

Volodymyr Nadykto *Editor*

Modern Development Paths of Agricultural Production

Trends and Innovations

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Effects of Different Dietary Selenium Sources Including Probiotics Mixture on Growth Performance, Feed Utilization and Serum Biochemical Profile of Quails



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1 Introduction

Poultry farming is an innovative and high-tech meat and egg production sector. In order to provide increased demand for animal protein, improve bird health and product quality, probiotics are an alternative to antibiotics [1]. Birds feedings include probiotics such as *Lactobacillus* and *Bifidobacterium*, which have a positive effect on the productivity of broilers, [2–4], regulate lipid metabolism [5] and processes of immunomodulation [6]. Selenium, which is inducted into feed, improves bird productivity and health and has a positive effect on the immune system and fatty acid composition of meat and eggs [7]. There are several different forms of selenium that are used as additives to fodder: inorganic forms (sodium selenite and sodium selenate) and organic forms (selenomethionine). However, new forms of selenium have appeared in recent years, such as 2-hydroxy-4-methylisobutanoic acid (HMSeBA) and nanoselens that have better bioavailability, efficacy and low toxicity [8]. Selenite and selenate are considered to be the most toxic forms of selenium, and then seleno-cysteine, whereas methyl compounds of selenium and nanoselenium have the lowest levels of toxicity. The addition of 0.3–0.5 mg/kg of nanoselen proved to be effective and beneficial in improving the protection against oxidative stress, and the maximum dosage of nanoselen should not exceed 1.0 mg/kg [8].

Selenium nanoparticles (SeNPs) are considered as new forms of selenium supplemented with high biological activity and low toxicity. However, the molecular mechanism by which they exhibit biological action for today is not fully understood. SeNPs are known to exhibit biomimetic activity, in particular, oxidase-like, what is also a characteristic of other nanoparticles [8–10].

Regarding the mechanism of action of nanoselen, the work [11] doubts the interpretation that nanoselen has a direct antioxidant effect, since it has been established

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that, as in various forms of selenium, its antioxidant effect is associated with the expression of selenoprotein genes and the synthesis of the corresponding proteins [12]. The studies show the positive effects of nanoselen on the activity of glutathione peroxidase (GSH-Px) and on direct expression of enzyme.

The most prominent biological role of selenium is due to its presence as a functional component in selenium enzymes such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR). SeNPs, as compared to selenium, selenomethionine, and methyl selenocysteine, have been shown to increase the activity of selenium enzymes in mice and rats more effectively, with significantly lower acute toxicity [13–15].

As the main effects of selenium implemented with selenomethionine, selenocysteine and selenoproteins that are not synthesized in humans, animals and birds, this biotransformation is implemented by plants or bacteria [16].

From this point of view, it is interesting to assume that the intestinal microbiota can transform nano-Se into selenite/selenate or recover in H_2Se with further synthesis of selenoproteins [17, 18]. This has been proven that selenium nanoparticles can be oxidized to inorganic selenium oxyanions in the intestine in the presence of bacteria presence for their absorption [17]. Recently, the genes encoding the YedE and YedF proteins are considered as new gene candidates participating in the Se metabolism in prokaryotes, including bacteria [19]. Thus, additional studies are needed to find out the mechanisms for the possible transformation of nano-Se into the gastrointestinal tract of animals and the possible synergy of action by the use of certain strains of probiotics and selenium nanoparticles. The metabolic action of SeNPs depends on the composition of the nanoparticles, including the nanoparticle coating agent [20].

Thus, selenium plays a very important role in feeding birds due to polyfactor action, and its incorporation into compound feed is necessary, especially in areas with soils that are scarce in selenium. In addition, considering the possible toxic effects and the more limited properties of inorganic forms of selenium, there is a need for the development of new compositions, in particular in nanoform.

In order to assess the impact of complex probiotic preparation Probifilact (Probiotic Containing *Lactobacillus*, *Bifidobacterium*), which is added to animal feed, along with various forms of selenium on performance, retention and blood biochemical parameters quail conducted the survey data.

2 Materials and Methods

In this work there was used a complex of strains of *Bifidobacterium animalis* VKB, *Lactobacillus casei* IMV B-7280, *Bifidobacterium animalis* VKL (Probifilact), what were obtained from the collection of the Institute of Microbiology and Virology named by D.K. Zabolotny National Academy of Sciences of Ukraine. A nanoselenium with spherical shape with a particle size of 7–60 nm (average size of 34 nm) stabilized with citrate was obtained at the Ukrainian State Research Institute “Resurs.”

The nanoselenium was tested for purity, morphology and size using an electron microscope and UV spectrophotometry. Electronic microscopy of the samples was performed according to the generally accepted method [21] using an electron microscope JEM-1400 (Institute of Microbiology and Virology named by DK Zabolotny National Academy of Sciences of Ukraine) at 80 kV. The research was conducted at the Bila Tserkva National Agrarian University, Ukraine. According to the scheme, a number of 600 one-day-old quails were used, of which six groups (with four replication subgroups in each) were formed on the basis of analogues: control and five experimental ones. The research lasted 35 days. The quail diet consisted of full-fodder feed, responsible for the content of energy and other nutrients standards (Standard organization of Ukraine, 2006). Birds of group 1 (control) were fed with standard diet (SD), experimental quails of second group—SD + 0.3 mg of sodium selenite/kg feed, third group—SD + 0.3 mg (SeNPs)/kg feed, fourth group—SD + Probifilact, fifth group—SD + 0.3 mg sodium selenite/kg feed + Probifilact, sixth group—SD + 0.3 mg (SeNPs)/kg feed + Probifilact. The conditions for birds keeping in all groups were the same.

Complex probiotal preparation Probifilact was added to the mixed fodder (150 g/t). Feed intake and body weight were determined at weekly intervals. The weight gain and feed conversion ratios of birds were calculated then. During the experiment records of the preservation of livestock, live weight of quails, feed consumption, calculating average daily and relative increments of live weight, feed costs per 1 kg of growth were kept.

Blood samples were taken from two birds (♂ + ♀) from each replication subgroup at the end of the experiment (35th day), after 4-h-length fasting after decapitation. The samples were held in an oblique position to form a blood clot and then centrifuged at 3000 rpm for 10 min. The serum was poured into sterile test tubes to determine the biochemical parameters. Biochemical blood parameters such as total protein, albumin, uric acid, total bilirubin, cholesterol, triglyceride, creatinine, calcium, phosphorus, AST and ALT were determined in the research laboratory of biochemical methods of research of Bila Tserkva National Agrarian University using standard sets in accordance with the manufacturer's instructions.

The statistical analysis was conducted using the Microsoft Office Excel software. Numerical data were presented as mean arithmetic values and their standard deviations ($M \pm m$). For single comparisons, values of P were determined using Student's t test. Differences between groups were significant at $P < 0.05$.

3 Results and Discussion

The results of experimental studies indicate that quails of experimental groups for live weight prevailed in control analogues (Table 1). In the young age, young poultry of the control and experimental groups did not differ significantly by live weight. However, in subsequent age periods, the live weight of quails varied depending on the type and level of feed supplement in the feed. The highest live weight was

Table 1 Growth performance and feed utilization of supplemented with different Se sources, under probiotics influence

Group	Parameters/treatments	Final weight (g) after fifth week
1	Standard diet (SD)	162.2 ± 2.86
2	SD + sodium selenite 0.3 mg/kg	169.5 ± 4.91
3	SD + SeNPs 0.3 mg/kg	172.2 ± 3.9
4	SD + Probifilact	174.5 ± 4.03*
5	SD + Probifilact + sodium selenite 0.3 mg/kg	177.6 ± 3.41**
6	SD + Probifilact + SeNPs 0.3 mg/kg	188.3 ± 4.23***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, the results are probably related to the values in the control part

observed in quail of sixth groups, which consumed feed containing Probifilact + SeNPs 0.3 mg/kg.

Quails of the fourth group that consumed feed from Probifilact practically did not concede to the weight of the fifth group young, probably exceeding the live weight of analogues of control in the period of 35-day age. The quail body weight of the second and third groups was similar, with some advantage of the bird of the third group. At the same time, quails of these groups also surpassed the peers of the control group by weight during the period of 35 days.

In this case, it was shown the reducing of feed intake in experimental groups of quails and poultry mortality during the experiment compared with quails receiving standard feed was shown (Fig. 1).

In studies [22], additives of nano-Se at a diet of 0.3 mg/kg, unlike sodium selenite, improve the growth efficiency, antioxidant and immune properties of broilers grown under high ambient conditions. In researches [23], there were the study of the comparative effects of nanoselenium (Nano-Se) and sodium selenite on growth, bioavailability, antioxidant activity, haematological and biochemical parameters, cellular and humoral immunity of birds. It was found that the body weight of all groups who got Nano-Se (to a dose of 0.3 mg/kg) was significantly ($p < 0.05$) higher than adding sodium selenite and control groups. However, the further increase in Nano-Se content in the feed had a negative impact on the body weight of the birds.

It had been established that selenium deficiency can lead to a decrease in the synthesis of triiodothyronine (T3) and inhibition of bird growth [24]. This is due to the fact that selenium is a component of the key enzyme 5'-deiodination, converting T4 to T3, a hormone that regulates body growth controls the energy and protein consumption. In addition, Se may be part of glutathione peroxidase, which prevents cellular lipid membrane destruction due to oxidative damage, which in general affects the growth rate of the organism [15].

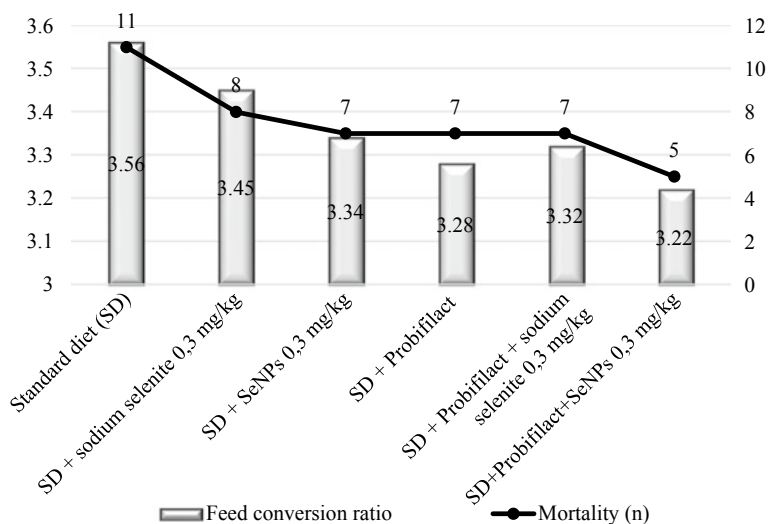


Fig. 1 Feed conversion ratio and mortality of supplemented with different Se sources, under probiotics influence

Saleh [15] believes that improving the bird growth rates by the use of the probiotic complex and nano-Se may be due to increased metabolizable feed energy. This improvement may be due to the balancing of the microbial population in the gastrointestinal tract, which plays an important role in maintaining the health and energy of bird growth.

We found that quails of the sixth group that consumed feed containing Probiolact + SeNPs 0.3 mg/kg were characterized by the lowest feed costs per 1 kg of body weight gain during the growing season (1–35 days), which was 9.6% less than in control group analogues. The death of quails for the use of various feed additives up to 5 weeks from age varied from 5 to 11% among different groups.

The concentration of total protein, albumin (Fig. 2) and bilirubin (Fig. 3) in serum reflects the main functions of the liver, such as the synthesis of proteins (total protein and albumin), withdrawal of anions and the formation of bile (bilirubin), as well as the degree of damage to hepatocyte membranes (AST, ALT) [25, 26]. Increasing of protein, albumin content, tendency to decrease the activity of AST, ALT (Table 2), absence of probable changes in total bilirubin suggests that the addition of selenium and probiotic compounds to the diet positively influenced the parameters of protein metabolism and had no negative influence on the liver.

Experimental groups (3–6) showed a tendency to decrease the concentration of uric acid and creatinine. The content of uric acid in birds reflects the use of protein, the withdrawal of nitrogen, as well as the antioxidant function of the body [27]. Concentration of uric acid (Fig. 3), as the main product of the catabolism of amino acids and purines in birds [28], correlates inversely with the process of protein degradation and reflects the balance between the consumption, use and degradation of proteins,

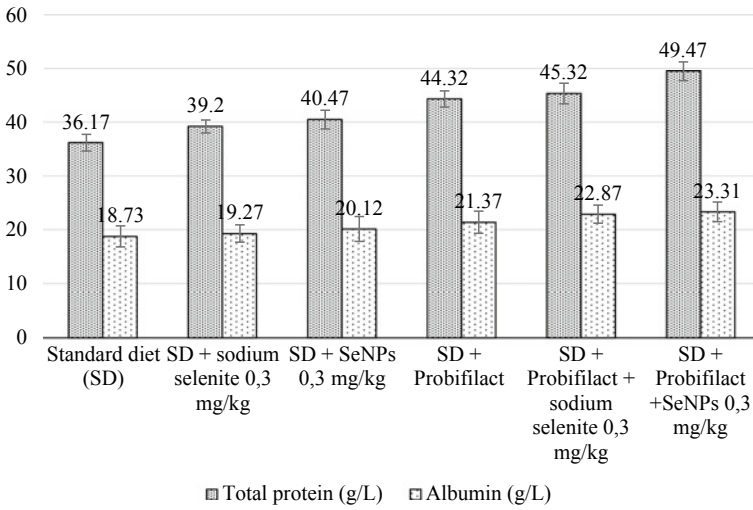


Fig. 2 The content of total protein and albumin in serum blood of 35-day quail

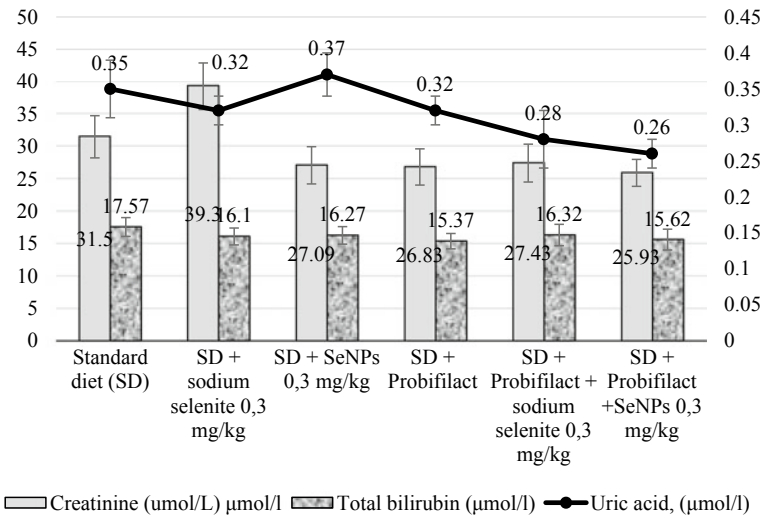


Fig. 3 Content of creatinine, total bilirubin and uric acid of blood of 35-day quail ($M \pm m$)

Table 2 Indicators of lipid metabolism of 35-day quail ($M \pm m$)

Group	Parameters/treatments	Total lipids (g/L)	Cholesterol (mmol/L)	Triglyceride (mmol/L)
1	Standard diet (SD)	17.57 ± 1.85	6.75 ± 0.72	5.63 ± 0.49
2	SD + sodium selenite 0.3 mg/kg	18.11 ± 1.30	5.98 ± 1.49	4.34 ± 0.61
3	SD + SeNPs 0.3 mg/kg	16.84 ± 2.03	4.84 ± 0.74	3.9 ± 0.99
4	SD + Probiolact	15.93 ± 1.22	4.35 ± 0.43*	3.61 ± 0.77*
5	SD + Probiolact + sodium selenite 0.3 mg/kg	15.27 ± 1.35	4.18 ± 0.52*	3.83 ± 0.41*
6	SD + Probiolact + SeNPs 0.3 mg/kg	14.37 ± 1.65	4.05 ± 0.95*	3.46 ± 0.87*

and the excretion of protein metabolites by the kidneys. Concentration of uric acid in serum is used to evaluate the function of the kidneys, with hyperuricemia (increased serum uric acid levels), often associated with kidney disease [28]. The trend towards lowering the concentration of uric acid and creatinine suggests that the addition of probiotics and various forms of selenium does not have a negative effect on the function of the kidneys.

The analysis of the lipid spectrum of blood established (Table 2) that the content of triacylglycerols and cholesterol decreased ($p < 0.05$) in quails of groups 4, 5 and 6, what is consistent with studies in which the reduction in their content in blood broilers for the introduction of probiotics [5].

It is known that microorganisms in the digestive tract can be involved in the metabolism of cholesterol in the body by acting on the host enzyme cell systems that synthesize endogenous cholesterol. Hypocholesterolemic activity of strains of lactic acid bacteria *L. casei* IBM-7280 [29] was established in vivo experiments on the model of mice. Researches [15] found that content in cholesterol and triglyceride plasma was lowered, while the number of high-density lipoproteins (HDL) was increased with the addition of a probiotic and selenium. Perhaps this is due to the activation of lipolysis for the supply of selenium [30, 31].

Researches [5] what added probiotics showed a decrease in the content of triglycerols, and the total level of cholesterol in the blood was also lower. There is an assumption that some bacterial probiotic strains can include cholesterol in bacterial cells and also hydrolyse 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 speed of cholesterologenesis and lowering the total pool of cholesterol in the body [2].

It is believed that probiotics containing specific microorganisms contribute to the transformation and assimilation of nano-Se in the intestine [11].

A significant increase in calcium content was found in experimental groups receiving probiotics and various forms of selenium (Table 3).

Table 3 The content of calcium, phosphorus and the activity of individual transaminases of blood of 35-day quails (M±m)

Group	Parameters/treatments	Calcium (mmol/L)	Phosphorus (mmol/L)	AST (U/L)	ALT (U/L)
1	Standard diet (SD)	2.71 ± 0.22	2.59 ± 0.34	247.2 ± 19.1	48.33 ± 5.74
2	SD + sodium selenite 0.3 mg/kg	2.70 ± 0.51	2.62 ± 0.32	279.7 ± 21.3	58.23 ± 5.04
3	SD + SeNPs 0.3 mg/kg	3.13 ± 0.24	2.84 ± 0.41	235.5 ± 11.9	43.67 ± 3.83
4	SD + Probiolact	3.51 ± 0.37	2.78 ± 0.28	214.1 ± 29.0	40.2 ± 3.03
5	SD + Probiolact + sodium selenite 0.3 mg/kg	3.39 ± 0.26*	3.05 ± 0.31	225.3 ± 18.03	45.2 ± 4.15
6	SD + Probiolact + SeNPs 0.3 mg/kg	3.81 ± 0.34*	3.10 ± 0.24	201.0 ± 19.2	40.2 ± 3.73

In the case of stimulation of the metabolic activity of lactic bacteria and bifidobacteria, low molecular weight fatty acids (acetate, lactate, propionate, butyrate), which lower the pH in the intestine, contribute to the absorption of calcium, magnesium and ferum [32].

It is believed that before this Se form can find itself in commercial poultry/livestock, it is necessary to find out how nanoselenium is converted into active selenoproteins. One of the possible mechanisms for the transformation of nano-Se may be mediated by the action of probiotics in the intestine, which is capable of converting nano-Se into selenite, H₂Se or Se-phosphate, followed by the synthesis of selenoproteins [11].

4 Conclusions

Analysing the data of the experiment, the effectiveness of the application of the studied feed additives in the feeding of quails has been established. The use of probiotics and various forms of selenium in poultry feeding has a positive effect on biochemical parameters, body weight gain, feed conversion rate and livestock retention, and nanoselen alone, and in combination with a probiotic, has a more effective effect compared to sodium selenite. Possibility of involvement of intestinal microbiota in assimilation of nano-Se and metabolism requires further study.

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