БІОТЕХНОЛОГІЇ ТА БІОІНЖЕНЕРІЯ

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Effect of using nanoselenium bioconjugates together with probiotics on metabolic parameters of quail

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Бітюцький В.С., Цехмістренко С.І., Харчишин В.М., Мельниченко Ю.О., Тимошок Н.О. Вплив використання біокон'югатів наноселену разом з пробіотиками на метаболічні показники організму перепелів. Збірник наукових праць «Технологія виробництва і переробки продукції тваринництва», 2024. № 1. С. 81–88.

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In the context of the modern industrialisation of poultry farming and the growing global demand for quail products, finding effective ways to improve bird productivity and health is extremely important. One of the most promising approaches is the use of nanoselenium bio-compounds with probiotics to improve the metabolic parameters of quail. Recent research has focused on the synthesis of selenium nanoparticles using probiotics as an environmentally friendly alternative to traditional methods of adding inorganic selenium to quail feed. The advantage of this approach is the production of a biocompatible and bioavailable form of selenium, which provides birds with the ability to effectively absorb and use selenium for various physiological processes.

The effect of innovative feed additives, such as selenium nanoconjugates and probiotics, on various metabolic parameters in quail was investigated. These include the activity of antioxidant defence enzymes, indicators of carbonyl oxidative stress, protein carbonyl levels and protein metabolism. By adding selenium nanoconjugates and probiotics to quail feed, an improvement in antioxidant defence mechanisms was observed, leading to a reduction in oxidative stress and an improvement in the overall health of the birds. In addition, improved protein metabolism as a result of these supplements has been shown to have a positive impact on the productivity and quality of quail products. In summary, investing in high quality feed additives such as selenium nanoconjugates and probiotics is a strategic approach to improving the productivity and profitability of poultry production. By taking advantage of the benefits of nanotechnology and probiotics, farmers can optimise the health and productivity of their poultry flocks, meeting the growing demand for quail products on the national market.

Keywords: bionanotechnology, nanoselenium conjugates, biogenic synthesis, quercetin, quail, blood, liver, biochemical parameters, oxidative modification of proteins.

Problem statement and analysis of recent research. The poultry sector is developing and industrialising in many parts of the world due to population growth, increasing purchasing power and urbanisation. The growth in global demand for poultry products, particularly quail, can be explained by their high nutritional value and the significant role of poultry. About 10% of all table eggs in the world come from quail, and their meat accounts for about 0.2% of global poultry production. The number of domestic quails used for meat and egg production is about 11.8% of all produc-

tive poultry, ranking them second only to laying hens. China, Spain, France, Italy, Brazil, the United States and Japan are the world leaders in quail production [20].

The addition of selenium to quail feed provides a variety of benefits that have a positive impact on health, productivity and reproductive capacity. Selenium supplementation has been shown to improve egg quality, including increased shelf life during storage periods [23]. Selenium plays a crucial role in antioxidant defence mechanisms, protecting against oxidative stress and supporting the overall immunity of poultry [26].

Selenium (Se) is an important microelement with a variety of physiological functions related to cellular homeostasis, body metabolism and antioxidant defence. Se is an integral component of at least 25 selenoproteins in the body, which regulate cellular redox and enzymatic antioxidant defence systems, control the content and activity of free radicals and reactive oxygen species [22].

One of the modern innovative solutions is the use of selenium nanoparticles obtained through green synthesis using probiotics. Traditional methods of selenium supplementation, such as inorganic selenium salts, have limitations in terms of bioavailability and potential toxicity. Therefore, the environmentally friendly synthesis of selenium nanoparticles using probiotics and plant extracts is a promising alternative [32].

Selenium nanoparticles (SeNPs) in poultry diets have been shown to improve performance, growth, immune response, antioxidant status, and overall health, providing advantages over traditional sources of selenium [22].

The dietary supplementation of chemical nanoselenium (Che-SeNPs 0.4 g/kg) in Japanese quail diets improves growth efficiency, antioxidant status, immunity and gut microbiota, while reducing feed intake [1].

Nano-selenium has a better feed effect than selenium yeast for quail, with an optimal supplementation level of 0.2 mg/kg [24]. The use of nano-Se (at a dose of 0.1 mg/kg) in the diet of Japanese quail has been shown to improve several parameters of reproductive function [11], but the best results are obtained with the addition of selenium at a dose of 0.3 mg/kg [29].

The use of probiotics in the synthesis of selenium nanoparticles not only increases the bioavailability of selenium, but also provides additional health benefits for poultry [33]. Probiotics are beneficial microorganisms that promote the development of a healthy gut microbiota, improve nutrient absorption, and enhance the immune response. The combination of selenium nanoparticles and probiotics can potentially synergise their effects, improving reproductive function, immunity, thyroid hormone metabolism, and antioxidant defence in animals [3].

Chronic stress and inflammation, also known as the "secret killers" of poultry, can lead to lipid peroxidation, protein oxidation and nitration, DNA damage and, ultimately, apoptosis. Systemic inflammation is associated with the release of cytokines, dysbiosis and leaky gut syndrome. This syndrome is mainly caused by oxidative stress reactions that disrupt the barrier function of the cells lining the intestinal wall [5].

The mechanisms of biological action underlying the positive effect of selenium nanoparticles obtained by green synthesis using probiotics are multifaceted. Firstly, selenium nanoparticles enhance the expression and activity of selenium enzymes, such as glutathione peroxidase and thioredoxin reductase, which are involved in antioxidant defence mechanisms. By reducing oxidative stress and scavenging free radicals, selenium nanoparticles protect the thyroid gland and other vital organs from damage. Secondly, the probiotics present in the green fusion process produce metabolites that promote gut health and modulate immune responses, further improving the overall health of broilers. Finally, the combination of selenium nanoparticles and probiotics improves nutrient absorption and utilisation, ensuring optimal metabolic processes in birds.

In summary, the addition of nanoselenium and probiotics in quail breeding is essential to ensure optimal metabolic activity, reproductive performance, egg quality and overall health of quail, making it a promising nutritional strategy for quail production.

The aim of the research was to investigate the effectiveness of the use of nanoselenium bioconjugates together with probiotics on markers of pro- and antioxidant processes, hepatocyte status and protein metabolism.

Materials and methods of research. Nanobioconjugates of selenium with probiotic exometabolites and onion peel flavonoids were synthesised jointly with scientists from the Department of Interferon and Immunomodulators of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine and in the laboratory of the Research Institute of Ecology and Biotechnology of the BNAU. The researchers used onion peel extract as a natural and affordable source of the flavonoid quercetin and its metabolites, as a reducing agent and stabiliser of selenium nanoparticles, and to improve the bioavailability of natural bioflavonoids [6].

Scientific and economic experiments to determine the effectiveness of using different forms of

selenium in combination with a probiotic on the metabolic parameters of quails were conducted in a vivarium. The study was performed using the method of analogue groups [17].

Quails of the Pharaoh breed of meat production were divided into 4 groups – control and three experimental, 100 birds in each. The duration of the study was 35 days.

The experimental quails were kept in groups of 25 birds in single-tiered cages. Quails of the control group received complete feed (CF). Biogenic nanoselenium (BnSe) (0.3 mg Se/kg feed + *B. Subtilis* (2 g); bioconjugate of nanoselenium with onion peel flavonoids (QSe) (0.3 mg Se/kg feed + *L. Plantarum* (3 g); (BnSe + QSe) 0.3 mg Se/kg feed + *B. Subtilis* IMB B-7392 + *L. Plantarum* (4 g). At the end of the scientific and economic experiment, quails were slaughtered (5 birds from each group). The dosage of probiotics and Selenium nanopreparations corresponded to the established effective amounts, according to previous scientific studies. Blood sampling and weighing of quails was carried out after the experiment (day 35). For biochemical studies, test kits from Filisit Diagnostics (Ukraine) were used. The content of total protein was determined in the blood serum by the Lowry method [19], total lipids, uric acid [27], transaminase activity (AST, CE 2.6.1.1 and ALP, CE 2.6.1.2) was determined using reagent kits "Aspartate aminotransferase" and "Alanine aminotransferase" [25]. The concentration of TBA-active products in liver homogenates was expressed as nmol MDA/g tissue [7]. The oxidative modification of proteins was studied in the liver by the content of carbonyls in proteins. To assess the intensity of oxidative modification of proteins, we used the method of spectrophotometric analysis of carbonyl groups formed by the interaction of reactive oxygen species with amino acid residues using 2,4-dinitrophenylhydrazine. The formation of 2,4-dinitrophenylhydrazone was recorded at a wavelength of 370 nm, and the level of carbonyl groups was calculated using a molar extinction coefficient of 21000 $M^{-1}cm^{-1}$ [34].

The research was conducted in accordance with the principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Official Journal of the European Union L276/33, 2010), as well as in accordance with the Law of Ukraine "On the Protection of Animals from Cruelty" of 28. 03.2006, No. 27, Art. 230, Order of the Ministry of Education and Science No. 416/20729 of 16 March 2012 "On Approval of the Procedure for Conducting Research and Experiments on Animals by Scientific Institutions" and approved by the Ethics Committee of Bila Tserkva National University (conclusion No. 2 of 31.05.18, Protocol No. 1). The research results were processed using standard statistical methods in Microsoft Excel.

Research results and discussion. Chronic stress and inflammation, also known as "secret killers" in animals, can lead to lipid peroxidation, protein oxidation and nitration, DNA damage, and ultimately apoptosis [14]. This is due to an imbalance between free radical generation and endogenous antioxidant protection, which in turn, negatively affects poultry health and productivity. In this study, we determined certain markers of pro- and antioxidant processes, hepatocyte status, and protein metabolism.

In this study, the level of protein carbonyl in the blood serum was measured as a biomarker of protein oxidation. Protein carbonylation is an irreversible oxidative modification of protein and has been confirmed to be an early marker of oxidative stress-related disorders. In our studies, the markers of the state of antioxidant defence and carbonyl oxidative stress (COS) were. The activity of the enzymes of the antioxidant defence system – superoxide dismutase (SOD) and catalase, as well as the content of the end product of lipid peroxidation – malondialdehyde (MDA) and the levels of oxidative modification products of protein molecules – aldehydephenylhydrazones (APH) and carboxyphenylhydrazones (CPH).

Protein carbonylation is the main sign of oxidative damage to proteins, which consists in the introduction of carbonyl groups, such as aldehyde, ketone and lactam groups, into the side chains of protein amino acids. To determine the level of oxidative stress in the context of cellular damage, aging, and some age-related disorders, protein carbonyls are commonly detected and quantified [21].

The level of SOD and catalase on day 35 in quails of the experimental groups statistically significantly increased by 28.9 % and 32.9 % ($p<0.05$), respectively, compared to the corresponding indicators of the control group (Table 1).

The study of MDA content, as well as aldehyde and carboxyl products of oxidative protein modification in liver tissue was characterised by a statistically significant decrease. In particular, on day 35 of the study, the decrease in AFH was 10.7 %, 26.5 % and 34.9 % ($p<0.05$) in the experimental groups, respectively. At the same time, a decrease in the concentration of CFGs, which are considered to be more toxic, was recorded at the level of 21.9 %, 23.4 and 43.2 % ($p<0.05$), respectively. The correlation between TBA-AP and CFH ($r=0.67$) established in this study indicates the relationship between the processes of carbonyl and oxidative modification of biocompounds.

Table 1 – **Evaluation of the state of oxidative protein modification and antioxidant status of quail liver in control (1), with the addition of BnSe + *B. Subtilis* (2), QuSe + *L. Plantarum* (3) and BnSe + QuSe + *B. Subtilis* IMV B-7392 + *L. Plantarum* (4),** M±m, n=6

Indicators	Poultry groups			
	1	2	3	4
APH, c.u./g protein	6,84±0,19	6,11±0,18	5,82±0,14*	5,24±0,12**
CPH, c.u./g protein	9,31±0,25	8,42±0,36	8,31±0,42*	7,22±0,24**
TBA-AP, nmol MDA/mg protein	4,82±0,46	3,96±0,32	3,35±0,38*	2,93±0,28**
SOD, act.unit/mg protein	968±181,6	1168±154,2	1188±153,8	1248±181,1
Catalase, unit/mg Protein	54,6±4,46	61,2±5,46	68,6±4,32	72,6±5,46*

The system of antioxidant enzymes consists of CAT, SOD, GSH-Px and others. The main enzyme of this system is CAT, which has anti-inflammatory and antioxidant effects and is widely distributed in microorganisms, animals and plants. It catalyses the decomposition of hydrogen peroxide (H_2O_2), thereby preventing iron chelates from using H_2O_2 and oxygen (O_2) to form more toxic hydroxyl radicals. This, in turn, prevents the oxidation of cell membrane lipids and reduces oxidative damage [30].

The results of our studies on the activation of the enzymatic link of antioxidant defence by functionalised selenium nanoparticles are confirmed by the data of other authors. It has been shown [4] that selenium nanoparticles conjugated with chitosan microparticles or chitooligosaccharides (SeNPs-CS/COS-Ms) are much safer than selenite and can protect mice from oxidative stress, induced by ethanol, reducing lipid and protein oxidation and enhancing the activity of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT).

The study by Khurana et al. (2019) presents the role of SeNPs in pharmacological protection against various inflammatory and oxidative stress conditions. Selenium (Se) differs from other essential micronutrients in that it is cotranslationally incorporated into the polypeptide chain as part of the 21st natural amino acid, selenocysteine (Sec), encoded by the UGA codon [16]. This unique characteristic distinguishes Se from other trace elements that interact with proteins as cofactors. Therefore, any protein containing Sec in its polypeptide chain is classified as a selenoprotein [35]. Selenoproteins often regulate the physiological redox balance through their oxidoreductase activity [16]. Inorganic selenium (Se) has a narrow therapeutic window, and the limits of toxicity are delicate. However, Se nanoparticles (SeNPs) have significantly lower toxicity [31].

Protein carbonylation is an irreversible oxidative modification of a protein and it is an early marker of oxidative stress-related disorders. Protein carbonyls can be formed as a result of metal-catalysed oxidation of lysine, proline, arginine and threonine residues, direct oxidation of tryptophan and reactive products of lipid peroxidation of cysteine. Since carbonylated proteins cannot be reduced by cellular enzymes, the cell's proteasome system must break down modified proteins [12]. The level of protein carbonyls is considered the gold standard for measuring protein oxidation [13].

Carbonyl derivatives of proteins are stable products that are formed with the participation of amino acid residues of proline, arginine, lysine, threonine with the formation of Michael adducts [8].

Proteins, lipids and nucleic acids are the main targets of oxidation reactions in humans and animals. Proteins are particularly sensitive to oxidation because of their rapid reaction with oxidants and their abundance in cells, extracellular tissues, and body fluids. This sensitivity makes proteins a primary target for oxidative damage and subsequent modifications. Reversible modifications are related to physiological processes and constitute signalling mechanisms ("redox signalling"), while irreversible modifications can contribute to pathological situations and a number of diseases [15].

Lipids and nucleic acids are also vulnerable to oxidation, leading to a wide range of oxidative modifications and potential consequences for cellular function and health [28].

Demasi et al. (2023) combined current knowledge of redox modifications of proteins with their role in redox signalling and human pathological conditions [10]. They explored the various ways in which protein oxidation can contribute to the development and progression of disease. Some oxidative modifications of protein contribute to loss of protein function, degradation or aggregation, and some lead to proteotoxicity and disruption of cellular homeostasis. However, not all

oxidative modifications are necessarily associated with damage, as is the case with the oxidation of protein residues Met and Cys. In these cases, changes in the redox state can alter protein structure, catalytic function, and signal transduction processes in response to metabolic and/or environmental changes [10].

Protein oxidation, which occurs as a result of the inevitable formation of reactive oxygen species (ROS) and other oxidants, leads to various post-translational modifications of proteins. These modifications include protein oxidation, glycoxidation, and lipoxidation, which can be either reversible, playing a role in physiological processes, or irreversible, contributing to pathological conditions and diseases. The products of protein oxidation are often chemically stable and abundant, making them likely biomarkers of oxidative damage.

Free radicals attack proteins along the entire length of the polypeptide chain, destroying all levels of their structural organisation, which causes aggregation and fragmentation of protein molecules. Protein aggregation is associated with the ability of ROS to form intermolecular cross-links. As a result of protein denaturation, their conformation is disturbed, and they become more sensitive to the action of proteolytic enzymes. The most susceptible to oxidation are lysine, arginine, proline, threonine, sulfur-containing (methionine, cysteine, cystine) and aromatic (histidine, tryptophan, tyrosine) amino acid residues of proteins, which are converted to carbonyl derivatives.

De Carvalho et al. (2022) conducted a comparative study of the antioxidant capacity of amino acids, including lysine, methionine, tryptophan, tyrosine, cysteine, proline, and arginine. They found that amino acids preferentially react with Fe(III)-1-nitroso-2-naphthol-3,6-disulfonic acid, indicating their reducing ability and potential susceptibility to oxidation [9].

Andrés et al. (2022) examined the effect of reactive species on amino acids. They described how tyrosine, methionine, cysteine, and tryptophan can react with harmful peroxynitrite or radicals such as •OH and $•NO_2$, leading to hydroxylation, nitration, or halogenation. This supports the idea that these amino acids are particularly susceptible to oxidative modifications [2].

Koshti et al. (2021) investigated unusual aggregates formed by the self-assembly of proline, hydroxyproline, and lysine. They found that these amino acids can form toxic self-assemblies, suggesting a role in metabolic disorders such as hyperprolinaemia, hyperhydroxyprolinaemia, and hyperlysinaemia. Research shows that these amino acids can undergo significant structural changes under certain conditions, potentially due to oxidative processes [18].

Thus, protein carbonylation is often used to quantify and qualitatively assess oxidative modification of proteins (OMP). The products of OMB have a longer half-life than lipid peroxidation (LPO) products, which makes them a promising marker for assessing the intensity of free radical oxidation in biological systems.

Proteins are targets of oxidative modification by reactive oxygen species (ROS). Their reactions with ROS often lead to the modification of certain amino acid residues, such as histidine, lysine, arginine, proline and threonine, to form carbonyl derivatives. Protein carbonylation is often used to quantify the total oxidation of proteins [34]. Regarding the advanced lipoxidation end products, recent studies have mainly focused on the reactive intermediates 4-Hydroxy-2-nonenal (4-HNE) and MDA, which are likely to correlate with the number of protein modifications formed by the reaction with this carbonyl species [15]. In addition, elevated levels of protein carbonyls have been observed in several human diseases and are associated with aging processes. Protein carbonyl content can be considered as a biomarker of global oxidative damage to protein, with the advantage of early formation and a long circulation period compared to other parameters of oxidative stress [15]. Studies have shown that oxidative modifications of proteins are affected by various reactive species, leading to changes in protein structure and function. These modifications can have both regulatory and deleterious effects, depending on the context and degree of oxidation. Understanding these mechanisms is crucial to elucidating the role of oxidative stress in various diseases. In summary, these studies provide insight into the susceptibility of specific amino acids to oxidation, highlighting their potential reactivity under oxidative conditions, which can lead to structural changes and affect their biological role.

The study of protein metabolism indicators revealed an increase in protein ($p \leq 0.05$), albumin, and a tendency to decrease the activity of aminotransferases, indicating that the addition of various selenium compounds to the diet had a positive effect on metabolic processes and did not have a negative effect on liver cells, as evidenced by the activity of liver transaminase marker enzymes – ALT and AST (Table 2).

The tendency to decrease the concentration of uric acid and creatinine in the experimental groups indicates the absence of a negative effect on renal function.

Table 2 – **Biochemical parameters of blood serum of quails in control (1), with the addition of BnSe + *B. Subtilis* (2), QuSe + *L. Plantarum* (3) and BnSe + QuSe + *B. Subtilis* IMV B-7392 + *L. Plantarum* (4),** M±m, n=6

Indicators	Poultry groups			
	1	2	1	4
Total protein, g/dm^3	27,7±1,95	31,5±1,76	29,8±1,42	38,6±1,75*
Albumin, %	45,9±3,4	47,2±1,57	46,02±0,49	48,02±2,03
AST, mmol/year× dm^3	2,06±0,28	1,52±0,13	1,64±0,18	1,83±0,16
ALT, mmol/year× dm^3	0,35±0,05	0,28±0,02	0,23±0,03	0,21±0,02
Uric acid, μM/dm^3	412,1 ± 23,9	394,7 ± 18,5	372,5 ± 20,34	351,9 ± 16,74
Creatinine, μM/dm^3	40,3±2,46	39,8±1,52	36,7±1,82	34,5±1,94

Conclusions. A study was conducted to determine the effectiveness of the use of new probiotic preparations. The "green" synthesis of Selenium nanoparticles was carried out, which has a wide prospect of application in agricultural production. The addition of probiotics and nanoselenium to feed leads to the activation of metabolic parameters and increases the profitability of quail production. Thus, investing in high-quality feed solutions that include alternative antibiotics is the only highly effective option to simultaneously ensure human health, animal and poultry welfare, increase their productivity and meet consumer requirements.

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Вплив використання біокон'югатів наноселену разом з пробіотиками на метаболічні показники організму перепелів

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У контексті сучасної індустріалізації птахівництва та зростаючого глобального попиту на перепелину продукцію пошук ефективних способів покращення продуктивності та здоров'я птахів

є надзвичайно важливим. Одним з перспективних підходів є використання біосполук наноселену з пробіотиками для покращення метаболічних параметрів перепелів. Останні дослідження були зосереджені на синтезі наночастинок селену з використанням пробіотиків як екологічно чистої альтернативи у противагу традиційним методам додавання неорганічного селену в корм для перепелів. Перевага цього підходу полягає в отриманні біосумісної та біодоступної форми селену, котра забезпечує птахам можливість ефективно поглинати та використовувати селен для різних фізіологічних процесів.

Досліджено вплив інноваційних кормових добавок, таких як нанокон'югати селену та пробіотики, на різні метаболічні параметри в організмі перепелів. До них належить активність ферментів антиоксидантного захисту, індикатори карбоніл-оксидативного стресу, рівні білкових карбонілів і білковий метаболізм. Додаючи до корму перепелів нанокон'югати селену та пробіотики, спостерігали покращення механізмів антиоксидантного захисту, що призвело до зниження оксидативного стресу та покращення загального стану здоров'я птахів. Крім того, було показано, що покращений метаболізм білка в результаті цих добавок позитивно впливає на продуктивність і якість перепелиної продукції. Підсумуємо, що інвестування у високоякісні кормові добавки, такі як нанокон'югати селену та пробіотики, є стратегічним підходом до підвищення продуктивності та рентабельності птахівництва. Використовуючи переваги нанотехнологій і пробіотиків, фермери можуть оптимізувати здоров'я та продуктивність стада птиці, задовольнити зростаючий попит на перепелину продукцію на вітчизняному ринку.

Ключові слова: біонанотехнології, кон'югати наноселену, біогенний синтез, кверцетин, перепели, кров, печінка, біохімічні показники, окисна модифікація білків.

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