*Agronomy Research* **19**(S1), 711–720, 2021
https://doi.org/10.15159/AR.21.075

# Comparative analysis of the use of biostimulants on the main types of soil

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Received: February 6th, 2020; Accepted: April 14th, 2021; Published: May 13th, 2021

**Abstract.** A plant requires certain physiological conditions for normal and productive development. The determining vital factor is the nutritional status of the soil and the environment. At present, the biologization of agriculture is becoming increasingly important. The use of biostimulants is one of the rapidly developing areas in the world practice of crop production and, in the cultivation of agricultural crops, contributes to the production of environmentally friendly products and the improvement of the environment.Such substances can be metal nanoparticles, as well as preparations with a high level of biogenicity, nutritional value and physiology,they are used to improve the growth and development of plants, as well as to activate soil-microbiological processes, which is a liquid-phase biological product (LPBP). The purpose of this work is to identify the effectiveness of the use of a liquid-phase biological product and cobalt nanoparticles in pre-sowing seed treatment against the background of a minimum dose of organic fertilizers, the effect on the productivity and quality indicators of green mass when growing a grass mixture with over-sowing oats on three main types of soil: sod-podzolic, gray forest and black soil. Used cobalt nanoparticles have the following characteristics 40–60 nm, phase composition - Co - 100%. A suspension of nanoparticles was obtained by dispersing with ultrasound in an aqueous solution. LPBP is a dark brown liquid with a specific odor, pH =6.5–7.5, contains N, C, P, K, Ca, Mg, tryptophan, microorganisms. Cobalt nanopowder in solution contained 0.01 g per hectare seeding rate; LPBP concentration was 1%. The seeds were soaked 30 min before sowing in double distilled water (control), in a suspension of nanoparticles and LPBP. The research was carried out according to generally accepted methods. Presowing seed treatment in combination with organic fertilizers in minimal doses provided an increase in yield on soddy-podzolic soil - by 5.69–21.71%, gray forest soil - 2.64–7.50%, black soil - 9.18–18.28%, while no decrease in nutritional value was observed: metabolizable energy, feed units, digestible protein. It is noted that the use of cobalt nanoparticles leads to an increase in the carotene content.

**Key words:** cobalt nanoparticles, liquid-phase biological product, grass mixture, oats, sod-podzolic soil, gray forest soil, chernozem.

## INTRODUCTION

The increase in crop yields and the production of grain, fodder and other crop products is carried out through intensive production methods. They provide for the widespread use of balanced doses of organic and mineral fertilizers, chemical agents for protecting plants from weeds, diseases and pests, and the use of super-heavy means of complex mechanization in the implementation of technological operations. All this increases the anthropogenic load on the ecological environment, leads to the pollution of soil and products by heavy metals, pesticides and nitrates (Matyuk et al., 2011).

The development of modern crop production in conditions of limited financial and material resources requires the improvement of the applied technologies to increase the yield and quality of the products, reduce the costs of its production, as well as ensure the restoration and preservation of soil fertility. One of the ways to solve these problems is the use of growth biostimulants (Efremova, 2016). The production and use of biostimulants is one of the most dynamic segments of agricultural products. Annually the segment is growing by 13%.

In order to improve the efficiency of agricultural production by increasing the yield and quality of grown products, nanotechnologies have been obtained in recent years, as well as technologies with using biological products (Sabirova & Sabirov, 2018).

The elucidation of the biological basis of biostimulant function is a prerequisite for the development of science-based biostimulant industry and sound regulations governing these compounds (Yakhin et al., 2017). Many applied studies have been devoted to the study of the use of biological products and metal nanoparticles as biostimulants, which show the effectiveness and expediency of their use (Ruzzi & Aroca, 2015; Arora et al., 2016; Madbouly, 2018; Youssef & Colla, 2020). At the same time, it should be noted that there is still a need for further research on this issue.

Control of biological processes in agrocenoses is possible through the introduction of agronomically valuable strains of microorganisms into the rhizosphere of plants, enhances the beneficial or weakens the negative impact of undesirable phenomena for the realization of their potential. Today, on the basis of numerous experiments, it can be argued that the symbiosis and association of microorganisms with plants is the basis of the latter's vital activity.

The main mechanisms of the beneficial effect of microorganisms on plants include: improving plant nutrition (increasing the utilization of nutrients from fertilizers and soil); optimization of phosphorus nutrition of plants; fixation of atmospheric nitrogen (improvement of nitrogen nutrition); stimulation of plant growth and development (faster plant development and crop maturation); suppression of the development of phytopathogens (control over the development of diseases and reduction of plant infestation by them, improvement of product storage); increasing plant resistance to stressful conditions (the possibility of increasing plant productivity against the background of water deficit, unfavorable temperatures, high acidity, salinity or soil pollution) (Zavalin, 2005). Unlike chemical preparations, biological preparations have a more pronounced selectivity of action, they are also recognized as harmless to humans and animals, and rapidly decompose in the soil.

All-Russian Research Institute of Reclaimed Lands has developed a fermentation-extraction technology for obtaining various liquid-phase biological products, including a liquid-phase biological product LPBP for plant growing and agriculture. LPBP production includes the stage of fermentation of a peat-manure mixture to obtain a solid-phase fermentation product, then its extraction by a saline solution, followed by filtration. The number of microorganisms (ammonifying, amylolytic, phosphate-mobilizing, amino-synthesizing, etc.) in a fresh biological product reaches n $10^9$ – n $10^{12}$CFU $mL^{-1}$, which makes it possible to refer it to microbial biological products. It lacks pathogenic microflora and parasites. In LPBP, the total nitrogen content is 0.2–0.5 g $L^{-1}$, mobile forms of potassium ($K_2O$) and phosphorus ($P_2O_5$) - 9.5 and 10 g $L^{-1}$, respectively. Also, it contains trace elements (copper, zinc, manganese, iron) and various metabolites of microorganisms (sugars, enzymes, amino acid tryptophan) (Rabinovich et al., 2009, 2015).

Nanosized micronutrient fertilizers are used as biostimulants to increase plant productivity in the direction of sustainable development of the environment. In this case, nanostructured plant trace elements such as Cu, Fe, Ni, Mn, Si, Co, Se and Zn play a crucial role in plant disease resistance by activating enzymes and in increasing the efficiency of energy production by photosynthetic processes for defense mechanisms (Vuong, 2019; Chernikova et al., 2019; Churilov et al., 2020; Seregina et al., 2020).

In plants, cobalt is necessary for fixing molecular nitrogen; it promotes the formation of bacteria in the nodules and leaves of legumes. Cobalt accumulates in pollen and accelerates its germination, participates in auxin metabolism, i.e. stimulates plant growth processes (incl. promotes stretching of cell membranes). This metal is involved in cell reproduction of leaves (an increase in the thickness and volume of the mesophyll, the size and number of cells in the columnar and spongy leaf parenchyma). In addition, cobalt increases the total water content of plants, thereby increasing the drought tolerance of crops.

The influence of cobalt on the formation and functioning of the photosynthetic apparatus of plants through the concentration of chloroplasts and pigments in the leaves was established. And although the amount of this microelement required for plants is very small (up to 12 mg $kg^{-1}$of dry weight), and its indispensability for plants has not been strictly proven, cobalt fertilizers still contribute to an increase in crop yields and improve product quality (Polishchuk et al., 2015).

Thus, under the influence of cobalt, the development of plant tissues containing bacteroids is stimulated, the number of ribosomes in both plant and bacteroid cells increases, and the mobility of bacteroids in the nodules of leguminous plants increases.

The purpose of this work is to determine the effectiveness of the use of a liquid-phase biological product and cobalt nanoparticles in the pre-sowing treatment of seeds against the background of a minimum dose of organic fertilizers, the effect on the productivity and quality indicators of green mass when growing a grass mixture with over-sowing of oats on three main types of soil: sod-podzolic, gray forest and black soil.

## MATERIALS AND METHODS

The research was carried out in lysimeters in four replicates (Dospekhov, 2012) on three types of soil. The soil was characterized by the following agrochemical indicators:

– sod-podzolic: $pH_{KCl}$ 6.0; humus content - 2.3%, $P_2O_5$ - 200 mg $kg^{-1}$, $K_2O$ - 198 mg $kg^{-1}$ of soil;

– gray forest: $pH_{KCl}$ 6.2; humus content - 2.6%, $P_2O_5$ - 204 mg $kg^{-1}$, $K_2O$ - 219 mg $kg^{-1}$ of soil;

– chernozem: $pH_{KCl}$ 6.2; humus content - 3.2%, $P_2O_5$ - 229 mg $kg^{-1}$, $K_2O$ - 250 mg $kg^{-1}$ of soil.

As it can be seen from the agrochemical indicators, all three types of soil are poorly supplied by nutrients.

The size of cobalt nanoparticles was 40–60 nm, the phase composition Co - 100%. A suspension of nanoparticles was obtained by dispersing with ultrasound in an aqueous solution (Dzidziguri et al., 2000). LPBP is a dark brown liquid with a specific odor, pH =6.5–7.5, contains N, C, P, K, Ca, Mg, tryptophan, and microorganisms.

In all variants, compost was introduced as a background at a dose of 20 t $ha^{-1}$, which included 90% of cattle manure and 10% of rotted poultry manure. On the control, the seeds were soaked in distilled water for 60 minutes, in the other two in 1% liquid-phase biological product and a solution of cobalt nanoparticles at the rate of 0.01 g per hectare seed rate. The experiment scheme is presented in the 1st Table.

**Table 1.** The scheme of the experiment

| No of variant | Experimental variants | Abbreviations of variant names in tables |
|---|---|---|
| 1 | Control (sod-podzolic soil) | Control (SPS) |
| 2 | Liquid-phase biological product (sod-podzolic soil) | LPBP (SPS) |
| 3 | Cobalt nanoparticles (sod-podzolic soil) | NPCo (SPS) |
| 4 | Control (gray forest soil) | Control (GFS) |
| 5 | Liquid-phase biological product (gray forest soil) | LPBP (GFS) |
| 6 | Cobalt nanoparticles (gray forest soil) | NPCo (GFS) |
| 7 | Control (chernozem) | Control (Ch) |
| 8 | Liquid-phase biological product (chernozem) | LPBP (Ch) |
| 9 | Cobalt nanoparticles (chernozem) | NPCo (Ch) |

An annual crop (oats) was sown as an experimental crop with perennial grasses overseeding on green fodder. After establishing a stable water table, early spring plowing was carried out, composting was applied to the soil immediately before sowing, then soil cultivation, soaking and sowing of seeds, and rolling. Soil cultivation, care of crops were carried out on the plots at the same time, taking into account the agrotechnical methods used on the farm. The harvest was carried out in the phase of milk ripeness of the grain. The plants were cut at a height of 1–2 cm from the root neck, placed in bags with the indication of the variant number and repetition.

In the laboratory, the above-ground crop was dried to a constant weight. The samples were taken from the crop according to the repetitions, numbered, according to the registration log, for sending to the laboratory. The dry matter content was determined according to GOST 31640 (Interstate standard, 2012). The essence of the method is to dry the feed weight to a constant mass at a temperature of 105 °C. The amount of exchange energy, feed units, and digestible protein was determined according to the guidelines for assessing the quality and nutritional value of feed (Guidelines, 2002). The carotene content was determined according to GOST 13496.17 by the photometric method, the essence of which consists in the dissolution of carotene in petroleum ether or gasoline and photometric measurement of color, the intensity of which depends on the carotene content (Interstate standard, 1997). Nitrates were determined by the ionometric method, which is based on the extraction of nitrates with an extraction solution and the subsequent measurement of their molar concentration using an ion-selective electrode (Interstate standard, 2015).

Statistical data processing and correlation analysis were performed using the analysis packages 'STATISTICA' and 'VARIANCE'.

## RESULTS AND DISCUSSION

It is well known that the basis for strengthening the fodder base is to increase the yield and biological value of fodder crops, hayfields, pastures, which is especially important when intensifying livestock raising and transferring it to an industrial basis.

The research has shown that the use of biostimulants against the background of the introduction of organic matter contributes to an increase in productivity (Table 2, Fig. 1).

The best effect was obtained when using a liquid-phase biological producton sod-podzolic soil and chernozem. Thus, there is an increase in yield compared to the control by 8.32 c ha$^{-1}$ (21.71%) and 8.01 c ha$^{-1}$ (18.28%) on sod-podzolic soil and chernozem, respectively. Earlier studies also showed that the use of LPBP biologics on spring wheat (Smirnova, 2015) grown on a mineral background (N50P50K50) increased grain yield by 15%, and organic background-by 27%. LPBP contributed to an increase in the mass of 1,000 grain high by 13%, productive tillering by 10%. There was a significant increase in the content of crude protein in spring wheat grain on both backgrounds of the main fertilizer.

**Table 2.** The yield of cereal hay on the main types of soil when using biostimulants

| No. | Experimental variants | Yield (c ha$^{-1}$) | | |
|---|---|---|---|---|
| | | average | change | |
| | | | % | c ha$^{-1}$ |
| 1 | Control (SPS) | 30.00 | - | - |
| 2 | LPBP (SPS) | **38.32** | 21.71 | 8.32 |
| 3 | NPCo(SPS) | 31.81 | 5.69 | 1.81 |
| | LSD $_{0.95}$ | 1.43 | | |
| 4 | Control (GFS) | 32.83 | - | - |
| 5 | LPBP (GFS) | 33.72 | 2.64 | 0.89 |
| 6 | NPCo (GFS) | **35.49** | 7.50 | 2.66 |
| | LSD $_{0.95}$ | 1.04 | | |
| 7 | Control (Ch) | 35.80 | **-** | **-** |
| 8 | LPBP (Ch) | **43.81** | 18.28 | 8.01 |
| 9 | NPCo (Ch) | 39.42 | 9.18 | 3.62 |
| | *LSD* $_{0.95}$ | 1.82 | | |

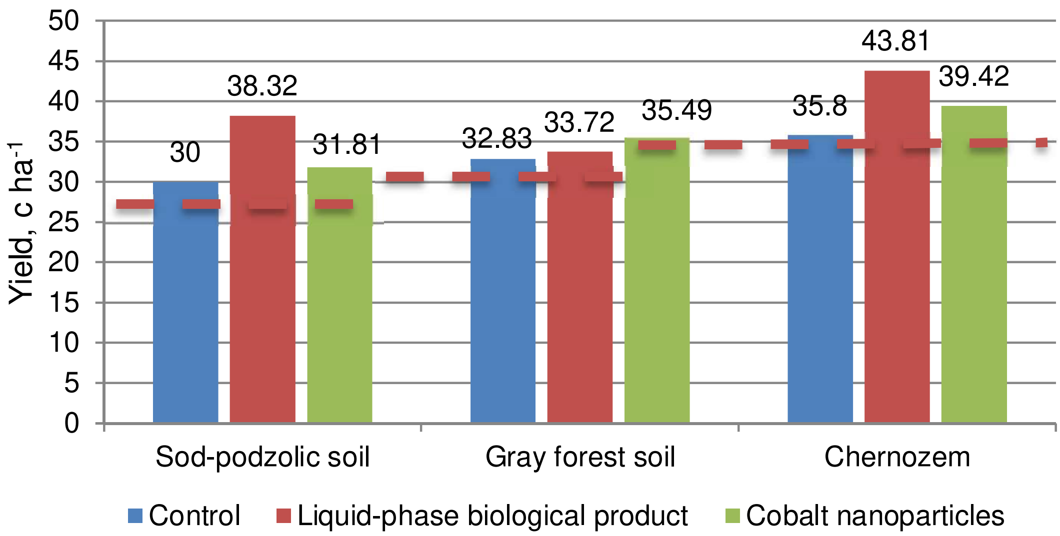


**Figure 1.** The yield of cereal hay on the main types of soil.

Presowing treatment of seeds by cobalt nanoparticles gave the best effect on gray forest soil: the increase in comparison with the control variant was 2.66 c ha$^{-1}$ (7.50%). Studies conducted by Polishchuk and co-authors (Polishchuk et al., 2015) in the field showed that pre-sowing treatment of wheat seeds with cobalt nanoparticles gave an increase in yield on average up to 14%, respectively, compared with the control.

Currently, the nutritional value of feed is characterized by almost seventy different indicators. When studying nutritional value, first of all, the content of moisture and dry matter in it is determined (Table 3). The water content in the studied samples was within the normal range from 10.58 to 11.75%.

The mass fraction of dry matter ranged from 88.22% to 89.42% with a norm of at least 83.0%.

It is known that feed productivity depends not only on the protein content, but also on the supply of energy nutrients. With intensive livestock farming, feed should have an average energy nutritional value of at least 9 MJ of exchange energy (0.70 c.u.) in 1 kg of dry matter.

So, according to the analyzes, the following variants meet these requirements: on sod-podzolic soil, when using a liquid-phase biological product, it contains 9.33 MJ of exchange energy and 0.74 c.u .; on gray forest soil and chernozem with the use of cobalt nanoparticles in the pre-sowing treatment of seeds contains 9.31 MJ of exchange energy and 0.70 c.u. (Table 3). In general, it should be noted that the energy value of the presented samples is quite high.

**Table 3.** Nutritional value

| No. | Samplename | Moisture, % | Drymatter, % | Exchangeable E, MJ | Feedunits, kg |
|---|---|---|---|---|---|
| 1 | Control (SPS) | 10.62 | 89.38 | 9.03 | 0.68 |
| 2 | LPBP (SPS) | 10.84 | 89.16 | **9.33** | **0.74** |
| 3 | NPCo(SPS) | 11.50 | 88.50 | 9.16 | 0.71 |
| | $LSD_{0.95}$ | 0.51 | - | - | 0.03 |
| 4 | Control (GFS) | 10.58 | 89.42 | 9.11 | 0.68 |
| 5 | LPBP (GFS) | 11.55 | 88.45 | 9.17 | 0.68 |
| 6 | NPCo (GFS) | 11.38 | 88.62 | **9.31** | **0.70** |
| | $\mathrm{LSD}_{0.95}$ | 0.55 | - | - | - |
| 7 | Control (Ch) | 11.78 | 88.22 | 9.13 | 0.68 |
| 8 | LPBP (Ch) | 11.40 | 88.60 | 9.23 | 0.69 |
| 9 | NPCo (Ch) | 11.75 | 88.25 | **9.31** | **0.70** |
| | $LSD_{0.95}$ | - | - | - | - |

As you know, the main limiting nutrient in animal diets is protein. The lack of protein substances in animal diets always leads to overspending of feed, underproduction and a decrease in product quality. Therefore, the value of feed is determined not only by the gross yield, but also by the protein content. The portion of the protein that is digested (used by the animal) is called digestible protein. This concept is applicable to monogastric animals, since there it is possible to clearly determine how much of the protein taken from the feed was absorbed.

According to the analysis results, the amount of digestible protein in the control variants on all three types of soil is lower than when using biostimulants when growing crops. The largest amount of vegetable protein is contained in LPBP (SPS) NPCo (SPS) variants and amounted to 30 g per kg, which is 56.7% higher than the control. In the

LPBP variant (GFS), this indicator was 25 g per kg, which is 12% higher than the Control (GFS) variant. The difference on chernozem was 24.1% when a liquid-phase biological product was used in the pre-sowing treatment of seeds. The use of cobalt nanoparticles contributed to an increase in this indicator by 15.4% (Table 4).

Nitrates are an intermediate form in the digestion process from nitrogen to protein and are available in low concentrations in herbs. In the body of the animal, it is processed into nitrites. High nitrite levels are dangerous because nitrites attach to red blood cells (corpuscles), which can lead to sudden death of the cow. Optimum value < 7.5 g kg$^{-1}$ of dry matter. As can be seen from the results of the analyzes, all options meet the requirements and are safe for animal feeding.

**Table 4.** Chemical composition

| No. | Experimental variants | Digestible protein, g kg$^{-1}$ | Carotene, mg kg$^{-1}$ | Nitrates, g kg$^{-1}$ |
|---|---|---|---|---|
| 1 | Control (SPS) | 13 | 7 | 0.588 |
| 2 | LPBP (SPS) | **30** | 8 | 1.005 |
| 3 | NPCo(SPS) | **30** | **12** | 0.413 |
| | *LSD* 0.95 | 1.51 | 1.3 | 0.04 |
| 4 | Control (GFS) | 20 | 8 | 2.961 |
| 5 | LPBP (GFS) | **25** | **11** | 0.399 |
| 6 | NPCo (GFS) | 22 | **11** | 0.636 |
| | *LSD* 0.95 | 1.85 | 1.3 | 0.07 |
| 7 | Control (Ch) | 22 | 8 | 2.631 |
| 8 | LPBP (Ch) | **29** | **10** | 4.619 |
| 9 | NPCo (Ch) | 26 | **10** | 0.563 |
| | *LSD* 0.95 | 1.3 | 1.3 | 0.12 |

According to Smirnova Yu. D. (2017), the use of LPBP on sod-podzolic soils of light granulometric composition provides an increase in biological indicators (the coefficient of mineralization for nitrogen, various groups of microorganisms) and increases the content of mobile phosphorus and nitrates in the soil. Observations showed that LPBP had a positive effect on activity ammonifying microorganisms that contribute to the additional accumulation of nitrogen. The increased content of ammonifiers in the soil is associated with their high content in the applied biological product (up to $10^{12}$ CFU mL$^{-1}$). The maximum total number of ammonifiers for the entire vegetation period of plants was observed from the use of LPBP in the rate of consumption of the working solution of 0.1 l m$^2$ (in the same dosage that was used in our studies). During the studied vegetation period, the accumulation of nitrates in the soil. Such an increased content of nitrates in the soil was reflected in the content of nitrates in cucumber fruits. It can be assumed that our research shows a similar trend and the excess of nitrates in the soil led to an increase in their green mass of plants.

Carotene is an important indicator that characterizes the quality of feed, therefore, when compiling the diet of animals, its content in feed should be known and taken into account. Once in the animal's body, carotene is converted into vital substances- retinoids (Vitamin A, retinoic acid, etc.). Vitamin A regulates the functional activity of epithelial tissue. With its lack, the skin and mucous membranes become dry and corneous, which leads to dermatitis, bronchitis, catarrh of the respiratory tract, gastrointestinal tract, etc. Vitamin A is involved in the formation of retinal visual purple. Visual purple is a combination of vitamin A with proteins. In the light, it breaks down into vitamin A and protein, in the dark it is restored again. With a lack of vitamin A in the body, the restoration of visual purple is slow, which disrupts the eye's adaptability to darkness. Vitamin A is also involved in the exchange of phosphorus, in the formation of cholesterol.

The research has shown that the use of cobalt nanoparticles in pre-sowing seed treatment contributes to an increase in carotene in all three types of soil. The content of this provitamin was higher in comparison with the control variant on sod-podzolic soil by 41.7%, on gray forest soil - 27.3%, on chernozem - 20%.

Cobalt in plant tissues is found in ionic and complexes compounds. In optimal concentrations, this trace element contributes to an increase in the thickness and volume of the mesophyll in the leaves. Cobalt affects the formation and functioning of the photosynthetic apparatus of plants. This trace element contributes to the concentration of chloroplasts and pigments in the leaves, which is associated with an increase in the volume of the plastid apparatus due to the replication and growth of organelles. Carotenoids are a widespread class of structurally and functionally distinct natural pigments, usually yellow, orange, and red. They are synthesized in yeast, fungi, and all photosynthetic organisms, from bacteria and microalgae to higher plants (Armstrong, 1994; Eds Britton et al., 1998). The influence of cobalt on the formation and functioning of the photosynthetic apparatus of plants by the concentration of chloroplasts and pigments in the leaves was established (Bityutsky, 1999). This is due to an increase in the volume of the plastid apparatus due to the growth of organelles. Cobalt can activate the biosynthesis of chlorophyll by stimulating the synthesis of cytoplasmic protein and chloroplasts. Under the influence of chloroplasts, the protein content increases in parallel with the increase in them. The binding of cobalt to protein synthesis can be carried out by regulating the structure and stability of ribosomes, as well as the functioning of RNA. Ultrasonic action on the powder of cobalt nanoparticles and the aqueous medium leads to the creation of a disordered system, in which the presence of many free active reagents is detected, entering into direct reactions occurring in the cell, or serving as catalysts for some of them. In addition, due to the high diffuse mobility of the particles, unsaturated metal valences and the formation of a large set of chelated compounds, it provides high biological efficiency. Thus, it can be assumed that cobalt nanoparticles contributed to the activation of carotene synthesis processes in the green mass of perennial grasses.

## CONCLUSIONS

Growth biostimulants used in pre-sowing seed treatment (liquid-phase biological product or cobalt nanoparticles) against the background of a minimum dose of organic fertilizers provide an increase in yield on sod-podzolic soil by 5.69–21.71%, gray forest soil - 2.64–7.50%, black soil - 9.18–18.28%. A liquid-phase biological product works better on sod-podzolic soil and chernozem, and treatment by cobalt nanoparticles works on gray forest soil.At the same time, there is no decrease in nutritional and energy value: exchange energy, feed units, digestible protein, and the products themselves are safe. The use of cobalt nanoparticles leads to an increase in the carotene content, regardless of the type of soil, from 20.0% to 41.7%, then the liquid-phase biological product has the best effect in the accumulation of provitamin on gray forest and sod-podzolic soil.

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