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Biological effects of iodine, selenium, sulfur citrates in broiler chickens

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This study aimed at the assessment of the I, Se, S citrate effect on the organism of broiler chickens. Feeding of chickens of all groups was carried out by using standard balanced feeds – starter, grower, and finish with the use of group feeders placed at the appropriate height from the floor depending on the poultry height. For the chickens of experimental groups, I, Se, S citrate was added to drinking water daily during the 48th day. To the drinking water of chickens of experimental groups different amounts of the citrate solution were added I (200 mg/L), Se (50 mg/L), S (300 mg/L), prepared by the nanotechnology method. Chickens of the II group received the lowest dose of I, Se, S at the rate of 5 µg I/L, 1.25 µg Se/L, 7.5 µg S/L of drinking water, and for poultry of other experimental groups, it was increased by 2 (III), 4 (IV), 6 (V) and 8 (VI) times compared to II group. Coccidiostat "Kokcisan 12%" (KRKA, Slovenia) was added to the starter and grower feed at the stages of its manufacture in quantities of 0.5 kg/t of feed. Coccidiostat was not added to the final feed. During the 48-day technological period, the clinical condition of the poultry was monitored daily by forage and motor activity, safety, and every 7 days – for growth intensity with the individual determination of body weight on a laboratory scale. The investigation of the blood was carried out from the 35th or 48th day during the growing period and also investigation of the internal organs – liver, heart, spleen, stomach, thymus, and bursa of Fabricius. The increase of cholesterol, triacylglycerols, and albumin in the blood of II, III, and VI chicken groups was established on the 35th day of the experiment, and the reduction of creatinine in III and VI groups. By that time, the effect of highest dose of I, Se, S caused a decrease in the T3 concentration in the blood of chickens in the VI group compared to the I and II groups. In particular, in the blood of chickens of IV, V and VI groups compared to the I, II, III group under these conditions there was a significant increase in the content of urea, Ca, P, cholesterol, as well as albumin – only in IV group on the 48th day. Triacylglycerols' content increased in the blood serum of chickens in III and IV groups compared to the II and V groups, but in V group – decreased compared to the III, IV groups. The increase in liver, thymus, and heart mass in the II and V chicken groups was noticed and their decrease in the VI group was revealed compared with the control group. Reliable lower coefficients of the body mass of chickens of III and VI groups may indicate a less notable stimulating biological effect of I, Se, S citrate in these doses on liver development than on the whole organism. The addition of I, Se, S citrate to broiler chickens during the period of growing revealed the difference in their biological action in the presence and absence of the Kokcisan 12% in a mixed fodder. A more notable stimulating effect of low dose of I, Se, S citrate on the growth and development on the chickens' organism was established during the withdrawal of coccidiostatics from their diet. The effect may be due to its inhibitory influence on the biological activity of these elements in the first 35 days of growing. In the final period of chicken growing and withdrawal of ionophore Kokcisan from the diet more noticeable changes were revealed in the studied parameters.

Keywords: physiological activity; poultry; blood; nanomaterials; macro/trace elements; thyroid regulation.

Introduction

An important problem at present for medicine is the lack of intake of I (Milanesi & Brent, 2017; Malavolta & Mocchegiani, 2018; Sarcar et al., 2018) and Se (Schomburg & Köhrle, 2008; Duntas, 2010; Malavolta & Mocchegiani, 2018) in humans. Optimisation of population iodine intake is an important component of preventive health care to reduce the prevalence of thyroid disorders. Severe iodine deficiency causes goitre and hypothyroidism because, despite an increase in thyroid activity to maximise iodine uptake and recycling in this setting, iodine concentrations are still too low to enable production of thyroid hormone. In mild-to-moderate iodine deficiency, increased thyroid activity can compensate for low iodine

intake and maintain euthyroidism in most individuals, but at a price: chronic thyroid stimulation results in an increase in the prevalence of toxic nodular goitre and hyperthyroidism in populations (Zimmermann & Boelart, 2015; Aakre et al., 2020). Hypothyroidism caused by iodine insufficiency in chicken broilers may cause the ascites syndrome with hypoxemia, impaired cardiac and pulmonary function, which were approved by other authors' studies (Luger et al., 2002). As a component of thyroid hormones, iodine has a crucial role in prenatal and early postnatal ontogenesis due to its involvement in the regulation of neurodevelopment, maturation of the musculoskeletal and respiratory systems and the formation of cognitive function. Adequate iodine status is also an important factor in preventing thyroid disorders and maintaining proper mental and physical health

in adulthood (Malavolta & Mocchegiani, 2018). Thyroid hormone is secreted from the thyroid predominantly as the prohormone thyroxine (T_4) and must be activated in tissues to the active form, triiodothyronine (T_3), through the action of the 5'-deiodinase enzymes, type 1 (D1) and type 2 (D2), both of which require the trace element selenium. Iodine is essential for thyroid hormone synthesis and must be provided through dietary sources. Thyroid hormone is required for normal growth and development. The regulation of thyroid hormone production and metabolism allows for adequate thyroid hormone action in tissues, despite significant fluctuations in iodine supply (Milanesi & Brent, 2017). Iodine ($Ca(IO_3)_2 \times 6H_2O$; $Ca(IO_3)_2$) and iodides (NaI, KI) (Antonyak & Vlizlo, 2013; Oliva & Gorshkov, 2014) are approved as animal feed additives in animal husbandry (European Community, 2005), and organic compounds of iodine – ethylenediamine dihydroiodide (EDD). Also, iodinated casein (KI + casein powder), pentacalcium orthoperiodate is used as iodine feed organic additive (Smyth, 2003; Beckett & Arthur, 2005; Islamov et al., 2020).

The stimulating effect of organic iodine compounds, introduced in different doses into the dietary regime of broiler chickens and its influence on the growth, safety, and quality of meat has been revealed (Oliva & Gorshkov, 2014). However, excess of iodine in the diet is also harmful because it reduces the concentration of T_3 and T_4 in the blood, inhibits the puberty of the poultry and the development of embryos (Drozdova & Shatskikh, 2009; Dolińska et al., 2011; Hunchak & Ratych, 2014). Excess iodine exposure (3.5 $\mu\text{g KI/100 g}$ of body weight) for a long duration causes the development of a biochemical state of hypothyroidism. Developed hypothyroidism was found responsible for the hyperglycaemic and hypercholesterolaemic status evidenced by high blood glucose and cholesterol levels and the depletion of glycogen at its storage sites in the liver and skeletal muscle, though there was extra deposition in the cardiac muscle and kidney (Sarcar et al., 2018). It has been shown that repeated administration of KI at 1 mg/kg/24h doesn't cause modification of thyroid hormones' level, but leads to a reversible modification of the expression of genes involved in the synthesis and secretion of thyroid hormones (Lebsir et al., 2018). Excess iodine induced public health problems are now emerging in many iodine sufficient regions due to indiscriminate intake of iodine through various iodized products (Chandra & Chakraborty, 2017). The US Food and Drug Administration (FDA) and the Nutrient Data Laboratory (NDL) of the USDA Agricultural Research Service have worked independently on determining the iodine content of foods and dietary supplements and are now harmonizing their efforts. The FDA and the NDL are working to combine their data on iodine in foods and to produce an online database that can be used for estimating iodine intake from foods (Perhsson et al., 2016). The primary source of iodine is the diet via consumption of foods that have been fortified with iodine, including salt, dairy products and bread, or that are naturally abundant in the micronutrient, such as seafood. However, excess iodine exposure or ingestion can result in thyroid gland dysfunction. Hypothyroidism or hyperthyroidism as a result of supra-physiologic iodine exposure might be either subclinical or overt, and the source of the excess iodine might not be readily apparent (Luger et al., 2002; Leung & Braverman, 2014). The addition to the animal feed of seaweed *Ascophyllum nodosum* and products containing the elevated level of I, enhanced the reproduction and activity of beneficial microbiota of the digestive tract of animals (Braden et al., 2004). Feeding it to pigs in the amount of 20 g/kg of feed optimized the composition of the beneficial microorganisms in the gastrointestinal tract with an increase in the amount of lactobacillus and inhibition of *Escherichia coli* (Dierick et al., 2009). Acidification of drinking water had a positive effect on the growth rate during the finisher phase and the reduction of crude protein in feces. The mechanism of how organic acids provide their positive effect on animal health and productivity, as reported by many studies, might be due to their ability to acidify the content of the digestive tract and regulate gut microbial flora (Samudovska et al., 2018).

It is well-known that in the tissues of the thyroid gland, there is more Se than in the brain and other organs (Schomburg & Köhrle, 2008; Drozdova & Shatskikh, 2009; Malavolta & Mocchegiani, 2018). Important biological functions of selenium are associated with selenoproteins that contain it in the form of selenocysteine (SeC), known as the 21st amino acid in the genetic code. Human selenoproteome contains 25 selenoproteins, including glutathione peroxidases, thioredoxin reductases and iodo-

thyronine deiodinases. As a component of selenoproteins, selenium participates in defence against oxidative stress, maintenance of cellular redox status, redox signaling and thyroid hormone metabolism (Malavolta & Mocchegiani, 2018).

The ability of the thyroid gland to accumulate not only I, but also Se indicates their significant role for the physiological functioning of this organ and the vital functions of the organism (Beckett & Arthur, 2005; Duntas, 2010; Mohapatra et al., 2014). Selenium is a cofactor for enzymes and proteins with vital importance in antioxidant defence, thyroid hormone and insulin function and regulation of cell growth (Strand et al., 2018). Selenium-dependent enzymes catalyze the process of T_4 to T_3 conversion and determine the level of triiodothyronine formation and regulation of metabolism in thyroid cells and other organs (Kohrle et al., 2005). It has been proved that the lack of Se increases the apoptotic response of the gland to the action of H_2O_2 , while with a sufficient supply of Se these enzymes protect thyrocytes from the effects of peroxides (Song et al., 2011; Antonyak & Vlizlo, 2013). Selenium has multiple roles in human health and is crucial for the proper functioning of the immune system. Adequate Se levels can potentially impact positively human life span and health of older persons. In contrast, a decrease in selenium content during aging may be accompanied by an increased susceptibility to diseases common in the elderly, a reduced efficiency of immune system, and a deficiency in the conversion of thyroxine to T_3 , which influences general metabolism (Malavolta & Mocchegiani, 2018).

A no less important role in the body belongs to S, which is involved in redox reactions, tissue respiration, energy production, the transmission of genetic information. Transfer of the sulfane sulfur from a sulfide quinone oxidoreductase (SQR) bound cysteine persulfide is catalyzed by a flavoprotein. Nanodisc-incorporated human SQR exhibits enhanced catalytic performance, and pre-steady-state kinetics characterization of the complete SQR catalytic cycle indicates that GSH serves as the physiologically relevant sulfur acceptor. The kinetic analysis of ndSQR is consistent with glutathione rather than sulfite being the predominant acceptor at physiologically relevant concentrations of the respective metabolites (Landry et al., 2017).

Transsulfuration allows conversion of methionine into cysteine using homocysteine (Hcy) as an intermediate. This pathway produces S-adenosylmethionine (AdoMet), a key metabolite for cell function, and provides 50% of the cysteine needed for hepatic glutathione synthesis. The route requires the intake of essential nutrients and is regulated by their availability (Pajares & Pérez-Sala, 2018). It is proved that S protects cells, tissues, and the whole body from the toxic effects of various microorganisms and substances, forms endogenous H_2SO_4 , which is involved in the disinfection of phenols, indole, and medicinal products (Landry et al., 2017; Halliwell et al., 2018; Veeravalli et al., 2020). Sulfur contributes significantly to nature chemical diversity and thanks to its particular features allows fundamental biological reactions that no other element allows. Sulfur natural compounds are utilized by all living beings and depending on the function are distributed in the different kingdoms (Landry et al., 2017; Pajares & Pérez-Sala, 2018; Francioso et al., 2020).

The thyroid gland, like hormones of the pituitary gland, is involved in the regulation of S metabolism. Animals are not able to fix inorganic sulfur into biomolecules and are completely dependent on preformed organic sulfurous compounds to satisfy their sulfur needs. However, some higher species such as humans are able to build new sulfur-containing chemical entities starting especially from plants' organosulfur precursors. Sulfur metabolism is very complicated and plays a central role in redox biochemistry. The chemical properties, the large number of oxidation states, and the versatile reactivity of the oxygen family chalcogens make sulfur ideal for redox biological reactions and electron transfer processes (Francioso et al., 2020). Experimental studies with hyperthyroidism established the inhibition of the sulfate inclusion in the cartilage of the bones of young animals. However, S compounds can inhibit the biosynthesis of the thyroid hormone (Antonyak & Vlizlo, 2013; Hunchak & Ratych, 2014; Pajares & Pérez-Sala, 2018). It has been proved that the synthesis of androgens and estrogens takes place with the participation of dehydroepiandrosterone sulfate (DHEAS), which is formed in the adrenal glands from endogenous cholesterol (Migues et al., 2002). Transsulfuration presents multiple interconnections with epigenetics, adenosine triphosphate (ATP),

and glutathione synthesis, polyol and pentose phosphate pathways, and detoxification that rely mostly in the exchange of substrates or products (Pajares & Pérez-Sala, 2018). Monogastric animals and poultry can synthesize from methionine sulfur-containing compounds of their tissues, in addition to thiamine and biotin. Biotin and lipoic acid are two universally conserved cofactors essential for intermediary metabolism, their synthetic pathways have become known only in recent years. Both pathways have unusual features. Biotin synthesis requires a methylation that is later removed whereas lipoic acid is assembled on the enzymes (Cronan, 2018). The deficiency of sulfur inhibits the synthesis of sulfur-containing amino acids, which is accompanied by a decrease in the productivity of poultry with a decrease in the metabolism of nitrogen compounds (Faridi et al., 2016; Gasparino et al., 2018; Suzuki et al., 2020). Current work (Rehman et al., 2019) evaluated the utilization of different sources of methionine either from DL-methionine (DL-Met) or L-methionine (L-Met) using different concentrations of dietary methionine plus cystine (Met + Cyst) in broiler chickens. Results showed that a better edible meat yield could be obtained by supplementing Met + Cyst at the rate of 80% of the digestible lysine. Broiler chickens responded to different dietary proportions of sulfur amino acids by altering their sulfur amino acid metabolism, and diets containing 50:50 Met:Cys is recommended for broilers of age 42 to 56 days (Suzuki et al., 2020). The results showed that the gain: feed ratio increased with dietary glycine equivalent supplementation. Very high nitrogen-utilization efficiency with low variation among treatments was found. The findings indicate that small differences in nitrogen-utilization efficiency caused low glycine equivalent dissipation for nitrogen excretion, likely resulting in small interactive effects among dietary glycine equivalent, cysteine, and choline (Hofmann et al., 2020). Solving the problem of deficiency of I and Se in animal husbandry leads to the development of new effective compounds of these elements (Celi et al., 2013; Mohapatra et al., 2014; Emara, 2019).

Taking into account the broad spectrum of biological activity of mineral and organic compounds of I, Se, and S, research on the influence of these citrates and other trace elements on the organism of animals was begun in Ukraine (Dolaychuk et al., 2015; Iskra et al., 2017; Stoika et al., 2017). The results of earlier studies indicate the pronounced biological effect of these compounds in low concentrations without manifestations of toxic reaction in the body of rats (Dolaychuk et al., 2015; Fedoruk et al., 2018) and cows, pigs, rabbits, bees (Iskra et al., 2017; Malavolta & Mochegiani, 2018). Studies by other authors (Mohapatra et al., 2014; Yausheva et al., 2016; Landry et al., 2017) stipulate a stimulating metabolic effect of nanosized compounds of biotic mineral elements. Adequate physiological ratios of I, Se, Cr, Ge citrates, for their combined use in laboratory animals were experimentally determined (Dolaychuk et al., 2015; Iskra et al., 2017). Feeding mature, 4–8-month-old, rats (feeding started at 4 months and lasted till the end of the 8th month) and young, 0–4-month-old, rats (feeding started at birth and lasted for 4 months) with “nanoaquacitrates” of germanium, chromium, and selenium causes an increase of physiological reactivity in their bodies (Dolaychuk et al., 2015). The study of the biological activity of new derivatives of organic compounds I, Se, S, including those obtained on the basis of nanotechnology, indicates their high physiological activity (Yausheva et al., 2016; Iskra et al., 2017; Stoika et al., 2017). Environmental considerations and regulations limiting trace mineral supply and improvements in analytical methods to detect putative contaminants in mineral sources have led to a need for a re-examination of trace mineral requirements. For the past 15 years, there has been an interest in optimising trace mineral (mainly I, Se, Zn, Cu, Fe, Mn) nutrition considering other parameters than solely birds’ performance (Nys et al., 2018). Therefore, this research aimed to study the biological effects of nanotechnology of I, Se, S citrate in different doses, by feeding it to broiler chickens with water during the full technological growth cycle in the presence and absence of coccidiostats in the diet.

Materials and methods

All manipulations with animals were carried out in accordance with the “European Convention for the Protection of Vertebrate Animals Used for Research and Scientific Purposes” (Strasbourg, 1986) and “General Ethical Principles of Animal Experiments” adopted by the First National

Congress on Bioethics (Kyiv, 2001). Protocol of the Bioethics Committee meeting at the Institute of Animal Biology No. 88 dated July 3, 2020.

The research was carried out on broiler chickens cross ROSS-308, formed from cockerels and chickens at the age of one day in the control (I) and 5 experimental (II–VI) groups, 7 in each. The chickens were kept in the vivarium of the State Scientific-Research Control Institute of Veterinary Medicinal Products and Feed Additives, using sawdust litter during the research. The total area of the production premises was 32.5 m², with a volume of 81 m³, and was divided into six sections. According to the technological map, the lighting was 18–23 h/day, using both natural and artificial sources, forced ventilation with air movement regulation depending on the age and weight of the poultry by periods of growth. The temperature regimen was 33–31 °C at 1–7 days and decreased in the following periods according to the scheme: 8–14 days – 31–29 °C; 15–21 days – 29–27 °C; 22–28 days – 27–25 °C; 29–35 days – 25–22 °C; 36–42 days – 22–19 °C; 43–48 days – 19 °C. The maintenance of the specified temperature was provided by centralized heating with the use of “Infra-Rush” electric lamps for local heating of chickens on t 33–27 °C in the first 3 weeks. Feeding of chickens of all groups was carried out by using standard balanced feeds – starter, grower, and finish with the use of group feeders placed at the appropriate height from the floor depending on the poultry height. The poultry were watered with drinking bowls of 2 and 6 liters according to their age, with the addition of different amounts of I, Se, S citrate solution to the drinking water, prepared by the method Kaplunenko & Kosinov (2017, Ukraine patent No. 119570).

To the drinking water of chickens of experimental groups different amounts of the citrate solution of I (200 mg/L), Se (50 mg/L), S (300 mg/L) were added, prepared by the method nanotechnology. Chickens of the II group watered the lowest dose of I, Se, S at the rate of 5 µg I/L, 1.25 µg Se/L, 7.5 µg S/L of drinking water, and for poultry of other experimental groups, it was increased in 2 (III), 4 (IV), 6 (V) and 8 (VI) times compared to II group.

Coccidiostat “Kokcisan 12%” (KRKA, Slovenia) was added to the starter and grower feed at the stages of its manufacture in quantities of 0.5 kg/t of feed. Coccidiostat was not added to the final feed. During the 48-day technological period, the clinical condition of the poultry was monitored daily by forage and motor activity, safety, and every 7 days – for growth intensity with the individual determination of body weight on a laboratory scale. Blood for research was obtained from the subcranial vein from 7 chickens from the group on the 35th day of growth (during the period of the coccidiostat activity), as well as on the 48th day (12 days after its withdrawal from the diet). The slaughter period was on the 48th day of growing, with compliance with the bioethical requirements (European Convention, 1986). From the poultry of the control and experimental groups during the slaughter period, were taken the internal organs – liver, heart, spleen, thymus, bursa of Fabricius, stomach. Their weight (g) was determined by electronic weight type TBE-0.21 and the mass coefficients of these organs calculated (g/kg) – the ratio of organ mass in (g) to body weight (kg) (Vlizo et al., 2012).

The blood serum was received by centrifugation. The concentration of I (except for 20 µg, E III, not set on for technical reasons) in blood serum was obtained by the method of capillary electrophoresis and the system “Capel-105m” (Vlizo et al., 2012); the thyroid hormone free triiodothyronine (free T₃) – by the immune enzyme method, using the instructions for the “TIA IFA-T3” (RF, 2019). Determination of albumin, creatinine, triacylglycerol (TAG), urea, cholesterol, Ca, and P was performed using the biochemical analyzer Humalyzer 2000 (Germany).

Statistical processing the survey results was performed using the computer software package Statistica 8 (StatSoft Inc., USA, 2014). The arithmetic mean value and the standard error of the arithmetic mean ($\bar{x} \pm SE$) were determined. The differences between the values in the control and experimental groups were determined using the ANOVA, where the differences were considered significant as P-value less than 0.05 (with Bonferroni correction).

Results

Drinking an aqueous solution of I, Se, S citrate in different concentrations caused changes in the content of total iodine in the serum of the

broiler chickens. Really low doses of the mixture of I, Se, S citrate reliably increased the concentration of I in the blood of chickens of II (98.9 ± 25.7 pmol/L) and III (111.9 ± 13.7 pmol/L) groups. However, higher doses of I, Se, S citrate caused a less pronounced but slight increase in the concentration of I in the blood of chickens of V (53.2 ± 1.6 pmol/L) and VI (57.9 ± 6.7 pmol/L) groups. The established differences in the activity of I, Se, S of citrate in the used concentrations on the content of I in the blood did not reveal a direct correlation with the functional activity of the chickens' thyroid gland and the amount of T_3 . In particular, the concentration of free T_3 in the blood serum of chickens of the II–V groups not reliably differ from the control (Fig. 1). By that time, the effect of the highest dose of I, Se, S caused a decrease in the T_3 concentration in the blood of chickens in the VI group compared to the I and II ($P < 0.001$) groups.

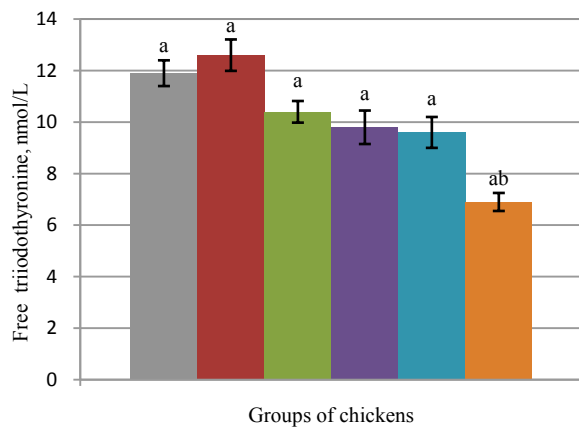


Fig. 1. The concentration of free T_3 in blood serum of broiler chickens on 48th day under the influence of different doses of I, Se, S citrate ($x \pm SE$, $n = 7$): ■ – I (I – 0 $\mu\text{g/L}$ of water, Se – 0 $\mu\text{g/L}$, S – 0 $\mu\text{g/L}$); ■ – II (I – 5.0 $\mu\text{g/L}$, Se – 1.25 $\mu\text{g/L}$, S – 7.5 $\mu\text{g/L}$); ■ – III (I – 10.0 $\mu\text{g/L}$, Se – 2.5 $\mu\text{g/L}$, S – 15.0 $\mu\text{g/L}$); ■ – IV (I – 20.0 $\mu\text{g/L}$, Se – 5.0 $\mu\text{g/L}$, S – 30.0 $\mu\text{g/L}$); ■ – V (I – 30.0 $\mu\text{g/L}$, Se – 7.5 $\mu\text{g/L}$, S – 45.0 $\mu\text{g/L}$); ■ – VI (I – 40.0 $\mu\text{g/L}$, Se – 10.0 $\mu\text{g/L}$, S – 60.0 $\mu\text{g/L}$): different letters indicate the values significantly differing one from another between groups on the results of comparison using the Tukey test ($P < 0.05$) with Bonferroni correction

Table 1

Biochemical blood serum parameters of broiler chickens on 35th and 48th day under the influence of different doses of I, Se, S citrate ($x \pm SE$, $n = 7$)

Indicator	Day	Group/Doses of I, Se, S, $\mu\text{g/L}$ of water					
		I–I(0), Se(0), S(0)	II–I(5.0), Se(1.25), S(7.5)	III–I(10.0), Se(2.5), S(15.0)	IV–I(20.0), Se(5.0), S(30.0)	V–I(30.0), Se(7.5), S(45.0)	VI–I(40.0), Se(10.0), S(60.0)
Creatinine, mmol/L	35	42.6 \pm 2.2 ^a	44.5 \pm 4.4 ^a	35.2 \pm 3.8 ^b	43.0 \pm 1.6 ^a	43.1 \pm 3.9 ^a	31.5 \pm 1.6 ^b
	48	36.8 \pm 2.1 ^a	35.9 \pm 1.5 ^a	34.9 \pm 1.9 ^a	38.6 \pm 3.8 ^a	34.8 \pm 3.6 ^a	36.6 \pm 1.4 ^a
Urea, mmol/L	35	2.08 \pm 0.11 ^a	2.52 \pm 0.22 ^b	1.60 \pm 0.16 ^b	2.24 \pm 0.30 ^a	2.02 \pm 0.13 ^{ab}	1.66 \pm 0.17 ^b
	48	1.48 \pm 0.11 ^a	1.60 \pm 0.07 ^a	1.38 \pm 0.15 ^a	2.65 \pm 0.68 ^b	2.70 \pm 0.24 ^b	3.10 \pm 0.25 ^b
Calcium, mmol/L	35	–	–	–	–	–	–
	48	2.18 \pm 0.13 ^a	2.32 \pm 0.18 ^a	2.32 \pm 0.19 ^a	3.00 \pm 0.35 ^b	2.72 \pm 0.08 ^b	2.58 \pm 0.36 ^b
Phosphorus inorganic, mmol/L	35	1.94 \pm 0.36 ^a	2.22 \pm 0.46 ^a	1.90 \pm 0.23 ^a	2.62 \pm 0.95 ^a	2.44 \pm 0.21 ^a	2.40 \pm 0.48 ^a
	48	2.50 \pm 0.31 ^a	2.74 \pm 0.21 ^a	2.40 \pm 0.22 ^a	4.58 \pm 0.36 ^b	3.32 \pm 0.32 ^{ab}	3.82 \pm 0.78 ^a
Triacylglycerols, mmol/L	35	0.614 \pm 0.096 ^a	0.730 \pm 0.096 ^a	1.596 \pm 0.153 ^b	0.426 \pm 0.100 ^a	0.414 \pm 0.051 ^a	1.274 \pm 0.387 ^b
	48	1.314 \pm 0.359 ^a	0.920 \pm 0.105 ^a	1.516 \pm 0.501 ^a	1.478 \pm 0.308 ^a	1.106 \pm 0.097 ^a	1.280 \pm 0.134 ^a
Cholesterol, mmol/L	35	2.72 \pm 0.32 ^a	4.23 \pm 0.41 ^b	4.10 \pm 0.46 ^b	2.85 \pm 0.52 ^a	3.28 \pm 0.41 ^a	5.00 \pm 0.20 ^{bc}
	48	2.87 \pm 0.52 ^a	2.72 \pm 0.32 ^a	3.50 \pm 0.47 ^a	5.49 \pm 0.88 ^b	5.63 \pm 0.68 ^b	6.03 \pm 1.40 ^b
Albumin, g/L	35	10.64 \pm 1.93 ^a	15.70 \pm 2.33 ^b	16.50 \pm 1.21 ^b	9.92 \pm 1.38 ^a	12.38 \pm 1.06 ^{ab}	14.74 \pm 1.63 ^b
	48	16.34 \pm 1.42 ^a	15.84 \pm 1.46 ^a	13.80 \pm 0.41 ^a	20.60 \pm 1.39 ^b	15.02 \pm 2.21 ^a	18.05 \pm 3.44 ^{ab}

Note: 35 day – for actions Kokcisan, 48th day – without Kokcisan; different letters indicate the values significantly differing one from another within a line of the Table 1 on the results of comparison using the Tukey test ($P < 0.05$) with Bonferroni correction; the mark “–” means that calcium determinations were not performed on the 35th day.

It should be noted that the intensive growth of the body weight of the chickens of the V group during the withdrawal of the coccidiostats from the diet caused the lagging development of their liver, whose weight factor was lower from the I, II and IV groups (Fig. 3a).

Among other changes in ontogenetic parameters, it is necessary to note the decrease in the liver weight factor in chickens of the III ($P < 0.001$) compared to the II group and VI ($P < 0.05$) group compared to the

The applied doses of I, Se, S caused intergroup differences in the biochemical parameters of the chickens' blood. There are differently directed changes in the metabolism of proteins, lipids, and mineral elements of the blood in the presence (35 days) and absence (48 days) of coccidiostats in the diet (Table 1).

In particular, the concentration of cholesterol and albumin increased in the serum of chickens of II, III and VI ($P < 0.001$) groups compared with group I, and triacylglycerols in III and VI groups – compared to the I, II, IV and V ($P < 0.001$) groups on the 35th day of growing and cholesterol in VI group compared to the I, III, IV and V groups ($P < 0.001$) and with the presence of a coccidiostats in the diet. While under these conditions, birds in the IV and V groups underwent no significant changes in the cholesterol and albumin content compared to the I group, but had lowered the level of triacylglycerols in the blood of chickens of the V ($P < 0.001$) group compared to the I group. There is also typically a reliable decrease in the content of creatinine in the blood of chickens of groups III ($P < 0.05$; $P < 0.01$) and VI ($P < 0.001$) groups compared to the I, II, IV, V groups in this period with pronounced to lower urea levels in III, and VI groups compared to the I, II, IV, V groups.

Changes in the biochemical indicators of blood on the 48th day during the withdrawal of the coccidiostat compound indicate a more pronounced effect of the doses: mean and high I, Se, S on the metabolism of lipids, proteins, and minerals. In particular, in the blood of chickens of IV, V and VI groups compared to the I, II, III groups under these conditions the content of urea, Ca, P, cholesterol significantly increased, as well as albumin – only in IV group. Triacylglycerols' content increased in the blood serum of chickens in III and IV groups compared to the II and V groups, but in V group this decreased compared to the III, IV groups. Differences in the concentration of triacylglycerols and creatinine in the blood of chickens of the experimental and control groups were maintained within the statistical deviations of the average values of these indicators.

The analysis of ontogenetic parameters of chickens indicates a reliable not pronounced effect of I, Se, S citrate on body weight gain of chickens of II–VI groups in the first 35 days of growing. In the technological period from the 36th to 48th days of growing under the exclusion of coccidiostats from the diet, the growth rate of chickens in the experimental groups was higher than in the control group. A more pronounced weight gain was observed in this period in chickens II and V groups compared to the I group. Higher body weights of chickens II and V groups was confirmed by an increase in the mass of their liver, heart, thymus compared to those of the I, III, IV and VI groups (Fig. 2).

I and II groups ($P < 0.05$ and $P < 0.001$). These changes may be due to the higher body mass of these chickens by 9.2% and 3.2% in the III and VI groups, respectively. In the chickens of group V ($P < 0.05$) a reliable decrease in the weight of the bursa of Fabricius was noted compared to the I group, and its weight ratio. Reliable lower coefficients of the mass of liver in the body of chickens of III ($P < 0.05$) and VI ($P < 0.05$) groups may indicate a less notable stimulating biological effect of I, Se, S citrate

in these doses on liver development than on the whole organism. In the chickens of group V ($P < 0.05$) a reliable decrease in the coefficient of the mass of the bursa of Fabricius was noted compared to the I group. These data may indicate some differences in the ontogenetic effects of doses of I, Se, S citrate the applied by us in chicken broilers of the II, III, V and VI groups. A more pronounced biological effect of I, Se, S citrate was

observed in the lower (II and III) and high (V and VI groups) doses. The average dose in the chickens of the IV group caused a slight inhibition of their growth during the addition to the diet of coccidiostats and a small stimulating metabolic effect after its exclusion at the end of the growing period. Diseases and deaths of chickens during the study were not noted.

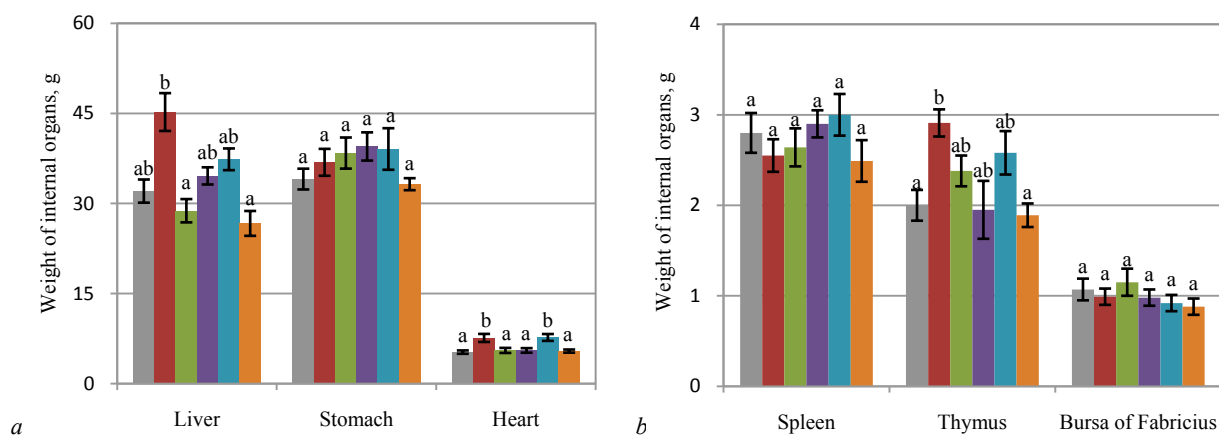


Fig. 2. Weight of internal organs of broiler chickens on 48th day under the effects of various doses of I, Se, S citrate ($x \pm SE$, $n = 7$): a – liver, stomach, heart; b – spleen, thymus, bursa of Fabricius (see Fig. 1)

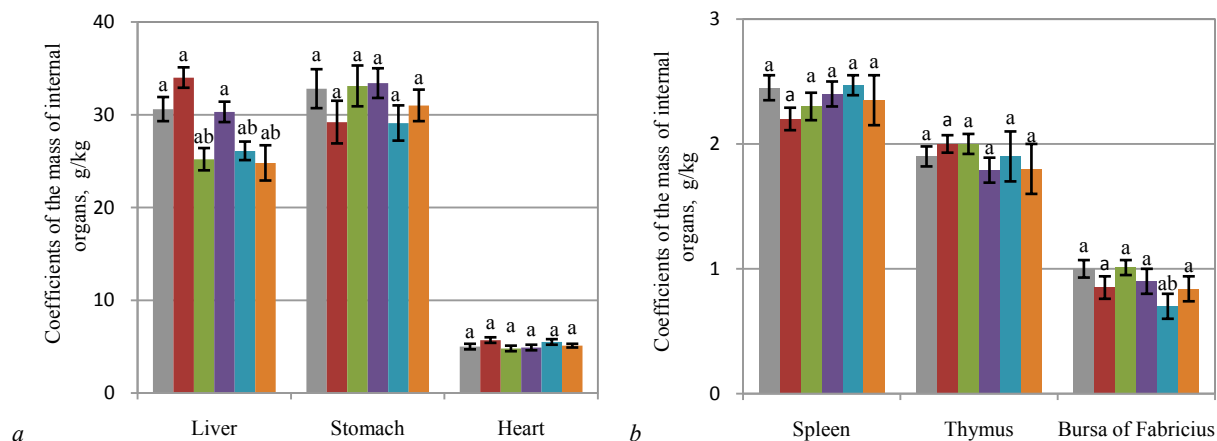


Fig. 3. Coefficients of the mass of internal organs (the ratio of the mass of internal organs (g) to body weight (kg)) of broiler chickens ($n = 7$) on 48th day under the effects of various doses of I, Se, S citrate ($x \pm SE$, $n = 7$): a – liver, stomach, heart; b – spleen, thymus, bursa of Fabricius (see Fig. 1)

Discussion

The biological effect of different I, Se, S doses in the form of citrate obtained by nanotechnology may be expressed both by the chemical properties of each element and the complex biological action of this compound in the presence and absence of coccidiostats in the diet. Changes in the concentrations of I and T_3 in the blood, as well as other biochemical and ontogenetic parameters of the poultry organism, indicate the unequal metabolic effect of the applied doses of I, Se, S citrate on the metabolism of lipids, minerals, proteins with some differences in the presence and removal of Kokcisan.

Other researchers noted the established features of the metabolic action of various doses and forms of I and Se compounds (Schomburg & Köhrle, 2008; Chen et al., 2016; Yuldasheva et al., 2016). According to Oliva & Gorshkov (2014), drinking water with iodine-casein increased the concentration of I in the blood of broiler chickens during slaughter on the 42nd day to 14.53 $\mu\text{g}\%$ against 9.92 $\mu\text{g}\%$ in control group. The content of Ca and P in the blood of chickens in the experimental group was not significantly different from the control. These results may indicate an insufficient supply of broilers with iodine from the standard diet and the active assimilation of this element from the iodine casein supplement, as noted by the authors of the article. A significant increase in the concentration of I in the liver and chest muscles of the poultry in the experimental group confirms this event. The increase in the level of I in the blood of chickens

of II and III groups due to the effect at low doses of I, Se, S did not significantly affect the concentration of T_3 . However, the use of higher doses of I, Se, S caused a reliable decrease in the content of total I and T_3 in the blood of chickens of the V and VI groups. The established differences in the effects of the applied doses of I, Se, S on the supply of I and T_3 to the blood may be determined by the regulatory mechanisms of the influence of I on the thyroid gland function, as evidenced by the literature data (Lu & Cheng, 2010; Song et al., 2011; Phan, 2017). Iodine is a crucial micronutrient for thyroid hormone synthesis and the only source is dietary. Iodide (I^-) is absorbed in the stomach and small intestine, and is concentrated from the blood stream into the thyroid follicular cell through the action of the sodium/iodide symporter (NIS). Iodide is incorporated into thyroglobulin, a process referred to as organification (Milanesi & Brent, 2017). It is proven that the excess of I inhibits the synthesis of thyroid hormones, and physiological levels stimulate these processes (Joanta et al., 2006; Lebsir et al., 2018). Twelve rats were taken, six were fed with iodine through gavage at a dose of 3.5 mg potassium iodide (KI)/100-g body weight, which corresponded to 500 times of the physiological daily dosage of iodide for a period of 60 days, while the other six formed the control group. KI-treated rats presented high body weight and urinary iodine with low levels of thyroid hormones (TH), suggesting a primary thyroid dysfunction. There was an increase in blood glucose, cholesterol, triglycerides, low density lipoprotein (LDL), and very low density lipoprotein (VLDL), while high density lipoprotein (HDL) levels decreased. Tissue

glycogen content in the liver and skeletal muscle was decreased and was increased in the heart and kidney. It may be concluded that excess iodine exposure for a long duration causes the development of a biochemical state of hypothyroidism (Sarcara et al., 2018). To a greater extent, this refers to the liver, skeletal and cardiac muscles, the kidneys (Kohrle et al., 2005; Liu et al., 2006). Changes in the content of triacylglycerols, cholesterol, albumin, Ca, and P in the blood of chickens in experimental groups indicate stimulating effects of citrate I, Se, and S in the body by adjusting the biochemical processes, synthesis of thyroid hormones, and functional activity of the thyroid gland. It is known that the level of I and Se depends on the concentration of thyroid hormones, proteins, lipids in the blood, the processes of cell proliferation and differentiation associated with the growth and development of the body (Joanta et al., 2006; Malavolta & Mocchegiani, 2018). Thyroid hormones are recognized as principal factors that determine the total energy expenditure, the level of basal metabolism, O₂ consumption, and production.

Commonly thyroid hormones regulate the processes of both lipid synthesis and lipolysis. In particular, T₃ stimulates the process of lipogenic in hepatocytes (Lu & Cheng, 2010; Antonyak & Vlizo, 2013; Milanese et al., 2017). In the adipose tissue cells, this hormone stimulates both lipogenesis and lipolysis (Song et al., 2011). This process is due to the activity of I, Se, S in low and high doses as assessed in our studies. In particular, the use of low dose of I, Se, S (II group) caused only a tendency to increase the concentration of T₃ in the blood, and the higher – III–VI group – to decrease. The level of cholesterol and triacylglycerols in the blood was higher, affected by both low and high doses of I, Se, S in the presence of a coccidiostat in the feed. But a higher level of cholesterol in the III–V groups was preserved under the conditions of Kokcisan exclusion from the diet.

The stimulating and inhibitory effect of I, Se, S on the transformation of T₄ into T₃ and on the processes of lipid metabolism caused dose-dependent changes in the content of certain classes of lipids in the blood of chickens. The higher levels of triacylglycerols, cholesterol, and albumin in the blood of chickens in experimental groups indicate the activation of lipid metabolism and blood transport function.

Due to the effect of various doses of I, Se, S citrate, the established changes in the lipid metabolism parameters were analyzed in connection with the concentration of T₃ and activity of the thyroid gland of poultry for the experimental groups. In the adipose tissue cells, T₃ hormone produces the expression of main lipogenic enzymes (acetyl CoA carboxylase, fatty acid synthase) and also, consistently regulates lipolysis with lipogenesis and lipid reservations (Lu & Cheng, 2010; Surai et al., 2018; Surai & Kochish, 2019). Three experiments were conducted with broiler chickens to evaluate the effects of digestible total sulphur amino acid (TSAA) on their performance at three different phases of starter (1–14 days), grower (15–28 days) and finisher (29–42 days). Optimization results showed decreases in optimal dietary TSAA values with increasing age for all traits, while the reverse was observed for intake values and requirements were increased as birds aged. The highest TSAA requirement (7.9, 7.2 and 6.6 g/kg and 283, 585 and 1150 mg/bird per d for starter, grower and finisher, respectively) were achieved for minimum body lipid BL and lowest (5.8, 5.2 and 4.9 g/kg and 201, 444 and 873 mg/bird per bird for starter, grower and finisher, respectively) were suggested for minimum nitrogen excretion (NE) (Faridi et al., 2016). The supplementary organic sulfur (OS) diet affected the performance, egg quality and stimulated immune response in laying hens. A total of 360 laying hens at the age of 31 weeks were distributed into four treatments having five replicates of 18 hens each until 54 weeks. The hens were fed four levels (0.0, 0.1, 0.2 and 0.4%) of OS with basal diet. The result of the study showed that egg production tended to increase with 0.4% OS in diet after 39 weeks of age and, there was a significant effect ($P < 0.05$) from 47 to 54 weeks of age (Chun et al., 2018). At physiological concentrations of I and Se, thyroid hormones play an important role in regulation the organism's resistance and productivity. In particular, studies of the effectiveness of the use of high doses of organic I (0.252 g/t) and Se (0.032 g/t) for broiler chickens established an increase in safety by 2%, and body weight for 21 days – by 5.0% and 6.1%. However, a 2-fold increase in the dose of these elements led to a decrease in the average daily gain of chickens by 1%, and the productivity index – by 1.9% at the age of 38 days (Ponomarenko, 2014). Studies by

other authors have shown the signs of premature involution of the bursa of Fabricius and thymus in broiler chickens, which were introduced a combination of I and Se inorganic and organic compounds (in the form of selenite – Na₂SeO₃, sel-plex, KI, iodine-casein) in pre-starter mixed fodder (Drozdova & Shatskikh, 2009). The full physiological development of these organs was established under the conditions of introduction into the diet of organic Se compounds alone in the form of sel-plex 0.2 g/t and I – iodine-casein – 0.7 g/t.

An approximately equally notable positive effect of Se at a concentration of 0.5 mg/kg of feed from organic drugs Se-BIONyc, Tor-Sel, and Sel-Plex has been shown on the growth and development of broiler chickens (Celi et al., 2013). The advantage of biological effect of nanoselen at a concentration of 0.3 mg/kg of feed compared to sodium selenite in broiler chickens with an increase in body weight and Se content in the muscles up to eight weeks of growing was proved (Mohapatra et al., 2014).

The more notable stimulating effect of I, Se, S citrate on weight gain of chickens of experimental groups at 42–48 days may be due to the lack of anabolic action of Kokcisan withdrawn from the diet from 36 days of growing. Several publications indicate a positive effect on the growth and development of broiler chickens of anti-coccidial products, which include Kokcisan and their combination with other preparations (Chand et al., 2016; Kieronczyk et al., 2017). In particular, research established a stimulating effect of coccidia products – ionophores (salinomycin, monensina) on the weight gain of broiler chickens during 35 days of growing. The combination of ionophores with *Nisinea* enhanced their action without suppressing the metabolism and immune status of the organism. Salinomycin from *Nisinea* did not significantly affect the concentration T₃ in the blood of broiler chickens on the 35th day of growing (Kieronczyk et al., 2017). The use of the prebiotic mannan oligosaccharide in the amount of 0.8 g/kg of feed for experimentally induced coccidiosis in broiler chickens increased body weight, feed consumption, and its conversion (FCR) compared with control and other experimental groups (Chand et al., 2016).

The reliable increase of Ca and P levels in the blood of chickens of IV–VI groups by the 48th-day indicates an increase in connection with the applied doses of I, Se, S, with the entry of these elements into the blood, when the coccidiostat is excluded from the diet. The established changes in the concentration of Ca and P in the blood may be due to their synergistic effect. Only this is confirmed by the tendency to a higher content of P in the blood of chickens of these groups on the 35th day of growing.

The feeding of coccidiostat caused changes in the effect of I, Se, S citrate on the blood transport function. The significantly higher level of albumin in the blood of chickens is evident in both low (II and III groups) and high (V and VI groups) doses under the action of I, Se, S for 35 days of growing. However, the exclusion of Kokcisan from the diet changed this effect because the level of albumin in the blood of the III chickens group for the 48th day was lower, and IV – higher compared to the control.

Therefore, the stimulating effect of I, Se, S citrate on albumin levels, and blood transport function of chickens was more notable when the coccidiostats was introduced to the feed. It may be due to the complex stimulating effect of I, Se, S in low and high doses, in combination with Kokcisan, their influence on the biosynthesis of the albumin fraction of proteins and their circulation in the peripheral blood of chickens.

Enhancement of the stimulating metabolic effect of I, Se, S citrate caused increase in the growth rate of poultry of II and V experimental groups for 42–48 days. The coccidiostat was excluded from the feed during this period, missing its antagonistic action. The established ontogenetic differences in the dynamics of body weight in chickens of the experimental and control groups during the period of withdrawal of Kokcisan from the diet are confirmed by an increase in the mass of the liver, heart, stomach, and thymus (II, V groups). According to Kieronczyk et al. (2017), feeding broiler chickens with ionophore *Monesinea* and its combination with the lactobiotic *Nisinea* did not reliably change the mass of immune organs but reduced the mass of the intestine and pancreas compared to the control. Administration of iodine-casein with water to broiler chickens increased body mass gain, development and weight of the liver (by 10.2%), heart (14.6%), breast muscles (15.2%, $P < 0.05$), bursa of Fabricius (15.3%), and safety of the poultry by 5% and reduced the content of abdominal fat by two times (Oliva & Gorshkov, 2014). Reliably higher

liver, heart, and thymus mass parameters in the II chickens' group showed positive connection with an increase in their body weight, which indicates the activation of metabolism, immune function, and blood circulation at the lowest dose of I, Se, S citrate. For poultry of group V a similar tendency in changes in these parameters indicates the possibility of increasing the stimulating effect of Se citrate at a higher dose, on the growth and development of these organs and the whole organism, as the concentration of Se in the applied solution I, Se, S is the lowest. The significant increase in the body weight of chickens in group V during the withdrawal of Kokcisan from the diet confirms this tendency, which may be due to enhanced Se action by the I citrate.

Thus, the use of I, Se, S citrate during the full technological cycle of growing broiler chickens indicates unequally directed regulatory biological action of the small, medium, and large doses of this compound with reliably determined changes in lipid and mineral metabolism in the blood, growth dynamics, and development of the body and internal organs. In the final period of chicken growing and withdrawal of ionophore Kokcisan from the diet more noticeable changes were revealed in the studied parameters.

Conclusions

In the presence of coccidiostat and of I, Se, S citrate in the chickens' diet, there was an increase in blood transport function with increasing albumin concentration in II, III, V, VI groups and renal filtration capacity with decreasing creatinine level in III and VI groups. There was also an adjustment of lipid metabolism with increasing of blood content of triacylglycerols in III and VI groups and cholesterol in II, III, V, VI groups.

Exclusion from the chickens' diet of coccidiostat in the final period of growing increased the metabolism of Ca, P and albumin and cholesterol under the action of both medium and higher doses of I, Se, S citrate on the background of increasing the content of total iodine in the blood. During this period, there was a reliably lower content of triiodothyronine in the blood of chickens of VI group, as well as the an increase in weight of the liver and thymus in II group, heart in II and V groups on the background of decreasing coefficients of the mass of liver in III, V and VI groups compared to the I group. There was a decrease in the mass of the liver, heart and thymus in chickens of III and VI groups compared to the II group.

The study estimated a more notable stimulating effect of low dose of I, Se, S citrate on the growth and development of chickens during the withdrawal of coccidiostats from the diet. The effect may be due to its inhibitory influence on the biological activity of these elements in the first 35 days of growing.

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