

THE ANTIOXIDANT SYSTEM IN RABBIT UNDER COMBINE ACTION OF SEVERE HEAT STRESS AND NANOPARTICLES OF ZINC, SELENIUM, AND GERMANIUM CITRATE

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It is generally known that rabbits cannot effectively ensure thermoregulation of their body at temperatures above 18-21°C due to the absence of sweat glands and thick fur. Heat stress negatively affects the metabolic processes and reproductive function of rabbits. One of the approaches to mitigating the adverse effects of heat stress is using organic compounds of trace elements created using nanotechnology. Our study aimed to evaluate the antioxidant protection indicators in the blood of animals under conditions of severe heat stress and the introduction of nanoparticles of zinc citrate, selenium, and germanium as a feed additive. Young rabbits of the Termonska Bila breed aged from 35 to 78 days were divided into groups of 6 animals. The control group received the main diet and water without restrictions. Rabbits of experimental groups I, II, and III consumed the same food as the control. Still, within 24 h, they received water: group I – 12 mg Zn/kg, group II – 60 µg Se/kg, group III – 12.5 µg Ge/kg. Within 15 days, the temperature in the room was increased from 28.9 to 30°C for 4 h per day using an electric air heater to provide severe heat stress according to the temperature-humidity index. It was found that the addition of zinc citrate and selenium nanoparticles contributed to positive changes in the functioning of the antioxidant defense system, which was changed due to severe heat stress. In contrast, the protective effect of germanium citrate was less pronounced.

Key words: rabbits, nanoparticles, severe heat stress, antioxidant system, oxidative stress, reduced glutathione, glutathione peroxidase, lipid hydroperoxides, TBA-active products.

The optimum ambient temperature for rabbits is 18 to 21°C, and the humidity level is 55 to 65% [1, 2]. Under heat stress conditions, rabbits cannot effectively provide thermoregulation of their bodies, as their thick hair coat and lack of sweat glands in the skin make it difficult to remove excess heat from the body [3]. Heat stress disrupts the oxidation-reduction balance in animals, reduces immune function, and disrupts estrogen secretion, which causes irregular polyovulation in rabbits and leads to abnormal egg morphology. Elevated temperatures damage the DNA of rabbit sperm, which causes changes in chromatin conformation and DNA methylation and affects embryos' reproductive capacity and development [4]. To reduce the adverse effects of elevated environmental temperatures, modern scientific research is focused on the possibility of using mineral compounds made by

nanotechnology since nanocompounds of mineral elements due to their nanoscale part can significantly improve the bioavailability of these substances, which ensures more efficient absorption by the body of animals.

Adding selenium to animals' diets ensures the functioning of antioxidant mechanisms, hormonal metabolism, and immune system activity and reduces damage to sperm DNA. It is an integral component of at least 25 selenoproteins and acts as an important cofactor in the system of antioxidant enzymes [6]. Zinc helps to increase body weight, participates in the biosynthesis of nucleic acids and cell division processes, provides antioxidant protection, stimulates testosterone synthesis, and neutralizes reactive oxygen species [7]. Germanium neutralizes free radicals and increases the body's antioxidant activity [8]. An appropriate germanium lactic acid cit-

rate concentration can increase reduced glutathione content in rat hepatocytes [9]. Organic germanium increases superoxide dismutase activity in the blood and reduces the content of lipid peroxides in the blood serum [10].

Our study aimed to determine changes in the antioxidant defense of rabbits after feeding zinc, selenium, and germanium citrate nanoparticles under severe heat stress conditions.

Materials and Methods

The study was carried out on young rabbits of the Thermon White breed from 35 to 78 days of age in the vivarium of the Institute of Animal Biology of the NAAS. Permission to conduct the research was obtained from the Bioethics Committee of the Institute of Animal Biology of the NAAS, Lviv. Lviv (protocol No. 162 of 11.12.2024). All manipulations with experimental animals were carried out by 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). During the experiment, the temperature in the room was increased from 28.9 to 30°C for 4 h a day using adjustable electric air heaters, which created severe heat stress conditions. The temperature and humidity parameters were determined using an electronic air analyzer (Patent No. 127047) and a Trotec BL30 thermohygrometer [11]. Animals were formed into groups of 6 with an average body weight of 1200 ± 50 g. Rabbits of the control group received an elemental diet, which included standard balanced pelleted feed and water without restriction. The rabbits of groups I, II, III of the experimental groups consumed the same feed and water without restrictions as in the control group but received water for 24 h a day: group I – zinc citrate – 60 mg Zn/l or 12 mg Zn/kg; group II – selenium citrate – 300 µg Se/l or 60 µg Se/kg; group III – germanium citrate – 62.5 µg Ge/l or 12.5 µg Ge/kg. Using separate drinking bowls for each animal and the placement of animals individually allowed us to control the amount of water consumed and the given compound by each animal.

The blood parameters of rabbits were studied on day 14 of the preparatory period and days 14 and 29 of the study under conditions of severe heat stress. On the 14th day of the preparatory period, blood sam-

ples were taken from rabbits to study biological material without the effect of an elevated temperature and humidity index and in the absence of supplements in the diet. The preparatory period lasted 14 days and was necessary to adapt the animals to the conditions of the vivarium and ensure the stability of the studied antioxidant system parameters. This is important in order to avoid the effects of stress, which can be caused by changes in environmental conditions (e.g., transition to new housing conditions). The choice of 14 days for preparation provided enough time for the animals to adapt to the new conditions and allowed for stable indicators to compare the data during the experimental period. In the following experimental periods, on days 14 and 29 of the study, the effect of heat stress and its mitigation by feeding a citrate compound of micronutrient nanoparticles were investigated.

Our results served as the basis for further comparison with those of the experimental groups exposed to elevated temperatures. In 6 animals from the groups, whole blood samples were taken from the marginal ear vein into tubes with the anticoagulant ethylenediaminetetraacetate (EDTA), in which the content of lipid hydroperoxides, TBA-active products, superoxide dismutase, catalase, reduced glutathione, glutathione peroxidase, and glutathione reductase were determined.

The solutions of zinc, selenium, and germanium citrate nanoparticles for the study were manufactured by Nanomaterials and Nanotechnologies LLC, Kyiv (Patent No. UA 38391) [12]. The size of nanoparticles was studied at the Franzevich Institute of Materials Science of the National Academy of Sciences of Ukraine.

Method for determining the size of zinc, selenium, and germanium nanoparticles. Coated with a thin carbon layer, a standard copper grid for electron microscopic studies was placed on filter paper. Using a micropipette, 1 to 2 drops of the compound under study were applied to the grid. After drying, the grid was placed in the microscope sample holder, which, after being vacuumed in the airlock, was transferred to the microscope column for examination. The appearance of the metal particles was studied using a JEM 100CX II transmission electron microscope (Japan) at a magnification of 100,000x. Additionally, microdiffraction lattice photographs were taken with subsequent calculation of the crystal lattice parameters and comparison with the table values [13]. It was not possible to obtain an image of selenium citrate nanoparticles. This is because Selenium has

a low saturated vapor pressure, which leads to its intense evaporation when placed in a vacuum. As a result, selenium citrate nanoparticles evaporate rapidly during microscopic studies, making it difficult to obtain clear images. Therefore, we used the developer's information on the size of selenium nanoparticles from 20 to 40 nm. It is worth noting that some nanoparticles did not give precise reflexes, which may be due to their amorphous state (Fig.).

Determination of lipid hydroperoxides. The content of lipid hydroperoxides in biological material was determined by precipitating proteins with a trichloroacetic acid solution and extracting lipids with ethanol. The resulting extracts interact with ammonium thiocyanate to produce a color reaction. The optical density was measured spectrophotometrically (Unico 1205, USA) ($\lambda = 480$ nm). The level of GPP was calculated in conventional units per 1 ml of blood plasma (U/ml). The content of lipid hydroperoxides was calculated as the difference between control and test samples [14].

Determination of the content of TBA-active products. The principle of the method is based on the color reaction between malondialdehyde (MDA) and thiobarbituric acid (TBA) in an acidic environment and at high temperatures. The reaction produces a trimethine complex consisting of one MDA molecule and two TBA molecules. The absorbance of the formed color reaction was measured spectrophotometrically ($\lambda = 535$ and $\lambda = 580$ nm). The content of TBA-active products was calculated as nmol of MDA per 1 ml of blood plasma (nmol/ml) [15].

Determination of superoxide dismutase activity in erythrocytes. The activity of superoxide dismutase was determined by assessing the level of inhibition of the process of nitroblue tetrazolium reduction by the enzyme in the presence of NADPH and phenazine methosulfate. The intensity of nitroblue tetrazolium reduction inhibition indicates the level of super-

oxide dismutase activity. The more pronounced the inhibition, the higher the enzyme's activity, which indicates its effectiveness in neutralizing superoxide radicals. Absorbance measurements were determined spectrophotometrically ($\lambda = 540$ nm). Superoxide dismutase activity was calculated in standard units per 1 mg of protein [14].

Determination of catalase activity. The catalase activity is measured in the presence of molybdenum salts that react with hydrogen peroxide. The reaction produces a colored product. The absorbance of the colored product was determined spectrophotometrically ($\lambda = 410$ nm). Catalase activity was calculated in mmol/min per 1 mg of protein [14].

Determination of glutathione peroxidase activity. The activity of glutathione peroxidase is determined by the oxidation rate of glutathione in the presence of tertiary butyl hydroperoxide. The concentration of reduced glutathione is measured colorimetrically before and after the reaction. The color reaction is based on the interaction of SH-groups with 5,5'-dithiobis-2-nitrobenzoic acid, which leads to the formation of a colored product, the thionitrophenyl anion. The absorbance of the colored product was recorded spectrophotometrically ($\lambda = 412$ nm). The glutathione peroxidase activity was calculated in $\mu\text{mol glutathione/min} \times \text{per mg of protein}$ [15].

Determination of glutathione reductase activity. The rate of glutathione reduction in the presence of NADPH determines glutathione reductase activity. The absorbance was recorded spectrophotometrically ($\lambda = 340$ nm) for 1 min at 37°C. The activity of glutathione reductase was calculated as $\mu\text{mol NADPH/min} \times \text{mg protein}$ [14].

Determination of reduced glutathione level. The content of reduced glutathione is determined based on the principle of formation of thionitrophenyl anion after the interaction of SH-groups of glutathione with 5,5-dithiobis,2-nitrobenzoic acid. The

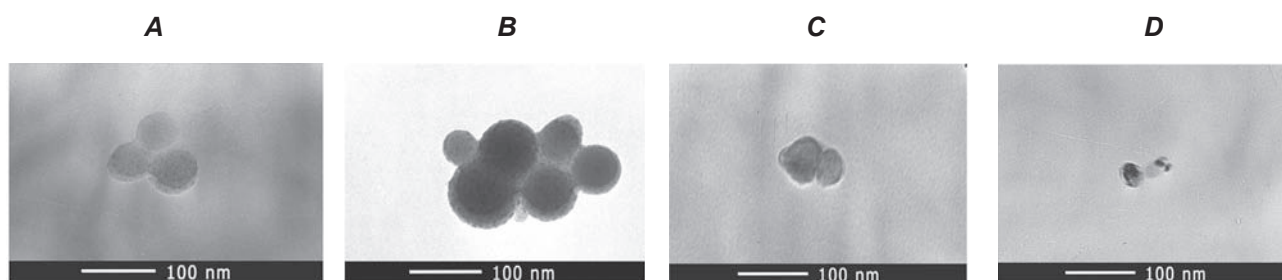


Fig. Electron microscopic studies of images of zinc nanoparticles **A** – 55.4-61.2 nm; **B** – 47-95.1 nm and germanium citrate **C** – 52.2 nm; **D** – 37.2 nm

absorbance was determined spectrophotometrically ($\lambda = 412$ nm). The reduced glutathione content in erythrocytes was calculated as mmol/l [15].

Determination of protein concentration. The concentration of total protein in erythrocytes was measured by the Lowry method [15] using Simco LTD kits (Ukraine, Lviv). All absorbance values were measured using a spectrophotometer.

Determination of the temperature and humidity index (THI). The THI index determined the limits of comfortable conditions for rabbits. The average daily air temperature and relative humidity were determined by the formula [2]:

$$\text{THI} = T - ((0.31 - 0.31 - \text{RH}/100) - (T - 14.4)),$$

where THI – temperature and humidity index; T – temperature (°C); RH – relative humidity in percentage (%).

The obtained THI values were classified as follows: <27.8°C – no heat stress; 27.8–28.9°C – moderate heat stress; 28.9–30.0°C – severe heat stress; >30.0°C – very severe heat stress [2].

The results were statistically processed using Statistica 7.0 (Statsoft, USA) and Microsoft EXCEL software and presented as mean (M) $\pm$ standard deviation (SD). The results were calculated by analysis of variance (ANOVA). To determine statistical differences between the control and experimental groups, the Tukey HSD post hoc method was used, and differences were considered significant at $P \leq 0.05$ [16].

Results and Discussion

To assess heat stress, the temperature and humidity in the vivarium were monitored daily during the study. In the preparatory period, the average temperature and humidity were 19.8°C and 56.3%, corresponding to 19.0 according to the THI calculations. During the 14-day experimental period, the average room temperature was 29.9°C, and the humidity was 86.5%. The calculated THI was 29.9°C, indicating severe heat stress according to the classification of Marai et al. (2002). At the final stage of the study, the average humidity and temperature were 84.3% and 29.9°C. According to the THI formula, the value during this period was 29.1, indicating severe heat stress parameters. The obtained THI values during the preparatory period indicate the absence of heat stress and its presence during the 29 days of the experimental period. Taking into account the results obtained, we used zinc, selenium, and germanium

citrate nanoparticles in the diet as corrective compounds to minimize the negative effects of elevated temperatures on the body of animals and correct oxidative processes. This made it possible to assess their effectiveness in reducing the level of oxidative stress caused by heat load and determine their potential in activating the antioxidant activity of the body of rabbits exposed to stress. As a result of the study, it was found that feeding zinc and selenium citrate nanoparticles reduced the content of lipid hydroperoxides in the blood plasma of rabbits by 42.5 and 37.0% ($P < 0.001$) and 34.7 and 27.6% ($P < 0.001$) on days 14 and 29 of the study compared to the control group, which indicates a positive effect of the compounds used (Table 1). When calculating changes in the content of LHP between the 14th and 29th day, the level of LHP increased by 33.65% ($P < 0.05$), with the addition of zinc citrate and selenium citrate by 35.05% ($**P < 0.01$) compared to the control. The increase in the level of LHP on day 29 can be explained by the fact that prolonged exposure to severe heat stress leads to the accumulation of reactive oxygen species during the experiment. However, the administration of nanoparticles (in comparison with the control groups) allows reducing the level of LHP on the 14th and 29th days. Under conditions of severe heat stress, the amount of formed ROS increases the activity of superoxide dismutase and catalase, which break down superoxide radicals and water peroxide, but their compensatory capacity is limited. As a result, with prolonged exposure to stress, antioxidant mechanisms reach a critical limit, which leads to the accumulation of LHP as a marker of oxidative stress.

The analysis of the data obtained on the TBARS content shows that no statistically significant differences between the groups were found, but there is a positive general trend towards a decrease in the level of these indicators in the experimental groups compared to the control. This may indicate a certain protective effect of the applied nanoparticles on the antioxidant system of the rabbit organism under conditions of severe heat stress, which is manifested in a less intense accumulation of lipid peroxidation products.

Since zinc is a component of the body's antioxidant defense, it protects cells from oxidative processes, interacts with sulfhydryl groups in biomolecules, and reduces the oxidation of DNA and protein macromolecules, which contributes to the reduction of reactive oxygen species [17]. The current results of our study are consistent with the findings

of Ahmed AA Abdel-Wareth et al., who showed that the addition of zinc oxide nanoparticles to the diet of rabbits in the amount of 20 and 80 mg/kg body weight mitigates the adverse effects of heat stress on their body and is reflected in higher animal performance [18]. Studies by Alissa et al. found that additional zinc intake in the amount of 0.5 % of the dry weight of the diet reduces the concentration of TBA-active products in the blood plasma of rabbits [19]; the researchers conclude that the addition of zinc nanoparticles helps to reduce the level of lipid hydroperoxides in the body of rabbits. The positive effect of selenium nanoparticles on the animal body can be explained by the fact that Selenium is an integral part of the active center of the glutathione peroxidase enzyme, which protects cell membranes from lipid peroxides and hydrogen peroxide (H_2O_2). Studies by Hosny et al. have shown that the addition of organic Selenium in the diet of 0.3 mg/kg provides glutathione peroxidase activity, affects the physiological function of Selenium to enhance antioxidant protection and help reduce the content of lipid hydroperoxides [20]. Thus, the results of our study confirm the effectiveness of feeding zinc and selenium citrate nanoparticles to enhance the antioxidant effect in rabbits, which is confirmed by a decrease in the content of lipid hydroperoxides in the blood plasma of rabbits.

Heat stress activates oxidation processes in the body, increasing reactive oxygen species levels. Superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) cause oxidative stress. In response to elevated environmental temperatures, the rabbit body activates protective mechanisms regulated by the transcription

factor Nrf2 (NF-E2-Related Factor 2), which activates genes encoding antioxidant enzymes, catalase, and superoxide dismutase [21]. The results of the study showed that zinc citrate and Selenium citrate feeding in rabbit erythrocytes increased superoxide dismutase activity by 66.7% ($P < 0.01$) and 46.6% ($P < 0.05$) on day 29 of the experiment (Table 2). Zinc nanoparticles activate the transcription factor Nrf2, which encodes antioxidant enzymes, including superoxide dismutase [7]. Zinc is a cofactor of superoxide dismutase, which converts two superoxide anions (O_2^-) into oxygen (O_2) and hydrogen peroxide (H_2O_2) molecules in a dismutation reaction and affects Nrf2 activity due to the high concentration of oxidized molecules [22]. Zinc ensures the integrity of cell membranes, inhibits prooxidant enzymes, and affects the synthesis of the protein metallothionein, which protects cells from hydroxyl radicals (OH), superoxide anions (O_2^-), and hydrogen peroxide (H_2O_2) under conditions of heat stress. Studies by Homma et al. [23] have shown that zinc deficiency leads to a mutant superoxide dismutase. This causes chronic stress of the endoplasmic reticulum and disrupts protein synthesis and induction of the zinc transporter Zip-14. Transcription factor (MTF-1) is a zinc-dependent transcription factor that affects the expression of the genes for metallothionein and zinc transporter-1 (ZnT-1), which is a mechanism against elevated zinc levels in the body [24]. MTF-1 protects cells during changes in the cell's redox state and promotes the expression of antioxidant genes. MTF-1 activates the selenoprotein 1 (Sepw1) gene, which affects the synthesis of a glutathione-binding protein that can neutralize free radicals and reduce cell oxi-

Table 1. Peroxidation products in rabbit plasma ($M \pm SD$), $n = 6$

Blood indicators	Group of animals	Study period age of the animal/day of supplementation		
		Preparatory period	Study period	
			63/14	78/29
LHP, U/ml	Control	0.90 ± 0.08	1.81 ± 0.16	2.13 ± 0.42
	Group I	0.78 ± 0.04	$1.04 \pm 0.21^{***}$	$1.39 \pm 0.25^{***}$
	Group II	0.80 ± 0.06	$1.14 \pm 0.24^{***}$	$1.54 \pm 0.12^{**}$
	Group III	0.90 ± 0.13	1.17 ± 0.08	1.85 ± 0.10
TBARS, nmol/ml	Control	1.40 ± 0.19	1.99 ± 0.22	2.22 ± 0.38
	Group I	1.50 ± 0.25	1.72 ± 0.43	1.93 ± 0.25
	Group II	1.67 ± 0.24	1.82 ± 0.37	1.91 ± 0.15
	Group III	1.42 ± 0.21	1.63 ± 0.43	2.19 ± 0.46

Note. Statistically significant differences compared to the control group: $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$

ductive stress [25]. Selenium affects the activity of the superoxide dismutase enzyme through its effect on selenoproteins, in particular through glutathione peroxidase and selenoprotein P (SeP), which ensures a balance between redox processes in cells, increasing the activity of superoxide dismutase in neutralizing superoxide anions [26]. Considering the above, it can be concluded that feeding zinc and selenium citrate nano compounds is more bioavailable to the rabbit body, which in turn enhances the antioxidant activity of superoxide dismutase under severe heat stress conditions.

After feeding zinc and selenium citrate nanoparticles, the activity of catalase was 32.6 and 26.3% ($P < 0.05$) on day 29 of the study compared to the control (Table 2). Catalase is a metalloenzyme containing heme groups in its structure and acts as a catalytic center in reactions that neutralize reactive oxygen species. The primary function of catalase is to break down hydrogen peroxide (H_2O_2) into water and oxygen, which is necessary to neutralize the toxic effects of hydrogen peroxide and the formation of hydroxyl radicals, a consequence of the Fenton reaction [27]. Catalase is a part of the phase II detoxification enzymes that protect the cell from oxidative stress. The enzyme's mechanism of action is to activate the expression of genes encoding antioxidant enzymes. The activation process occurs through the interaction of the transcription factor Nrf2 with antioxidant response elements (AREs) in the promoters of genes encoding these enzymes. Under conditions of heat stress, Zinc affects the activity of ERK1/2 (Extracellular signal-regulated kinases), which is part of the MAPK (Mitogen-

activated protein kinase) signaling pathway and activates Nrf2 and its translocation to the nucleus, where it initiates the transcription of antioxidant enzymes, including catalase, which protects the cell from oxidative stress [28]. Selenium is not directly a component of catalase, but activating glutathione peroxidase helps to reduce the amount of hydrogen peroxide that catalase needs to neutralize. The group of rabbits fed with germanium citrate showed relatively stable superoxide dismutase and catalase activity during the experiment, which may indicate a certain protective effect of germanium citrate on the antioxidant system, although without statistically significant changes. This indicates a possible need to adjust the dose or duration of use to achieve a more pronounced effect. Thus, our study's results have shown significant changes in the intake of zinc and selenium citrates compared to the control group. These trace elements affect gene expression under severe heat stress conditions and encode antioxidant enzymes, including catalase, which protects cells from oxidative stress.

After feeding selenium nanoparticles, the activity of glutathione peroxidase in the blood of rabbits was significantly higher by 73.0% ($P < 0.001$) and 63.2% ($P < 0.01$) compared to the control group on days 14 and 29 of the study (Table 3). Glutathione peroxidase belongs to the class of selenoproteins. Each monomer of this enzyme has a selenium atom in the active center and replaces sulfur in the cysteine amino acid to form selenocysteine (R-SeH), a catalyst for peroxide detoxification reactions [27]. The active center of glutathione peroxidase neutralizes reactive oxygen species, hydrogen peroxi-

Table 2. Activity of antioxidant enzymes in rabbit erythrocytes ($M \pm S D$), $n = 6$

Blood indicators	Group of animals	Study period age of the animal/day of supplementation		
		Preparatory period	Study period	
			63/14	78/29
SOD, U/mg prot.	Control	6.20 $\pm$ 1.49	5.58 $\pm$ 1.09	4.93 $\pm$ 1.22
	Group I	7.36 $\pm$ 1.18	6.14 $\pm$ 1.09	8.22 $\pm$ 1.37**
	Group II	8.00 $\pm$ 1.54	5.72 $\pm$ 0.90	7.23 $\pm$ 1.40*
	Group III	6.96 $\pm$ 1.03	6.82 $\pm$ 1.28	5.53 $\pm$ 1.03
CAT, mmol H_2O_2 /min $\times$ mg prot.	Control	90.08 $\pm$ 5.76	97.29 $\pm$ 13.26	74.94 $\pm$ 4.40
	Group I	91.80 $\pm$ 4.12	100.6 $\pm$ 9.61	99.44 $\pm$ 8.11*
	Group II	97.71 $\pm$ 4.76	98.69 $\pm$ 13.34	94.67 $\pm$ 17.88*
	Group III	92.63 $\pm$ 5.17	101.60 $\pm$ 3.98	91.80 $\pm$ 13.52

Note. Statistically significant differences compared to the control group: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

de, and organic peroxides that cause cell oxidative stress. During the catalytic reaction, the selenium-containing form of the enzyme, selenol (protein-SeH), interacts with peroxide molecules and leads to the formation of selenic acid (protein-SeOH), which is reduced to selenol by two molecules of reduced glutathione [29]. During the reactions, the selenium-containing glutathione peroxidase uses reduced glutathione as an electron donor, oxidizing it to glutathione disulfide, and organic peroxides are reduced to alcohol or water. Oxidized glutathione is reduced to reduced glutathione by the enzyme glutathione reductase, which uses electrons obtained from NADPH. This cycle of reactions is necessary for the homeostasis of antioxidant enzymes in cells, which regulates the level of reactive oxygen species and protects cells from oxidative stress [30]. Studies by Zhang et al. found that the addition of 0.24 mg/kg of Selenium to the diet of rabbits increased the activity of glutathione peroxidase and catalase in the blood serum [31], which, in our opinion, under conditions of severe heat stress, increases the expression of glutathione peroxidase after selenium citrate feeding, and reduces the level of toxic peroxides, thereby protecting rabbit cells from oxidative stress.

When zinc citrate was administered, the probable content of reduced glutathione was increased by 72.5 and 80% ($P < 0.01$) and selenium citrate by 58.3 and 79.1% ($P < 0.001$) on days 14 and 29

compared to the control group of animals (Table 3). Reduced glutathione is a tripeptide with no catalytic activity, and its action depends on the interaction with enzymes that affect its activity [32]. The main enzyme with which reduced glutathione interacts is glutathione peroxidase, which uses glutathione as an electron donor to reduce hydrogen peroxide and organic peroxides, thus reducing the toxic effects on cells. Zinc participates in glutathione synthesis by activating the enzyme glutamate cysteine ligase, which catalyzes the formation of reduced glutathione and regulates cellular antioxidant defense processes. A comparison of the consensus DNA binding sites of CNC (cap 'n' collar) and bZIP (basic leucine zipper) proteins revealed that NRF2 is a transcription factor that regulates the cell's response to oxidative stress [33, 34]. Zinc's mechanism of action affects the activity of Nrf2, which provides the cell with a response to oxidative stress. The activity of the transcription factor Nrf2 triggers the transcription of genes encoding antioxidant enzymes and protects cells from reactive oxygen species. Zinc activates the Keap1 protein (Kelch-like ECH-associated protein 1), which interacts with Nrf2 in the cytoplasm and controls its destruction through the proteolysis system. When the cell is exposed to oxidative stress, Zinc changes the conformation of Keap1, which leads to the separation of Nrf2 from this protein and transports it to the cell nucleus, where it activates

Table 3. Indicators of the glutathione link of antioxidant defense in rabbit erythrocytes ($M \pm SD$), $n = 6$

Blood indicators	Group of animals	Study period age of the animal/day of supplementation		
		Preparatory period	Study period	
			63/14	78/29
GR, $\mu\text{mol NADPH}/\text{min} \times \text{mg prot.}$	Control	0.84 ± 0.10	0.63 ± 0.06	0.48 ± 0.11
	Group I	0.78 ± 0.06	0.67 ± 0.17	0.45 ± 0.04
	Group II	0.75 ± 0.14	0.61 ± 0.17	0.50 ± 0.14
	Group III	0.80 ± 0.09	0.74 ± 0.13	0.51 ± 0.06
GSH, $\mu\text{mol/l}$	Control	0.058 ± 0.009	0.040 ± 0.006	0.024 ± 0.005
	Group I	0.066 ± 0.004	$0.069 \pm 0.016^{**}$	$0.038 \pm 0.008^{*}$
	Group II	0.059 ± 0.010	$0.072 \pm 0.018^{**}$	$0.043 \pm 0.006^{***}$
	Group III	0.063 ± 0.007	0.051 ± 0.005	0.034 ± 0.006
GPx, $\text{nmol GSH}/\text{min} \times \text{mg prot.}$	Control	33.59 ± 2.66	10.35 ± 2.06	5.88 ± 1.32
	Group I	35.74 ± 4.54	13.24 ± 3.26	6.17 ± 1.61
	Group II	37.95 ± 1.93	$17.91 \pm 2.27^{***}$	$9.60 \pm 1.58^{**}$
	Group III	32.81 ± 4.31	12.32 ± 1.24	7.27 ± 1.89

Note. Statistically significant differences compared to the control group: $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$

the transcription of genes encoding antioxidant enzymes, protecting cells from oxidative damage [35, 36]. Selenium is involved in the synthesis and functioning of selenoproteins, such as peroxidases and reductases, which have oxidoreductase activity and regulate the redox balance of cells. In the form of the amino acid selenocysteine, Selenium is a part of selenoproteins, which are part of the active center of these enzymes. The antioxidant effect of Selenium and reduced glutathione is exerted through interaction with the enzyme glutathione reductase, which uses reduced glutathione as an electron donor to reduce hydrogen peroxide and organic peroxides [37].

The absence of significant changes in the level of reduced glutathione, glutathione peroxidase and glutathione reductase indicates that, despite the tendency to increase them with germanium citrate, it is not statistically significant. This may indicate that the stimulating effect of germanium citrate on the glutathione link of antioxidant defense is moderate or compensated by the body's adaptive mechanisms, which allows maintaining the redox balance even under conditions of severe heat stress. Thus, it is possible to assume a potential increase in the ability of the rabbit organism to neutralize peroxide compounds. However, the study data indicate that further analysis is needed to definitively confirm this effect.

Thus, feeding zinc and selenium citrate nanoparticles increases the content of reduced glutathione in rabbits, which indicates the activation of cells' antioxidant defense under severe heat stress conditions. Zinc activates the enzyme glutamate cysteine ligase, which catalyzes the formation of glutathione. It regulates the activity of the transcription factor Nrf2, which initiates gene expression and encodes antioxidant enzymes. Selenium is a component of selenium-dependent enzymes, particularly glutathione peroxidase, which restores and neutralizes hydrogen peroxide and lipid hydroperoxides using reduced glutathione.

Conclusions. Thus, given the results obtained we can conclude the following:

1. Feeding micronutrient nanoparticles in the diet of rabbits after weaning under conditions of severe heat stress contributed to positive changes in the functioning of the antioxidant defense system with a pronounced effect of zinc citrate (60 mg/l) and selenium citrate (300 µg/l). This was reflected in lower levels of lipid hydroperoxides in blood plasma and higher activity of superoxide dismutase, cata-

lase, reduced glutathione and glutathione peroxidase in erythrocytes on days 14 and 29 of the study compared to the control group.

2. The use of germanium citrate at a dose of 12.5 µg/l on the body of rabbits under conditions of severe heat stress had no significant effect on superoxide dismutase, catalase and glutathione system of the rabbit body under conditions of severe heat stress. The absence of statistically significant changes may indicate a weak or indirect antioxidant effect of germanium citrate under the proposed experimental conditions and requires further evaluation of the effectiveness at other dosages or a longer period of use.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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АНТИОКСИДАНТНА СИСТЕМА В ОРГАНІЗМІ КРОЛІВ ЗА КОМБІНОВАНОЇ ДІЇ СИЛЬНОГО ТЕПЛОВОГО СТРЕСУ ТА НАНОЧАСТИНОК ЦИТРАТУ ЦИНКУ, СЕЛЕНУ І ГЕРМАНІЮ

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Як відомо, кролі не можуть ефективно забезпечувати терморегуляцію свого тіла за температури вище 18-21°C через відсутність потових залоз і густого хутряного покриву. Тепловий стрес негативно впливає на обмінні процеси та репродуктивну функцію кролів. Одним із підходів до пом'якшення негативних наслідків теплового стресу є використання органічних сполук мікроелементів, створених за допомогою нанотехнології. Метою нашого дослідження було оцінити показники антиоксидантного за-

хисту в крові тварин за умов сильного теплового стресу та введення наночастинок цитрату цинку, селену та германію як кормової добавки. Молодняк кролів породи Термонська біла віком від 35 до 78 діб було розділено на групи по 6 тварин. Контрольна група отримувала основний раціон і воду без обмежень. Кролі дослідних груп I, II, III споживали той самий корм, як у контролі, проте впродовж 24 год з водою отримували: I група – 12 мг Zn/кг, II група – 60 мкг Se/кг, III група – 12,5 мкг Ge/кг. Упродовж 15 діб температуру в приміщенні підвищували з 28,9 до 30°C на 4 год на добу для забезпечення сильного теплового стресу. Встановлено, що додавання наночастинок цитрату цинку та селену сприяло позитивним змінам у функціонуванні системи антиоксидантного захисту, зміненої внаслідок сильного теплового стресу, тоді як захисна дія цитрату германію виявилася менш вираженою.

Ключові слова: кролі, наночастинок, сильний тепловий стрес, антиоксидантна система, оксидативний стрес, відновлений глутатіон, глутатіонпероксидаза, глутатіонредуктаза, гідропероксиди ліпідів, ТБК-активні продукти.

References

1. Szendrő K, Szabó-Szentgróti E, Szigeti O. Consumers' attitude to consumption of rabbit meat in eight countries depending on the production method and its purchase form. *Foods*. 2020; 9(5): 654.
2. Marai IFM, Habeeb AAM, Gad AE. Rabbit's productive, reproductive and physiological performance traits as affected by heat stress: a review. *Livest Prod Sci*. 2002; 78(2): 71-90.
3. Oladimeji AM, Johnson TG, Metwally K, Farghly M, Mahrose KM. Environmental heat stress in rabbits: implications and ameliorations. *Int J Biometeorol*. 2022; 66(1): 1-11.
4. Lesyk Y, Boiko O, Bashchenko M, Honchar O, Ivanikiv N. Blood parameters of rabbits given different amounts of iodine citrate. *Sci Horizons*. 2022; 25(5): 40-47.
5. Liang ZL, Chen F, Park S, Balasubramanian B, Liu WC. Impacts of heat stress on rabbit immune function, endocrine, blood biochemical changes, antioxidant capacity and production performance, and the potential mitigation strategies of nutritional intervention. *Front Vet Sci*. 2022; 9: 906084.
6. Abu Hafsa SH, Centoducati G, Hassan AA, Maggiolino A, Elghandour MMY, Salem AZM. Effects of dietary supplementations of vitamin C, organic selenium, betaine, and pomegranate peel on alleviating the effect of heat stress on growing rabbits. *Animals (Basel)*. 2024; 14(6): 950.
7. Qi Y, Li X, Guo S, He F, Liu R. Insights into the potential toxicity of Zn(II) to catalase and their binding mechanisms. *J Mol Liq*. 2024; 394: 123760.
8. Fedoruk RS, Kovalchuk II, Mezentseva LM, Tesarivska UI, Pylypets AZ, Kaplunenko VH. Germanium compounds and their role in animal body. *Anim Biol*. 2022; 24(1): 50-60.
9. Kotyk BI, Iskra RYa, Slivinska OM, Liubas NM, Pylypets AZ, Lubenets VI, Pryimych VI. Effects of ethylthiosulfanylate and chromium (VI) on the state of pro/antioxidant system in rat liver. *Ukr Biochem J*. 2020; 92(5): 78-86.
10. Li LJ, Ruan T, Lyu Y, Wu BY. Advances in effect of germanium or germanium compounds on animals – a review. *J Biosci Med*. 2017; 5(7): 56-73.
11. Nebylytsia MS, Onyshchenko RO, Vashchenko OV, Boyko OV. Electronic air environment analyser. Patent of Ukraine for utility model No. 127047. Bull No. 13 from 29.03.2023. Available from: <https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=284535>. (In Ukrainian).
12. Kosinov MV, Kaplunenko VG. Process for the preparation of metal carboxylates nanotechnology of metal carboxylates preparation. Patent of Ukraine for utility model No. 38391. Bull No. 1/2009. Available from: <https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=128062&chapter=description>. (In Ukrainian).
13. Salyha YT, Snitynskyi VV. Electron microscopy of biological objects. Lviv: Svit, 1999. 152 p.
14. Salyha N, Salyha Y. L-glutamic acid mitigates carbon tetrachloride-induced acute tissue injury by reducing oxidative stress in a rat model. *Curr Chem Biol*. 2022; 16(2): 130-137.
15. Salyha NO. Effect of glutamic acid and cysteine on oxidative stress markers in rats. *Ukr Biochem J*. 2020; 92(6): 165-172.
16. Petrovska IP, Salyha YuT, Vudmaska IV. Statistical methods in biological research: a

- teaching and methodological manual. Kyiv: Ahrarna nauka, 2022. 172 p. (In Ukrainian).
17. Boiko O, Lesyk Ya, Bashchenko M, Honchar O, Denys H, Grabovska O, Luchka I. Zinc citrate influence on the concentration of some macro- and microelements in rabbit body tissues. *Biol Stud.* 2022; 16(4): 45-58.
 18. Abdel-Wareth AAA, Amer SA, Mobashar M, El-Sayed HGM. Use of zinc oxide nanoparticles in the growing rabbit diets to mitigate hot environmental conditions for sustainable production and improved meat quality. *BMC Vet Res.* 2022; 18(1): 354.
 19. Alissa EM, Bahijri SM, Lamb DJ, Ferns GA. The effects of coadministration of dietary copper and zinc supplements on atherosclerosis, antioxidant enzymes and indices of lipid peroxidation in the cholesterol-fed rabbit. *Int J Exp Pathol.* 2004; 85(5): 265-275.
 20. Hosny NS, Hashem NM, Morsy AS, Abo-Elezz ZR. Effects of organic selenium on the physiological response, blood metabolites, redox status, semen quality, and fertility of rabbit bucks kept under natural heat stress conditions. *Front Vet Sci.* 2020; 7: 290.
 21. Sheiha AM, Abdelnour SA, Abd El-Hack ME, Khafaga AF, Metwally KA, Ajarem JS, Maodaa SN, Allam AA, El-Saadony MT. Effects of dietary biological or chemical-synthesized nano-selenium supplementation on growing rabbits exposed to thermal stress. *Animals (Basel).* 2020; 10(3): 430.
 22. Yang F, Smith MJ, Siow RCM, Aarsland D, Maret W, Mann GE. Interactions between zinc and NRF2 in vascular redox signalling. *Biochem Soc Trans.* 2024; 52(1): 269-278.
 23. Homma K, Fujisawa T, Tsuburaya N, Yamaguchi N, Kadowaki H, Takeda K, Nishitoh H, Matsuzawa A, Naguro I, Ichijo H. SOD1 as a molecular switch for initiating the homeostatic ER stress response under zinc deficiency. *Mol Cell.* 2013; 52(1): 75-86.
 24. Basak P, Sadhukhan P, Sarkar P, Sil PC. Perspectives of the Nrf-2 signaling pathway in cancer progression and therapy. *Toxicol Rep.* 2017; 4: 306-318.
 25. Bonaventura P, Benedetti G, Albarède F, Miossec P. Zinc and its role in immunity and inflammation. *Autoimmun Rev.* 2015; 14(4): 277-285.
 26. Yuzviak M, Lesyk Ya, Luchka I, Denys H, Salyha Yu. The effects of zinc citrate, selenium citrate, and germanium citrate on hematological parameters of rabbits under heat stress. *Biol Stud.* 2024; 18(3): 69-86.
 27. Day BJ. Catalase and glutathione peroxidase mimics. *Biochem Pharmacol.* 2009; 77(3): 285-296.
 28. Nguyen T, Sherratt PJ, Pickett CB. Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. *Annu Rev Pharmacol Toxicol.* 2003; 43: 233-260.
 29. Kondaparthi P, Flora SJS, Naqvi S. Selenium nanoparticles: an insight on its pro-oxidant and antioxidant properties. *Front Nanosci Nanotech.* 2019; 6: 1-5.
 30. El-Deep MH, Shabaan M, Assar MH, Attia KM, Sayed MAM. Comparative effects of different dietary selenium sources on productive performance, antioxidative properties and immunity in local laying hens exposed to high ambient temperature. *J Anim Poult Prod Mansoura Univ.* 2017; 8: 335-343.
 31. Zhang Y, Zhu S, Wang X, Wang C, Li F. The effect of dietary selenium levels on growth performance, antioxidant capacity and glutathione peroxidase 1 (GSHPx1) mRNA expression in growing meat rabbits. *Anim Feed Sci Technol.* 2011; 196(3-4): 259-264.
 32. Hübner C, Haase H. Interactions of zinc- and redox-signaling pathways. *Redox Biol.* 2021; 41: 101916.
 33. Dzen Ye, Rosalovsky V, Shtapenko O, Slypaniuk O, Salyha Yu. Effect of zinc methionine supplementation on biochemical and hematological indices of growing rabbits. *Bulg J Agric Sci.* 2023; 29(4): 714-722.
 34. Ngo V, Duennwald ML. Nrf2 and oxidative stress: A general overview of mechanisms and implications in human disease. *Antioxidants (Basel).* 2022; 11(12): 2345.
 35. Sharan O, Stefanyk V, Bartlewski PM, Sharan M. The effect of supplementing freezing extender with Mn^{2+} -, Zn^{2+} - or Cu^{2+} -nanosuccinate on select post-thaw characteristics of ram semen. *Reprod Biol.* 2024; 24(3): 100932.

36. Kim HJ, Nel AE. The role of phase II antioxidant enzymes in protecting memory T cells from spontaneous apoptosis in young and old mice. *J Immunol.* 2005; 175(5): 2948-2959.
37. Lubos E, Loscalzo J, Handy DE. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal.* 2011; 15(7): 1957-1997.