

## Original researches

### Efficiency of application of inorganic and nanopreparations of selenium and probiotics for growing young quails

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**Abstract.** Modern industrial poultry farming occupies a leading position in the production of dietary foods, for which it is appropriate to use biologically active substances and probiotics. The study compared the effectiveness of compound feed with the addition of sodium selenite, bio-nano-selenium and *L. plantarum* in terms of body weight, weight gain, feed consumption and safety of experimental quail. The quails of the Pharaoh meat breed were kept in the vivarium of Bila Tserkva National Agrarian University and by the method of analogues were divided into 4 groups - control and three experimental ones. The duration of the study was 35 days. The results of the study indicate that the use in the quail's diet of probiotics (*L. plantarum*) and selenium preparations (sodium selenite and bio-nano-selenium) leads to a predominance of poultry experimental groups in live weight over control analogs. It was found that the growth of bird's live weight during the experiment differed depending on the use of the Selenium medication or probiotic, and the most intense effect on live weight of quails was the use of bio-nanoselenium in the diet of poultry (11.8% at the end of the experiment). Changes in absolute and average daily body weight gains of experimental birds have been clarified. The use of sodium selenite in the diet of quail caused a tendency for an increase in gains compared with control analogs at the beginning of the study and a downward trend during the fifth week of the study. It was found that the use of probiotics and bio-nano-selenium caused an increase in quail growth, which was more pronounced and reliable when feeding bio-nano-selenium. The obtained results show that probiotic and selenium preparations reduce feed consumption by 1 kg of weight gain, body weight increase by 3.05–11.8% and by 3.3–6.6% increase the safety of the experimental livestock with the best indicators in the group, receiving bio-nano-selenium.

**Keywords:** quails; sodium selenite; nano-selenium; *L. plantarum*; body weight; feed conservation ratio; conservation

### Ефективність застосування неорганічних сполук і нанопрепаратів селену та пробіотиків у вирощуванні молодняку перепелів

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**Анотація.** Сучасне промислове птахівництво займає чільні позиції у виробництві дієтичних продуктів харчування, для отримання яких доречно застосовувати біологічно активні речовини та пробіотики. У роботі проводилося порівняння ефективності згодовування комбікорму з додаванням селеніту натрію, біонаноселену та *L. plantarum* за масою тіла, приростами, витратами корму та збереженістю поголів'я дослідних перепелів. Перепели породи фараон м'ясного напрямку продуктивності утримувались в умовах віварію Білоцерківського національного аграрного університету та методом аналогів були розподілені на чотири групи – контрольну і три дослідні. Тривалість дослідження становила 35 діб. Результати експерименту свідчать, що використання у раціоні перепелів пробіотика (*L. plantarum*) та препаратів селену (селеніт натрію та біонано-Se) викликають до переважання птиці дослідних груп за масою тіла контрольних аналогів. Установлено, що зростання маси тіла птиці впродовж експерименту різнилося залежно від використання препарату Селену чи пробіотика, найінтенсивніше вплинуло на масу тіла перепелів використання у складі раціону птиці біонаноселену (11,8% наприкінці експерименту). З'ясовано зміни абсолютних та середньодобових приростів маси тіла дослідної птиці. Використання селеніту натрію у раціоні перепелів викликало тенденцію до збільшення приростів порівняно з контрольними аналогами на початку дослідження та тенденцію до зниження впродовж п'ятого тижня дослідження. Встановлено, що використання пробіотика та біонаноселену спричинило зростання приростів маси тіла перепелів, що більш яскраво та достовірно проявлялося за згодовування біонаноселену. Отримані результати свідчать, що пробіотик та препарати селену сприяють зменшенню витрат корму на 1 кг приросту, зростання маси тіла на 3,05–11,8% та на 3,3–6,6% підвищують збереженість дослідного поголів'я з найкращими показниками у групі, що отримувала біонаноселен.

**Ключові слова:** перепели; селеніт натрію; наноселен; *L. plantarum*; маса тіла; витрати корму; збереження

## Introduction

Modern industrial poultry production, as well as other livestock industries, occupies a leading position in the production of dietary foods. Due to the high demand for poultry products, it is necessary to apply the latest technologies for poultry farming, increase its productivity without reducing the quality of the final product, ensure intensive metabolism in farmed livestock, and increased stress resistance and resistance to adverse environmental factors (Bityutskyy et al., 2019a; Tsekhmistrenko et al., 2020a). That is why it is appropriate to use biologically active substances (Tsekhmistrenko et al., 2018) and probiotics (Tymoshok et al., 2019) in a balanced diet, which allow obtaining high-quality products from healthy livestock without the use of feed antibiotics (Tsekhmistrenko et al., 2020b).

An important component of the diet are products which combine probiotic microflora and raw plant and animal origin materials. In recent years, the use of probiotic supplements is aimed at correcting of the gastrointestinal tract microbiocenosis (Artyuhova & Antonyuk, 2014) under adverse animal husbandry conditions.

Selenium plays a very important role in feeding poultry due to its multifactorial action and its inclusion in feed is necessary, especially in areas with soils deficient in selenium. In addition, taking into account the possible toxic effects and more limited properties of inorganic forms of selenium, there is a need to develop new compositions, in particular in nanoform. Laboratory studies have shown that selenium nanoparticles (SeNPs) do not act exclusively as antioxidants: not only redox-regulating activity of SeNPs has been established, which may enable them to act as non-traditional antibacterial, immunomodulatory and growth-promoting agents through various metabolic regulatory mechanisms. The addition of probiotic additives, in a complex with nano-selenium, to forages will promote increase of growth rates, and antioxidant protection activation of an organism, positively influence bird's preservation.

Adding probiotic supplements to feed increases nutrient bioavailability, health, immunity, productivity, and poultry conservation. Selenium supplements using inorganic and organic forms are used to increase growth rates and increase the body's antioxidant defenses. However, these forms have limitations: a narrow safety interval, nonspecific binding to tissue proteins. An alternative form may be selenium nanoparticles (SeNPs), which have better bioavailability, relatively high safety margin, and low toxicity.

The approach of green nanoparticle synthesis is based on 12 principles of «green» chemistry, which include the development and design of nanoparticles using non-toxic chemicals, renewable materials, environmentally friendly solvents, and finally, waste that decomposes to form non-toxic compounds (Xiao et al., 2018). The introduction of «green chemistry» and «nanotechnology» is one of the revolutionary developments in science that has contributed to the safety research and the reduction of object size. Combining these two fields has paved the way for a new “green” and nanoscale science called «green nanotechnology» or bionanotechnology (Tsekhmistrenko et al., 2020c).

Three important stages in the preparation of nanoparticles for the perception of «green» chemistry include a harmless solvent environment, a non-toxic reducing agent, and environmentally friendly stabilizers.

The advantage of nanostructures synthesized by the green approach is that specific bio objects (plants, microorganisms) contain a wide variety of biomolecules that cover the surface of the synthesized nanoparticles, thus forming coating layers around it, which further provide stability, biocompatibility, and unique specificity actions. Selenium nanoparticles synthesized by probiotic bacteria can be effectively used as an alternative to other forms of selenium as a feed additive due to the synergism of Se and probiotics to reduce oxidative stress, increase resistance and productivity of poultry.

Oxidative stress is a serious detrimental factor for cell integrity due to the constant release of reactive oxygen species, mediated by various biotic (bacteria, viruses, fungi, etc.) and abiotic stressors. A fundamental way to maintain cellular redox homeostasis is the redox-sensitive Keap1 / Nrf2 / ARE signaling system, which plays a key role in stressful, inflammatory, carcinogenic and proapoptotic conditions. Recently, there have been reports that biogenic selenium nanoparticles obtained by «green» chemistry with lactobacilli affect the redox-sensitive transcription factor Nrf2 (Keap1 / Nrf2 / ARE signaling), which activates the transcription and synthesis of a number of antioxidants (Xu et al., 2018; Xiao et al., 2018; Qiao et al., 2020).

Recent publications have widely covered research on the effects of mineral, organic and selenium nano preparation and probiotics on the efficiency of poultry farming (Bityutskyy et al., 2019a; 2019b; Xiao et al., 2019). These studies show a positive effect of selenium and probiotics on the growth and development of poultry and body weight, weight gain, feed consumption and livestock safety. The positive and negative effects of feed additives use on the environment (Tsekhmistrenko et al., 2020a), the possibility of restoring the environment with selenium preparations and due to selenium contamination, were also studied.

Selenium (Se) is an essential element, a component of selenoproteins that regulate the metabolism of thyroid hormones, enzymatic antioxidant protection and the immune system (Wu et al., 2019). Its deficiency in the diet causes the development of cardiovascular disease, susceptibility to viral infections, and an increased risk of mortality (Tsekhmistrenko, 2008). Low serum levels of this element is a symptom of acute kidney and liver damage (Wu et al., 2019), which is associated with a risk of death from coronary heart disease.

Selenium is part of glutathione peroxidase (Wrobel et al., 2016), thyroreductase (Mehdi et al., 2013) and selenocysteine, the 21st amino acid. Unlike other important trace elements that interact with proteins in the form of 4 cofactors, selenium becomes co-translationally incorporated into the polypeptide chain as part of the 21st amino acid selenocysteine (Sec), which is encoded by the UGA triplet. Each protein that includes Sec in its polypeptide chain is defined as a selenoprotein (Zoidis et al., 2018; Khalak t al., 2020).

Normal intestinal microflora forms a relatively stable system in animals and birds (Mehdi et al., 2013; Alvarez, 2019), but nutrient deficiencies that occur during intensive farming and various environmental stressors can upset the balance of the system (Alvarez, 2019; Andrade et al., 2019; Gupta & Ayan, 2019). Therefore, one of the effective ways to preserve the qualitative and quantitative composition and biochemical activity of normal microflora is the use of diets enriched with lactobacilli, which prevent the development of diseases and activate the immune response.

Lactobacilli are involved in the formation of systemic and local immune resistance (Artyuhova & Antonyuk, 2014) due to their universal immunomodulatory properties. *L. plantarum* forms associations with the intestinal mucosa, interacts with epithelial and immunocompetent cells and M-cells of Peyer's patches, stimulates the immune system through activation of humoral mechanisms and cell-mediated immune response (Kleerebezem et al., 2003), and endothelial system of the intestinal tract and cytokine production.

Lactobacilli activate the proliferation and differentiation of immunocompetent cells (Jang et al., 2020), lead to the induction of immunoglobulin synthesis at the local and systemic levels (Xie et al., 2019), stimulate intestine contractile functions, promoting digestion. Lactobacilli exhibit anticancer properties, enhancing the cytotoxic functions of T lymphocytes, macrophages, and killer cells (Ryu et al., 2019; Saadat et al., 2019), participate in the formation of the phenomenon of «oral tolerance» to food antigens.

**Table.** Scheme of scientific and economic experiment

Group	Poultry, number of individuals	Characteristics of feeding young quails in the period of 1–35 days
1 – control	60	BD (basic diet)
2 – experimental	60	BD + 0.3 mg Se/kg of feed (as Na <sub>2</sub> SeO <sub>3</sub> additive)
3 – experimental	60	BD + 0.3 mg Se/kg of feed (as Na <sub>2</sub> SeO <sub>3</sub> additive) + <i>L. plantarum</i> IMB B-7679 (2.5×10 <sup>6</sup> CFU per head/day)
4 – experimental	60	BD + 0.3 mg Se (SeNPs)/kg of feed + <i>L. plantarum</i> IMB B-7679 (2.5×10 <sup>6</sup> CFU per head/day) which grew in the presence of biologically synthesized nanoselenium

Note: probiotic (*L. plantarum* IMB B-7679 and biogenic nanoselenium for research were provided by the Department of Interferon and Immunomodulators of the D. K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Kyiv, Ukraine.

The aim of the research was to study the effect of feed probiotic additives, biogenic selenium nanoparticles, compared to the inorganic form of selenium and their complex on growth, feed conversion, biochemical parameters of blood, and preservation of quails.

### Material and methods

Experimental studies were conducted in the vivarium of Bila Tserkva National Agrarian University. Research on birds was carried out in accordance with the documents governing the organization of work with experimental animals and compliance with the principles of the «European Convention for the protection of vertebrate animals used for experimental and other scientific purposes» (Strasbourg, 1986) and Art. 26 of the Law of Ukraine № 5456-VI of 16.10.2012 «On protection of animals from cruel treatment». The material for the study was the quail of the Pharaoh breed of meat productivity. At the age of one day, 240 chicks were selected and divided into 4 groups by the method of analogs – control and three experimental ones, with 60 birds in each (Table).

The duration of the study was 35 days with a division into 5 subperiods of 7 days. Birds received a complete feed, to which probiotic, sodium selenite and biogenic nano-selenium were added by the method of multistage mixing. The dosages of probiotics and selenium preparations listed in Table correspond to the established effective amounts according to previous scientific studies (Tsekhmistrenko, 2008; Virkutyte & Varma, 2011).

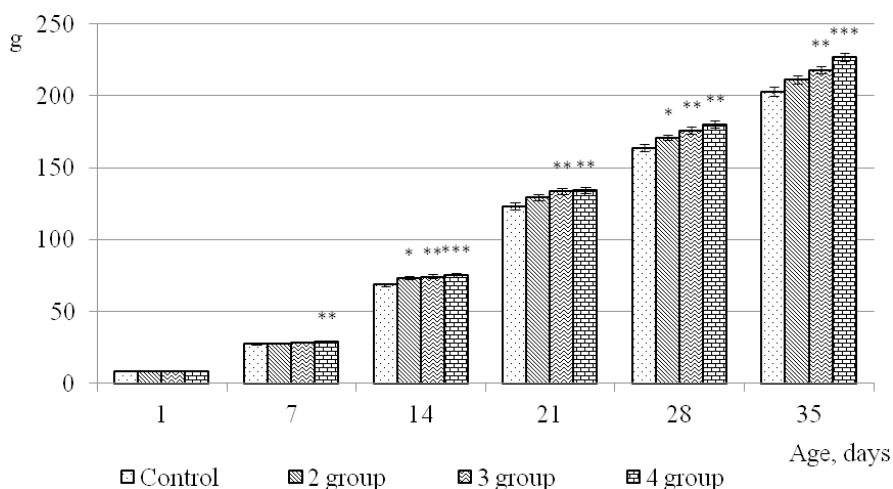
The experiment examined the bird's body weight, feed consumption, livestock safety, calculated weight gain, and feed consumption per 1 kg of gain. Biometric processing of the obtained results was carried out according to standard methods (Plohinskij, 1969).

### Results

The results of the study show that the use in the quails' diet of probiotics (*L. plantarum*) and selenium preparations (sodium selenite and bionano-Se) lead to a predominance of poultry experimental groups by body weight of control analogs (Fig. 1). Diurnal quails of the control and experimental groups did not have a significant difference in body weight, which served to select them and form them by the method of analogs into the groups for research.

The increase in birds' body weight during the experiment varied depending on the use of the Selenium preparation or probiotics. The lowest weight gain was shown by the use of sodium selenite in the diet (group 2), which was significant at 14-, 21-day-old quails ( $p < 0.05$ ) and on the 28<sup>th</sup> day of the experiment ( $p < 0.01$ ). The absolute weight gain of this group exceeded the control indicators by 1.7–10.9%. The use of probiotics in the diet (group 3) significantly increased the body weight of quails from the 14<sup>th</sup> day of the experiment till the end of the experiment, which exceeded the control indicators of growth with a difference in body weight relative to control by 4.2–8.3%.

The most intense effect on the quails body weight of was exerted



**Fig. 1.** Body weight of quails with the addition of inorganic selenium (group 2), inorganic selenium with probiotic (group 3) and nano-selenium with probiotic (group 4), g ( $M \pm m$ ,  $n = 12$ )

Note: hereinafter \* –  $p < 0,05$ , \*\* –  $p < 0,01$ , \*\*\* –  $p < 0,001$  compared to the control group.

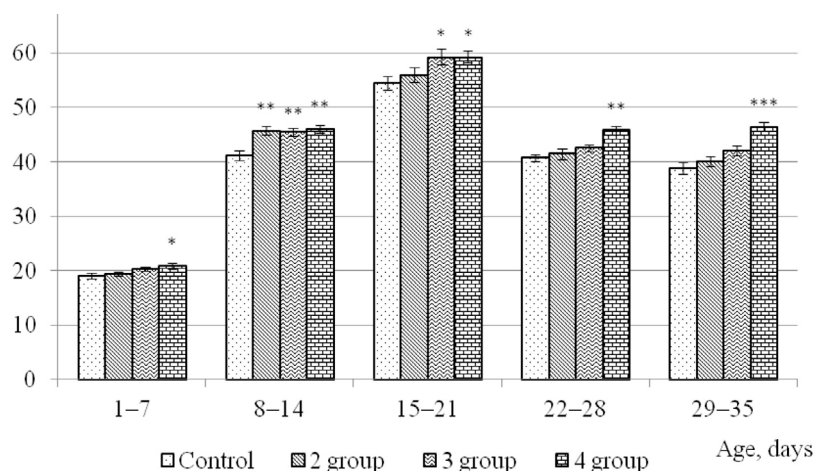


Fig. 2. Absolute weight gain of quails, g ( $M \pm m$ ,  $n = 12$ )

by the use of bio nano-selenium in combination with a probiotic in the diet of birds. The body weight of quails from this experimental group significantly exceeded the control indicators during the experiment with a final indicator of 11.8%.

According to body weight, the absolute gains of experimental quails also changed (Fig. 2). The use of sodium selenite in the diet of poultry caused a tendency to increase the quails' absolute weight gain of the second experimental group compared with control analogs with a reliable indicator during the second ( $p < 0.001$ ) and sixth ( $p < 0.05$ ) weeks of rearing, which may be due to juvenile plumage and restructuring the body before laying eggs, which is a stress factor for the bird's body.

Absolute weight gain in group 3, which together with compound feed received *L. plantarum* in the amount of  $2.5 \times 10^6$  CFU per head/day, by 4.4–10.5% prevailed over the control group. The indicators were reliable during the first two weeks of the study and at the end of the experiment.

Birds, which received bio nano-Se in the diet, in absolute increments, significantly outperformed the control group by 8.8–19.8%. In this case, the indicators were probable in the quails of the 4<sup>th</sup> group throughout the experiment.

A similar trend was observed with respect to the average daily weight gain of young quails (Fig. 3). The highest rates were characterized by quails of the 4<sup>th</sup> experimental group, which received bio nano-Se as part of the feed in the amount of 0.3 mg / kg of feed. Similar to the absolute increments, the changes in the average daily increments were significant throughout the experiment period. These dynamics allowed the average daily gains of the 4<sup>th</sup> experimental group to significantly exceed the control indicators on average during the study period by 11.8%.

Significantly lower rates were in quails treated with probiotics (group 3), reliably during the second ( $p < 0.01$ ) and third ( $p < 0.05$ ) weeks of the experiment. The control indicators of quails' average daily gains that received sodium selenite in the diet in the amount of 0.3 mg/kg  $\text{Na}_2\text{SeO}_3$  (group 2) prevailed the least. The trend was observed throughout the experiment with a significant advantage ( $p < 0.05$ ) during the second week of the study.

Simultaneously with the study of the effects on the quails' body of selenium preparations and probiotics added to the diet, changes in weight, absolute and average daily weight gain of poultry, were also studied changes in feed consumption per unit weight gain and preservation of experimental livestock (Fig. 4).

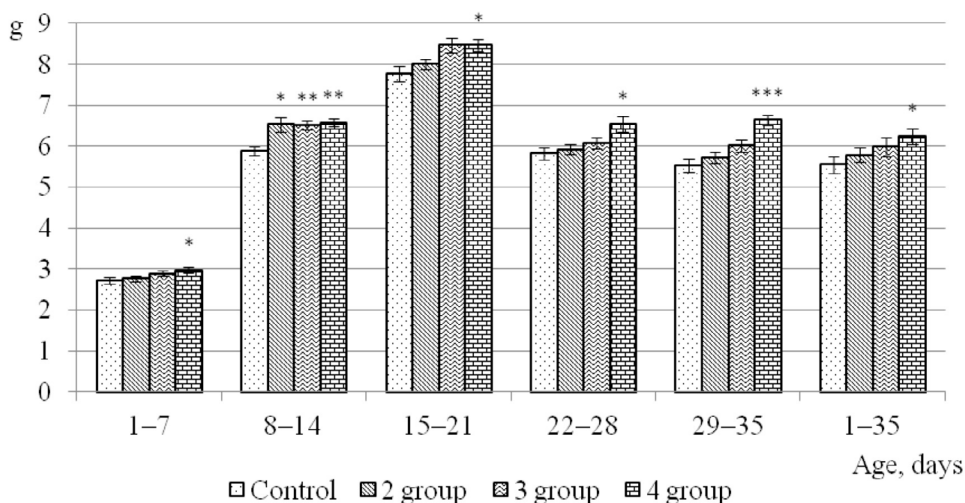


Fig. 3. Average daily quails' weight gain, g ( $M \pm m$ ,  $n = 12$ )

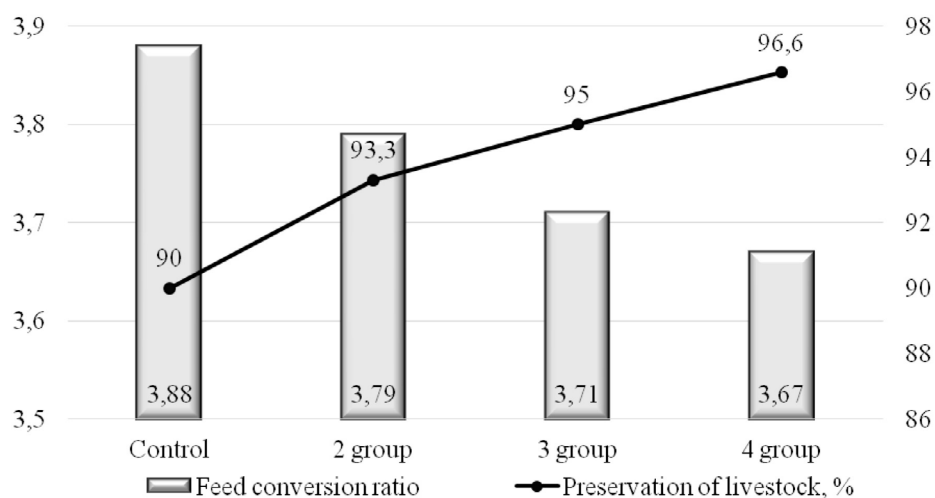


Fig. 4. Feed consumption per 1 kg of body weight gain, kg and survival of quails

The results of the study show that the quails of the experimental groups receiving sodium selenite, *L. plantarum* and bio nano-Se showed lower feed consumption per 1 kg of body weight gain compared to control counterparts by 2.72%, 4.40, and 5.4%, respectively and higher livestock safety by 3.4%; 5 and 6.7%.

### Discussion

A number of studies highlight the results of studies about the effects on the body of inorganic (sodium selenite) (Mehdi et al., 2013; Tsekhmistrenko et al., 2019), organic (Sel-Plex) (El-Deep et al., 2016), selenium nano- and bionanopreparations (Bityutsky et al., 2017; Tsekhmistrenko et al., 2018). However, there is a narrow range of physiological effects between Se concentrations without causing a toxic effect. At high concentrations (900 µg / day), selenium becomes extremely toxic (Staicu & Barton, 2017). Se preparations in the form of L-selenomethionine, in contrast to sodium selenite, are quickly and completely absorbed by organisms and cause minimal side effects. Selenomethionine (Se-Met) included in the methionine site in various proteins provides regulation of the endocrine system, but Se-Met is not synthesized in the body (Chauhan et al., 2019). That is why much attention is now paid to bioavailable forms of Se, in particular nano-selenium and biogenic nano-selenium, which can be obtained using probiotic microorganisms (Ahmadi et al., 2013).

Selenium is known for its antioxidant activity and involvement in redox potential optimization, reproductive processes, thyroid hormone metabolism, muscle development, and anticancer genesis (Senthil Kumaran et al., 2015; Mao & Lien, 2017). In the form of Nano-Se it leads to higher Se content due to smaller size and greater bioavailability (Bai et al., 2017). Nano-Se showed better results in terms of weight gain compared to sodium selenite in the broilers' diets (Senthil Kumaran et al., 2015). Similar results were also observed when 0.3 mg / kg Se in the form of nanoelement Se, sodium selenite, or selenium-containing yeast was added to the main diet (Boostani et al., 2015; Tsekhmistrenko et al., 2015; Ahmadi et al., 2018). The addition of nano-Se (0.2, 0.3, 0.4 and 0.5 mg / kg) in the broilers' diet improved growth, immune function and post-mortem performance of poultry without affecting the internal organs (Ahmadi et al., 2018). The combination of probiotics and Se nanoparticles have also been shown to improve growth, skeletal muscle fatty acid profile, and serum α-tocopherol content (Saleh,

2014). In addition, nano-Se optimized antioxidant status by influencing the activity of antioxidant enzymes and increased IgG and IgM levels compared to organic and inorganic Se compounds under oxidative stress (Boostani et al., 2015) in chickens and during thermal stress (El-Deep et al., 2016) in broilers while improving growth and immunity, activating the expression of cytokine genes.

*L. plantarum* belongs to the normal microflora of the group of gram-positive lactic acid organisms that ferment 25 carbohydrates to lactic acid (Saadat et al., 2019). Antimicrobials produced by *L. plantarum* are an alternative to antibiotics because they block receptors for gram-negative and gram-positive bacteria (Mehdi et al., 2013). *L. plantarum* exhibits enzymatic properties which can reduce the number of pathogenic microorganisms, including *Candida Albicans*, *Candida Enteritis*, *Shigella*, *Salmonella*, *Listeria* etc. (Alvarez, 2019; Gupta & Ayan, 2019). *L. plantarum* is stable at high temperatures (98 °C) and high acidity (pH 1.2-2.0) (Andrade et al., 2019; Vaziri et al., 2019; Shah et al., 2020), which allows to use them as in vivo transporting agents of therapeutic compounds or proteins (Niedzielin et al., 2001).

*L. plantarum* has antioxidant activity, the ability to maintain intestinal permeability (Mehdi et al., 2013; Ryu et al., 2019; Yang et al., 2019), inhibit the growth of gas-forming bacteria, reduce the symptoms of irritable bowel syndrome (Saadat et al., 2019), normalize the balance of the microbiota and stabilize the structure of digestive enzymes (Yang et al., 2019). *L. plantarum* is able to induce interferon, reduce tumor activity (Saadat et al., 2019; Yang et al., 2019), increase brain-derived neurotrophic factor, which allows their use in the treatment of the nervous system diseases (Gharaei-Fa & Eslamifard, 2011).

*L. plantarum* shows antiviral activity. Panetta cells, by attacking viruses, produce interleukin (IL-1β), which leads to exfoliation of the intestinal lining and secondary microbial/fungal infection (March et al., 1985). *L. plantarum* is able to destroy IL-1β, relieving inflammation, and accelerating the recovery of the intestine for several hours. Experiments have shown that biogenic BNSe2 synthesized by *L. casei* ATCC 393, protects intestinal epithelial barrier function from the effects of oxidative damage, normalizes mitochondrial function, involving the Nrf2 / ARE signaling pathway (Qiao et al., 2020).

Thus, according to the quails' growth rates, it was found that birds, which consumed nano-selenium and probiotic together with compound feed, exceeded the analogs of the control and other

experimental groups in terms of body weight, absolute and average daily gain. Such results are associated with easier absorption of bio nano-Se, in contrast to sodium selenite, in the quails' gastrointestinal tract and more intensive inclusion of the former in metabolism, in particular in the enzymes of antioxidant protection (glutathione-dependent enzymes) and thyroid hormones. receiving bio nano-Se, show higher gains and body weight (Bityutskyy et al., 2019b; Tymoshok et al., 2019; Tsekhmistrenko et al., 2020a; 2020b).

### Conclusion

The use of various forms of selenium and probiotics in the feeding of young quails has a positive effect on their productivity and preservation. Feeding compound feed to quails with the introduction of biogenic nano-selenium and probiotics is the most effective according to the growth and productivity of young quails of Pharaoh breed during the rearing period of 1–35 days (increase in quail's pre-slaughter weight by 11.8% ( $p < 0.01$ ) compared to control group, increasing the safety of livestock to 96.6% and reducing feed costs per 1 kg of body weight gain by 5.4%.

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