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BIOLOGICAL EFFICIENCY OF CITRATES OF MICROELEMENTS IN ANIMAL BREEDING

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To ensure normal functioning of the organism of animals, the maintenance of their vital functions, the growth and development of the young animals, their productive and reproductive capacities, it is necessary to ensure their full nutrition. Unbalanced mineral nutrition in the diet of the animals leads to the impairments of mineral metabolism in their body. One of the most promising way of obtaining micronutrients with guaranteed safety and bioticity is the use of the achievements of nanotechnology and bioorganic chemistry for the synthesis of organometallic biocomplexes, in particular, citrates. The Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine conducts studies to find out the physiological and biochemical mechanisms of the action of nanoaquacitrate minerals in the organism of animals in different periods of ontogenetic development and productive use. It has been established that the trace elements of microelements are biologically active and safe for health, and their use in livestock breeding leads to increased animal vitality and productivity.

Keywords: citrates, trace elements, livestock, nanotechnology.

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The health condition of animals, their performance and reproduction features are considerably defined by the degree of supplying the organism with the energy and a number of chemical elements, first and foremost, the essential, irreplaceable, ones. Any deviation from the balanced diet leads to the impairment of organism functions, especially if these deviations are rather clearly expressed and longstanding. Thus, the rationing of their content for animals, especially highly productive ones, is a relevant condition for the manifestation of a high level of genetic potential of performance [1, 2].

Modern studies in the animal breeding industry show (prove) established that the most promising way of obtaining micronutrients with guaranteed safety and bioticity is the use of achievements of nanotechnology and bioinorganic chemistry for the synthesis of metalloorganic biocomplexes. Nanobiomaterials, used in animal breeding, should correspond to such main requirements as ecological purity, biocompatibility with this biological object (including the compatibility on the cellular level) and programmed productive effect [3]. It is clear that obtaining such nanobiomaterials is a complicated task, but only then it is possible to qualify them as functional nanobiomaterials. Their further successful application in animal breeding is also manifested by the fact that these materials should be obtained at sufficient amount and at the affordable cost.

The functional nanobiomaterials, currently of most immediate interest for animal breeding, are, first and foremost, microelement compositions, as microelements are incomparable to all the elements, present in various biological objects, including the cells of humans, animals, microorganisms by the ratio of "element content – its effect on vital functions of the biological object" [3].

A group of researchers in Ukraine [4] developed a nanotechnology method to obtaine carboxylates of super purity of the main food acids and biotic elements (Zn, Mg, S, Mn, Fe, Cu, Co, Mo, Cr, I, Se). The method elaborated, aimed at, aimed at enriching food additives with microelements in the form of citrates of biotic elements, obtained using nanoaquatechnology [3, 5, 6].

The Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine conducts the studies in determining physiological-biochemical mechanisms of the effect of nanoaquacitrates of microelements in the organism of animals in different periods of ontogenetic development and productive use [7, 8]. The toxic doses of nanoaquacitrates of 18 elements were determined (Cr, Cu, Fe, Zn, Se, Mn, Mo, I, V, Bi, Ni, Mg, S, Ge, Al, Ag, Ce, Si) along with those of their combinations, which were found to be 6–8-fold lower than their mineral salts. This information was used to elaborate food additives, veterinary medicinal products and preparations based on the citrates of microelements with high biological activity [9]. The introduction of different amounts of nanoaquacitrates of microelements to the diet of animals allowed determining the minimal physiologically active and optimal doses for cattle, pigs, rabbits, bees. The impact of these compounds was studied in terms of the content of macro- and microelements in the tissues and fluids, the formation of immunobiological reactivity of the organism, the condition of antioxidants, disintoxication, reproductive and immune systems, the growth and development of calves, piglets and infant rabbits as well as the role of citrates in treatment and prevention of microelementosis in animals. The impact of nanoaquacitrates of specific elements on the biological value of animal breeding products was studied in terms of indices of the chemical composition of milk, meat, content of fatty acids, microelements and proteins. These results promoted the elaboration of the methodology of studying the biological effect of nanoaquacitrates in the organism of animals as well as their impact on the biological value and quality of animal breeding products. Some differences in the effect of nanoaquacitrates were revealed for Se, Ge, Cr, Fe in the organism of animals compared to other compounds of these microelements which are conditioned by their increased physiological activity and absorption intensity in the digestive tract.

The introduction of citrates of Cr, Se, Co and Zn to the diet of cows improved their liver function, the exchange of Ca, P and vitamin E [10]. The introduction of citrates of these elements promoted the activation of exchange processes in the organism, enhancing the catalase, superoxide-dismutase and glutathione-peroxidase activity and the decrease in the number of leukocytes, the content of hydroperoxides of lipids and TBA-active products during the 1st and 2nd months of feeding with the additive. The mineral additive stimulated the secretory activity of the mammary gland, increasing the average daily milk yield of cows by 3.3–7.8 % [11].

It was established that the capability of males to produce functional sperm depends on the content of microelements, which belong to the active centers of numerous enzymes of glycolysis, pentose phosphate way and antioxidant protection. For instance, the effect of citrates of Mn^{2+}, $Cu^{2+}$and Zn^{2+} on the intensity of oxidative processes in sperm and survival of sperm cells of bulls was investigated. It was established that microelements in the form of citrates penetrate the germ cells and have their regulatory impact on the intensity of oxygen consumption and the restoring capability of sperm, the activity of succinate dehydrogenase and the survival of sperm cells in diluted ejaculates of bulls. To balance the composition of the diluting agent to the physiological boundaries of native ejaculates, the normalization of oxidative metabolism, and the provision of long survival of sperm cells, the researchers recommend adding 10-20-fold lower doses of Mn-, Cu- and Zn-citrates into the dilution environment compared to their analogues in the form of salts. The optimal concentrations, at which the intensity of oxidative processes normalized in the diluted sperm of bulls, are as follows: 0.01 mg/l Mn-, 0.004 mg/l Cu- and 0.06 mg/l of Zn-citrates. At the abovementioned concentrations of citrates of microelements the survival of sperm cells of bulls at the temperature of 0–4 ºC was 127.2–132.0 hours. The increase in the concentrations of microelements in the form of citrates exceeding the optimal values resulted in the inhibition of the respiratory activity of sperm and the accumulation of protons in the extracellular environment, and in case of the maximal dose of Cu-citrate (0.4 mg/l) – in the activity of succinate dehydrogenase and the survival of sperm cells as well [12].

There was a study on the impact of analogous citrates of microelements in the diluted ejaculates of boars, diluted with phosphate-saline buffer, on the intensity of oxidative processes and survival of sperm cells. It was established that the increase in the content of citrates of microelements in the diluted ejaculates of boars leads to the reliable decrease in the respiratory activity of sperm as well as to the redistribution of the flow of protons between sperm cells and the diluting agent towards their increase in the extracellular environment. High survival rate of the sperm of boars (132.7–141.2 hours in the phosphate-saline buffer at the temperature of 2–4 ºC is manifested at the same concentrations of citrates of microelements, noted for bulls: 0.01 mg/l Mn-, 0.004 mg/l Cu- and 0.06 mg/l of Zn-citrates [13].

The experimental studies have proven that the citrates of microelements affect the metabolism processes in the organism of sows. It was established that there was an expressed complex impact of the citrates of microelements (Fe, Zn, Mn, Cu, Co) in the concentration which was several times lower compared to their inorganic salts, on the metabolism indices in the blood of piglets during the weaning period [8, 14]. For instance, the effect of the citrates of microelements was found to induce the increase in the antioxidant activity of erythrocytes which conditioned the increase in adaptive properties of the organism during the period of stress-factor of weaning. The increase in the number of erythrocytes and the concentration of hemoglobin at the effect of the citrates of microelements in piglets during this period is of great relevance for the prevention of iron-deficient anemia. The activation of the hematopoietic system towards hemopoiesis process intensification is likely to be the adaptive reaction of the organism, ensuring the realization of the protective function of blood and promoting the formation of the immune status of animals during the early postnatal period of development. The citrates of microelements enhance this capability of the organism, and improve the resistance of animals to diseases during the weaning period. The consumption of the citrates of microelements by piglets also affected the protein exchange in the organism, in particular, promoted the increase in the content of total protein and the decrease in the activity of alanine aminotransferase in their blood. At the same time, an insignificant decrease in the glucose concentration in the blood of piglets at the effect of the citrates of microelements on the 30th day of life is a positive effect, caused by the increased use of this substrate in their organism as energy material during the period of action of the stress-factor of weaning. The results obtained are in good agreement with the literature data on the fact that some microelements in the citrate form affect the hormonal activity. As zinc regulates insulin secretion, and chromium increases its effect, promoting the binding of the hormone to the receptors on the surface of the cells, these elements affect the whole spectrum of insulin-dependent processes [1]. The intensification of exchange processes in the organism of piglets at the effect of the citrates of microelements is conditioned by the better consumption of the latter, compared to inorganic salts. It was determined that the application of the citrates of microelements while feeding piglets promoted the 33.6 % increase in the average daily bodyweight gain which were 131 g in the control group, and 175 g in the experimental one [14].

There was a complex study of the impact of ferrum citrate in the composition of the Nanoferotsyt preparation (TU U 21.2-30995014-009:2014), elaborated at the Institute of Animal Biology NAAS, on ferrum- and oxygen-transporting functions of blood and on the metabolism processes in the organism of piglets. It was established that this preparation was efficient in preventing alimentary ferrum-deficient anemia. It was determined that the introduction of ferrum citrate promotes the increase in the number of erythrocytes and hemoglobin concentration in blood, has positive effect on ferrum-binding function of transferin, stabilizes blood proteins, the content of Fe, Cu, Co, Mn, vitamins A and E, lipid peroxidation products and the indices of antioxidant system [15].

The citrates of microelements, consumed by female animals during their pregnancy, may influence the metabolism processes both in their organism and in their fetuses and new-born progeny. It was found that citrate chromium, fed to the sows 10–15 days prior to their littering down and for 20 days after that in the doses of 0.5 and 2 μg Cr(III)/kg of bodyweight, stimulated the erythropoietic function both in the sows and in newborn piglets in the first days after their birth [16]. In addition, at the effect of citrate chromium in the amount of 2 μg Cr(III)/kg, the functional activity of leukocytes and the number of lymphocytes in the blood of sows increased with the decrease in the level of segmented neutrophils during the last month of pregnancy and in the first days after birth. This testifies to enhancing the cellular link of non-specific resistance, specific factors of protection, the increase of reserve possibilities of redox metabolic processes of leukocytes in the organism of animals. Therefore, the changes in hematopoietic and immunological indices of blood of the sows and newborn piglets at the introduction of citrate chromium to the diet are likely to be the adaptive reactions of their organism during the period of action of the stress-factor – the birth.

The correction of different links of metabolism is conducted for pregnant sows and doe-rabbits at the effect of citrate chromium, in particular, involving the stabilization of carbohydrate, lipid and protein exchange and normalization of antioxidant and immune systems of the organism. Metabolically efficient amounts of citrate chromium, which were additionally introduced to the diet of animals, may be used as recommended doses for the regulation of metabolism processes and prevention of Cr(III) deficiency in the organism [8].

The effect of citrate chromium in the amount of 3 μg/kg of bodyweight on the indices of T- and B-cellular immunity was studied in rats. It was established that there was an increase in total T-lymphocytes, T-active lymphocytes, T-helpers, B-lymphocytes and immunoregulatory index in their blood which testifies to the activation of the functional activity of lymphocytes and induces the increase in the cellular and humoral links of the immunity of rabbits at the effect of citrate chromium [17].

The experiments proved high efficiency of the action of citrate chromium in the organism of animals on the indices of carbohydrate, protein, and lipid exchanges, the activation of antioxidant, NO-synthase, endocrine and immune systems [8]. It was determined that the introduction of citrate chromium to the diet of animals is accompanied with the regulatory impact on the function of suprarenal, thyroid and pancreas glands. For instance, the experiments proved that the content of insulin in the blood serum at the effect of citrate chromium in the amount of 5 μg Cr(III)/kg of bodyweight tended to increase, with the reliable increase in the content of triiodothyronine – by 53.1 % and thyroxine – by 21.0 %, whereas the content of cortisol decreased by 20.8 % [18].

It was proven that citrates of Cr, Zn, Mg and Va have a normalizing effect on metabolism processes in animals in the hyperglycemia conditions. It was established that at the effect of these compounds in the blood of animals with experimental hyperglycemia there was a decrease in the levels of glucose, glycolized hemoglobin, products of peroxide oxidation of lipids, lactate, the activity of lactate dehydrogenase, inducible NO-synthase, with the simultaneous increase in the activity of glucoso-6-phosphate dehydrogenase, the activity of AOS enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase) and the content of reduced glutathione [19]. The obtained results may be practically used while treating animals from hyperglycemia, which occurs at their excessive consumption of easily digestible carbohydrates which simultaneously leads to obesity, in case of insular hypofunction of the gland in the newborn animals as well as during the pregnancy of females. The application of Cr, Zn, Mg and Va in the citrate form causes the normalization of the functional state of animals due to the increase in secretion and improvement in the binding of insulin to receptors on the surface of cells.

The disintoxicating function and activity of enzymes of antioxidant protection in blood and tissues of young rats was studied while feeding mothers-rats with the solution of citrates of Cr, Se and Ge. It was established that feeding citrates of these microelements for a long time has a direct effect on the increase in the level of phenols in liver tissues and skeletal muscles of the progeny of female rats. Generally, these changes were conditioned with the increase in the concentration of phenol sulfates and phenol glucuronides. In addition, while feeding with the solution of citrates of Cr, Se and Ge there was an increase in the activity of antioxidant protection enzymes – catalase and superoxide dismutase in liver tissues and skeletal muscles of the progeny of female rats [20, 21].

There was a remarkable positive dose-dependent (10, 20, 200 μg Ge/kg of bodyweight) impact of Ge citrate on the growth and development of the organism of rats, its hematological and immunophysiological indices [22]. There was expressed impact of Ge citrate in the dose of 20 μg Ge/kg of bodyweight on the growth and development of the male organism. The effect of Ge citrate stimulated the immunophysiological response of the organism with the increase in the content of total Ig, average weight molecules, sialic acids, the number of leukocytes due to the lymphocytes and granulocytes at the background of the decrease in the level of circulating immune complexes and hexoses, bound to proteins.

The scientists of the Bila Tserkva National Agrarian University investigated the effect of the Ge aquachelate solution on the level of peroxide oxidation of lipids, the enzymatic link of the antioxidant system and the condition of endogenous intoxication of quail embryos. It was established that 5.0 μg/kg of eggs of Ge aquachelate had a positive effect on the activity of the enzymes of antioxidant protection of embryos, not causing any intoxication [23].

The studies on goslings which were fed with combined feeds, enriched with Ge citrate in different doses during the period of their growth, established the intensification of the growth of young birds, improvement of their vital functions and efficiency of using the feeds. The dose of Ge of 0.20 mg/kg was found to be the most efficient as its introduction to the combined feeds promoted the growth of live weight of goslings by 2.3 %, their survival – by 3.0 % and the decrease in the feed amounts per unit of product gain by 2.5 % [24].

It was determined that local feeding of sows with Ge citrate in the dose of 11.16 μg/kg from 1 to 9 days till littering down led to obtaining a higher number of normally developed newborn piglets without any consid-

erable changes in their bodyweight. In addition, feeding with Ge citrate induced the tendency to the increase in the weight of newborn male piglets [25].

It was proven that "nanoaquacitrates" of mineral elements are both biologically active and safe for health, permissible for the purpose of enriching the feeds, raw materials and food products and demonstrate protective properties regarding heavy metals [4, 6]. For instance, the use of citrates of Cr, Se, Co and Se to feed bees demonstrated the decrease in the content of heavy metals (Cd, Pb) both in the tissue of the whole organism and in separate anatomic areas of bees [26]. It was determined that there was some dynamics in the content of lipids in the tissues of bees which promoted the processes of metabolic accumulation of energetic and plastic components. The obtained data confirm the reasonability of using the additives of the citrates of microelements while feeding bees. This ensures the increase in their viability, the increase in the content of essential microelements, lipid and carbohydrate components in the organism and the products of bee-keeping.

The effect of separate and combined application of different doses of Co and Ni citrates in spring feed additives for honey-bees on the intensity of queen bee laying eggs was investigated. The obtained results of studies indicate a considerable stimulating effect of separately applied Co and Ni citrates on the intensity of egg laying of queen bees in spring [27].

The estimation of biological activity of citrates of Fe, Cu, Zn, Mg, obtained by the nanotechnology method, was conducted using *in vitro* conditions. Their impact on cell cultures and blood proteins (albumins, immunoglobulins) was studied. It was established that the highest cytotoxic activity regarding the cell culture was demonstrated by Cu and Zn citrates and the lowest activity – by Mg citrates. The highest denaturating activity regarding blood plasma proteins was determined for Fe compounds and the lowest one – for Mg [28].

The specialists of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine" of NAAS determined the dynamics and degree of the effect of nanoaquacitrates of metals Ge, Se, Mg, Cu and Ag in different doses on the factors of non-specific resistance of the organism of birds [29]. The addition of nanoferrum citrate to the complex preparation "metalloglobulin" facilitated obtaining an improved "nanometalloglobulin" which allows using it to treat anemia of newborn piglets and chickens. It was established that there was a positive effect of complex probiotic-metalloprotein preparation with the addition of nanoferrum citrate on the bodyweight gain, the indices of congenital immunity and expression of genes, encoding cytokines IL-17α, IL-2, IFN-γ in chickens [29].

CONCLUSIONS

To preserve the health of animals, to improve productive and reproductive features, their organism should be supplied with a number of chemical elements, starting with the essential ones. The physiological-biochemical mechanisms of the action of the citrates of microelements, obtained using the achievements of nanotechnology, are studied at the Institute of Animal Biology NAAS. It was established that there was a positive effect of these compounds on the formation of immunobiological reactivity of the organism, the condition of antioxidant, disintoxication, reproductive and immune systems, the growth and development of newborn animals as well as the role of citrates in treatment and prevention of microelementosis in animals. It was proven that the application of the citrates of microelements in animal breeding as biologically active compounds, safe for health, leads to the improvement of vital functions of animals and to the increase in their performance.

Біологічна ефективність цитратів мікроелементів у тваринництві

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Для забезпечення нормального функціонування організму тварин, підтримання його життєвих функцій, росту і розвитку молодняку, продуктивної та репродуктивної здатності необхідне забезпечення повноцінного їх живлення. Незбалансоване мінеральне живлення у раціоні тварин призводить до порушень мінерального обміну в їхньому організмі. Одним з найбільш перспективних шляхів одержання мікронутрієнтів з гарантованою безпечністю та біотичністю є використання досягнень нанотехнології та біонеорганічної хімії для синтезу металоорганічних біокомплексів, зокрема цитратів. В Інституті біології тварин НААН проводяться дослідження із з'ясування фізіолого-біохімічних механізмів дії наноаквацитратів мікроелементів в організмі тварин у різні періоди онтогенетичного розвитку та продуктивного використання. Встановлено, що цитрати мікроелементів є біологічно активними та безпечними для здоров'я, а їх застосування у тваринництві призводить до підвищення життєздатності тварин та продуктивності.

Ключові слова: цитрати, мікроелементи, тваринництво, нанотехнології.

Биологическая эффективность цитратов микроэлементов в животноводстве

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Для обеспечения нормального функционирования организма животных, поддержания его жизненных функций, роста и развития молодняка, продуктивной и репродуктивной способности необходимо обеспечение полноценного питания. Несбалансированное минеральное питание в рационе животных приводит к нарушениям минерального обмена в их организме. Одним из самых перспективных путей получения микронутриентов с гарантированной безопасностью и биотичностью является использование достижений нанотехнологии и неорганической химии для синтеза металлоорганических биокомплексов, в частности цитратов. В Институте биологии животных НААН проводятся исследования по выяснению физиолого-биохимических механизмов действия наноаквацитратов микроэлементов в организме животных в разные периоды онтогенетического развития и продуктивного использования. Установлено, что цитраты микроэлементов являются биологически активными и безопасными для здоровья, а их применение в животноводстве приводит к повышению жизнеспособности животных и продуктивности.

Ключевые слова: цитраты, микроэлементы, животноводство, нанотехнологии.

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