate in the synthesis of triglycerides, phospholipids, hormones, ketone bodies, are a blood vehicle, the main component of natural skin lubrication and the most important form of energy storage. Fatty acids are the basis for the formation of phospholipids, form cell membranes, and are the precursors of prostaglandins and leukotrienes.

Our previous studies had established that the addition of selenium preparations to the quails' diet causes a tendency to decrease the content of lipid spectrum indicators, as well as total lipids, while the use of nanoselenium provokes a more pronounced drop in the content of total lipids and their fractions, in particular, cholesterol and triacylglycerols in the blood of quails, which is consistent with data from the literature [13].

It is known that microorganisms in the digestive tract can take part in the metabolism of cholesterol in the body, acting on the cellular systems of the host enzyme that synthesize endogenous cholesterol. The hypocholesterolemic activity of strains of lactic acid bacteria L. casei IBM-7280 [19] was established in in vivo experiments on a mouse model. Studies [17] found that plasma cholesterol and triglycerides were reduced, while high-density lipoprotein (HDL) was increased with probiotic and selenium supplementation. Perhaps this is due to the activation of lipolysis to supply Selenium.

The use of Selenium preparations also resulted in a tendency to increase the content of Calcium and Phosphorus in the blood serum of experimental quails in our previous studies, a significant change was the use of sodium selenite ($p<0.05$). In the case of stimulation of the metabolic activity of probiotic preparations, low-molecular fatty acids (acetate, lactate, propionate, butyrate) are formed, which lower the pH in the intestines, promote the assimilation of Calcium, Magnesium, and Ferrum [6].

Research [10], in which probiotics were added, showed a decrease in the content of triglycerides and cholesterol in the blood. It has been suggested that some bacterial probiotic strains can incorporate cholesterol into bacterial cells and hydrolyze bile salts that inhibit the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which plays a key role in the synthesis of this sterol. limiting the rate of cholesterologenesis and reducing the total pool of cholesterol in the body.

It is believed that probiotics containing specific microorganisms contribute to the transformation and assimilation of nano-Se in the intestine [21]. One of the possible mechanisms of nano-Se transformation may be mediated by the action of microbiotics in the intestine, which are able to transform nano-Se into selenite, H2Se or Se-phosphate with further synthesis of selenoproteins [21].

Polyenoic fatty acids (FA) are not synthesized in the body, but are irreplaceable vitamin-like factors that enter with feed. Their normal content depends on the mobility of membranes and their performance of physiological functions (differential permeability, active transport of ions and metabolites, protective and support functions, participation in the transmission of excitation, contractility, etc.). Damage to structural components of membranes in the process of LPO negatively affects their functions.

The aim of our current research was to determine the composition of fatty acids of lipids extracted from erythrocytes of the quails blood, which were injected with the studied drugs. Since both the traditional mineral preparation of Selenium and the newest nanoforms of the element showed a positive effect on the metabolic processes in the body of quails, it was decided to compare the intensity of their effects. Table 1 shows data on the content of individual fatty acids in the membranes of erythrocytes of birds of the experimental and control groups. In birds of the 1st group (received sodium selenite), compared to the 2nd group (nanoselenium reseived), the percentage content of acids 14:0, 16:0 and 18:0 probably decreases. At the same time, the level of acids 20:4 (ω-3), 22:3, 22:5 (ω-3) and 22:6 (ω-3) increases significantly. Moreover, the percentage of 22:6 (ω-3) in the erythrocyte membranes of quails of the 1st group is 3.2 times higher than that of the 2nd group, and 20:4 (ω-6) by 33.6%. Therefore, in the bird that was injected with pro-oxidant nanoselenium, there is a decrease in the level of NFAs, as well as a significant increase in the level of the main PUFAs.

It is known that erythrocytes do not have their own genetic apparatus that regulates the biosynthesis of fatty acids, which are transferred to these cells with the help of very low-density lipoproteins, mainly from the liver, where their synthesis takes place. Only NFAs and monoene fatty acids can be synthesized de novo in the body of humans and animals. A decrease in the content of polyunsaturated fatty acids during oxidative stress can occur due to a decrease in the intensity of their de novo synthesis. As for the main PUFAs of the n-3 and n-6 series, they are not synthesized in the animal body from NFAs and MNFAs.

The detected imbalance of fatty acids due to the introduction of sodium selenite may be associated with a violation of the formation of fatty acids under the influence of elongases and desaturases. After the introduction of the complex drug, a normalization of lipid parameters is observed in the fatty acid composition of erythrocyte lipids, primarily due to a change in the level of palmitic, arachidonic, stearic, eicosate, and docosapolyene fatty acids.

The lack of compensatory accumulation of docosapolyene fatty acids in the cell membranes of erythrocytes of birds of the 2nd group can be considered as a factor of the beneficial effect of the administered drug, as well as as a justification for the need to prescribe complex drugs to birds capable of modulating the fatty acid composition of cell membranes.

Oxidative stress is accompanied by the release of catecholamines and glucocorticoids from the adrenal glands into the blood. Catecholamines, when interacting with β-adrenoceptors on the surface of the plasma membrane, activate signal transduction reactions. Under the influence of phospholipase, the release of PUFAs from phosphatidyllinositol and phosphatidylcholine occurs. These fatty acids are able to cause repression of genes that control β-oxidation of fatty acids and the expression of proteins involved in the synthesis of *de novo* lipids [9]. Endogenic fatty acid imbalance can be preceded by changes in other lipid classes, including cholesterol. Since PUFAs are ligands for some transcription factors that regulate the genetic level of synthesis and lipid catabolism, fatty acids may be an independent factor in various diseases.

Table 1 – **Distribution of fatty acids in the membranes of erythrocytes of quails of the experimental groups, mol % to the total content of fatty acids,** M±m

Fatty acids and their codes	1. Sodium selenite	2. For the addition of bionano-selenium
Myristic $C_{14:0}$	0,39±0,05**	0,78±0,08
Pentadecanoic $C_{15:0}$	0,18±0,01	0,17±0,03
Isopalmitic $C_{16:0}$	0,61±0,08	0,56±0,06
Palmitic $C_{16:0}$	30,1±1,2*	37,8±2,6
Palmitic $C_{16:0}$	5,76±1,1	6,28±0,5
Margaric $C_{17:0}$	0,45±0,05	0,48±0,04
Stearic $C_{18:0}$	12,0±1,2*	17,1±1,4
Oleic $C_{18:1}$	25,2±4,3	24,2±2,5
Linoleic $C_{18:2}$	12,0±2,5	11,1±1,6
Eicosatrienoic $C_{20:3}$	0,68±0,05**	0,19±0,05
Arachidonic $C_{20:4}$	2,74±0,3*	1,82±0,2
Docosatrienoic $C_{22:3}$	0,28±0,04*	0,15±0,03
Docosapentaenoic $C_{22:5}$	0,28±0,06*	0,12±0,02
Docosahexaenoic $C_{22:6}$	0,16±0,04*	0,05±0,01

Conclusions. Increasing percentage content of 22: 5 (ω-3), 22: 6 (ω-3) in erythrocyte erythrocyte membranes are a factor in protection against peroxide damage. The main intracellular depot of some other PUFAs are phosphatidyllinositol and phosphatidyletanolamine. The imbalance of fatty acids in the erythrocytes of the birds, which were administered sodium selenitis, is characterized by a decrease in the number of major saturated fatty acids, as well as an increase in the content of polyunsaturated fatty acids (arachidonic and docosahexaenoic). The effectiveness of the use of a complex drug is to normalize the level of fatty acids and restore their metabolism at the stage of formation of eicosanoids.

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Жирнокислотний склад мембран еритроцитів крові перепелів

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Значною проблемою у птахівництві є інфекційні захворювання, адже вони суттєво уповільнюють темпи зростання галузі та призводять до збитків. Для продуктивного розвитку галузі та уникнення небезпеки для здоров'я споживачів за неконтрольованого застосування препаратів варто використовувати альтернативні методи захисту птиці та поліпшення якості отриманої продукції, зокрема препарати, отримані нанотехнологічним шляхом. Нині у птахівництві використовуються наночастинки різних мінералів, мінеральний антагонізм яких у кишківнику знижується, порівняно із препаратами звичайного розміру, що сприяє модуляції механізмів всмоктування, оптимізації імунної відповіді та підвищенню ефективності травлення.

Оксидативний стрес є згубним фактором для клітинної цілісності внаслідок постійного вивільнення реактивних форм оксигену. Se має широке застосування як добавка для зменшення оксидативного стресу. Елемент зустрічається в органічних та неорганічних сполуках, заміщує сірку в білкових молекулах і є важливою частиною селенопротеїнів. Se відомий своєю антиоксидантною дією, відіграє головну роль в оптимізації редокс-потенціалу, репродуктивних процесах, метаболізмі гормонів, розвитку м’язів та антиканцерогенезі. Nano-Se зумовлює вищу активність утримання Se внаслідок менших розмірів та більшої біодоступності.

Біогенні наночастинки Селену (SeNPs), синтезовані за участю бактерій, мають унікальні фізико-хімічні та біологічні властивості порівняно з неорганічними і органічними сполуками, а збагачені наноселеном пробіотичні бактерії можуть ефективно застосовуватися як альтернатива для інших форм Селену у складі харчових і кормових добавок.

У роботі порівнювався склад жирних кислот ліпідів, екстрагованих з еритроцитів крові перепелів, яким вводили селеніт натрію та прооксидантний нано-Se. За введення наноселену спостерігали зниження рівня НЖК, а також суттєве зростання рівня основних ПНЖК. Відсутність компенсаторного накопичення докозаполієнових жирних кислот у клітинних мембранах еритроцитів птиці 2-ої групи можна розглядати як фактор сприятливого впливу введеного препарату, а також як обґрунтування необхідності призначення птиці комплексних препаратів, що здатні модулювати склад жирних кислот мембран клітин. Ефективність використання комплексного нанопрепарату полягає в нормалізації рівня жирних кислот і відновленні їх метаболізму на стадії утворення ейкозаноїдів.

Ключові слова: жирні кислоти, перепели, пероксидне окиснення, селен, селеніт натрію, наноселен, наночастинки, ліпіди.

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