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CONTENT

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An effect of cerium dioxide nanoparticles on metabolic processes in the body of broiler chickens

Tsekhmistrenko O.[1], **Bityutskyy V.**[1], **Tsekhmistrenko S.**[1],, **Demchenko O.**[2], **Spivak M.**[2]

[1] *Bila Tserkva National Agrarian university*
[2] *D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine*

Tsekhmistrenko Oksana E-mail: tsekhmistrenko-oksana@ukr.net

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The scope of application of cerium dioxide and its special physical and chemical properties are considered in the work. The effect of the size factor on the properties of nanodispersed cerium dioxide determines the biological activity of the material, low toxicity and high oxygen non-stoichiometry. Specific properties of CeO_2 include the ability to regenerate oxygen non-stoichiometry, which is expressed in the ability of cerium dioxide nanoparticles to return to their initial state after participating in the redox process in a relatively short period of time, which enables their repeated use.

Nanoparticles, due to their small size, easily penetrate into the body through the respiratory, digestive, and skin organs and exhibit more pronounced biological activity due to the large surface area per unit mass. The change in the physical and chemical mechanisms of action of nanoparticles is due to the fact that most of the atoms are on the surface. Such an arrangement changes the physical, chemical, biological, toxicological properties of the substance and facilitates the interaction of nanoparticles with a living organism. Once inside a biological system, nanoparticles come into contact with a number of physical and chemical features of the organism, which affect their properties and can change the response. These features are largely due to the ability to pass through the redox cycle between two natural oxidation states (Ce^{3+} and Ce^{4+}).

The influence of cerium dioxide nanoparticles on metabolic processes in the body of broiler chickens has been established. Their introduction contributed to an increase in the content of total lipids in the blood by 24.6–31.3 %, albumins – by 16–22 %, and a decrease in the content of uric acid to the level of 63–67 % of the control. Non-toxicity of poultry meat treated with nano-cerium for consumers was established.

The high degree of biocompatibility, low toxicity and catalytic activity of nanodispersed cerium dioxide make it possible to consider it as a promising nanobiomaterial for use in biology, medicine and agriculture.

Key words: nanobiotechnologies, nanoparticles, ceriumdioxide, layinghens, lipids.

Problem statement and analysis of recent research. In the list of ten priority nanomaterials, experts of the interdepartmental program on the correct management of chemical preparations (IOMC) included nanodispersed cerium dioxide [13]. Cerium is the most common rare earth metal and is an element of industrial importance. Cerium dioxide (CeO2), which is also called cerium, is the most famous compound of the element, due to its redox properties and ability to guarantee excellent oxygen mobility [22]. It is a powerful oxidizing agent used in catalysis, biology, and medicine. Nanodispersed cerium dioxide is a promising material that is widely used in modern high-tech industries [1].

Considerable interest in the study of cerium dioxide is due to the fact that upon transitioning to the nanocrystalline state, this compound significantly changes its physicochemical properties in a rather unusual way [1]. In particular, as the particle size decreases, the unit cell parameter of CeO_2 increases. At the same time, there is a change in the oxygen non-stoichiometry of cerium dioxide due to an increase in the proportion of atoms located on the surface of the particles, which causes a change in its electronic and electrophysical properties.

The pronounced influence of the size factor on the physical and chemical properties of nanodispersed cerium dioxide determines the unique biological activity of the material, low toxicity and high oxygen non-stoichiometry. The first factor ensures the comparative safety of using cerium dioxide nanoparticles *in vivo*. The second determines the activity of nanodispersed CeO_2 in redox processes in a living cell, especially in the case of inactivation of active forms of oxygen [19, 27]. Specific properties of CeO_2 include the ability to regenerate oxygen non-stoichiometry, which is expressed in the ability of cerium dioxide nanoparticles to return to their initial state after participating in the redox process in a relatively short period of time, which provides the possibility of their multiple use [12, 18].

Nanoparticles, due to their small size, easily penetrate into the body through the respiratory, digestive, and skin organs and exhibit more pronounced biological activity due to the large surface area per unit mass. The change in the physical and chemical mechanisms of action of nanoparticles is due to the fact that most of the atoms are on the surface. This arrangement changes the physical, chemical, biological, toxicological properties of the substance and facilitates the interaction of nanoparticles with a living organism [11, 19].

Engineered nanoparticles (from 1 to 100 nm) may have different physical and chemical properties than those found in nature, their impact on human health should be evaluated depending on their size and shape [16]. The modern strategy for obtaining nanoparticles of cerium dioxide involves the use of the principles and approaches of "green chemistry" [3, 29].

Once inside the biological system, nanoparticles come into contact with a number of physical and chemical features of the body, which affect their properties and can change the response [2, 21, 23]. These features are largely due to the ability to pass through the redox cycle between two natural oxidation states (Ce^{3+} and Ce^{4+}). However, CeO_2 nanoparticles were previously thought to be stable and sparingly soluble [15] or insoluble in environmental conditions, depending on the carrier, pH, and particle size [16]. The dissolution of nanoparticles depends on the ratio between Ce^{3+} and Ce^{4+} on their surface layer [2]. As the size of the nanoparticles decreases, oxygen vacancies in the lattice are increasingly freed, which leads to a local decrease in the amount of Ce^{4+}.

In recent years, there have been reports in the literature about the use of metal nanoparticles, in particular cerium, in animal husbandry, since the use of antibiotics as growth promoters is prohibited in the European Union. It is reported that rare earth elements (REEs) can be successfully used as new natural feed additives to increase animal productivity [5, 7].

REE can activate the metabolism of proteins and other nutrients by stimulating the activity of hormones (growth hormone and T_3), induce the synthesis of metallothioneins and increase the content of glutathione in the liver. The antimicrobial and antioxidant effects of REE for animals have been established. In the case of their use in the diet of pigs (100 mg/kg), a positive effect on the feed conversion ratio and growth indicators was found.

Changes in the pro-oxidant-oxidant status of the blood of cows with hypogonadism and after their treatment when using the drug caplaestrol, which contains CeO_2 (cerium dioxide) nanoparticles, were established. The efficiency of ovarian repair and restoration of reproductive capacity of cows was also determined. The use of cerium nanoparticles in combination with drugs makes it possible to normalize the structure and function of the mammary gland and increase the level of colostralimmunoglobulins [25].

The use of REE had positive results for poultry [5, 13, 28], contributing to a significant increase in egg production, egg weight and fertilization rate of hatching eggs of 6-month-old laying hens and significantly improving egg production rate and egg weight.

Addition of REE citrate to the diet helps to increase the productivity of broilers [5]. One of the mechanisms of influence is increased secretion of digestive juices. The addition of REE to the diet contributed to a significant increase in SOD activity in the blood of fish and chickens.

Addition of different amounts of cerium dioxide to laying hens did not have a significant effect on feed consumption and egg weight, however, the feed conversion ratio improved and egg production increased ($p<0.05$). Egg quality criteria, with the exception of shell breaking strength, did not change. In particular, the addition of 200 and 300 mg/kg of cerium dioxide to the feed of laying hens led to a significant ($p<0.01$) increase in the breaking strength of the eggshell. The concentration of Calcium and Phosphorus in blood serum increased significantly ($p<0.05$) after the administration of 100 mg/kg of cerium oxide [5]. It was also noted that the superoxide dismutase activity and concentration of malondialdehyde (MDA) in blood serum significantly decreased with the addition of cerium dioxide. Different doses of cerium dioxide addition had no significant effect on the activity of aminotransferases, the content of glucose, triglycerides, total cholesterol, high and low density lipoproteins in blood serum [7]. When cerium dioxide was included in the diet of chickens, a significant decrease in the content of TBC-AP in egg yolk was observed. At the same time, the addition of cerium dioxide improves the oxidative stability of eggs, and this may have a favorable effect on their shelf life. In the applied dose, nanocrystalline cerium dioxide does not accumulate in eggs and parenchymal organs of poultry [29].

Drinking nanocrystalline cerium dioxide to quail has a positive effect on egg productivity. When using nanocerium in a dose of 1 mM/l of drinking water, the laying capacity of quails increased by 7.8 %, the mass of eggs – by 16.9 %, and the intensity of laying – by 6.7 %. In doses of 0.1–10 mM/l of drinking water, nanocerium does not accumulate in eggs and parenchymal organs of poultry [26, 20]. The influence on the intensity of growth and feed consumption of young quails was revealed [29, 30].

The effect of nanocrystalline cerium dioxide was studied and lethal and semi-lethal doses of the drug were determined. The Ld_{50} of nanocrystalline cerium dioxide is greater than 2000 mg/kg, which confirms that this compound belongs to the V class of toxicity and indicates very low toxicity [29]. The positive antibacterial potential of CeO_2 nanoparticles against poultry pathogens, namely *Klebsiella sp., E. coli*, *Staphylococcus sp*. and *Salmonella sp*.was revealed. [9].

The high degree of biocompatibility, low toxicity and catalytic activity of nanodispersed cerium dioxide makes it possible to consider it as a promising nanobiomaterial for use in biology, medicine and agriculture. However, currently all possible mechanisms of its biological activity are poorly studied and require further research [4].

Target organs and reaction-response development mechanisms differ for different metal nanoparticles. They are able to induce reactive oxygen species, disrupt membrane structures, penetrate tissue barriers, enter cells and interact with intracellular components [6, 8, 16]. The issue of researching the positive effect and toxicity of metal nanoparticles is ambiguous and multifaceted, requiring a complex approach. This especially applies to nanoparticles that are used in pharmacology, medicine and agriculture, which contributes to their direct entry into the human body.

The aim of the research – to study of the effect of cerium dioxide nanoparticles on metabolic processes in the body of broiler chickens and the non-toxicity of the obtained poultry meat.

Material and methods of research. The synthesis of nanodispersed cerium dioxide was performed at the Department of Interferon and Immunomodulators of the D.K. Zabolotny Institute of Microbiology and Virology National Academy of Sciences of Ukraine. In the case of studying the effect on the body of nanodispersed cerium dioxide in the conditions of the educational research center of the Ukrainian National Academy of Sciences, 3 groups of broiler chickens of the Ross 308 cross were formed – control and 2 experimental groups of 100 heads each. Broilers were kept in BKN-3 cage batteries with free access to feed and water. The main parameters of the microclimate corresponded to zootechnical standards. Determination of live weight of broilers was carried out weekly by individual weighing. For 58 days, the research groups were given the preparation of nanodispersed cerium dioxide orally with drinking water at a dose of 8.6 mg/dm3 during the first 14 days, after the 7^{th} (2^{nd}Nanocerium group) and 14^{th} (3^{rd}Nanocerium group) day break, the course was repeated.

Blood stabilized with EDTA was used for hematological studies, and blood serum was used for biochemical studies. In blood serum, the following were determined: total protein, activity of enzymes (AlAT, AsAT, LDH), urea, total lipids, total cholesterol, calcium and phosphorus content with the help of a semi-automatic biochemical analyzer-HumaLyzer 3000 using standard sets of the company Human.

The biological value and toxicity of poultry meat was evaluated in the research laboratory "Veterinary and sanitary examination of livestock products" by the express method on the test culture *Tetraximena Piriformis*, laboratory strain WH-14.This test culture reacts to the action of chemical and biological factors adequately to higher animals, which makes it possible to quickly obtain reliable information. Toxicity was assessed by the express method, by detecting dead ciliates or altered forms, by motility and growth inhibition of *Tetrachimena Piriformis* ciliates [30]. The criterion of relative biological value was the number of ciliate cells, expressed as a percentage, which grew in three days on the studied object in relation to the cells in the control product [30].

Results and discussion. The study of the positive effect and toxic effect of metal nanoparticles is ambiguous and multifaceted, requiring a complex approac [1, 14, 17, 24].This especially applies to nanoparticles used in agriculture, which contributes to their direct entry into the human body [10].

The results of the study show that blood indicators of broilers, which characterize the main types of metabolism (protein, lipid, mineral) during the period of the experiment, were within the physiological norm. The morpho-functional development of animals and poultry and the adaptive capabilities of their organism at an early age depend significantly on the intensity of synthesis and the degree of use of structural lipids (phospholipids, cholesterol) and reserve lipids (triacylglycerols) in energetic tissue processes [10]. Therefore, the tendency to a higher level of these indicators of lipid metabolism in the blood serum of chickens under the influence of the studied additive characterizes the intensive growth of young broilers. During the study of the indicators, no significant differences between the groups were found, but throughout the experiment there was a tendency to increase the content of total lipids and cholesterol in the experimental groups of chickens (Fig. 1). Thus, in the case of the introduction of cerium dioxide nanoparticles for 14 days with a seven-day break, the content of total lipids increased by 24.6 %, and in the case of a 14-day break – by 31.3 %.

During the study of the total protein content, no significant differences between the groups were found, but a significant increase in the albumin content in the blood of the broilers of the experimental groups was observed – inthe first group by 16 %, in the second – by 22 %. Regarding the content of uric acid, this content was lower in the experimental groups and amounted to 63 and 67 % of the control, respectively (Fig. 2).

It can be stated that in the case of the introduction of nanoparticles of cerium dioxide into the bird's body, activation of protein metabolism was observed, which contributed to assimilation processes and a decrease in the number of catabolism products.

In clinical studies, the activity of transaminases, in particular, alanine and aspartate aminotransferases, which characterize the degree of liver damage, are determined as marker enzymes. It was found that the birds of the 2nd research group showed a tendency to decrease the activity of AsAT in blood serum (Fig. 3). Such a change in the activity of aminotransferases is due to a certain hepatoprotective effect of cerium dioxide nanoparticles.

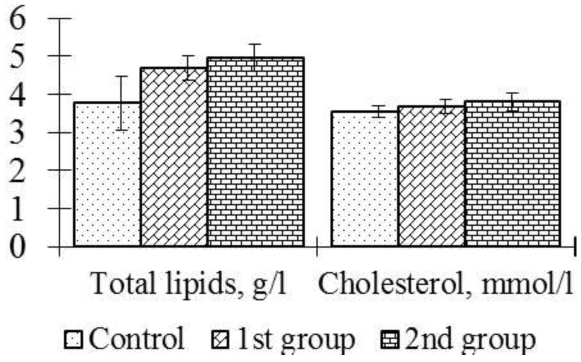

Fig. 1. **Content of total lipids and cholesterol in the blood serum of ROSS 308 broiler chickens after the addition of cerium dioxide nanoparticles.**

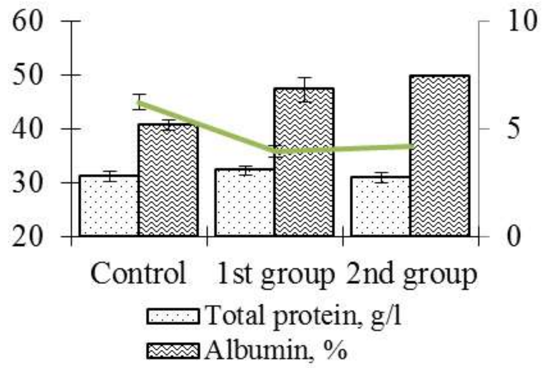

Fig. 2. **Content of total proteins, albumin and uric acid in the blood serum of ROSS 308 broiler chickens with the addition of cerium dioxide nanoparticles.**

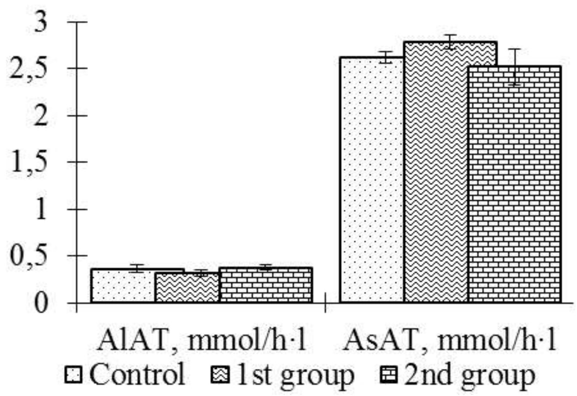

Fig. 3. **Activity of blood serum transaminases of ROSS 308 broiler chickens with the addition of cerium dioxide nanoparticles.**

It has been proven that a specific property of nanocerium is the ability to return to its original state after participating in the redox process in a relatively short period of time, which provides the possibility of its repeated use [6, 8, 13, 19]. Probably, such a unique property of nanocerium caused certain changes in the exchange of proteins and lipids.

As a result of the conducted research, an increase in the ratio of Calcium to Phosphorus was established, which makes it possible to assert the positive effect of cerium dioxide nanoparticles on mineral metabolism in the bird's body (Fig. 4).

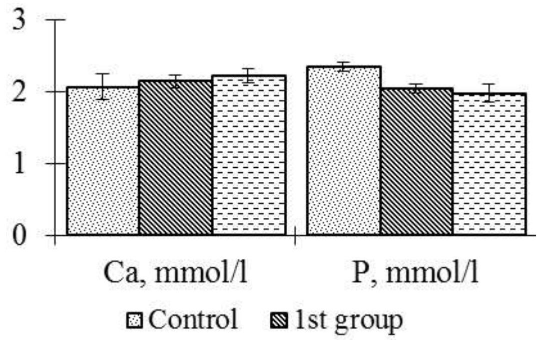

Fig. 4. **Calcium and Phosphorus content in the blood serum of ROSS 308 broiler chickens with the addition of cerium dioxide nanoparticles.**

The introduction of the drug into the diet of broilers from 1 day to 58 days of age with different intervals provides a tendency to a stable increase in their growth intensity, while the introduction of the supplement at an interval of 14 days probably ($p<0.05$) affected the increase in body weight.

Biochemical indicators of blood reflect metabolic processes, and also characterize the effect on the body of feed additives, antioxidants and other biologically active substances [19]. In further research, it is advisable to study the effect of the addition of cerium dioxide nanoparticles on the quality of poultry products.

The final stage of checking the use of any additive in animal husbandry and poultry farming is checking the product's quality. The main product of broiler chickens is meat. Studies were conducted to determine the toxicity and biological value of the meat of broiler chickens of different experimental groups. 0.56 % sea salt solution and glucose-peptone medium were used as controls.

Toxicity assessment was carried out by an express method, by detecting dead or changed forms, by mobility and inhibition of growth of *Tetraximena Piriformis* ciliates.

Tetraximena Piriformis ciliates in the 1st control group, which contained a 0.56 % sea salt solution, were active, but their growth was inhibited, which is explained by the neutrality of the medium. In the 2nd control group, the ability of ciliates to active movement and reproduction was noted. In all experimental groups, *Tetraximena Piriformis* cilia were mobile, reproduced well, pathological and dead cilia were absent. The obtained results indicate the non-toxicity of the meat of broiler chickens under the conditions of exposure to cerium dioxide nanoparticles.

The criterion of relative biological value was the number of ciliate cells, expressed as a percentage, which grew in three days at the studied object in relation to the cells in the control product (Table1).

Table 1 – **Results of the express method on the test culture of the ciliate *Tetraximena Piriformis* on the relative biological value of broiler chicken meat,** %; M±m; n=10

Indicators	Meat of broiler chickens		
	1st group	2nd group	3rd group
The number of cells, $\times 10^6$ in 1 cm^3 of medium	2,83±0,18	2,78±0,13	2,75±0,21
Relative biological value, % of control	100	98,2	97,2

The relative biological value of the meat of broiler chickens of the experimental groups was at the same level as that of intact birds. The noted decrease in biological value is insignificant (1.8–2.8 %) and is not probable.

Conclusion. The paper examines the effect of cerium dioxide nanoparticles on metabolic processes in the body of broiler chickens. Their introduction contributed to an increase in the content of total lipids in the blood by 24.6–31.3 %, albumins – by 16–22 %, and a decrease in the content of uric acid to the level of 63–67 % of the control, which indicates the activation of protein metabolism. Non-toxicity of poultry meat treated with nano-cerium for consumers was established. In the future, it is appropriate to study the effect of the drug in the diet of poultry on economic indicators.

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Вплив наночастинокдіоксиду церію на метаболічні процеси в організмі курчат-бройлерів

Цехмістренко О.С., Бітюцький В.С., Цехмістренко С.І., Демченко О.А., Співак М.Я.

У роботі розглянутосферу застосування діоксиду церію та його особливі фізико-хімічні властивості. Вплив розмірного фактору на властивості нанодисперсногодіоксиду церію обумовлює біологічну активність матеріалу, низьку токсичність і високу кисневу нестехіометрію. Специфічними властивостями CeO_2 є і здатність до регенерації кисневої нестехіометрії, яка виражається у здатності наночастинокдіоксиду церію після участі в окисно-відновному процесі за порівняно невеликий проміжок часу повертатися до вихідного стану, що забезпечує можливість їх багаторазового використання.

Наночастинки, завдяки маленьким розмірам, легко проникають у організм через органи дихання, травлення, шкіру та проявляють більш виражену біологічну активність внаслідок великої площі поверхні на одиницю маси. Зміна фізико-хімічних механізмів дії наночастинок зумовлена тим, що більшість атомів знаходиться на поверхні. Таке розташування змінює фізичні, хімічні, біологічні, токсикологічні властивості речовини та сприяє полегшенню взаємодії наночастинок з живим організмом. Потрапивши у біологічну систему, наночастинки стикаються з низкою фізичних і хімічних особливостей організму, які впливають на їх властивості та здатні змінити відповідь. Ці особливості значною мірою обумовлені здатністю до проходження у окисно-відновному циклі між двома природними станами окиснення (Ce^{3+} і Ce^{4+}).

Встановлений вплив наночастинок діоксиду церію на метаболічні процеси в організмі курчат-бройлерів. Їх введення сприяло підвищенню у крові вмісту загальних ліпідів на 24,6–31,3 %, альбумінів – на 16–22 % та зниженню вмісту сечової кислоти до рівня 63–67 % від контролю. Встановлена не токсичність м’яса птиці, що отримувала нано-церій, для споживачів.

Висока ступінь біосумісності, низька токсичність і каталітична активність нанодисперсногодіоксиду церію дає змогу його розглядати як перспективний нанобіоматеріал для застосування у біології, медицині та сільському господарстві.

Ключові слова: нанобіотехнології, наночастинки, діоксид церію, кури-несучки, ліпіди.

ORCID iD:
Tsekhmistrenko O. https://orcid.org/0000-0003-0509-4627
Bityutskyy V. https://orcid.org/0000-0002-2699-3974
Tsekhmistrenko S. https://orcid.org/0000-0002-7813-6798
Demchenko O. https://orcid.org/0000-0003-1457-143X
Spivak M. https://orcid.org/0000-0002-4394-7275