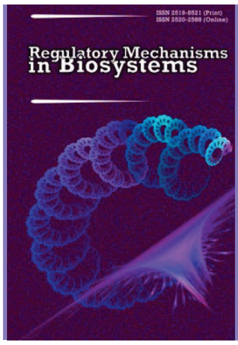

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Changes in the blood parameters of rabbits consuming a complex of citrates of zinc, selenium, and germanium under the conditions of heat stress

M. I. Bashchenko*, O. V. Boiko*, Y. V. Lesyk**, O. F. Honchar*, O. M. Havrysh*, B. V. Gutyj***, N. K. Hoivanovych****, H. V. Krechkivska****

Cherkassy Experimental Station of Bioresources of National Academy of Agricultural Sciences of Ukraine, Cherkassy, Ukraine
***Institute of Animal Biology of NAAS, Lviv, Ukraine*
****Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Lviv, Ukraine*
*****Drohobych Ivan Franko State Pedagogical University, Drohobych, Ukraine*

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Institute of Animal Biology of NAAS, V. Stus st., 38, Lviv, 79034, Ukraine, Tel.: +38-068-503-46-25. E-mail: yaroslav_lesyk@inenbiol.com.ua

Cherkassy Experimental Station of Bioresources National Academy of Agricultural Sciences of Ukraine, Pasterivska st., 76, Cherkassy, 18036, Ukraine. Tel.: +38-047-231-40-52. E-mail: bioresurs.ck@ukr.net

Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, Pekarska st., 50, Lviv, 79010, Ukraine. Tel.: +38-068-136-20-54. E-mail: bvh@ukr.net

Drohobych Ivan Franko State Pedagogical University, Ivana Franka st., 24, Drohobych, 82100, Ukraine.

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The negative effect of heat stress on rabbit productivity can be alleviated through technological solutions that enhance maintenance practices and improve nutrition, which are more effective and profitable. As of now, there is a growing interest in citrates of microelements that can extenuate the heat stress in rabbit farming, but the efficacy varies depending on the compound applied and its amount in the diet. Therefore, this study was aimed at the effects of a complex citrate compound of microelements on the morphological and biochemical parameters of blood of rabbits subject to heat stress, which received the drug with water. The study was conducted in a vivarium, on young analogue-paired Termonde rabbits, aged 35 to 78 days. During the experimental period, we raised the air temperature in the room from 28.9 to 30.0 °C from 12 to 16 (four hours a day). During the study, we controlled the temperature according to the temperature-humidity index. Rabbits of Group 1: Control were given the main diet consisting of a standard balanced granular mixed feed and had free access to water. Rabbits of Group 2 and Group 3 consumed the same granular mixed feed as in the control, but throughout the day received a complex compound of citrates of microelements. Besides the main diet, throughout the day, Group 2 received 20 mg/L of zinc citrate, 100 µg/L of selenium citrate, and 20 µg of germanium citrate, and Group 3, consumed drinking water with zinc citrate in the dose of 40 mg Zn/L, selenium nitrate in the dose of 200 µg Se/L, and germanium citrate in the dose of 40 µg Ge/L. Blood for the assay was collected on day 14 of the preparation period (Collection 1) and on days 28 and 43 (collections 2 and 3, respectively) of the experiment, when the animals were subject to heat stress. It was found that the rabbits of groups 2 and 3 had higher numbers of erythrocytes on day 28 and day 43, respectively. The hemoglobin concentration in the blood of the rabbits correlated with the numbers of erythrocytes in animals of groups 2 and 3, and was higher on day 28, and increased further on day 43. In the blood of rabbits of experimental groups 2 and 3, we found higher contents of hematocrit and significant increase in the numbers of monocytes on days 28 and 43 of the experiment. Erythrocyte and platelet indices and the analyzed biochemical blood parameters correlated with the content of those cells in blood of the rabbits according to period of the study. The statistical calculations revealed a relationship between the effects of the microelements used and the duration of their intake, as well as their impact on alleviating heat stress in rabbits. The conducted studies indicate the practical benefits of using a complex of citrates of zinc, selenium, and germanium in industrial rabbit farming.

Keywords: nanotechnologies; mineral compounds; erythrocyte indices; temperature-humidity index.

Introduction

Over recent decades, the most acute problem in rabbit breeding has been the impact of heat stress, and therefore an array of studies searched for ways to improve the productivity and reproductive function of rabbits under those conditions (Gonzalez Rivas et al., 2020; Thornton et al., 2021). Rabbits are characterized by high parameters of productivity and reproductive ability, which are negatively impacted by high ambient temperatures (Lamarca et al., 2018). This problem is being exacerbated by the climate change (Chipo et al., 2019). Rabbits are very sensitive to extreme environmental conditions, especially high temperatures, due to dense fur and absence of the sweat glands, which makes it hard for them to release excessive heat from the body. Rabbits are economically significant in animal husbandry and are also used as laboratory animals in research (Anjum et al., 2019; Lesyk et al., 2022). In veterinary medicine, there is a problem of obtaining reference values due to the limited number of experiments, especially those dealing with various stress conditions in rabbit breeding (Moore et al., 2015). The physiological parameters of the environment for optimal growth and development of rabbits are within the range of 16 to 21 °C (Ashour et al., 2017). Subject to high temperatures, measuring over 25–30 °C, they try to disperse the excessive heat using various physiological mechanisms.

The thermoregulation reaction impacts the rabbits, impairing their physiological functions and behavior, in particular, reduce appetite, food metabolism, productivity, lactation, and reproduction, causing great industrial and economic losses (El Sabry et al., 2021; Mutwedu et al., 2021; Liang et al., 2022). Jimoh et al. (2017) reported that rabbits usually use body position and increase respiratory rate for thermoregulation. It is known that in the conditions of heat stress, reducing the body temperature by 1 °C inhibits the physiological parameters and dairy productivity of cattle to a lesser degree (Mylostyvyi et al., 2021). Rabbits are known to be sensitive to high surrounding temperature (Maertens & Gidenne, 2016; Boiko et al., 2020). Continuous exposure to heat impairs the rabbits' homeostatic mechanisms, imposing a negative impact (Farghly et al., 2021). In laboratory conditions and on industrial rabbit farms, there is ongoing development of methods of relieving the animals from heat stress using technological solutions of introducing improved technologies that reduce temperature in the room (Farghly et al., 2021). Currently, studies are concentrated on approaches to adjusting the diet, in particular, using organic compounds of microelements so as to alleviate the action of high temperatures towards the rabbits (Sheiha et al., 2020; Bojko et al., 2020; Boiko et al., 2021).

Today, there are a limited number of studies dealing with the effects of heat stress on the productivity and reproductive abilities of rabbits. The scientific literature provides data on heat stress effects on the zootechnical parameters, overlooking the adaptability to thermoregulation, and also the physiological, biochemical, and hematological reactions in rabbits. Changes in the blood parameters under heat stress and mitigating them by supplementing water with citrate microelement compound complex are important for scientific studies, as well as the practice of rabbit farming under the conditions of industrial rabbit farms. Therefore, the objective of our study was analyzing the effects of supplementing water with the complex of citrate microelement compounds on the morphological and biochemical blood parameters of rabbits subject to heat stress.

Materials and methods

The studies were conducted in a vivarium of the Lviv Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine. The animals were kept in a room with regulated microclimate, in 50 × 120 × 30 cm gridded cages. The experiment was allowed by the Bioethics Commission of the Institute (Protocol No. 146 as of February 19, 2024). All the procedures with the experimental animals were performed according to the provisions of the General Ethical Principles of Experiments on Animals, approved by the First National Congress of Bioethics (Kyiv, 2011), and the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986).

The studies were carried out on the young white Termonde rabbits aged 35 to 78 days, which were analogues by age, body mass, and clinical condition. During the experiment, we increased the air temperature in the room from 28.9 to 30 ºC between 12 and 16 PM (four hours a day). The temperature and humidity were controlled using a thermohygrometer, with data record using Trotec BL30. The humidity and temperature were measured using an electronic analyzer of the air environment (Patent No. 127047). During the study, we controlled the temperature according to the temperature-humidity index (THI). The thresholds of comfortable surrounding conditions for the rabbits were determined as the indices of temperature and humidity. The mean daily temperature and relative humidity were determined using the formula (Marai et al., 2002): temperature-humidity index (THI) = T − ((0.31−0.31×B/100)×(T−14.4)), where T = temperature (ºC), B = relative humidity as a percentage (%)/100.

To identify the air temperature in the vivarium, we controlled the temperature to humidity ratio each day, and calculated it according to the formula: THI = 19.8 − ((0.31−0.31×56.3%/100%)×(19.8−14.4)) = 19.0.

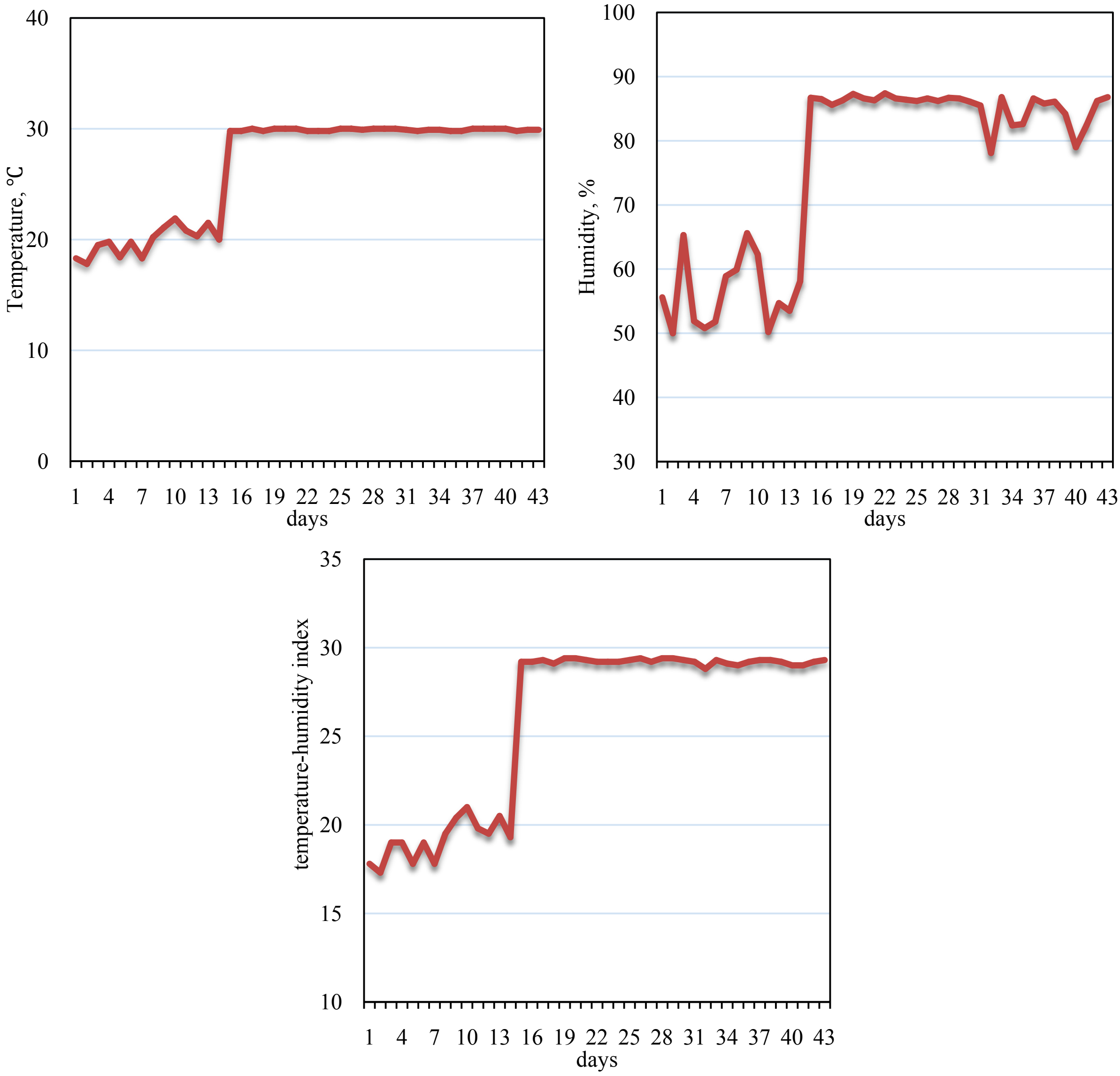

Fig. 1. Temperature, humidity, temperature-humidity index during the preparation and experimental periods

The obtained THI value was 19.0, indicating the absence of heat stress in the preparation period. Following the objective of the experiment, the room temperature was increased from 12:00 to 16:00. Therefore, the mean temperature in the room during the 14 days of experiment was 29.9 ºC and the humidity was 86.5%. The THI for the indicated period measured 29.2 ºC, which, according to Marai et al. (2002), indicates strong heat stress. Similar mathematical calculations were performed at the final stage of the experiment, lasting 15 days. Therefore, the mean values of humidity and temperature measured 84.3% and 29.9 ºC, respectively (Fig. 1). According to the said formula, the THI equaled 29.1, indicating powerful heat stress at the final stage of the research.

Thus, the obtained values of the temperature-humidity index in the preparation and experimental periods were in line with the experiment's goal, suggesting the absence of heat stress during the preparation period and its presence during 29 days of intake of the compound.

The animals for the research, males and females, were divided into three groups of 6 animals in each (3 males, 3 females), of an average body mass of 1200 ± 50 g. Rabbits of the control group, Group 1: Control, received the main diet with a standard balanced granulated mixed feed and water in free access. Rabbits of experimental groups 2 and 3 consumed the same granulated mixed feed as in Group 1: Control, but throughout the day consumed water containing the complex compound of citrates of microelements. Besides the main diet, Experimental Group 2 rabbits consumed the following microelements together with water: zinc citrate – 20 mg Zn/L, selenium citrate – 100 µg Se/L, and germanium citrate – 20 µg Ge/L, whereas Group 3 received zinc citrate in the amount of 40 mg Zn/L, selenium citrate – 200 µg Se/L, and germanium citrate – 40 µg Ge/L.

Blood for the assays was collected on day 14 of the preparation period (Collection 1 – day 14) and on days 14 and 29 of intake of the supplements in the conditions of heat stress (days 28 and 43 of the entire experiment, respectively) we made collections 2 and 3, respectively. The samples of whole blood were collected from the marginal ear vein of 6 animals of each group into test tubes with the anticoagulant ethylenediaminetetraacetic acid (EDTA-K2+) for hematological studies to determine the general number of erythrocytes and erythrocyte indices: red blood cells (RBC), hemoglobin (HGB), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), white blood cell count (WBC), and their forms – lymphocytes (LYM), monocytes (MON), granulocytes (GRA); numbers of platelets and platelet indices (absolute content platelets (PLT), mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW) using an automatic hematology analyzer Orphee Mythic 18 (Switzerland).

To study the biochemical parameters, we collected blood using 1% solution of heparin and determined the total content of protein, albumin, the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the activity of alkaline phosphatase (ALP), content of triacylglycerols, cholesterol, total calcium, and inorganic phosphorus using a biochemical analyzer Hymalyzer 2000 (Vlislo et al., 2012).

The obtained data were analyzed using Statistica 7.0 (StatSoft Inc., USA). The data are presented in tables as x ± SD (mean ± standard deviation). The differences between the control and experimental groups were determined using Tukey's Test, indicating significance at $P < 0.05$.

Results

Against the background of heightened ambient temperature, the supplement produced positive effects on the rabbits' blood cells, manifesting in changes in Group 2 and Group 3, in particular, increases in the numbers of erythrocytes, measuring 22.2% and 8.0% ($P < 0.05$) on day 14, and 20.4% and 20.2% increases ($P < 0.05$) on day 28 of the study, respectively, compared with Group 1 (Table 1). The hemoglobin concentration in blood of the rabbits correlated with the numbers of erythrocytes in Group 2 and Group 3, measuring 25.6% and 20.8% higher ($P < 0.05$) at the first stage of the study and increased by 9.1% ($P < 0.05$) and 11.4% ($P < 0.01$) at the final stage of the study, compared with Group 1. It has to be noted that the hematocrit concentration in blood of rabbits of groups 2 and 3, against Group 1, underwent the most significant changes during intake of the microelement citrate supplement, which was reflected in the blood of rabbits of those experimental groups as 4.1% and 39.1% ($P < 0.001$) and 36.7% and 33.2% ($P < 0.001$) higher content of hematocrit on days 28 and 43 of the experiment, respectively.

Table 1
Numbers of erythrocytes, hemoglobin and hematocrit concentrations in the blood of rabbits consuming the compound of citrates of zinc, selenium, and germanium, against the background of heat stress (x ± SD, n = 6)

Parameters	Group	Period of the study: Preparation	Experimental	
		Day 14	Day 28	Day 43
Absolute content of red blood cells (RBC), 10^{12}/L	1	5.65 ± 0.64^a	5.61 ± 0.43^a	5.09 ± 0.20^a
	2	5.67 ± 0.54^a	6.86 ± 0.45^b	6.13 ± 0.69^{ab}
	3	5.43 ± 0.36^a	6.06 ± 0.52^{ab}	6.12 ± 0.21^{ab}
Hemoglobin concentration (HGB), g/L	1	136.8 ± 9.6^b	115.8 ± 2.9^a	128.8 ± 7.2^b
	2	131.0 ± 9.9^b	145.5 ± 3.8^c	140.7 ± 3.7^c
	3	132.0 ± 6.7^b	140.0 ± 6.8^{bc}	143.6 ± 8.8^{bc}
Hematocrit (HTC), L/L	1	0.433 ± 0.041^{ab}	0.388 ± 0.023^a	0.403 ± 0.042^{ab}
	2	0.474 ± 0.063^b	0.546 ± 0.072^{bc}	0.551 ± 0.061^{bc}
	3	0.492 ± 0.042^b	0.574 ± 0.041^c	0.537 ± 0.042^{bc}

Note: in this and the other tables, different letters for each characteristic (within three columns and three lines) indicate samplings that are significantly different one from another according to the Tukey test ($P < 0.05$).

The mean corpuscular hemoglobin underwent no significant changes in the preparation period, whereas after 28 days of intake of the supplements, groups 2 and 3 were observed to have significant improvement in this parameter, higher by 7.1% and 8.3% ($P < 0.05$) and it had increased by 7.3% ($P < 0.05$) and 9.9% ($P < 0.01$) by day 43 of the experiment, compared with the control (Table 2).

Table 2
Erythrocyte indices in blood of the rabbits consuming the compounds of citrates of zinc, selenium, and germanium against the background of heat stress (x ± SD, n = 6)

Parameters	Group	Period of the study: preparation	experimental	
		day 14	day 28	day 43
Mean corpuscular volume (MCV), f/L	1	94.23 ± 3.48^a	93.38 ± 2.33^a	95.85 ± 4.06^a
	2	94.68 ± 3.07^a	91.86 ± 4.00^a	96.61 ± 2.96^a
	3	96.95 ± 4.76^a	95.04 ± 5.57^a	93.61 ± 4.67^a
Mean corpuscular hemoglobin (MCH), pg	1	22.15 ± 1.64^{ab}	21.90 ± 1.01^a	22.08 ± 0.48^{ab}
	2	23.13 ± 0.72^{ab}	23.46 ± 0.83^{ab}	23.70 ± 1.10^{ab}
	3	24.05 ± 1.47^{ab}	23.73 ± 1.53^{ab}	24.28 ± 1.17^b
Mean corpuscular hemoglobin concentration (MCHC), g/L	1	243.62 ± 6.97^a	245.51 ± 6.97^{ab}	243.02 ± 7.94^a
	2	244.34 ± 4.92^a	250.12 ± 3.54^{ab}	264.01 ± 6.19^b
	3	248.13 ± 7.70^{ab}	246.31 ± 3.38^{ab}	260.00 ± 3.40^b
Red blood cell distribution width (RDW), %	1	9.35 ± 0.30^a	10.30 ± 0.47^{ab}	10.26 ± 0.52^{ab}
	2	9.51 ± 0.48^a	11.06 ± 0.28^b	10.98 ± 0.51^b
	3	9.70 ± 0.74^a	10.48 ± 0.93^{ab}	10.16 ± 0.87^{ab}

Note: see Table 1.

Significant changes occurred in the final stage of the experiment. Therefore, in blood of rabbits of groups 2 and 3, the mean corpuscular hemoglobin concentration was 8.6% and 6.9% ($P < 0.001$) higher on day 43 of the experiment, compared with Group 1. In the blood of rabbits of groups 2 and 3, the absolute number of monocytes significantly increased by 22.2% ($P < 0.01$) and 18.5% ($P < 0.05$), respectively, during the second stage of the experiment, and was characterized by insignificant changes at the final stage of the experiment (Table 3).

In the blood of Group 3 rabbits, the relative content of lymphocytes and granulocytes increased by 37.8% and 58.9% ($P < 0.01$), respectively, on day 14 of the experiment. The highest number of significant changes occurred in the content of monocytes. In particular, in rabbits of groups 2 and 3, their content had increased by 20.4% ($P < 0.05$) and 39.6% ($P < 0.001$) and 68.0% and 69.9% ($P < 0.001$) by days 28 and 43 of experiment, respectively, compared with Group 1.

In blood of Group 3 rabbits, we saw an increase in the number of platelets, measuring 43.7% ($P < 0.001$) on day 14 of the experiment (Table 4).

Table 3
The numbers of leukocytes and their forms in blood of the rabbits consuming the compounds of citrates of zinc, selenium, and germanium against the background of heat stress (x ± SD, n = 6)

Parameters	Group	Period of the study		
		preparation	experimental	
		day 14	day 28	day 43
White blood cell count (WBC), 10^9/L	I	9.05 ± 0.44^{ab}	9.05 ± 0.68^{ab}	8.96 ± 0.37^{a}
	II	9.21 ± 1.14^{ab}	10.00 ± 1.54^{ab}	9.41 ± 0.96^{ab}
	III	9.98 ± 1.09^{ab}	9.55 ± 1.03^{ab}	9.91 ± 0.63^{b}
Lymphocytes (LYM), 10^9/L	I	3.33 ± 0.81^{a}	4.41 ± 0.25^{ab}	4.43 ± 0.36^{ab}
	II	4.20 ± 0.67^{ab}	4.58 ± 0.49^{ab}	4.50 ± 0.76^{ab}
	III	4.36 ± 1.04^{ab}	4.71 ± 0.87^{ab}	5.16 ± 0.64^{b}
Monocytes (MON), 10^9/L	I	1.43 ± 0.08^{ab}	1.35 ± 0.10^{a}	1.36 ± 0.15^{ab}
	II	1.51 ± 0.17^{ab}	1.65 ± 0.16^{b}	1.56 ± 0.20^{ab}
	III	1.55 ± 0.16^{ab}	1.60 ± 0.12^{b}	1.55 ± 0.16^{ab}
Granulocytes (GRA), 10^9/L	I	3.63 ± 0.47^{b}	3.48 ± 0.50^{ab}	3.28 ± 0.74^{ab}
	II	2.43 ± 0.64^{a}	3.83 ± 1.26^{ab}	3.73 ± 0.60^{b}
	III	2.96 ± 1.31^{ab}	2.68 ± 0.94^{ab}	4.21 ± 0.80^{b}
Lymphocytes (LYM), %	I	36.66 ± 3.20^{a}	35.58 ± 7.56^{ab}	41.05 ± 3.77^{ab}
	II	45.18 ± 3.93^{b}	42.83 ± 6.46^{ab}	47.70 ± 6.16^{ab}
	III	43.63 ± 4.48^{ab}	49.03 ± 5.68^{b}	46.80 ± 5.78^{ab}
Monocytes (MON), %	I	31.58 ± 5.61^{c}	16.65 ± 2.05^{ab}	18.88 ± 2.47^{b}
	II	28.41 ± 6.01^{c}	20.05 ± 2.26^{b}	12.85 ± 1.85^{a}
	III	26.88 ± 6.01^{bc}	23.25 ± 1.70^{bc}	13.21 ± 1.98^{a}
Granulocytes (GRA), %	I	34.60 ± 3.77^{b}	46.95 ± 9.58^{c}	39.35 ± 5.31^{bc}
	II	27.73 ± 4.05^{a}	37.11 ± 7.52^{ab}	39.45 ± 5.67^{bc}
	III	29.38 ± 6.00^{ab}	27.70 ± 4.49^{a}	37.25 ± 3.85^{bc}

Note: see Table 1.

Table 4
The numbers of platelets and their indices in blood of the rabbits consuming the compound of citrates of zinc, selenium, and germanium against the background of heat stress (x ± SD, n = 6)

Parameter	Group	Period of the study		
		preparation	experimental	
		day 14	day 28	day 43
Absolute content of platelets (PLT), 10^9/L	1	404 ± 40^{ab}	344 ± 37^{a}	431 ± 59^{b}
	2	455 ± 66^{b}	351 ± 38^{a}	389 ± 74^{ab}
	3	472 ± 89^{b}	495 ± 120^{ab}	425 ± 51^{ab}
Mean platelet volume (MPV), fL	1	5.31 ± 0.23^{ab}	5.11 ± 0.37^{a}	5.18 ± 0.39^{a}
	2	5.36 ± 0.25^{ab}	5.66 ± 0.20^{ab}	5.81 ± 0.19^{b}
	3	5.48 ± 0.19^{ab}	5.55 ± 0.16^{ab}	5.53 ± 0.33^{ab}
Plateletcrit (PCT), %	1	0.204 ± 0.032^{ab}	0.151 ± 0.017^{a}	0.245 ± 0.018^{c}
	2	0.252 ± 0.028^{bc}	0.199 ± 0.014^{b}	0.246 ± 0.021^{bc}
	3	0.283 ± 0.071^{bc}	0.175 ± 0.063^{ab}	0.244 ± 0.009^{c}
Platelet distribution width (PDW), %	1	14.15 ± 0.81^{ab}	13.30 ± 1.33^{ab}	13.06 ± 0.78^{a}
	2	15.13 ± 0.67^{b}	14.96 ± 0.54^{b}	14.36 ± 1.23^{ab}
	3	14.70 ± 0.98^{ab}	14.53 ± 0.90^{ab}	14.93 ± 0.75^{b}

Note: see Table 1.

In the platelet indices, the highest number of changes occurred in the mean platelet volume, which in blood of Group 2 rabbits increased by 10.7% and 12.1% during the study and was increased by 8.6% in Group 3 on the 28th day of the experiment.

We observed a relationship between the amount of the administered supplement and the percentage of platelet distribution width. In Group 2 rabbits, it was 12.4% ($P < 0.05$) higher on day 28 and exceeded that of the control by 14.3% ($P < 0.05$) on day 43 of the experiment.

Significant differences were determined in the activity of alkaline phosphatase in the blood of rabbits of groups 2 and 3, being 24.3% and 23.6% higher than control, respectively, on day 28 of the experiment, and 19.8% and 11.9% lower on day 43 of the experiment, compared with the control (Table 5).

The content of albumin in blood of Group 2 rabbits increased 20.0% during the final period of the study, compared with Group 1 (Table 6). The duration of intake of the complex of microelements affected the changes in the parameters of lipid metabolism, manifesting in 33.8% higher cholesterol in Group 3 on day 43 and 48.4% and 29.6% declines in triacylglycerols in groups 2 and 3, respectively, on day 28 of intake of the supplements, compared with the control.

Table 5
Contents of protein and the activity of enzymes in blood of the rabbits consuming the compounds of citrates of zinc, selenium, and germanium against the background of heat stress (x ± SD, n = 6)

Parameter	Group of animals	Period of the study		
		preparation	experimental	
		day 14	day 28	day 43
Total protein, g/L	I	54.31 ± 2.09^{a}	57.08 ± 5.76^{ab}	54.73 ± 3.42^{a}
	II	55.83 ± 3.57^{ab}	63.25 ± 5.94^{b}	57.11 ± 5.24^{ab}
	III	56.55 ± 2.16^{ab}	55.43 ± 5.29^{ab}	57.51 ± 2.33^{ab}
Aspartate aminotransferase, U/L	I	16.0 ± 1.5^{a}	16.0 ± 2.5^{ab}	20.1 ± 1.9^{b}
	II	19.0 ± 3.5^{ab}	19.1 ± 3.8^{ab}	18.7 ± 1.8^{ab}
	III	17.9 ± 3.1^{ab}	17.6 ± 4.4^{ab}	22.1 ± 2.8^{b}
Alanine aminotransferase, U/L	I	54.0 ± 5.2^{ab}	67.0 ± 7.0^{b}	65.4 ± 4.9^{b}
	II	54.5 ± 5.0^{ab}	60.9 ± 10.7^{ab}	59.5 ± 6.6^{b}
	III	48.2 ± 2.2^{a}	60.8 ± 10.6^{ab}	62.3 ± 5.7^{b}
Alkaline phosphatase, U/L	I	402 ± 68^{a}	289 ± 30^{ab}	316 ± 35^{b}
	II	439 ± 81^{bc}	359 ± 51^{b}	253 ± 22^{a}
	III	456 ± 51^{c}	358 ± 43^{b}	278 ± 13^{ab}

Note: see Table 1.

Table 6
Changes in the individual biochemical parameters in blood of the rabbits consuming compounds of citrates of zinc, selenium, and germanium against the background of heat stress (x ± SD, n = 6)

Parameters	Group of animals	Period of the study		
		preparation	experimental	
		day 14	day 28	day 43
Albumin, g/L	1	36.7 ± 3.4^{ab}	48.7 ± 6.8^{bc}	41.8 ± 2.9^{b}
	2	34.7 ± 3.0^{a}	53.9 ± 3.4^{c}	50.1 ± 5.5^{c}
	3	41.2 ± 6.7^{ab}	51.4 ± 3.5^{c}	38.2 ± 1.5^{ab}
Cholesterol, mmol/L	1	0.57 ± 0.17^{ab}	0.67 ± 0.09^{ab}	0.59 ± 0.05^{a}
	2	0.55 ± 0.09^{a}	0.81 ± 0.20^{bc}	0.66 ± 0.04^{b}
	3	0.75 ± 0.12^{bc}	0.65 ± 0.17^{ab}	0.79 ± 0.06^{c}
Triacylglycerols, mmol/L	1	0.90 ± 0.10^{d}	0.64 ± 0.09^{c}	0.50 ± 0.07^{b}
	2	0.80 ± 0.10^{cd}	0.33 ± 0.07^{a}	0.46 ± 0.08^{ab}
	3	0.81 ± 0.09^{cd}	0.45 ± 0.13^{ab}	0.41 ± 0.03^{ab}
Creatinine, µmol/L	1	87.3 ± 4.7^{a}	105.4 ± 9.4^{ab}	104.4 ± 5.2^{b}
	2	92.0 ± 5.0^{ab}	106.3 ± 6.4^{b}	108.5 ± 7.7^{b}
	3	92.3 ± 7.0^{ab}	105.0 ± 7.5^{ab}	106.2 ± 4.1^{b}
Urea, mmol/L	1	5.48 ± 0.88^{b}	3.90 ± 0.43^{a}	4.61 ± 0.42^{ab}
	2	5.65 ± 0.99^{b}	4.21 ± 0.69^{ab}	4.18 ± 0.34^{ab}
	3	6.01 ± 0.60^{b}	4.33 ± 0.38^{ab}	4.41 ± 0.71^{ab}
Ca, mmol/L	1	3.05 ± 0.23^{a}	3.15 ± 0.24^{a}	2.81 ± 0.27^{a}
	2	3.06 ± 0.30^{a}	2.83 ± 0.25^{a}	2.78 ± 0.20^{a}
	3	3.01 ± 0.14^{a}	2.91 ± 0.13^{a}	2.81 ± 0.14^{a}
P, mmol/L	1	1.95 ± 0.17^{b}	1.90 ± 0.12^{b}	1.95 ± 0.15^{b}
	2	1.91 ± 0.13^{b}	1.85 ± 0.16^{ab}	1.63 ± 0.16^{a}
	3	2.18 ± 0.42^{ab}	1.75 ± 0.27^{ab}	1.73 ± 0.15^{ab}

Note: see Table 1.

Discussion

Heat stress is a problem in animal farming, and especially in the fur industry, leading to changes in the physiological parameters of rabbits as a result of impaired homeostatis (Jaén-Téllez et al., 2020). Rabbits are homeothermal animals; they are able to regulate their body temperature within a narrow range (Szendrő et al., 2018).

The temperature of rabbits under laboratory and semi-industrial conditions is measured using thermography, which reveals the external temperatures of the ear and eyeball, allowing the identification of the animal's overall temperature (Jaén-Téllez et al., 2020, Agea et al., 2021). A more common and practical method to assess the effects of heightened temperatures in a room considers the parameters of temperature and humidity in the environment. This method fully reveals the temperature that affects the rabbits (Jimoh et al., 2018).

The reaction of animals to heightened ambient temperature, which exceeds their range of thermal comfort, is called heat stress (Kang et al., 2020). According to the generalized results, the mean daily values of humidity and temperature, equaled 56.3% and 19.8 ºC, respectively, which in calculation according to the temperature-humidity index (THI) equaled 19.0, suggesting the absence of heat stress during the 14 day preparation period. The obtained THI values were classified (Marai et al., 2002) as follows: <27.8 – no heat stress; 27.8 to <28.9 – moderate heat stress; 28.9 to <30.0 – strong heat stress; and 30.0 and more – severe heat stress. Similar studies were conducted at the first and second stages of the experimental period, revealing 29.2 and 29.1 ºC, respectively, thus indicating strong heat stress. Therefore, our estimates of the mean values of temperature and humidity indicate the absence of heat stress during the preparation period and the parameters of strong heat stress during the 29 days of the experiment. The administered physiological amounts of microelements in one complex compound mitigated the negative effects of heat stress on the rabbits in different ways.

The blood parameters are a means of early diagnostics of an animal, evaluating the heath and detecting diseases (Barkakati et al., 2015). During the 14 day preparation stage, the blood parameters of the rabbits were within the reference values and within a close range. However, at the heightened ambient temperature, we observed a positive effect of the used compounds of microelements on the hemapoiesis parameters in the animals that drank the compound of citrates of zinc, selenium, and germanium in higher and lower doses. In rabbits of groups 2 and 3, we observed significantly higher number of erythrocytes ($P < 0.05$) on days 28 and 43 ($P < 0.05$) of the experiment, compared with Group 1. The hemoglobin concentration in blood of the rabbits correlated with the numbers of erythrocytes in Group 2 and Group 3 animals and was significantly higher ($P < 0.001$) at the first and the final stages of the experiment, compared with the control. It has to be noted that the hematocrit percentage in blood of rabbits of experimental groups 2 and 3 – against the control – underwent the most significant changes during intake of the microelement citrate supplement, which was reflected in higher hematocrit content in their blood ($P < 0.001$).

The research found significant values of the factor of the duration of intake of the supplements, the factor of influence of the microelement citrates and their combined effect on the studied parameters of erythrocytes in blood of the rabbits. This may indicate indirect actions of both the applied microelements and the duration of their stay on the processes in the body that soothed the negative impact of high surrounding temperatures. The dietary strategy such as natural compounds or microelements can play a crucial role in alleviating those adverse effects. Recently, the use of nanomaterials in the diet of animals has been recognized as a promising method of maintaining homeostasis in their bodies as a result of correction by microelements. Also, it was found that nanocompounds of microelements, particularly zinc, can be a powerful antioxidant and exert anti-inflammatory action through increasing the antioxidant protection of enzymes and decreasing inflammation mediators (Rodriguez-Amaya, 2019).

The studies of the erythrocyte indices revealed the correlation dependency on the parameters of both the number of erythrocytes and content of hemoglobin in them. The mean corpuscular hemoglobin in blood of the rabbits of groups 2 and 3 increased on days 28 ($P < 0.05$) and 43 ($P < 0.05$-0.01) days, respectively, as compared with the control. Inclusion of the complex of microelements, zinc in particular, in the diet reduced heat stress in the rabbits. Abdel-Wareth et al. (2022) found that using Zn in the amount of 80 mg/kg of the diet improved the blood parameters of the rabbits. The authors assumed that the nanoform of Zn has increased bioavailability and is better absorbed than its organic and inorganic forms. Higher parameters of blood cells can be related to the activation of growth, and synthesis and division of cells through enhanced metabolism of nutrients and improved synthesis of protein and lipids (Chrastinová et al., 2015; Li et al., 2021). Our data are consistent with several studies, for example Abdel-Wareth et al. (2022), who reported that Zn augmented the productivity of the rabbits held in high surrounding temperatures (Hassan et al., 2021).

The studies of the number of leukocytes and their forms in blood of the rabbits revealed significant changes in the absolute content of monocytes and relative number of all the studied parameters in blood of the rabbits depending on the amount of the supplement. The absolute number of monocytes increased in the blood of Group 2 ($P < 0.01$) and Group 3 ($P < 0.05$) rabbits on day 14 of the study. As with the relative level of forms of leukocytes, they depended on the amounts of consumed citrates of microelements and had been affected to a higher degree. In the blood of Group 3 rabbits, the relative contents of lymphocytes and granulocytes increased ($P < 0.01$) on day 28 of the experiment. This may indicate indirect action of the consumed amount of the supplement on the processes of activation of the cellular and humoral non-specific immunity during the short period of intake. In the blood of Group 2 and Group 3 rabbits, the contents of monocytes reliably increased during the study. Platelets are the cells that are responsible for the thrombus formation and tissue regeneration, and their quantity significantly declines as a result of heat stress. The used supplements of citrates of microelements promoted alleviation of the negative impact of heat stress on the rabbits, which manifested in increase ($P < 0.001$) in the number of platelets in Group 2 rabbits on day 28 of the experiment and an insignificant change in this parameter in this and the other group during intake of the supplement. Our results are consistent with other studies.

The number of platelets increased in the blood of Group 3 rabbits ($P < 0.001$) on day 28 of the experiment. Interaction of the time factor and citrates of microelements had a statistically significant effect on platelets, as seen in their indices. The mean platelet volume in the blood of Group 2 and Group 3 rabbits was observed to be increased on day 28 of the experiment. The mechanism of activation of organic zinc, selenium, and germanium is determined by enrichment of the oxygen reserves, as reported by Li et al. (2017), detoxification of heavy-metal elements, absorption of active oxygen species, and increase in the level of reduced glutathione (Wada et al., 2018). Platelets, blood cells that can react to oxidative stress, have protective mechanisms that may be influenced by heat stress. The utilized supplements in respective amounts had an indirect effect on the course of those reactions in the rabbits, manifesting in the physiological processes in their bodies (Lesyk et al., 2020; Lesyk et al., 2022).

We determined a dependence on the applied amount of the supplement according to the percentage of platelet distribution width. This parameter was higher in Group 2 on day 28 and in Group 3 on day 43 of the experiment.

Waltz et al. (2014) studied raising of swine that had undergone heat stress, revealing results that are consistent with ours. They observed an increase in the levels of erythrocytes, hemoglobin, and hematocrit. Those authors attributed it to the fact that heat stress enhanced the blood flow in the skin, contributing to loss of heat, which can lead to decrease in blood flow in other tissues and thus tissue hypoxy. Therefore, intensification in the synthesis of reticolocytes and their release occur in order to increase the hemoglobin level and protect the tissues from hypoxy, which causes a high level of hematocrit. They also found that the increase in those parameters manifested in the positive correlation with the physiological parameters. Other authors consider this increase a result of heightened blood viscosity due to excessive loss of water caused by hyperventilation (respiration enhancement) and loss of bile, which caused dehydration and hemoconcentration in the rabbits (Nakyinsige et al., 2013). However, Askar & Ismail (2012) noted considerable declines in the levels of hemoglobin, erythrocytes, and leukocytes in the New Zealand rabbits that were raised in the conditions of chronic heat stress (7%, 4%, 9%, respectively). Similarly, Mostafa et al. (2007) and Okab et al. (2008) observed declines in the levels of hemoglobin, number of erythrocytes, and hematocrit, and also pointed out increase in leukocytes in summer compared to winter.

On the other hand, Ondruska et al. (2011) observed no impact of heat stress on the numbers of erythrocytes, leukocytes, and monocytes in the young rabbits, but saw a significant decline in lymphocytes in the males. The studies by Khalil et al. (2014) and Dyavolova et al. (2014) found no significant effect of acute heat on the number of erythrocytes, hemoglobin level, and hematocrit, except lymphocytes in blood.

Decline in leukocytes observed during heat stress can be considered an indicator of stress, as described by Dhabhar et al. (1995).

The studies of the biochemical blood parameters revealed positive changes at the level of tendency for the content of total protein and the activities of AST and ALT, compared with the control. The activity of alkaline phosphatase in the blood of Group 2 and 3 rabbits increased on day 14 of the intake and decreased on day 29 of intake of the supplements. Such changes in the activity of alkaline phosphatase can indicate peculiarities of influence of various amounts of microelement compounds in the conditions of heat stress. Obviously, the activity of alkaline phosphatase depended on the duration of consumption of the supplement and the impact of heat stress on the rabbits.

The duration of consumption of the complex of microelements promoted changes in the parameters of lipid metabolism, which manifested in the blood of rabbits of Group 3, in particular, in higher content of cholesterol on day 43 of the experiment and decline of the level of triacylglycerides in animals of both Group 2 and Group 3 on day 28 of the experiment. Those results are similar to those obtained by Ondruska et al. (2011) and Okab et al. (2008), who reported that plasma cholesterol and the general concentration of lipids were much higher in summer than in winter. Ondruska et al. (2011) explained this increase by higher activity of hydroxymethyl-glutaryl-coenzyme reductase and the stimulation of cholesterol synthesis.

Conclusion

Consumption of the complex of citrates of zinc, selenium, and germanium by the post-weaning rabbits in a higher dose was accompanied by more pronounced effects on the numbers of erythrocytes, leukocytes, and monocytes, and their indices and the analyzed biochemical parameters, as compared with lower numbers of them in the control, which may indicate the practical benefits of using them in the industrial rabbit farming.

The authors declare that they have no conflict of interests.

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