Biol. Stud. 2024; 18(3): 69–86 • doi: https://doi.org/10.30970/sbi.1803.790
www.http://publications.lnu.edu.ua/journals/index.php/biology

UDC: 636.92.053.112.385.4

THE EFFECT OF ZINC CITRATE, SELENIUM CITRATE, AND GERMANIUM CITRATE ON HEMATOLOGICAL PARAMETERS OF RABBITS UNDER HEAT STRESS

Marian Yuzviak [1], ***Yaroslav Lesyk*** [2],
Ivan Luchka [1], ***Halyna Denys*** [1], ***Yuriy Salyha*** [1]

[1] *Institute of Animal Biology NAAS, 38 V. Stusa St., Lviv 79034, Ukraine*

[2] *Drohobych Ivan Franko State Pedagogical University, 3 Stryiska St., Drohobych 82100, Ukraine*

Yuzviak, M., Lesyk, Ya., Luchka, I., Denys, H., & Salyha, Yu. (2024). The effects of zinc citrate, selenium citrate, and germanium citrate on hematological parameters of rabbits under heat stress. *Studia Biologica*, 18(3), 69–86. doi:10.30970/sbi.1803.790

Background. The environmental crisis has affected the annual ambient temperature increase, adversely affecting the mammalian body. Due to their lack of sweat glands, Rabbits are more sensitive to heat stress than other animals. The effect of elevated ambient temperatures on the rabbit body leads to violations of blood parameters, endocrine regulation, immune and reproductive function, which reduces their productivity and increases animal mortality. Particular attention is now paid to the study of organic compounds of trace elements, which are characterized by high physiological activity, are non-toxic, have a wide range of biological effects, and have a positive impact on reducing the negative effect of elevated ambient temperatures on animals. However, their action depends on the element and its applied quantity. Therefore, the main objective of this study was to investigate the effect of zinc citrate, selenium citrate, and germanium citrate on changes in the number of blood cells in rabbits to mitigate the effects of heat stress.

Materials and methods. The studies were conducted on young analog rabbits of the Termon White breed from 35 to 78 days of age. The rabbits were kept indoors at elevated ambient temperatures from 28.9 to 30 °C and relative humidity from 78.1 to 87.4 %. Animals of the control group were kept on the main diet with feeding of standard balanced granulated compound feed and water without restriction. Rabbits of groups I, II, and III of the study groups consumed the same compound feed as in control, but within 24 hours, they received water: group I – zinc citrate – 60 mg Zn/L or 12 mg Zn/kg

of body weight; group II – selenium citrate – 300 μg Se/L or 60 μg Se/kg of body weight; group III – germanium citrate – 62.5 μg Ge/L or 12.5 μg Ge/kg of body weight. Using individual drinkers for each animal and placing the animals in different cages allowed us to control the amount of water consumed by each rabbit.

Blood for the study was selected for supplementation on the 14th day of the preparatory period and the 14th and 29th days of the study period. During the study period, the room temperature was monitored, taking into account the temperature and humidity index.

Results. The addition of micronutrient citrates to the diet of rabbits during 29 days of study under heat stress caused haematological changes in indicators compared to the control: the number of erythrocytes in the blood of rabbits of experimental groups I and II increased by 16.4 and 13.6 % and 19.9 and 14.5 % on day 14 and 29, respectively, in group III by 15.3 % on day 14; the haemoglobin content of groups I, II and III increased by 20.8, 21.6 and 19.5 % on day 14 and 11.1, 12.5 and 9.7 % on day 29; haematocrit value of groups I and II increased by 24.1 and 15.7 % and 21.1 and 16.5 % during the study, group III by 18.6% on day 14; the number of leukocytes of groups I, II and III decreased by 13.1 and 8.3 %; 11.2 and 10.4 % and by 11.4 and 9.3 % on days 14 and 29; the number of lymphocytes of groups I, II and III decreased by 25.9, 27.3 and 29.0 % on day 14 and by 20.4, 21.7 and 16.0 % on day 29; the number of monocytes of groups I and II increased by 14.8 and 21.3 % and 17.0 and 18.3 % over 29 days; the number of platelets in animals of group II decreased by 29.4 % on day 29, the average volume of red blood cells increased by 11.6 and 14.6 % on days 14 and 29 of the experiment.

Conclusions. Adding micronutrient citrates to the rabbit diet mitigated the effects of heat stress on the body. The effect of these additives on animals resulted in significant changes in the hematological parameters of the rabbits' blood, of which the best results were observed under the influence of selenium citrate (60 μg Se/kg body weight) and zinc citrate (12 mg Zn/kg body weight): red blood cell count ($p < 0.05–0.01$), leukocyte ($p < 0.05–0.01$), lymphocyte ($p < 0.05–0.01$), monocyte ($p < 0.05–0.01$), haemoglobin content ($p < 0.01–0.001$), haematocrit value ($p < 0.01$), compared to the control. Feeding germanium citrate led to less pronounced changes in these blood parameters.

Keywords: rabbits, blood, zinc citrate, selenium citrate, germanium citrate, heat stress

INTRODUCTION

Heat stress is a severe issue in the livestock industry that negatively affects animal health (Liang *et al.*, 2022). The thermoneutral zone for rabbits covers a temperature range of 18 to 21 °C (Marai *et al.*, 2001). The optimal moisture values for the body of rabbits are from 55 to 65 % (Liang *et al.*, 2022). Exposure to elevated ambient temperatures reduces the activity of the immune function, and the antioxidant defense system inhibits the reproductive ability, growth, and development of the rabbit organism (Farghly *et al.*, 2021). Organic compounds of trace elements, characterized by high bioavailability in the body, surface activity, catalytic and adsorption properties, and lower toxicity, are used in animal feeding (El-Ratel *et al.*, 2023). It is known that organic compounds of trace elements used in rabbits' diets are better absorbed in the digestive system than their salts (Konkol *et al.*, 2018).

Selenium plays a vital role in the functioning of the reproductive system, immunity, antioxidant protection, stress resistance, and endocrine regulation of the body (Boiko *et al.*, 2021; El-Ratl *et al.*, 2023). Compared to inorganic selenium, selenium citrate has higher absorption and catalytic activity and is less toxic (Alagawany *et al.*, 2021). The addition of selenium citrate to the diet at the rate of 25 and 50 mg/kg to the feed increases the average daily gains, glutathione activity, and catalase, reduces the level of TBA-active products in the serum of rabbits under the influence of heat stress (Sheiha *et al.*, 2020). Toxicological studies have shown that SeNPs 20–60 nm and Se-methionine in amounts (30 and 70 μg Se/kg body weight) improve Se accumulation in whole blood, liver, and kidneys in a dose-dependent manner compared to the control (Zhang *et al.*, 2008).

The application of selenium in the form of a chelated compound in rabbit diets is a critical factor in ensuring the antioxidant functions of their body (Wang *et al.*, 2007).

Zinc plays a vital role in the metabolism of the synthesis of carbohydrates, proteins, and lipids due to its activating effect on enzymes that regulate the processes of digestion and absorption and ensure the structural integrity of proteins (Chrastinová *et al.*, 2015; Boiko *et al*., 2022; Dzen *et al*., 2023). There are virtually unlimited possibilities for adjusting zinc doses. More research is needed to identify optimal concentrations and new sources of Zn in rabbit diets in hot climates. Nanoparticles can be used at lower doses and provide better results than conventional sources (Abdel-Wareth *et al.*, 2022). Different concentrations of organic zinc can activate different biochemical pathways or mechanisms of action in the body. For example, low doses of zinc nanoparticles may significantly impact antioxidant production or inflammation prevention.

In comparison, higher doses of organic zinc may significantly impact tissue growth or immune response. It was noted that applying organic zinc oxide at the rate of up to 80 mg/kg of feed gave higher indicators of the rabbit's body development and a higher coefficient of diet digestibility under heat stress (Abdel-Wareth *et al.*, 2022). Adding 100 mg/kg zinc oxide to the diet of rabbits contributes to an increase in the productivity of rabbits (Hassan *et al.*, 2021). Feeding zinc oxide improves antioxidant properties and reduces the negative effect of heat stress on embryo hepatocytes in broilers. (Lee *et al*., 2018). Feeding zinc citrate or lactate increases the activity of superoxide dismutase, glutathione, and glutathione transferase in the blood, thereby inhibiting the action of free radicals in animals (Lee *et al.*,2018; Liang *et al.*, 2022).

Studies have shown that organic germanium can adsorb free radicals and increase the body's antioxidant activity, especially under stress (Oh *et al.*, 2011; Fedoruk *et al.*, 2022). Once in the body, Germanium interacts with hemoglobin and provides cellular metabolism (Li *et al.*, 2017). Germanium citrate has an inhibitory effect on lipid peroxidation in the body (Zheng, 2011). Studies have found that the organic compound germanium Ge-132 can absorb and prevent damage to cells by reactive oxygen species (Nakamura *et al.*, 2010). The action of germanium citrate at concentrations of 200 and 300 μg Ge/L of water revealed immunomodulatory properties, which were manifested in an increase in the level of hemoglobin, circulating immune complexes in the blood of rats, a decrease in the activity of lipid hydroperoxides and TBA-activeproducts (Khrabko *et al.*, 2016). Given the above material, the aim of this study to determine the effect of zinc citrate, selenium citrate, and germanium citrate on changes in the number of blood cells in rabbits to mitigate the effects of heat stress is highly relevant.

MATERIALS AND METHODS

The study was conducted in the vivarium of the Institute of Animal Biology of the NAAS in Lviv. The animals were kept in a room with a controlled microclimate in mesh cages measuring 50×120×30 cm. The permission to conduct research was received from the Bioethics Commission of the Institute of Animal Biology of the NAAS in Lviv (No. 146 of 19.02.24). All manipulations with experimental animals were carried out following the provisions of the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001) and the guidelines of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986). The studies were conducted on young rabbits from 35 to 78 days of age, the Termon White breed, analogs by age, body weight, and clinical condition. During the experimental period, the ambient temperature in the room was increased from noon to 4 p.m. (four hours a day), using electric adjustable heaters, from 28.9 to 30 °C. Temperature and humidity were monitored using a thermohydrometer with Trotec BL30 data logging. The Electronic Air Analyzer measured Humidity and temperature (Nebylytsia *et al.*, 2023. Patent No.127047). The temperature was monitored by the temperature and humidity index (THI). Animals were selected for the control and study groups I, II, and III of 6 animals (3 males, 3 females), with an average body weight of 1200 ± 50 g. The control group rabbits were kept on the main diet, with a standard balanced granulated feed and water without restriction. Rabbits of the experimental groups I, II, and III consumed granulated compound feed, like in the control, but received citrates of trace elements with water for 24 hours. Experimental group I – zinc citrate – 60 mg Zn/L or 12 mg Zn/kg of body weight; group II – selenium citrate – 300 μg Se/L or 60 μg Se/kg of body weight; group III – germanium citrate – 62.5 μg Ge/L or 12.5 μg Ge/kg of body weight. Solutions for the study were manufactured by Nanomaterials and Nanotechnologies LLC, Kyiv (Kosinov & Kaplunenko, 2009. Patent No. UA 38391). Blood parameters of rabbits were studied on the 14th day of the preparatory period and the 14th and 29th days of supplementation under conditions of heat stress. On the 14th day of the preparatory period, blood was taken from rabbits to study the characteristics of the selected biological material without exposure to an elevated temperature-humidity index and in the absence of additives in the diet. These data served as the basis for further comparison with the results of the experimental groups exposed to elevated temperatures. Whole blood samples were taken from the marginal auricular vein in 6 animals from the group into tubes with anticoagulant ethylenediaminetetraacetate (EDTA) for hematological studies and the total number of red blood cells (absolute red blood cell (RBC), average red blood cell volume (HGB), hematocrit (HCT), white blood cell (leukocyte) (WBC) and their forms – lymphocytes (LYM), monocytes (Mon), granulocytes (GRA); platelet count and platelet indices (absolute platelet count (PLT), median platelet volume (MPV), platelet count (PCT), relative width of platelet distribution by volume (PDW) were determined using the Orphee Mythic 18 automatic hematological analyzer (Switzerland), (Vlizlo *et al.*, 2012).

The limits of comfortable living conditions for rabbits were defined as temperature and humidity index**.** The average daily air temperature and relative humidity were determined by the formula (Marai *et al*., 2002):

$$THI = T - ((0.31 - 0.31 \cdot RH/100) \cdot (T - 14.4)),$$

where: THI = temperature-humidity index; T = temperature (°C); RH = relative humidity in percent (%).

The resulting THI values were classified according to the literature (Marai *et al.*, 2002) as <27.8 – no heat stress; 27.8 to <28.9 – moderate heat stress; 28.9 to <30.0 – severe heat stress; 30.0 and more – very severe heat stress.

The study results were analyzed using the Statistica 7.0 software package (Statsoft, USA). The experimental data are presented as a 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 ANOVA, where factor A is time, factor B is microelement citrates, and AB is the interaction of time and microelement citrates. To identify 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 (Petrovska *et al.*, 2022).

RESULTS AND DISCUSSION

The average humidity and temperature in the preparatory period were 56.3 % and 19.8 °C, and THI was 19.0. The average room temperature in the experimental period on day 14 of the study period was 29.9 °C, and the humidity was 86.5 % (**Fig. 1**). THI for the specified period of the experiment was 29.2 °C, which, according to I. F. M. Marai *et al.* (2002), indicates a severe heat stress.

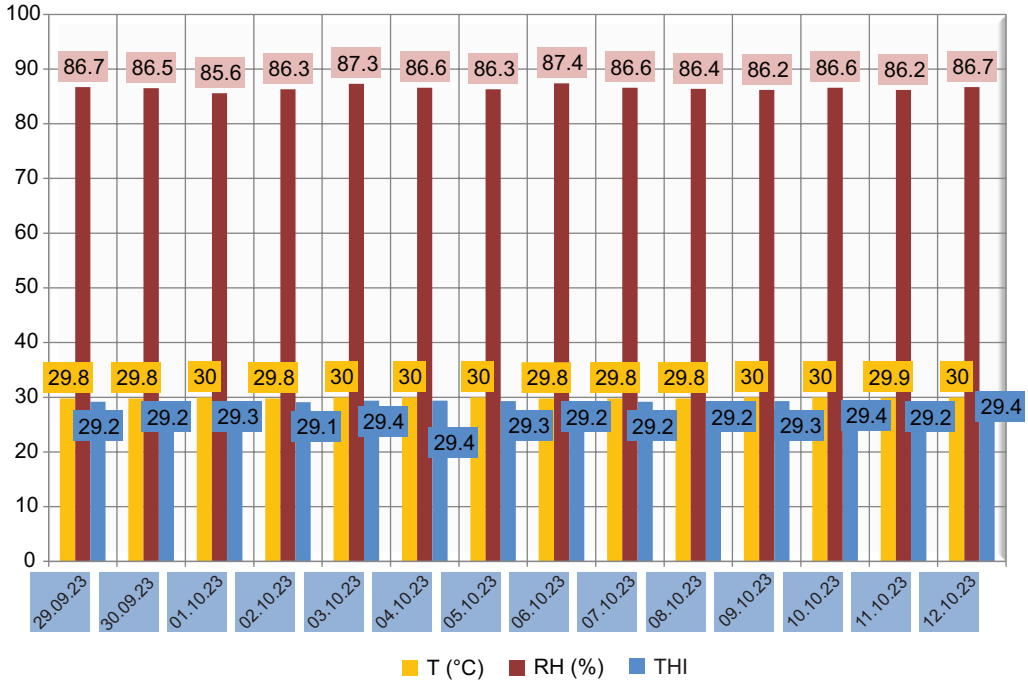

Fig. 1. Temperature – humidity index during the experimental period for 15 days (29.09.23 – 12.10.23)

On day 29 of the experimental period, the average values of humidity and temperature, respectively, were 84.3% and 29.9 °C (**Fig. 2**). THI, according to this formula, corresponded to 29.1, indicating the parameters of severe heat stress in the final period of the study.

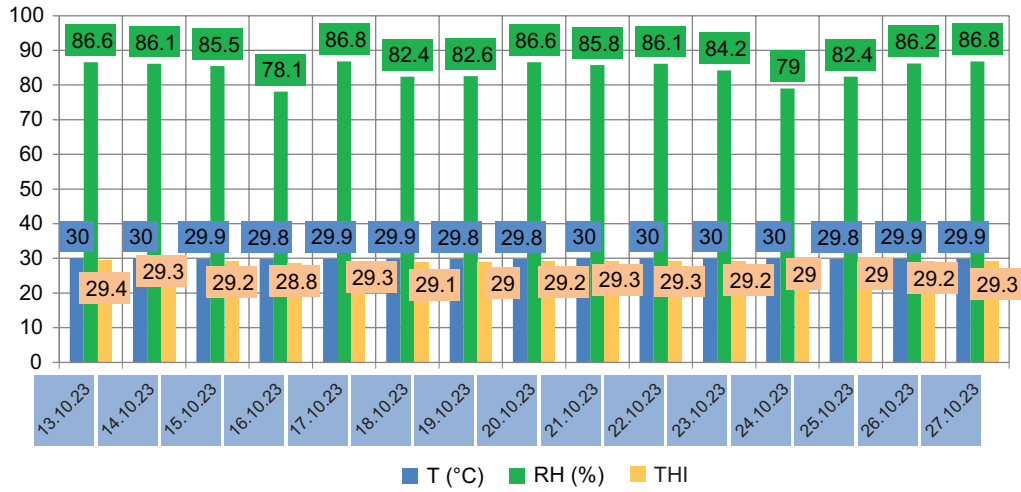

Fig. 2. Temperature – humidity index during the experimental period for 15 days (13.10.23 – 27.10.23)

Studies of the number of blood cells in rabbits indicate that the results of the upper and lower values were within physiological limits (Leineweber *et al.*, 2018). Analysis of the obtained results of the absolute content of erythrocytes shows that in the blood of rabbits of study groups I and II, their number was respectively higher by 16.4 % ($p < 0.05$) and 13.6 % ($p < 0.05$) and 19.9 % ($p < 0.01$) and 14.5 % ($p < 0.05$) on days 14 and 29 of the study compared with the control group of the I and II period of the study (**Table 1**). The analysis of the influence of the time factor (A) – ($p = 0.047546$) and the citrate trace element factor (B) – ($p = 0.000153$) showed a statistically significant effect on the number of erythrocytes during the experimental period and the absence of changes under the influence of a combination of factors (AB – $p = 0.248966$). Organic Zinc is an alternative source of inorganic compounds due to its better animal absorption and use (Hassan *et al.*, 2021). Adding 80 mg Zn/kg feed in zinc lactate increased the number of erythrocytes and hemoglobin concentration in rabbits (Yan *et al.*, 2017). Our results are consistent with the experiments by A. K. Sharaf *et al*. (2021), which found that vitamin E and organic selenium had a positive effect on blood counts and body weight of young rabbits and increased the consumption of food and water in their diet. Organic selenium at a dose of 0.3 mg/kg of feed reduced the negative impact of heat stress on the sperm quality of male rabbits (Hosny *et al.*, 2020). In the blood of animals of the III experimental group, which used germanium citrate in the amount of 12.5 μg Ge/kg of body weight, the absolute content of red blood cells was significantly higher by 15.3 % ($p < 0.05$), concerning the control group only on the 14th day of the experiment, which may indicate a pronounced effect of this supplement on the body of rabbits during its prolonged use under heat stress. We assume that the supplementation of germanium citrate had a rapid effect on the body and that the body adapted to the supplement. This is confirmed by other studies (Fedoruk *et al.*, 2017; Khrabko *et al.*, 2016) on rats during physiological maturation.

It is known that at elevated temperatures, the body of rabbits needs more oxygen due to more intensive metabolism (Farghly *et al.*, 2021). Our studies have established a positive effect of the applied trace element citrates on hemoglobin content, which was more pronounced in the first stage of the study in all study groups than in the second.

Table 1. **Red blood cell count, hemoglobin concentration, and hematocrit value in rabbit blood under the action of zinc citrate, selenium citrate, and germanium citrate under heat stress (M ± 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
RBC, 10^{12} /L	K	5.81±0.48	5.52±0.45	5.58±0.32
	D – 1	5.82±0.61	6.43±0.44*	6.34±0.33*
	D – 2	6.14±0.69	6.62±0.46**	6.39±0.68*
	D – 3	5.79±0.32	6.37±0.47*	6.21±0.21
HGB, g/L	K	141.1±10.62	117.6±3.32	131.8±8.10
	D – 1	138.3±11.11	142.1±4.75***	146.5±4.92**
	D – 2	138.8±5.15	143.1±4.75***	148.3±6.21**
	D – 3	135.3±5.71	140.6±3.93***	144.6±5.46*
HCT, L/L	K	0.390±0.03	0.402±0.02	0.418±0.04
	D – 1	0.346±0.03	0.499 ±0.03**	0.484±0.02**
	D – 2	0.370±0.04	0.487±0.04**	0.487±0.02**
	D – 3	0.406±0.05	0.477±0.03*	0.460±0.03

***Note*:** in this and the following tables, there are statistically significant differences compared to the control group: * – p <0.05; ** – p <0.01; *** – p <0.001

The effect of the time factor A (p = 0.0023328) and the effect of trace element citrates B (p = 0.000000) had a statistically significant effect on haemoglobin content. In particular, the combined effect of time and micronutrient citrates was statistically significant AB (p = 0.000012), which may indicate that the additional use of citrates indirectly affected the mechanisms of oxygen transport by red blood cells, which showed a better result on hemoglobin concentration. Thus, in the blood of rabbits of groups I, II, and III, the hemoglobin concentration was respectively higher by 20.8 % (p <0.001), 21.6 % (p <0.001), and 19.5 % (p <0.001) on day 14 of the study compared to the control. On day 29 of the study, the application of micronutrient citrate supplements in the blood of animals of the I, II, and III study groups was marked by a higher level of hemoglobin by 11.1 % (p <0.01), 12.5 % (p <0.01) and 9.7 % (p <0.05), respectively. It should be noted that the effect of germanium citrate was marked by slightly lower levels of the studied indicator. Central to cellular defense is the body's immune response, which adapts to protect against infection or tissue damage. Haemoglobin transports oxygen to tissues, including immune cells (Quaye, 2015). Therefore, a high concentration of haemoglobin can provide optimal oxygen levels for the immune system to function.

Experiments conducted on animals indicate a pronounced effect of this trace element on hemoglobin concentration (Li *et al.*, 2017; Khrabko *et al.*, 2016). In our opinion, the result depends on the amount used since organic micronutrient compounds have a higher activity, but it depends on the dose in the diet.

The study results show an increase in the number of red blood cells in the blood of rabbits, which is confirmed by the higher hematocrit level in the control groups. In the blood of rabbits of groups I and II, significant changes in haematocrit values were noted, respectively, by 24.1 % ($p < 0.01$), 15.7 % ($p < 0.01$), and 21.1 % ($p < 0.01$) and 16.5 % ($p < 0.01$) during the study. A less pronounced effect on the content of formed elements in the total volume of blood was observed when drinking germanium citrate, which in the blood of rabbits was marked by a significant increase by 18.6 % ($p < 0.05$) in the first stage of the study compared to the control group.

The determination of factor A ($p = 0.000000$) and factor B ($p = 0.001143$) was marked by a statistically significant effect on the percentage of haematocrit values, but the values of factor A indicate a more pronounced effect of the studied changes. The interaction of time factors and micronutrient citrates was statistically significant AB ($p = 0.004550$), which may indicate a positive effect of the duration of feeding organic micronutrients on the number of blood cells in rabbits.

Studies in pigs (Waltz *et al.*, 2014) and rabbits (Dahia *et al.*, 2022) subjected to heat stress indicate an increase in the level of red blood cells, hemoglobin concentration, and hematocrit due to an increase in blood circulation in the skin under the influence of heat.

An increase in the number of maturing reticulocytes leads to an increase in the content of formed elements in the total blood volume, which helps to protect tissues from hypoxia. Other researchers (Nakyinsige *et al.*, 2013) explain the increase in these parameters by increased blood viscosity due to excessive water loss caused by hyperventilation and fluid loss due to urination, which causes dehydration in rabbits. However, M. A. Ayoub *et al.* (2007) recorded a decrease in the level of red blood cells, hemoglobin, and hematocrit in the blood of rabbits under the effects of heat stress.

The studied citrates of trace elements can stimulate the process of erythropoiesis, which leads to an increase in the number of erythrocytes in the blood of rabbits under the influence of heat stress. Zinc affects the process of erythropoiesis through various mechanisms. Firstly, it acts as a catalyst for the enzyme alpha-aminolevulinic acid dehydratase, which is important in iron metabolism and heme synthesis. Secondly, zinc is incorporated into the structure of proteins such as Zinc finger 1B (Gfi-1B) and GATA-1, which regulate the growth of erythroid cells by controlling the expression of cell-specific genes. This may include the regulation of signaling pathways such as SMAD and TGF-β. In addition, zinc promotes the transcription of erythropoietin through signaling cascades such as the growth hormone and insulin-like growth factor-1 pathways, resulting in increased erythropoiesis. Zinc is essential in differentiating erythroid cells, contributing to their formation and function. Thus, the effect of zinc on erythropoiesis is complex and covers various aspects of this process (Hanson *et al.*, 2023).

Selenium is a component of selenoproteins (Hariharan *et al.*, 2020). Y. Kawatani *et al.* (2011) established the critical role of selenoproteins and Nrf2 in the regulation of erythropoiesis using a model of transgenic mice in which the $tRNA^{[Ser]Sec}$ (Trsp) gene, which controls selenoprotein synthesis, was deleted. Mice with deleted selenoprotein ($Trsp^{fl/del}$: Mx1-Cre) developed anaemia, which was manifested by a decrease in haematocrit, a decrease in serum haemoglobin content and an increase in the average red blood cell volume, which are pathological signs of erythropoiesis disorders. In addition, immature and damaged red blood cells were found in the bone marrow of genetically altered mice. Significantly, the genes associated with oxidative stress were

amplified. These data strongly suggest the importance of selenoproteins in maintaining redox homeostasis in erythroid cells (Kawatani *et al.*, 2011)

Studies have found that an organic germanium compound known as Ge-132 can promote the expression of the cellular stimulatory factor IL-3 in IL-3 in multifunctional haematopoietic stem cells (HSCs) and participate in the regulation of the differentiation and proliferation of multifunctional haematopoietic stem cells (MHSCs) and progenitor cells, thus regulating haematopoietic function in the body (Kong *et al.*, 2007). Analysis of the obtained results of the number of leukocytes indicates a likely decrease in their number in the blood of rabbits of groups I and II, respectively, by 13.1 % ($p < 0.05$) and 8.3 % ($p < 0.05$) and 11.2 % ($p < 0.05$) and 10.4% ($p < 0.01$) on days 14 and 29 of the study compared with the control (**Table 2**). When germanium citrate is fed, a decrease in the number of leukocytes is observed relative to the control group on days 14 and 29 by 11.4 % ($p < 0.05$) and 9.3 % ($p < 0.05$). The results of the analysis of the effect of time factor A ($p = 0.000000$), the factor of micronutrient citrates B ($p = 0.000000$), and the combination of factors AB ($p = 0.000000$) during the study period showed a statistically significant effect on the number of leukocytes during the study period.

Table 2. **The number of leukocytes and their forms in the blood of rabbits under the action of zinc citrate, selenium citrate, and germanium citrate under conditions of heat stress (M ± SD, n = 6)**

Blood indicators	Group of animal	Research period age of the animal/day of supplementation		
		Preparatory period	Study period	
			63/14	78/29
WBC (10^9/L)	C	9.16±0.52	8.73±0.84	8.78±0.49
	D – 1	9.11±0.65	7.58±0.44*	8.05±0.40*
	D – 2	8.55±0.54	7.75±0.53*	7.86±0.38**
	D – 3	8.46±0.30	7.73±0.49*	7.96±0.41*
LYM, 10^9/L	C	3.60±0.47	4.86±0.64	4.60±0.37
	D – 1	3.51±0.53	3.60±0.46*	3.66±0.55**
	D – 2	3.33±0.44	3.53±0.80*	3.60±0.50**
	D – 3	3.53±0.48	3.45±0.82*	3.86±0.28*
MON, 10^9/L	C	1.26±0.10	1.35±0.13	1.36±0.18
	D – 1	1.36±0.16	1.55±0.10*	1.65±0.10**
	D – 2	1.35±0.21	1.58±0.07*	1.61±0.07*
	D – 3	1.45±0.15	1.50±0.15	1.53±0.10
GRA, 10^9/L	C	3.63±0.62	3.58±0.71	3.38±0.82
	D – 1	2.66±0.70	3.48±0.92	3.56±0.47
	D – 2	3.36±0.61	2.51±0.69	3.38±0.50
	D – 3	2.68±0.65	3.51±0.77	3.71±0.93

The absolute content of lymphocytes in the blood of rabbits in study groups I, II, and III significantly decreased, relative to the control group, by 25.9 ($p < 0.05$), 27.3 ($p < 0.05$), and 29.0 % ($p < 0.05$) on day 14 of the study compared to the control group. A significant decrease in lymphocytes was also observed on day 29 of the study in the blood of rabbits in study groups I, II, and III, namely by 20.4 ($p < 0.05$), 21.7 ($p < 0.01$) and 16.0 % ($p < 0.01$) compared to the control group. The effect of time factor A ($p = 0.024344$) and the effect of micronutrient citrates B ($p = 0.000043$) was reflected in statistically significant changes in the number of lymphocytes, where the expressed effect was observed for factor B. The analysis of the combination of AB factors did not show a statistically significant result ($p = 0.075463$). Studies (Khalil *et al.*, 2014) have noted a decrease in the number of lymphocytes during acute heat stress. Heat stress has been found to cause a significant decrease in the percentage of lymphocytes due to an increase in neutrophils (Dhabhar *et al.*,1995). Studies (Viswanathan & Dhabhar, 2005) have found that corticosteroids and catecholamines increase the accumulation of lymphocytes in the spleen, lymph nodes, and mucous membranes, which can decrease their number in the blood. Heat stress leads to organ dysfunction and stress.

The absolute content of monocytes significantly increased in the blood of rabbits of study groups I and II by 14.8 % ($p < 0.05$), 21.3 % ($p < 0.01$), and 17.0 % ($p < 0.05$), and 18.3 % ($p < 0.05$) relative to the control. Determining factor A ($p = 0.000061$) and factor B ($p = 0.000135$) established statistically significant values for the number of monocytes. The interaction of time and trace element citrates was not statistically significant AB ($p = 0.405292$). Heat stress can lead to platelet depletion. Platelets' number and functional capacity play an essential role in developing coagulopathies, and the direct action of high temperature stimulates platelet activation (He *et al.*, 2022). Low platelet count correlates with platelet consumption in addition to their aggregation and reduced release from megakaryocytes to bone marrow due to high sensitivity to excessive heat (Jayaratne *et al.*, 2018).

The results of the analysis of the effect of time factor A ($p = 0.489500$), the effect of trace element citrates B ($p = 0.139516$), and the interaction of time factor and citrates AB ($p = 0.491788$) did not show statistically significant changes in the effect on granulocytes.

A significant decrease in the number of platelets in study group II by 29.5 % ($p < 0.05$) at the final stage of the study was detected (**Table 3**). The effect of time factor A ($p = 0.000560$) had a statistically significant impact on platelet count. The effect of trace element citrate B ($p = 0.373400$) did not reveal any significant changes. The interaction of time and micronutrient citrates is statistically significant ($p = 0.023193$) and indicates that the combined effect of these two factors significantly impacts platelet count. In the blood of animals of the second experimental group, there was an increase in the average platelet volume by 11.6 % ($p < 0.05$) on day 14 and by 14.6 % ($p < 0.05$) on day 29 of supplementation compared to the control. The results of the analysis of factor A ($p = 0.755470$) did not show statistically significant changes in the effect on the average platelet volume. The influence of the factor of microelement citrate B ($p = 0.000006$) 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 ($p = 0.853328$), indicating the absence of influence in the combination of these factors.

The determination of factor A ($p = 0.429977$), factor B ($p = 0.099840$), and the interaction of time factors and microelement citrates AB ($p = 0.199339$) did not show statistically significant changes in terms of their effect on thrombocrit.

The effect of time factor A (p = 0.345298) did not reveal statistical changes in the relative width of platelet distribution by volume. However, the effect of micronutrient citrate B (p = 0.017980) slightly affected the studied index. In the combined interaction of the two factors, there were no statistically significant changes in AB (p = 0.989848).

Table 3. **Platelet counts and platelet indices under the influence of zinc citrate, selenium citrate, and germanium citrate under heat stress (M ± SD, n = 6)**

Blood indicators	Group of animals	Research period age of the animal/day of supplementation		
		Preparatory period	Study period	
			63/14	78/29
PLT, 10^9/L	C	415.1±81.96	309.1±63.68	466.5±63.50
	D – 1	408.5±57.47	338.0±99.17	439.5±73.27
	D – 2	385.8±101.23	389.6±69.24	328.8±82.42*
	D – 3	460.3±82.29	329.3±51.30	445.5±53.22
MPV, f/L	C	5.03±0.40	4.98±0.25	4.93±0.34
	D – 1	5.10±0.33	5.28±0.33	5.00±0.35
	D – 2	5.50±0.45	5.56±0.40*	5.65±0.22*
	D – 3	5.68±0.40	5.55±0.40	5.48± 0.57
PCT, %	C	0.208±0.03	0.152±0.02	0.230±0.03
	D – 1	0.215±0.03	0.177±0.04	0.184±0.05
	D – 2	0.209±0.04	0.233±0.05	0.184±0.09
	D – 3	0.231±0.07	0.221±0.09	0.261±0.03
PDW, %	C	13.50±1.16	13.45±1.13	13.16±0.91
	D – 1	13.88±1.10	13.96±1.50	13.35±0.86
	D – 2	14.76±1.64	14.88±1.61	14.33±1.23
	D – 3	14.68±0.79	13.98±1.01	13.93±1.57

Consequently, the results of changes in the content of erythrocytes, leukocytes, and blood platelets indicate the physiological features of changes in their number under heat stress conditions. The additives used in the study, zinc citrate and selenium citrate to a greater extent, and germanium citrate to a lesser extent, affect the mitigation of the effect of elevated temperatures on the body of rabbits.

CONCLUSIONS

Adding micronutrient citrates to the rabbit diet mitigated the effects of heat stress on the body. The effect of these additives on animals resulted in significant changes in the

hematological parameters of rabbits' blood, where the best results were observed under the influence of selenium citrate (60 μg Se/kg body weight) and zinc citrate (12 mg Zn/kg body weight): red blood cell count ($p < 0.05–0.01$), leukocyte ($p < 0.05–0.01$), lymphocyte ($p < 0.05–0.01$), monocyte ($p < 0.05–0.01$), haemoglobin content ($p < 0.01–0.001$), haematocrit value ($p < 0.01$), compared to the control. Feeding germanium citrate led to less pronounced changes in these blood parameters.

ACKNOWLEDGMENTS AND SOURCES OF FUNDING

We thank Cherkasy Bioresources Research Station of NAAS for cooperation and assistance.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: the authors did not receive special funding for this work and declare no conflict of interest.

Human Rights: the article does not contain any experiments on humans.

Animal rights: all international, national, and institutional recommendations for the care and use of animals have been followed.

AUTHOR CONTRIBUTIONS

Conceptualization, [Y.M.; L.Y.; S.Y.]; methodology, [Y.M.; L.Y.]; research, [Y.M.; D.H.]; resources, [L.I.]; data processing, [Y.M.]; writing – preparation of the original project, [Y.M.]; writing – review and editing, [Y.M.; L.Y.; S.Y.]; visualization, [Y.M.; L.I.] supervision, [Y.M.; L.Y.; S.Y.]; project management, [L.Y.; S.Y.]; funding search, [-].

All authors have read and agreed to the published version of the manuscript.

REFERENCES

Abdel-Wareth, A. A. A., Amer, S. A., Mobashar, M., & El-Sayed, H. G. M. (2022). Use of zinc oxide nanoparticles in the growing rabbit diets to mitigate hot environmental conditions for sustainable production and improved meat quality. *BMC Veterinary Research*, 18(1), 354. doi:10.1186/s12917-022-03451-w
Crossref ● PubMed ● PMC ● Google Scholar

Alagawany, M., Qattan, S. Y. A., Attia, Y. A., El-Saadony, M. T., Elnesr, S. S., Mahmoud, M. A., Madkour, M., Abd El-Hack, M. E., & Reda, F. M. (2021). Use of chemical nano-selenium as an antibacterial and antifungal agent in quail diets and its effect on growth, carcasses, antioxidant, immunity and caecal microbes. *Animals*, 11(11), 3027. doi:10.3390/ani11113027
Crossref ● PubMed ● PMC ● Google Scholar

Ayoub, M. A., Okab, A., & Koriem, A. (2007). Effect of seasonal variations on some haematological and plasma biochemical parameters in Egyptian male and female baladi red rabbits. In *International Conference on Rabbit Production in Hot Climates* (Vol. 5, pp. 509–522), Hurghada, Egypt.
Google Scholar

Boiko, O. V., Honchar, O. F., Lesyk, Y. V., Kovalchuk, I. I., Gutyj, B. V., & Dychok-Niedzielska, A. Z. (2021). Effect of consumption of I, Se, S and nanoaquacitrates on hematological and biochemical parameters of the organism of rabbits. *Regulatory Mechanisms in Biosystems*, 12(2), 335–340. doi:10.15421/022145
Crossref ● Google Scholar

Boiko, O., Lesyk, Ya., Bashchenko, M., Honchar, O., Denys, H., Grabovska, O., & Luchka, I. (2022). Zinc citrate influence on the concentration of some macro- and microelements in rabbit body tissues. *Studia Biologica*, 16(4), 45–58. doi:10.30970/sbi.1604.697
Crossref ● Google Scholar

Chrastinová, Ľ., Čobanová, K., Chrenková, M., Poláčiková, M., Formelová, Z., Lauková, A., Ondruška, Ľ. Pogány Simonová, M., Strompfová, V., Bučko, O., Mlyneková, Z., Mlynár, R., & Grešáková, Ľ. (2015). High dietary levels of zinc for young rabbits. *Slovak Journal of Animal Science*, 48(2), 57–63. Retrieved from http://www.cvzv.sk/slju/15_2/2_chrastinova.pdf
Google Scholar

Dahmani, Y., Benali, N., Saidj, D., Chirane, M., Ainbaziz, H., & Temim, S. (2022). Effects of heat stress on growth performance, carcass traits, physiological components, and biochemical parameters in local Algerian growing rabbits. *World's Veterinary Journal*, 405–417. doi:10.54203/scil.2022.wvj51
Crossref ● Google Scholar

Dhabhar, F. S., Miller, A. H., McEwen, B. S., & Spencer, R. L. (1995). Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms. *The Journal of Immunology*, 154(10), 5511–5527. doi:10.4049/jimmunol.154.10.5511
Crossref ● PubMed ● Google Scholar

Dzen, Y., Rosalovsky, V., Shtapenko, O., Slypaniuk, O., & Salyha, Y. (2023). Effect of zinc methionine supplementation on biochemical and hematological indices of growing rabbits. *Bulgarian Journal of Agricultural Science,* 29(4), 714–722. Retrieved from https://www.cabidigitallibrary.org/doi/pdf/10.5555/20230358730
Google Scholar

El-Ratel, I. T., Elbasuny, M. E., El-Nagar, H. A., Abdel-Khalek, A.-K. E., El-Raghi, A. A., El Basuini, M. F., El-Kholy, K. H., & Fouda, S. F. (2023). The synergistic impact of Spirulina and selenium nanoparticles mitigates the adverse effects of heat stress on the physiology of rabbits bucks. *PLoS One*, 18(7), e0287644. doi:10.1371/journal.pone.02876444
Crossref ● PubMed ● PMC ● Google Scholar

Farghly, M. F. A., Mahrose, K. M., Peris, S. I., Abou-Kassem, D. E., Metwally, K. A., Abougabal, M. Sh., & Abd El-Aziz, A. (2021). Effects of lighting source as an environmental strategy for heat stress amelioration in growing Californian rabbits during summer season. *Animal Biotechnology*, 33(1), 159–166. doi:10.1080/10495398.2021.1895186
Crossref ● PubMed ● Google Scholar

Fedoruk, R. S., Kovalchuk, I. I., Mezentseva, L. M., Tesarivska, U. I., Pylypets, A. Z., & Kaplunenko, V. H. (2022). Germanium compounds and their role in animal body. *The Animal Biology*, 24(1), 50–60. doi:10.15407/animbiol24.01.050
Crossref ● Google Scholar

Fedoruk, R., Tesarivska, U., Khrabko, M., Tsap, M., & Denys, H. (2018). Impact of feeding male rats F2 with different doses of germanium citrate on the content of traceelements in their tissues and organs. *Agricultural Science and Practice*, 5(3), 40–46. doi:10.15407/agrisp5.03.040
Crossref ● Google Scholar

Hanson, Z. D., Mirshahidi, H., Brothers, J., Mirshahidi, S., Pham, B., Samaeekia, R., & Akhtari, M. (2023). Hemoglobin response to zinc supplementation in patients with zinc deficiency and chronic anemia. *Blood*, 142(Supplement 1), 5222–5222. doi:10.1182/blood-2023-191197
Crossref ● Google Scholar

Hariharan, S., & Dharmaraj, S. (2020). Selenium and selenoproteins: it's role in regulation of inflammation. *Inflammopharmacology*, 28*(*3), 667–695. doi.org/10.1007/s10787-020-00690-x
Crossref ● PubMed ● PMC ● Google Scholar

Hassan, F., Mobarez, S., Mohamed, M., Attia, Y., Mekawy, A., & Mahrose, K. (2021). Zinc and/or selenium enriched spirulina as antioxidants in growing rabbit diets to alleviate the deleterious impacts of heat stress during summer season. *Animals*, 11(3), 756. doi:10.3390/ani11030756
Crossref ● PubMed ● PMC ● Google Scholar

He, L., Lin, Q., Zhong, L., Zeng, Q., & Song, J. (2022). Thromboelastography maximum amplitude as an early predictor of disseminated intravascular coagulation in patients with heatstroke. *International Journal of Hyperthermia*, 39(1), 605–610. doi:10.1080/02656736.2022.2066206
Crossref ● PubMed ● Google Scholar

Hosny, S. H., Hashem, N. M., Morsy, A. S., & Abo-elezz, Z. R. (2020). 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. *Frontiers in Veterinary Science*, 7, 290. doi:10.3389/fvets.2020.00290
Crossref ● PubMed ● PMC ● Google Scholar

Jayaratne, B. R., & Uththara, S. L. H. (2018). Clinico-pathological evaluation of heat stroke induced disseminated intravascular coagulation. *Medico-Legal Journal of Sri Lanka*, 6(2), 73–77. doi:10.4038/mljsl.v6i2.7378
Crossref ● Google Scholar

Kawatani, Y., Suzuki, T., Shimizu, R., Kelly, V. P., & Yamamoto, M. (2011). Nrf2 and selenoproteins are essential for maintaining oxidative homeostasis in erythrocytes and protecting against hemolytic anemia. *Blood*, 117(3), 986–996. doi:10.1182/blood-2010-05-285817
Crossref ● PubMed ● Google Scholar

Khalil, H. A., Yaseen, M. A., & Hamdy, A. M. M. (2014). Behavioral activities, physiological body reactions, hematological parameters and hormonal profiles for bucks of New Zealand white and baladi red rabbits exposed to short term of high temperature. *Asian Journal of Poultry Science*, 9(4), 191–202. doi.org/10.3923/ajpsaj.2015.191.202
Crossref ● Google Scholar

Khrabko, M. I., Fedoruk, R. S., & Dolaichuk, O. P. (2016). Physiological and biochemical processes in the bodies of female rats F_0 and male rats F_1 under conditions of administration of "nano-germanium" citrate and chemically synthesized germanium citrate. *Visnyk of Lviv University. Biological series*, 73, 226–234. (In Ukrainian)
Google Scholar

Kong, T., Qu, Y. S., & Zhu, L. Q. (2007). Biological function of trace element-germanium. *Studies of Trace Elements and Health*, 24(1), 59–60.
Google Scholar

Konkol, D., & Wojnarowski, K. (2018). The use of nanominerals in animal nutrition as a way to improve the composition and quality of animal products. *Journal of Chemistry*, 2018, 1–7. doi:10.1155/2018/5927058
Crossref ● Google Scholar

Kosinov, M. V., & Kaplunenko, V. G. (2009). Process for the preparation of metal carboxylates nanotechnology of metal carboxylates preparation (Patent of Ukraine for utility model No. 38391). Bulletin No. 1/2009. Retrieved from https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=128062&cha pter=description (In Ukraine)
Google Scholar

Lee, S. R. (2018). Critical role of zinc as either an antioxidant or a prooxidant in cellular systems. *Oxidative Medicine and Cellular Longevity,* 2018, 9156285. doi:10.1155/2018/9156285
Crossref ● PubMed ● PMC ● Google Scholar

Leineweber, C., Müller, E., & Marschang, R. E. (2018). Blood reference intervals for rabbits (*Oryctolagus cuniculus*) from routine diagnostic samples. *Tierärztliche Praxis Ausgabe K: Kleintiere / Heimtiere*, 46(6), 393–398. doi:10.1055/s-0038-1677403
Crossref ● PubMed ● Google Scholar

Li, L., Ruan, T., Lyu, Y., & Wu, B. (2017). Advances in effect of germanium or germanium compounds on animals – a review. *Journal of Biosciences and Medicines*, 5(7), 56–73. doi:10.4236/jbm.2017.57006
Crossref ● Google Scholar

Liang, Z.-L., Chen, F., Park, S., Balasubramanian, B., & Liu, W.-C. (2022). 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. *Frontiers in Veterinary Science,* 9, 906084. doi:10.3389/fvets.2022.906084
Crossref ● PubMed ● PMC ● Google Scholar

Marai, I. F. M., Ayyat, M. S., & Abd El-Monem, U. M. (2001). Growth performance and reproductive traits at first parity of New Zealand white female rabbits as affected by heat stress and its alleviation under Egyptian conditions. *Tropical Animal Health and Production*, 33(6), 451–462. doi:10.1023/a:1012772311177
Crossref ● PubMed ● Google Scholar

Marai, I. F. M., Habeeb, A. A. M., & Gad, A. E. (2002). Rabbit's productive, reproductive and physiological performance traits as affected by heat stress: a review. *Livestock Production Science*, 78(2), 71–90. doi:10.1016/S0301-6226(02)00091-X
Crossref ● Google Scholar

Nakamura, T., Nagura, T., Akiba, M., Sato, K., Tokuji, Y., Ohnishi, M., & Osada, K. (2010). Promotive effects of the dietary organic germanium poly-trans-[(2-carboxyethyl) germasesquioxane] (Ge-132) on the secretion and antioxidative activity of bile in rodents. *Journal of Health Science,* 56(1), 72–80. doi:10.1248/jhs.56.72
Crossref ● Google Scholar

Nakyinsige, K., Sazili, A. Q., Aghwan, Z. A., Zulkifli, I., Goh, Y. M., & Fatimah, A. B. (2013). Changes in blood constituents of rabbits subjected to transportation under hot, humid tropical conditions. *Asian-Australasian Journal of Animal Sciences,* 26(6), 874–878. doi:10.5713/ajas.2012.12652
Crossref ● PubMed ● PMC ● Google Scholar

Nebylytsia, M. S., Onyshchenko, R. O., Vashchenko, O. V., & Boyko, O. V. (2023). Analizator povitrianoho seredovyshcha elektronnyi [Electronic air environment analyser] (Utility model patent of Ukraine No. 127047). Bulletin No. 13. Retrieved from https://base.uipv.org/searchINV/search.php?action=viewdetails&IdClaim=284535 (In Ukraine)

Oh, C., Li, M., Kim, E., Park, J. S., Lee, J., & Ham, S. W. (2011). Cheminform abstract: antioxidant and radical scavenging activities of ascorbic acid derivatives conjugated with organogermanium. *Cheminform*, 42(16), 3513–3514. doi:10.1002/chin.201116213
Crossref ● Google Scholar

Okab, A. B., El-Banna, S. G., & Koriem, A. A. (2008). Influence of environmental temperatures on some physiological and biochemical parameters of male New-Zealand rabbits. *Slovak Journal of Animal Science*, 41(1), 12–19.
Google Scholar

Petrovska, I., Salyha, Y., & Vudmaska, I. (2022). *Statystychni metody v biolohichnykh doslidzhenniakh* [*Statistical methods in biological research*]. Kyiv: Ahrarna nauka. Retrieved from https://www.inenbiol.com/images/stories/Rozrobky/Books/2022/Statistika_2022.pdf (In Ukraine)
Google Scholar

Quaye, I. K. (2015). Extracellular hemoglobin: the case of a friend turned foe. *Frontiers in Physiology,* 6, 96. doi:10.3389/fphys.2015.00096
Crossref ● PubMed ● PMC ● Google Scholar

Sharaf, A. K., El-Darawany, A. A., Nasr, A. S., & Habeeb, A. A. M. (2021). Alleviation the negative effects of summer heat stress by adding selenium with vitamin E or AD3E vitamins mixture in drinking water of female rabbits. *Biological Rhythm Research*, 52(4), 535–548. doi:10.1080/09291016.2019.1613796
Crossref ● Google Scholar

Sheiha, A. M., Abdelnour, S. A., Abd El-Hack, M. E., Khafaga, A. F., Metwally, K. A., Ajarem, J. S., Maodaa, S. N., Allam, A. A., & El-Saadony, M. T. (2020). Effects of dietary biological or chemical-synthesized nano-selenium supplementation on growing rabbits exposed to thermal stress. *Animals*, 10(3), 430. doi:10.3390/ani10030430
Crossref ● PubMed ● PMC ● Google Scholar

Viswanathan, K., & Dhabhar, F. S. (2005). Stress-induced enhancement of leukocyte trafficking into sites of surgery or immune activation. *Proceedings of the National Academy of Sciences*, 102(16), 5808–5813. doi:10.1073/pnas.0501650102
Crossref ● PubMed ● PMC ● Google Scholar

Vlizlo, V. V., Fedoruk, R. S., & Ratych, I. B. (2012). *Laboratorni metody doslidzhen u biolohiyi, tvarynnytstvi ta veterynarniy medytsyni* [*Laboratory methods of investigation in biology, stockbreeding and veterinary*]. Lviv: Spolom. (In Ukrainian)
Google Scholar

Waltz, X., Baillot, M., Connes, P., Bocage, B., & Renaudeau, D. (2014). Effects of hydration level and heat stress on thermoregulatory responses, hematological and blood rheological properties in growing pigs. *PLoS One*, 9(7), e102537. doi:10.1371/journal.pone.0102537
Crossref ● PubMed ● PMC ● Google Scholar

Wang, H. L., Zhang, J. S., & Yu, H. Q. (2007). Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: comparison with selenomethionine in mice. *Free Radical Biology & Medicine,* 42(10), 1524–1533. doi:10.1016/j.freeradbiomed.2007.02.013
Crossref ● PubMed ● Google Scholar

Yan, J. Y., Zhang, G. W., Zhang, C., Tang, L., & Kuang, S. Y. (2017). Effect of dietary organic zinc sources on growth performance, incidence of diarrhea, serum and tissue zinc concentrations, and intestinal morphology in growing rabbits. *World Rabbit Science,* 25(1), 43–49. doi:10.4995/wrs.2017.5770
Crossref ● Google Scholar

Zhang, J., Wang, X., & Xu, T. (2008). Elemental selenium at nano size (Nano-Se) as a potential chemopreventive agent with reduced risk of selenium toxicity: comparison with Se-methylselenocysteine in mice. *Toxicological Sciences*, 101(1), 22–31. doi:10.1093/toxsci/kfm221
Crossref ● PubMed ● Google Scholar

Zheng, H. P. (2011). Physiological function of organic germanium and its application in food. *Studies of Trace Elements and Health*, 28, 65–67. Retrieved from https://www.scirp.org/reference/ReferencesPapers?ReferenceID=2082944
Google Scholar

ВПЛИВ ЦИНКУ ЦИТРАТУ, СЕЛЕНУ ЦИТРАТУ ТА ГЕРМАНІЮ ЦИТРАТУ НА ГЕМАТОЛОГІЧНІ ПОКАЗНИКИ КРОЛІВ ЗА УМОВ ТЕПЛОВОГО СТРЕСУ

***Мар'ян Юзьвяк*[1], *Ярослав Лесик*[2], *Іван Лучка*[1], *Галина Денис*[1], *Юрій Салига*[1]**

[1] *Інститут біології тварин НААН, вул. В. Стуса, 38, Львів 79034, Україна*

[2] *Дрогобицький державний педагогічний університет імені Івана Франка вул. Стрийська, 3, Дрогобич 82100, Україна*

Обґрунтування. Екологічна криза позначилася на щорічному підвищенні температури довкілля, що негативно впливає на організм ссавців. Кролі через відсутність потових залоз є більше чутливими до теплового стресу, ніж інші тварини. Вплив підвищених температур довкілля на організм кролів призводить до порушень параметрів крові, ендокринної регуляції, імунної та репродуктивної функції, що знижує їхню продуктивність і збільшує кількість загиблих тварин. Особливу увагу тепер приділяють дослідженню органічних сполук мікроелементів, які характеризуються високою фізіологічною активністю, є нетоксичними, мають широкий спектр біологічного впливу і позитивно впливають на зниження негативної дії підвищених температур довкілля на організм тварин. Однак їхня дія залежить від елемента і його застосованої кількості. Тому головним завданням цього експерименту було дослідити вплив цинку цитрату, селену цитрату і германію цитрату на зміну кількості клітин крові з метою пом'якшення дії теплового стресу.

Матеріали та методи. Дослідження проводили на молодняку кролів-аналогів у період з 35- до 78-добового віку, породи Термонська біла. Кролів утримували у приміщенні за підвищених температур довкілля від 28,9 до 30 °C та відносної вологості від 77,1 до 87,4 %. Тварин контрольної групи утримували на основному раціоні зі згодовуванням стандартного збалансованого гранульованого комбікорму і води без обмеження. Кролі I, II і III дослідних груп споживали такі ж комбікорми, як у контролі, проте упродовж 24 год з водою отримували: I група: цинку цитрат – 60 мг Zn/л, або 12 мг Zn/кг маси тіла; II група: селену цитрат – 300 мкг Se/л, або 60 мкг Se/кг маси тіла; III група: германію цитрат – 62,5 мкг Ge/л, або 12,5 мкг Ge/кг маси тіла. Використання індивідуальних поїлок для кожної тварини та розміщення тварин у різних клітках дало нам змогу контролювати кількість води, яку отримував кожен кріль. Кров для дослідження відбирали до випоювання добавок на 14-ту добу підготовчого періоду та 14-ту і 29-ту доби дослідного періоду. Упродовж періоду дослідження контролювали температуру приміщення з урахуванням температурно-вологісного індексу.

Результати. Додавання цитратів мікроелементів до раціону кролів протягом 29 днів дослідження в умовах теплового стресу викликало гематологічні зміни показників порівняно з контролем. Кількість еритроцитів у крові кролів I та II дослідних груп підвищувалася на 16,4 і 13,6 % та 19,9 і 14,5 % на 14-й та 29-й день, у III групі – на 15,3 % на 14-ту добу. Вміст гемоглобіну I, II і III груп підвищувався на 20,8, 21,6 і 19,5 % на 14-ту добу та 11,1, 12,5 і 9,7 % на 29-ту добу. Гематокритна величина I і II груп підвищувалася на 24,1 та 15,7 % і 21,1 та 16,5 % упродовж дослідження, III групи на 18,6 % на 14-ту добу. Кількість лейкоцитів I, II і III груп знижувалася на 13,1 та 8,31 %; 11,2 та 10,4 % і на 11,4 і 9,3 % на 14-ту і 29-ту доби; кількість лімфоцитів I, II і III груп знижувалася на 25,9, 27,3 і 29,0 % на 14-ту добу і на 20,4, 21,7

і 16,0 % на 29-ту добу. Кількість моноцитів I і II груп підвищувалася на 14,8 та 21,3 % і 17,0 та 18,3 % за 29 діб. Кількість тромбоцитів у тварин II групи знижувалася на 29,4 % на 29-ту добу, середній об'єм еритроцитів підвищувався на 11,6 і 14,6 % на 14-ту та 29-ту добу експерименту.

Висновки. Додавання до раціону кролів цитратів мікроелементів пом'якшило наслідки негативного впливу теплового стресу на організм. Результатом впливу даних добавок на тварин стали достовірні зміни в гематологічних параметрах крові кролів, де найкращі результати спостерігали за впливу селену цитрату (60 мкг Se/кг маси тіла) та цинку цитрату (12 мг Zn/кг маси тіла): кількість еритроцитів ($p < 0,05–0,01$), лейкоцитів ($p < 0,05–0,01$), лімфоцитів ($p < 0,05–0,01$), моноцитів ($p < 0,05–0,01$), вміст гемоглобіну ($p < 0,01–0,001$), гематокритна величина ($p < 0,01$) порівняно з контролем. Випоювання германію цитрату позначилося менш вираженими змінами цих показників крові.

Ключові слова: кролі, кров, цинку цитрат, селену цитрат, германію цитрат, тепловий стрес

Received / Одержано 06 February, 2024 | Revision / Доопрацьовано 29 April, 2024 | Accepted / Прийнято 30 August, 2024 | Published / Опубліковано 25 September, 2024