https://doi.org/10.15407/animbiol26.02.047
UDC 661.8'074.5-022.532[546.47+546.23+546.289]:[616.151+577.12]:636.92:612.591
Received 14.03.2024 ▪ Revision 07.06.2024 ▪ Accepted 08.07.2024 ▪ Published online 10.07.2024

Influence of Zinc, Selenium and Germanium citrates nanoparticles on hematological and biochemical parameters of rabbits under moderate heat stress

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Authors' Contributions:
YM: Conceptualization; Project management; Methodology; Formal analysis; Research; Resources; Data curation; Writing — original draft, review & editing; Visualization.

Declaration of Conflict of Interests:
None to declare.

Ethical approval:
A permission to conduct the study was obtained from the Bioethics Committee of the Institute of Animal Biology NAAS of Ukraine (Protocol no. 152 from 10.04.2024, Lviv, Ukraine).

Acknowledgements:
None.

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Climate change leads to increased environmental temperatures, which negatively affects the bodies of animals. Rabbits do not have sweat glands and are not able to regulate their body temperature, which, under conditions of heat stress, disrupts the physiological processes of the body: body thermoregulation, respiration, immune function, endocrine regulation, blood parameters, reproductive capacity, which are critical factors in ensuring the functioning of the body. In recent years, in order to mitigate heat stress, considerable attention has been focused on compounds produced by nanotechnology, which have a positive effect on the animal body compared to organic and inorganic substances and have a wide range of biological effects. However, the effect of macro- and microelement nanoparticles on the mammalian body depends on the amount used. Therefore, the purpose of the experiment was to study the hematological and biochemical parameters of rabbits after weaning under moderate heat stress by feeding Zinc, Selenium and Germanium citrates produced by nanotechnology.

Key words: rabbits, blood, moderate heat stress, nanoparticles, Zinc citrate, Selenium citrate, Germanium citrate, hematological and biochemical parameters

Introduction

In the context of global warming, an increase in ambient temperature becomes a negative factor for the development of rabbit breeding [10, 17]. Heat stress has negative consequences for the health of rabbits, leading to a decrease in daily weight gain by 20–25%, a decrease in feed intake by 8–15%, an increase in mortality by 9–12%, and a decrease in reproductive capacity by 6–10% [19]. Under heat stress conditions, rabbits cannot maintain a balance between heat production and heat release [26]. The optimal physiological values of the rabbit organism's temperature and humidity range are 18 to 21°C and humidity 55–65% [21, 22]. To reduce the adverse effects of heat stress in animal feeding, organic compounds of trace elements of nanotechnological origin are used. Trace element nanoparticles, in combination with organic acids, are characterized by high bioavailability, surface activity, catalytic and adsorption properties, and low toxicity [9].

Zinc is a component of more than 300 enzymes. It is essential physiologically for the functioning of alcohol dehydrogenase, alkaline phosphatase, aldolase, lactate dehydrogenase, RNA and DNA polymerase, transcriptase, carboxypeptidase A, B, G, and superoxide dismutase [32]. It is involved in the biosynthesis of nucleic acids, cell division processes, and metabolism of proteins, lipids, and carbohydrates [4]. Zinc plays a vital role in the antioxidant defense system and inhibits the oxidation of DNA and protein macromolecules [5, 28]. It regulates proliferation, differentiation, apoptosis, and metallothionein gene expression [6]. The addition of zinc oxide nanoparticles to the diet of rabbits at doses of 20, 40, 60, and 80 mg/kg body weight reduces the level of alanine aminotransferase

and aspartate aminotransferase activity, creatinine, and urea, which may indicate an improvement in liver and kidney function [1]. A study by F. Hasan et al. found that rabbits treated with zinc oxide nanoparticles at 30 and 60 mg/kg body weight had better growth and feed intake than the control group [12]. Studies by D. A. Kamel et al. found that the addition of 50 mg of ZnO/kg or 30 mg of nano-Zn/kg in the diet of rabbits increased the levels of glutathione, glutathione-S-transferase, superoxide dismutase, IgG and IgM immunoglobulins, and high-density lipoprotein while reducing the concentration of triacylglycerols and TBA-active products in the blood serum of rabbits under heat stress [14].

Selenium is a critical element of the composition of selenoproteins, which regulate thyroid hormones, act as inhibitors of the nonspecific immune response, and neutralize inflammatory and phagocytic processes [11]. Adding 0.3 mg/kg of organic Selenium to the diet reduces rectal temperature, respiratory rate, and concentration of TBA-active products in the sperm plasma, increases the total number of spermatozoa, the number and body weight of the offspring of rabbits exposed to heat stress [13]. Studies by M. S. Ayyat et al. found that feeding organic Selenium at 0.03 mg/kg of rabbit diet mitigated the adverse effects of heat stress, which was reflected in a decrease in rectal temperature, respiratory rate, and heart rate in the summer [3]. Adding Selenium citrate to the diet at 25 and 50 mg/kg to rabbit feed increases the average daily weight gain, reduced glutathione, and catalase activity. It reduces the level of TBA-active products in the blood serum of rabbits under heat stress [30]. Toxicological studies have shown that selenium nanoparticles of 20–60 nm and Se-methionine in amounts of 30 and 70 μg Se/kg in the diet of mice improve Se accumulation in the blood, liver, and kidneys compared to the control [35].

It is known from the literature that organic germanium can adsorb free radicals and increase the body's antioxidant activity, especially during times of stress [15]. Once in the body, germanium interacts with hemoglobin and ensures cellular metabolism [18]. Germanium compounds improve the immunological characteristics of lymphocytes, including T-helper cells and cytotoxic T-suppressors, and stimulate the production of various cytokines, which can help strengthen the immune system in the fight against various diseases [31]. A significant interferon activity in the blood serum was found after oral administration of the organic germanium compound Ge-132 300 mg/kg body weight to mice [2]. Studies have shown that Ge-132 increases the cellular stimulatory factor IL-3 level in stem cells, which helps regulate the processes of their differentiation and proliferation and ensures regulation and balance in the processes of blood formation [18].

Given the above, the aim of the study was to determine the effect of Zinc, Selenium and Germanium Citrates obtained by nanotechnology on the morphological and biochemical parameters of rabbit blood after weaning under moderate heat stress.

Materials and Methods

The study was conducted in the vivarium of the Institute of Animal Biology, National Academy of Sciences (Lviv, Ukraine). Animals were kept in a room with a regulated microclimate in mesh cages measuring 50×120×30 cm. The study was conducted on young rabbits-analogues from 35 to 78 days of age, the Thermon White breed. For the study, groups of 6 animals were formed, 24 animals in total. During the experimental period, the room temperature was increased for 12–16 hours using electric adjustable heaters for 43 days. The temperature range during the study was from 27.8 to 28.9°C.

The temperature and humidity were monitored using a *Trotec BL30* thermo-hygrometer with a data logger. An electronic air analyzer measured humidity and temperature (patent No. 127047) [23]. The temperature-humidity index controlled the room temperature. Animals for the study were selected in the control and I, II, and III experimental groups, with an average body weight of 980±50 g. Rabbits of the control group were exposed to heat stress and kept on an essential diet of standard balanced granular feed and water without restriction.

Rabbits of I, II, and III experimental groups consumed pelleted feed as in the control but received citrates of trace elements with water for 24 hours daily for 43 days. Using individual drinkers for each animal and placing the animals in separate cages allowed us to control the amount of water each rabbit received. I experimental group received Zinc citrate in amount of 60 mg Zn/l or 12 mg Zn/kg body weight; II group — Selenium citrate, 300 μg Se/l or 60 μg Se/kg body weight; III group — Germanium citrate, 62.5 μg Ge/l or 12.5 μg Ge/kg body weight. "Nanomaterials and Nanotechnologies" LLC (Kyiv, Ukraine) manufactured the solutions for the study (patent no. 38391) [16]. The appearance of metal particles was studied using a *JEM 100CX II* transmission electron microscope. Permission to conduct the research was obtained from the Bioethics Committee of the Institute of Animal Biology of the National Academy of Sciences of Ukraine, Lviv (protocol No. 152 of 10.04.2024). All manipulations with experimental animals were carried out following the provisions of "The General Ethical Principles for Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001) and the rules of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986). The blood parameters of rabbits were studied on the 14th day of the preparatory period and the 14th and 29th days of supplementation in the experimental period under moderate heat stress conditions.

For hematological studies, blood was taken from the marginal ear vein of rabbits into tubes with the anticoagulant ethylenediaminetetraacetate (*EDTA-K2+*), and the total number of red blood cells and red blood cell indices were determined: absolute red blood cell count (RBC),

mean erythrocyte volume (HGB), hematocrit (HCT), mean erythrocyte corpuscular volume (MCV), mean erythrocyte hemoglobin content (MCH), mean erythrocyte hemoglobin concentration (MCHC), erythrocyte distribution width (RDW); the number of white blood cells (WBC) and their forms — lymphocytes (LYM), monocytes (MON), granulocytes (GRA); platelet count and platelet indices: absolute platelet count (PLT), mean platelet volume (MPV), thrombocrit (PCT), relative platelet distribution width (PDW) using an automatic hematology analyzer *Orphee Mythic 18* (Switzerland) [33]. Samples of whole blood were taken from the marginal ear vein of 6 animals from the group into tubes with 1% heparin for biochemical studies. The content of total protein, albumin, alanine aminotransferase (ALT) activity aspartate aminotransferase (AST), and alkaline phosphatase (ALP), triacylglycerols, cholesterol, total calcium, and inorganic phosphorus was determined using a *Hymalyzer 2000* biochemical analyzer [33].

The study results were analyzed using the *Statistica 7.0* software package (*Statsoft*, USA). The experimental data are presented as mean (M) ± standard deviation (SD). The study's quantitative data were tested for homogeneity of variances using the Lever test. Multiple comparisons were made using a two-factor analysis of variance (Two-way ANOVA), where factor A is time, factor B is trace element citrates, and AB is the interaction of time and trace element citrates. To detect statistical differences between the control and experimental groups, the *a posteriori* Tukey HSD method was used, and differences were considered significant at P≤0.05 [27].

Results and Discussion

Rabbits have the most negligible lung weight concerning the body weight of all farm animals, so they need a constant supply of clean air to ensure the body's functioning [4]. Studies have shown that feeding rabbits Zinc citrate and Selenium citrate in the blood, respectively, increased the number of red blood cells by 22.2 (P<0.01) and 20.2% (P<0.05) compared to the control at the final stage of the study, which may indicate a stimulating effect on erythropoiesis (table 1). The analysis of the results of hemoglobin concentration in animals of I, II, and III groups were respectively higher by 16.3% (P<0.01), 28.5% (P<0.001), and 21.9% (P<0.001) on the 29th day of the study. The percentage of hematocrit value in the blood of rabbits of the I and II experimental groups increased by 27.3% (P<0.01) and 20.7% (P<0.05), respectively, on the 29th day of the experiment. The increase in the concentration of hemoglobin and the percentage of hematocrit value in the blood of rabbits directly correlates with the number of red blood cells.

The effect of time factor A (P=0.020794) and the factor of trace element citrates B (P=0.019834) was reflected in statistically significant changes in the absolute red blood cell content. The analysis of the combination of AB factors did not show a statistically significant result (P=0.147556).

Table 1. Number of red blood cells, hemoglobin, and hematocrit in the blood of rabbits after feeding Zinc, Selenium, and Germanium citrates compounds under moderate heat stress (M±SD, n=6)

Indicators	Group	Period of research, age/day of supplementation		
		Preparatory period	Study period	
		49/1	63/14	78/29
RBC, 10^{12}/l	C	5.76±0.51	5.81±0.70	5.04±0.62
	I E	6.08±0.69	5.84±0.53	6.16±0.55**
	II E	6.28±0.55	6.02±0.56	6.06±0.63*
	III E	6.45±0.42	5.93±0.69	5.41±0.26
HGB, g/l	C	132.67±5.75	133.00±10.21	109.17±6.11
	C	135.17±8.47	134.50±12.56	127.00±5.25**
	I E	149.66±16.57	145.66±13.54	140.33±9.77***
	II E	150.00±10.82	134.67±8.01	133.16±5.63***
HCT, L/l	C	0.413±0.07	0.387±0.06	0.362±0.02
	C	0.467±0.06	0.433±0.04	0.461±0.03**
	I E	0.449±0.05	0.416±0.07	0.437±0.07*
	II E	0.502±0.06	0.467±0.04	0.442±0.01

Note. In this and the following tables, the statistically significant differences compared to the control group are: * — P<0.05; ** — P<0.01; *** — P<0.001.

Table 2. Erythrocyte indices in the blood of rabbits after feeding Zinc, Selenium, and Germanium citrates compounds under moderate heat stress (M±SD, n=6)

Indicators	Group	Period of research, age/day of supplementation		
		Preparatory period	Study period	
		49/1	63/14	78/29
MCU, f/l	C	97.43±2.30	98.23±2.31	97.23±3.46
	I E	96.31±2.53	101.10±2.74	98.53±2.28
	II E	98.00±2.52	102.57±2.57	99.46±2.45
	III E	95.03±1.36	100.15±4.38	100.37±3.14
MCH, pg	C	23.56±1.11	23.53±0.95	23.83±0.84
	I E	23.83±0.43	23.90±1.23	24.61±0.90
	II E	24.38±0.77	25.03±0.88	24.40±0.94
	III E	24.68±0.57	24.71±1.23	25.10±0.15
MCHC, g/l	C	241.67±2.87	225.83±6.49	231.17±5.98
	I E	247.17±3.54	228.17±3.18	243.00±2.96**
	II E	243.67±5.27	235.83±3.65*	245.50±3.53***
	III E	244.67±1.75	232.17±6.01	234.00±6.35
RDW, %	C	10.23±0.42	10.38±0.60	10.43±0.58
	I E	10.68±0.36	10.81±0.34	10.98±0.53
	II E	10.36±0.20	10.46±0.62	10.60±0.43
	III E	10.75±0.59	10.96±0.52	10.50±0.63

The analysis of the time factor A (P=0.000021) and the factor of trace element citrates B (P=0.000001) resulted in statistically significant changes in hemoglobin content, where the expressed effect was observed for factors A and B. The results of the analysis of the combination of AB factors did not show a statistically significant result (P=0.079910).

The effect of time factor A (P=0.051486) had no statistically significant effect on the hematocrit value, but

the effect of trace element citrates B (P=0.000400) had a significant effect on the studied index. In the combined interaction of the two factors, there were no statistically significant changes in AB (P=0.588368).

In the blood of rabbits, an increase in the average concentration of hemoglobin in the erythrocyte was found after feeding Zinc citrate by 5.11% (P<0.01) on the 29th day of the study and Selenium citrate by 4.42 and 6.19% (P<0.05–0.001), respectively, on the 14th and 29th days of the experiment (table 2). Zinc is a necessary coenzyme for many enzymes and participates in the synthesis of DNA, RNA, and proteins. In particular, it is essential for the activity of ribonucleic polymerase, which is necessary for the transcription of genes encoding hemoglobin proteins and other essential components of red blood cells [1]. Selenium is a component of enzymes such as glutathione peroxidase and thioredoxin reductase, which protect cells from oxidative stress, reducing the level of damage to erythropoietic cells in the bone marrow, allowing them to multiply more efficiently and differentiate into mature red blood cells [6].

In our opinion, due to their antioxidant properties and essential function in the processes of enzyme activation, Selenium and Zinc indirectly affect the number of red blood cells and red blood cell indices, which is an essential indicator of the body's hematopoietic function under moderate heat stress. The analysis of the influence of factor A (P=0.000000), factor B (P=0.000003), and the interaction of time factors and citrates of trace elements AB (P=0.001152) was marked by statistically significant changes in the effect on the average concentration of hemoglobin in the erythrocyte.

The influence of time factor A (P=0.000059) was reflected in statistical changes in the effect on the average erythrocyte volume, but the factor of trace element citrates B (P=0.089660) did not affect the studied indicator. In the combined interaction of the two factors, there were no statistically significant changes in AB (P=0.182130). The determination of factor A (P=0.468084) was not marked by statistical changes in the average hemoglobin content in a single erythrocyte. The factor of action of trace element citrates B (P=0.000730) had a significant effect on the studied indicator. In the combined interaction of the two factors, no statistically significant changes in AB were observed (P=0.778313). The results of the analysis of factor A (P=0.5578669) did not show statistically significant changes in the effect on the width of red blood cell distribution. The influence of the factor of trace element citrates B (P=0.021730) had a statistically significant effect on the studied variable. In the interaction of the two factors, the data obtained were unaffected by statistical changes in AB (P=0.734692), indicating the absence of influence in the combination of these factors.

Studies have shown that the number of leukocytes in the blood of rabbits of the I and II experimental groups decreased by 9.01 (P<0.05) and 7.95% (P<0.05), respectively, and lymphocytes by 11.3 (P<0.05) and 12.5% (P<0.05) at the final stage of the study (table 3). Leukocytes are cells of the body's defense system. They are within physiological values, but their decrease in the experimental groups may indicate the absence of inflammation or a reduced risk of inflammation with supplementation [1]. A reduction in the number of lymphocytes during the study period may indicate a lower risk of acute inflammation and a reduced risk of heat stress diseases under the influence of trace element citrates [19].

The effect of time factor A (P=0.829832) did not reveal statistical changes in leukocyte counts, but trace element citrates B (P=0.000245) significantly affected the studied index. In the combined interaction of the two factors, no statistically significant changes in AB were observed (P=0.597066). Analysis of the time factor A (P=0.000338) and the factor of micronutrient citrates B (P=0.000878) showed statistically significant changes in the absolute lymphocyte count, where the expressed effect was observed for factors A and B. The analysis of the combination of AB factors did not show a statistically significant result (P=0.634944).

The use of Zinc citrate supplementation led to an increase in the content of monocytes in the blood of rabbits by 23.5% (P<0.05) on the 29th day of the experiment. Zinc is an essential trace element involved in regulating the immune system. It promotes the development and functioning of immune cells, including monocytes [1]. An increased content of monocytes may indicate the activation of the immune system, which responds to stressful conditions, including heat stress. Therefore, a higher content of monocytes is involved in the immune response by producing various cytokines that activate the response of the immunophysiological system during exposure to elevated environmental temperatures.

Feeding citrates of trace elements in the blood of rabbits in I and III experimental groups increased the absolute content of granulocytes by 30 (P<0.01) and 66.3% (P<0.001), respectively, and the relative content of granulocytes by 13.5 (P<0.05) and 41.1% (P<0.001) during 29 days of the experiment. The addition of Selenium citrate to the diet of rabbits caused significant changes in the relative content of granulocytes by 17.1% (P<0.01) in the final period of the study compared to the control group (table 3). Germanium can activate the immune system by stimulating the formation of various types of immune cells, including granulocytes [24]. The intake of physiologically reasonable amounts of organic zinc and selenium compounds in the diet stimulates the activation of enzymes responsible for the synthesis of DNA and RNA, which promotes the proliferation and differentiation of cells, including granulocytes, which is an essential component of the immune system in protecting the body from infections and inflammation [19].

The effect of the time factor A (P=0.000000) and the factor of micronutrient citrates B (P=0.007262) was marked by statistically significant changes in the content of monocytes, where the expressed effect was observed for factor A. The results of the analysis of the combination of AB factors did not show a statistically significant

result (P=0.0442296). The study of the effect of factor A (P=0.000000), factor B (P=0.001213), and the interaction of time factors and citrates of trace elements AB (P=0.000180) were characterized by statistically significant changes in the effect on the number of granulocytes. The effect of time factor A (P=0.005293) and the effect of trace element citrates B (P=0.008958) was characterized by statistically significant changes in the relative content of lymphocytes. The analysis of the combination of factors AB did not show a statistically significant result (P=0.262943). The analysis of the time factor A (P=0.000000) was marked by statistical changes in the relative content of monocytes, but the factor of trace element citrates B (P=0.076088) had no effect on the studied indicator. In the combined interaction of the two factors, no statistically significant changes in AB were observed (P=0.100019). The determination of factor A (P=0.000000), factor B (P=0.002499), and the interaction of time and trace element citrates of AB (P=0.006297) were characterized by statistically significant changes in the effect on the relative content of granulocytes, where time is the predominant factor of influence.

In the case of micronutrient citrate supplementation, there was a tendency to increase in platelet count, mean platelet volume, thrombocrit value, and relative width of platelet distribution by volume during the study (table 4).

The effect of time factor A (P=0.000000) and the factor of trace element citrate B (P=0.001883) was marked by statistically significant changes in platelet count, where the expressed effect was observed for factor A. The analysis of the combination of AB factors did not show a statistically significant result (P=0.665514). The effect of the time factor A (P=0.000000) and the factor of influence of citrates of trace elements B (P=0.001883) was marked by statistically significant changes in platelet count, where a pronounced effect was observed for factor A. The data analysis shows that the combination of AB factors did not show a statistically significant result (P=0.665514). The influence of the factor of action of citrates of trace elements B (P=0.014762) had a statistically significant effect on the studied variable. In the interaction of the two factors, the data obtained were unaffected by statistical changes in AB (P=0.297532), indicating the absence of influence in the combination of these factors.

The results of the study of the biochemical parameters of rabbit blood, which characterize the state of metabolism and functioning of the body, indicate a positive trend in the feeding of Zinc citrate, Selenium citrate, and Germanium citrate compared to the control group. Feeding Selenium citrate to rabbits of the second experimental group in their blood, respectively, decreased the level of creatinine by 7.5% (P<0.05) and urea by 5.61% (P<0.01) on the 14th day and by 7.3% (P<0.05) and 12.3% (P<0.01). Germanium citrate feeding under conditions of moderate heat stress in III experimental group resulted in a decrease in urea by 14.7 (P<0.001) and 15.1% (P<0.01) on the 14th and 29th days of feeding compared to the control (table 5). Creatinine is formed in muscle tissue during

Table 3. The number of leukocytes and their forms in the blood of rabbits after feeding Zinc, Selenium, and Germanium citrates compounds under moderate heat stress (M±SD, n=6)

Indicators	Group	Period of research, age/day of supplementation		
		Preparatory period	Study period	
		49/1	63/14	78/29
WBC, 10^9/l	C	9.58±0.83	9.35±0.59	9.43±0.41
	I E	8.66±0.69	8.63±0.80	8.58±0.44*
	II E	8.73±0.35	9.11±0.59	8.68±0.37*
	III E	8.70±0.31	8.96±0.32	9.15±0.48
LYM, 10^9/l	C	5.26±0.63	5.16±0.47	5.75±0.27
	I E	4.66±0.58	4.88±0.29	5.10±0.34*
	II E	4.41±0.18	4.98±0.19	5.03±0.33*
	III E	4.43±0.95	4.80±0.40	5.23±0.42
MON, 10^9/l	C	1.43±0.10	1.33±0.12	1.06±0.05
	I E	1.55±0.25	1.51±0.11	1.31±0.19*
	II E	1.65±0.24	1.50±0.20	1.15±0.08
	III E	1.50±0.20	1.55±0.10	1.16±0.13
GRA, 10^9/l	C	1.81±0.56	2.11±0.54	2.20±0.14
	I E	2.38±0.27	2.25±0.24	2.86±0.57*
	II E	1.66±0.33	2.20±0.33	2.68±0.35
	III E	1.76±0.50	2.16±0.54	3.66±0.40***
LYM, %	C	54.96±6.14	54.87±4.70	57.31±3.35
	I E	48.55±4.68	54.00±1.96	55.38±2.10
	II E	55.61±2.62	53.91±2.39	56.33±2.29
	III E	49.98±4.56	52.80±2.49	54.21±3.32
MON, %	C	26.83±5.35	22.04±3.84	12.41±1.29
	I E	23.56±6.15	22.85±1.95	13.65±0.71
	II E	23.01±2.32	23.86±1.90	12.56±1.14
	III E	28.73±3.64	25.46±1.61	12.70±1.39
GRA, %	C	18.50±4.81	19.38±1.63	25.91±1.47
	I E	27.73±6.45	20.78±2.08	29.43±0.89*
	II E	21.36±4.43	21.20±4.78	30.35±2.85**
	III E	21.25±6.88	20.36±3.40	36.56±2.25***

Table 4. Platelet count and platelet indices in the blood of rabbits after feeding Zinc, Selenium, and Germanium Citrates compounds under moderate heat stress (M±SD, n=6)

Indicators	Group	Period of research, age/day of supplementation		
		Preparatory period	Study period	
		49/1	63/14	78/29
PLT, 10^9/l	C	303.00±35.18	368.00±13.88	376.50±14.87
	I E	263.33±46.24	373.00±25.28	385.67±25.81
	II E	349.83±57.70	390.66±23.43	390.33±12.78
	III E	331.33±34.82	386.67±25.19	397.83±28.83
MPV, fl	C	4.96±0.30	5.01±0.38	4.86±0.37
	I E	5.36±0.29	5.25±0.21	5.06±0.19
	II E	5.25±0.24	5.61±0.57	5.15±0.24
	III E	5.28±0.40	5.66±0.38	5.21±0.31
PCT, %	C	0.262±0.02	0.212±0.02	0.189±0.01
	I E	0.233±0.03	0.217±0.03	0.200±0.01
	II E	0.237±0.02	0.221±0.02	0.212±0.01
	III E	0.239±0.04	0.218±0.02	0.211±0.02
PDW, %	C	12.45±1.21	13.71±0.98	12.55±1.41
	I E	14.31±0.97	14.51±0.53	13.01±0.91
	II E	13.50±0.84	14.01±1.01	13.58±0.47
	III E	13.91±1.83	13.78±0.74	13.90±0.76

creatine metabolism, which is used to supply energy to the muscles. The kidneys filter creatinine and eliminate it from the body through the urine. The level of urea in the blood is an indicator of the functional state of the kidneys, as well as protein metabolism in the body [29]. Zinc citrate, Selenium citrate, and Germanium citrate have antioxidant properties that help reduce heat stress and protect the kidneys from the adverse effects of heat stress [8, 24]. The lower levels of creatinine and urea compared to the control group may indicate an improvement in renal function under moderate heat stress.

Table 5. The content of total protein, albumin, creatinine, and urea in the blood of rabbits after feeding Zinc, Selenium, and Germanium Citrates under moderate heat stress (M±SD, n=6)

Indicators	Group	Period of research, age/day of supplementation		
		Preparatory period	Study period	
		49/1	63/14	78/29
Total protein, g/l	C	60.78±5.60	57.20±4.54	54.01±3.29
	I E	62.31±4.55	58.08±3.36	55.51±5.24
	II E	63.40±2.11	62.76±3.09	59.81±3.15
	III E	60.33±2.73	60.06±4.59	57.33±2.56
Albumin, g/l	C	29.40±1.64	33.56±2.09	31.50±2.28
	I E	32.00±1.52	30.80±4.21	27.23±1.61
	II E	31.23±2.20	29.68±3.53	28.25±3.42
	III E	31.41±2.96	28.56±1.98	29.10±3.98
Creatinine, µmol/l	C	115.10±4.33	118.03±5.44	117.62±7.91
	I E	112.03±5.64	111.57±4.64	115.28±4.63
	II E	108.53±4.24	109.10±4.94*	108.95±3.45*
	III E	111.97±4.30	112.70±6.06	116.05±3.93
Urea, mmol/l	C	7.03±0.61	6.26±0.38	6.03±0.63
	I E	6.33±0.36	5.88±0.31	5.66±0.61
	II E	6.60±0.63	5.55±0.35**	4.96±0.30**
	III E	6.23±0.49	5.01±0.11***	4.80±0.33**

Table 6. The level of aminotransferase and alkaline phosphatase activity in the blood of rabbits after feeding Zinc, Selenium, and Germanium Citrates under moderate heat stress (M±SD, n=6)

Indicators	Group	Period of research, age/day of supplementation		
		Preparatory period	Study period	
		49/1	63/14	78/29
AST, u/l	C	24.70±2.14	31.90±1.40	29.48±0.96
	I E	22.83±2.38	20.71±1.79***	22.96±1.99***
	II E	26.01±3.93	27.05±2.30**	25.45±1.30**
	III E	25.53±3.36	29.96±3.10	28.85±2.43
ALT, u/l	C	58.75±5.80	74.73±4.52	63.45±3.67
	I E	51.38±4.90	62.31±3.83***	55.58±2.87**
	II E	53.00±4.82	66.63±2.70**	56.73±3.99**
	III E	56.51±6.26	70.05±2.07	60.91±4.00
Alkaline phosphatas, u/l	C	277.45±25.02	324.50±31.81	293.25±37.13
	I E	309.07±21.98	316.08±18.28	278.83±28.11
	II E	303.73±22.66	315.03±28.99	273.73±29.55
	III E	308.18±13.35	310.37±22.72	268.55±34.87

Determination of the time factor A (P=0.167163) revealed no statistical changes in creatinine content. The influence of the factor of trace element citrates B (P=0.000131) had a significant effect on the studied index. In the combined interaction of the two factors, no statistically significant changes in AB were observed (P=0.918005). The effect of time factor A (P=0.000000) and the effect of trace element citrates B (P=0.000000) was marked by statistically significant changes in urea content, where a pronounced effect is observed for two factors. The effect of the time factor A (P=0.000014) and the factor of trace element citrate (P=0.001055) was marked by statistically significant changes in total protein content, where the expressed effect was observed for factor A. The results of the analysis of the set of AB factors did not show a statistically significant result (P=0.627532).

The effect of the time factor A (P=0.000000) had a statistically significant effect on albumin content. The action factorof trace element citrates B (P=0.178466) did not show any significant changes. Interaction of time and trace element citrates is statistically significant (P=0.021085) and indicates a combined effect on mitigating the effects of moderate heat stress.

The addition of Zinc citrate and Selenium citrate to the diet of rabbits reduces AST activity by 35.0 (P<0.001) and 22.1% (P<0.001), respectively, and by 15.2 (P<0.05) and 13.6% (P<0.05) on the 14th and 29th days of the study compared to the control group. Significant values were also found in rabbits of the I and II experimental groups for ALT activity, where a decrease of 16.6 (P<0.001), 12.4% (P<0.01), and 10.8 (P<0.01) and 10.5% (P<0.01) was observed on the 14th and 29th days of supplementation (table 6). A decrease in the level of aspartate aminotransferase and alanine aminotransferase activity within physiological parameters may indicate an improvement in rabbit liver function, which was more pronounced with Zinc citrate and Selenium citrate under moderate heat.

The effect of time factor A (P=0.001088) and the effect of micronutrient citrate B (P=0.000000) had a statistically significant effect on the level of AST activity, where the effect of factor B was expressed. In particular, the combined effect of time and micronutrient citrates was statistically significant AB (P=0.000597), which may indicate a better result on the enzyme activity. The effect of the time factor A (P=0.000000) and the factor of trace element citrate B (P=0.000000) was marked by statistically significant changes in the level of ALT activity, where the expressed effect was observed for factors A and B. The analysis of the combination of factors AB did not show a statistically significant result (P=0.840587). The results of the analysis of the time factor A (P=0.000060) were marked by statistical changes in the level of alkaline phosphatase activity, but the factor of trace element citrates B (P=0.940835) had no effect on the studied indicator. In the combined interaction of the two factors during the experimental period, AB had no statistically significant effect (P=0.225183).

The study of cholesterol content in the blood of rabbits of I and II experimental groups showed a corresponding decrease of 27.7 (P<0.01), 22.2% (P<0.01), and 20.3 (P<0.05), and 16.6% (P<0.05) on the 14th and 29th days of the experiment. Cholesterol is the main component of cell membranes and is involved in forming structural integrity and cell permeability (table 7). Selenium and Zinc are components of glutathione peroxidase and thioredoxin reductase, which help protect cells from oxidative stress, including cholesterol oxidation [36]. Zinc is essential for functioning the HMG-CoA reductase enzyme, which ensures cholesterol synthesis in cells. By regulating the activity of this enzyme, Zinc can affect the synthesis and breakdown of cholesterol [7]. The influence of the time factor A (P=0.000000), the trace element citrates factor B (P=0.000002), and the combination of factors AB (P=0.000176) during the experimental period were characterized by statistically significant changes in cholesterol content during the experimental period.

Feeding Selenium citrate increased the content of inorganic phosphorus by 10.7% (P<0.05) on the 29th day of the study. Phosphorus is vital to many biochemical processes, including energy metabolism, DNA and RNA synthesis, and protein phosphorylation [20]. Heat stress causes an increase in free radicals in the body, which damages cell membranes and structures. As a part of glutathione peroxidase, Selenium reduces oxidative stress by neutralizing free radicals, which helps maintain the functionality of cell membranes and enzymes, phosphatase and phosphorylase, involved in the transport and metabolism of phosphorus. Under conditions of moderate heat stress, activation of phosphatases is essential to provide free phosphorus, which is necessary for ATP synthesis and repair of damaged cells. Phosphorylases add phosphate groups to organic molecules, which is essential for the regulation of metabolic pathways, in particular, the breakdown of glycogen to glucose-1-phosphate, which promotes the release of glucose into the blood and can be used for the energy needs of the cell, under conditions of heat stress on the body of rabbits [34]. The influence of the factor of action of citrates of trace elements B (P=0.001718) has a statistically significant effect on the studied variable. In the interaction of the two factors, the data obtained were unaffected by statistical changes in AB (P=0.334127), indicating the absence of influence in the combination of these factors.

Analysis of the effect of time factor A (P=0.021905) had a statistically significant effect on the content of triacylglycerols. The effect of trace element citrates B (P=0.069257) did not reveal any significant changes. The interaction of time and trace element citrates factors is statistically significant (P=0.030044) and indicates the combined effect of these two factors on the variable. The effect of time factor A (P=0.000000) and the effect of trace element citrates B (P=0.000000) was marked by statistically significant changes in urea content, where a pronounced effect is observed for two factors. The results of the analysis of the set of factors AB did not show statistically significant results (P=0.168903). The effect

Table 7. The content of cholesterol, triacylglycerols, total calcium, and inorganic phosphorus in the blood of rabbits after feeding Zinc, Selenium, and Germanium Citrates under moderate heat stress (M±SD, n=6)

Indicators	Group	Period of research, age/day of supplementation		
		Preparatory period	Study period	
		49/1	63/14	78/29
Triacylglycerols, mmol/l	C	0.63±0.07	0.79±0.07	0.71±0.06
	I E	0.69±0.14	0.67±0.10	0.62±0.05
	II E	0.78±0.06	0.76±0.06	0.66±0.09
	III E	0.76±0.06	0.72±0.09	0.67±0.05
Cholesterol, mmol/l	C	0.17±0.02	0.54±0.07	0.54±0.06
	I E	0.19±0.03	0.39±0.03**	0.42±0.04**
	II E	0.20±0.01	0.43±0.04*	0.45±0.02*
	III E	0.21±0.02	0.52±0.07	0.48±0.05
Total calcium, mmol/l	C	3.06±0.26	3.06±0.20	2.83±0.41
	I E	3.26±0.19	3.10±0.24	2.86±0.28
	II E	3.35±0.15	3.13±0.29	2.98±0.37
	III E	3.30±0.14	3.20±0.23	2.90±0.25
Inorganic phosphorus, mmol/l	C	1.61±0.07	1.93±0.15	1.78±0.17
	I E	2.08±0.23	1.96±0.16	1.95±0.22
	II E	2.01±0.24	2.11±0.07	2.16±0.12*
	III E	2.13±0.24	1.95±0.23	1.90±0.24

of the time factor A (P=0.000112) indicated statistical changes in total calcium content. However, the factor of trace element citrates B (P=0.254447) did not affect the studied indicator. In the combined interaction of the two factors, there were no statistically significant changes in AB (P=0.951526).

During the period of the study, it was found that feeding rabbits with Zinc citrate in the blood increased the number of red blood cells (P<0.01), hemoglobin concentration (P<0.01), percentage of hematocrit value (P<0.01), mean hemoglobin concentration in erythrocyte (P<0.05), monocyte count (P<0.05), absolute and relative granulocyte content (P<0.05) and reduced the number of leukocytes and lymphocytes (P<0.05) during 29 days of experiment. The addition of Selenium citrate to the diet of animals increased the number of red blood cells (P<0.05), hemoglobin concentration (P<0.001), percentage of hematocrit value (P<0.05), relative content of granulocytes (P<0.01), average hemoglobin concentration in erythrocyte (P<0.05–0.001) and a decrease in the number of leukocytes (P<0.05), the number of lymphocytes (P<0.05) was noted during the study. Germanium citrate feeding had less effect on the blood parameters of rabbits under heat stress, with an increase in hemoglobin concentration (P<0.001), absolute granulocyte content (P<0.001), and relative granulocyte content (P<0.001) during 29 days of the experiment.

The change in the biochemical parameters of rabbit blood was more influenced by the supplementation of

Zinc citrate and Selenium citrate, which were characterized by a decrease in AST activity (P<0.001 and P<0.001; P<0.05 and P<0.05), ALT (P<0.001 and P<0.01; P<0.01 and P<0.01), cholesterol content (P<0.01 and P<0.01; P<0.05 and P<0.05) on the 14th and 29th days of the experimental period, respectively. Selenium citrate decreased the level of creatinine (P<0.05) and urea (P<0.01) during the experiment and increased the content of inorganic phosphorus (P<0.05) on the 29th day of the study. The addition of Germanium citrate caused a decrease in urea content (P<0.001 and P<0.05) on the 14th and 29th days of the experiment, respectively.

Thus, feeding rabbits with Zinc citrate (12 mg Zn/kg body weight) and Selenium citrate (60 μg Se/kg body weight) under moderate heat stress caused pronounced positive changes in the hematological and biochemical parameters of rabbit blood during the study. The addition of Germanium citrate (12.5 μg Ge/kg) to the diet had less pronounced effect on the body of rabbits, but mitigated the negative effect of heat stress, with more pronounced positive changes in blood parameters on hematocrit value, absolute and relative content of granulocytes on the 29th day of the study compared to the control.

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Вплив наночастинок цитратів цинку, селену та германію на гематологічні та біохімічні показники організму кролів за умов помірного теплового стресу

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Зміни клімату призводять до підвищених температур довкілля, що негативно впливає на організм тварин. Кролі не мають потових залоз і не здатні регулювати температуру свого тіла, що за умов теплового стресу порушує фізіологічні процеси організму: терморегуляцію тіла, дихання, імунну функцію, ендокринну регуляцію, параметри крові, репродуктивну здатність, які є ключовими чинниками функціонування організму. В останні роки для пом'якшення теплового стресу значну увагу зосереджують на сполуках, виготовлених методами нанотехнології, які проявляють позитивний ефект на організм тварин порівняно з органічними і неорганічними речовинами та мають широкий спектр біологічної дії. Однак вплив наночастинок макро- та мікроелементів на організм ссавців залежить від застосованої кількості. Тому мета експерименту полягала у дослідженні гематологічних та біохімічних параметрів організму кролів після відлучення в умовах помірного теплового стресу за випоювання цитратів цинку, селену та германію, виготовлених методами нанотехнології.

Ключові слова: кролі, кров, помірний тепловий стрес, наночастинки, цинку цитрат, селену цитрат, германію цитрат, гематологічні та біохімічні показники

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