



Hematological and biochemical parameters and resistance of the organism of mother rabbits receiving sulfur compounds

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Nanotechnologies are developing continuously, and their use is becoming more diverse and concrete, with high potential to improve the products of animal husbandry and animals in general. Yet, there have been few studies of nanotechnologies in those spheres. However, to confirm the efficiency and, mainly, safety of nanotechnologies, to avoid any harm to the environment or especially humans and animals, substantiated studies are still needed. A promising, but barely studied, direction of research is dosage of nanocompounds of biogenic elements in diets of animals, both modern supplements and alternatives for correcting mineral nutrition. Therefore, the objectives of the study presented here were changes in hematologic, biochemical and immune-biological parameters of the organism of mother rabbits under the influence of sulfur citrate and sodium sulfate for 14 days until insemination and until day 20 of lactation. The research was conducted on mother rabbits of the *Hyla* breed which were giving birth for the second time in LLC Horlytsia in Dobriany village of Horodok district of Lviv Oblast. Mother rabbits of the control group were fed ad libitum with complete granulated mixed feed with free access to water. Animals of experimental group I were fed feeds of the diet of the control group and during the day were watered sulfate citrate in dosage of 8 µg of S/kg of body weight. Females of experimental group II were fed the diet feeds of the control group and given sodium sulfate (Na₂SO₄) in the amount of 40 mg of S/kg of body weight. The mother rabbits received the supplements for 14 days both until insemination and for up to the 20th day of lactation. In the preparation period, on day 10 after the beginning of the study and on day 20 of lactation in the experimental period (65th day of watering the supplements), blood samples were taken from the marginal ear vein of the mother rabbits for hematological and biochemical studies. The study revealed that watering sulfate citrate in the dosage of 8 µg of S/kg of the body weight in the diet of mother rabbits for 14 days until insemination and up to day 20 of lactation increased the amount of erythrocytes by 19.5%, leukocytes by 37.5%, granulocytes by 38.3%, concentration of hemoglobin by 21.0%, mean corpuscular hemoglobin by 15.6%, and red cell distribution width by 14.7%, and activated protein metabolism, which manifested in 8.5% higher content of protein and activity of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase respectively by 12.9%, 29.6%, 19.8% and 51.8% lower content of triacylglycerols in the blood on day 20 of lactation compared with the control group. Intake of sulfur citrate heightened the parameters of immunobiological reactivity of the organisms of mother rabbits on day 65 of the experiment with significantly higher level of phagocytic number, phagocytic activity, lysozymic and bactericidal activities of blood serum, protein-associated hexoses, sialic acids and ceruloplasmin and immune globulins, compared with the control. Giving mother rabbits sodium sulfate in the amount of 40 mg/kg of body weight for 65 days led to less notable changes in the blood, expressed in 20.0% increase in mean corpuscular hemoglobin, higher phagocytic value, phagocytic activity, lysozymic and bactericidal activities of blood serum, protein-associated hexoses and ceruloplasmin. The results of the studies indicate possibility of additional use of supplement sulfur citrate in the amount of 8 µg of S/kg in diet of mother rabbits to improve metabolism, reproductive ability and immunological resistance in the period of increased physiological load.

Keywords: nanotechnologies; mineral substances; erythrocyte indices; immune system; proteins.

Introduction

Proteins are composed of amino acids that become available from the end products of nutrition (Gidenne et al., 2017). There are two types of amino acids, those which animals can synthesize and those which they cannot synthesize by themselves (Delgado et al., 2019). Some of the overall consumed raw protein, which is absorbed by enzymes, converts into aminoacids and peptides and is absorbed in the stomach of rabbits, mostly in the small intestine, while the rest is absorbed in the large intestine with the help of microflora of the cecum (Trocino et al., 2013). As soon as amino acids reach the cell cytoplasm, they are used by transport RNA

inside the nucleus, depending on needs of the organism (Minguez et al., 2016). Sulfur, after calcium and phosphorus, is the commonest mineral element present in the organisms of mammals (de Blas & Gonzalez-Mateos, 2010). It is available in the nutrition of mammals, which is obtained exclusively from protein, and only two of 20 amino acids – which are usually present in protein molecule – contain sulfur. One of those amino acids, methionine, cannot be synthesized in the organism, and therefore should be provided in the diet (Alagón et al., 2016). Cysteine, another amino acid that contains sulfur, as well as a large amount of essential intermediate substances for metabolism, which are needed for life, are synthesized in the organism, but the process requires constant provision of

sulfur with diet feeds. Sulfur avoided attention until recent years. The increase in yield of agricultural crops, decrease in atmospheric precipitations, and larger amounts of plant products have made reducing the content of sulfur in feeds more likely. Additional introduction of sulfur is economically expedient in soils with significant deficiency of sulfur, but not all fields react to its application. Furthermore, the norms of introduction of nitrogen play a significant role in response to the rates of using sulfur (Sawyer et al., 2011).

Producers of modern diets for growing rabbits aim at reducing proteins in the diet and increasing the content of cellulose to avoid digestion impairments (Trocinio et al., 2013). In this context, highly-productive animals with high growth rates are fed with a moderate amount of protein during this period. Research has revealed decrease in the amount of amino acids when modern diets with moderate content of protein were used for fast-growing rabbits (Marín-García et al., 2020a). Usually, the first limiting amino acids in poultry, swine and rabbits are lysine, amino acids of sulfur and threonine (Van Milgen & Doumad, 2015). However, modern recommendations for the content of amino acids are mainly expressed in total units of diet or studies of feces of rabbits. Moreover, rabbit breeding has certain biological specifics such as coprophagy, and therefore its contribution to protein supply may impede the evaluation of the needs in protein itself (Marín-García et al., 2020b). In the conditions of modern industrial rabbit farming, for raising various age groups of rabbits, two, more rarely more components in diet are mostly used. Some studies of diet components, conducted on broiler chickens and different breeds of swine, revealed that animals may choose between different ways of feeding in order to obtain nutrients that meet their demands (Elahi et al., 2020). Research has been conducted on nutrition parameters of rabbits, focusing on choice of diet components. However, modern scientific studies show perspectives of utilization of organic compounds of biogenic elements that are prepared using methods involving nanotechnologies (Oloruntola et al., 2019).

Technologies of obtaining nanoparticles are used in different spheres, particularly medical treatment and nutrition. Nanoparticles are currently broadly used in animal husbandry due to their specific chemical and physical properties. Nanoparticles of biogenic elements are characterized by specific size measuring less than 100 nanometers. The conducted studies indicate that intake of nanoparticles of biogenic elements improves the efficiency of digestion, strengthens immunity and improves productivity of cattle and poultry. Because of their antibacterial and antifungal characteristics, silver nanoparticles have become broadly applied as a powerful disinfecting factor in animal husbandry (Ognik et al., 2016). Research has revealed that mice that had been intraperitoneally injected with AgNP had an ability to increase immune response in vivo and in vitro (Ognik et al., 2016). Rabbits intravenously injected with AgNP (0.6 mg/kg of body weight) exhibited higher reactive oxygen species in spermatozooids, less mobile spermatozooids and lower straightforward speed and oxygen consumption than in control animals. By contrast, libido, serum testosterone, concentration of sperm and volume of sperm hardly affected the level of AgNP (Castellini et al., 2014). Intake of AgNP with drinking water in the concentration of 50 ppm decreased the growth of broilers, worsened the immune functions and had no antibacterial effect on different intestinal bacterial groups (Vadalasetty et al., 2018). Introduction of these new materials would need safety assessment, as well as clear understanding of any potential effects on the health of humans and animals (Ema et al., 2017).

Great attention is paid to studies in the sphere of nanotechnologies because nanosized particles have innovative characteristics, high surficial and catalyst activities, large surface area, significant adsorbing ability and low toxicity (Samak et al., 2020). Recently, in animal husbandry and in human life, methods of nanotechnologies are being used more often, particularly in the sphere of biology, biotechnology, for purposes of mineral nutrition, reproduction and pharmacology. Research has determined the advantage of nanoparticles over salts of mineral elements, which may be effectively synthesized using any physical, chemical or biological techniques that are economically profitable (Reda et al., 2020).

This has been confirmed by research which has revealed positive stimulating effects of nanoaqua-chelates of separate biogenic microelements on biochemical processes in the animal organisms, their productivity and quality of obtained products (Abouelezz et al., 2019). Application of nano-

particles of biogenic elements in rabbit husbandry has positively influenced the parameters of the organism of rabbits, specifically different sources and levels of zinc, during additional introduction to their diet, positively influenced the immunobiological reactivity of their organisms compared with salts of this element (Meshreky et al., 2015). The main function of nanoparticles is support of normal functioning of the organism through bioavailability of components of the standard diet which cannot be absorbed sufficiently (Swelum et al., 2021). Nanoparticles of mineral substances may be used in small doses, producing better results than common sources of mineral compounds. The abovementioned data indicate positive effects of nanoparticles of biogenic elements and their potential use as mineral additives to rabbits' diet. Since the previous studies have physiologically substantiated the amounts of compounds of nanosulfur in the diet of young rabbits after weaning, this study was focused on influence of organic and inorganic compounds of sulfur on the organisms of mother rabbits in the period of physiological load. Therefore, the objectives of the study were the changes in hematological, biochemical and immunobiological parameters of the organisms of mother rabbits during the influence of sulfur citrate and sulfur sodium on their organisms 14 days before insemination and until the 20th day of lactation.

Materials and methods

All the experimental involvement and slaughter of animals were conducted according to the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasburg, 1985) and the Order of the First National Congress of the Bioethics (Kyiv, 2001).

The studies were carried out on *Hyla* mother rabbits giving birth for the second time in Horlytsia LLC in Dobriany village of Horodok district, Lviv Oblast. In the experiments, young rabbits were given organic and inorganic compounds of sulfur in physiologically substantiated amounts. The females were divided into three groups (control and two experimental), 20 animals in each, selected using pair analogue method. Mother rabbits of the control group (C) were fed an unrestricted diet of granulated mixed feed containing 18.5% raw protein, 8.0% raw cellulose, 3.0% raw fat and 2,250 ME kcal/kg with free access to water. Animals of experimental group (I) were fed the diet of the control group and were given sulfur citrate in the dose of 8 µg of S/kg of body weight. Solution of nanosulfur citrate (1.0 g/dm³, pH 1.38) was obtained by LLC Nanomaterials and Nanotechnologies, city of Kyiv. Females of experimental group II (II) received the diet of the control group and water with sodium sulfate (Na₂SO₄) in the amount of 40 mg of S/kg of body weight. Mother rabbits received supplements 14 days prior to insemination and for 20 days before the lactation. The experiment lasted for 95 days, including 10 days preparation period and 85 days experimental period. During the preparation period, on day 10 before the experiment and on day 20 of lactation (65th day of watering the supplements), blood samples were taken from the marginal ear vein of mother rabbits for hematological and biochemical studies. Blood for hematological study was taken into test tubes containing ethylenediamine dipotassium salt – tetraacetic acid (EDTA-K²⁺), which was anticoagulant, and 1% heparin was used as an anticoagulant for biochemical studies. In the blood, we determined the overall amount of red blood cells (RBC), hemoglobin concentration (HGB), hematocrit (HCT) and mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cells (WBC) and their forms – lymphocytes (LYM), monocytes (MON), granulocytes (GRA) and the number of platelets (PLT) and platelet index (mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PWD) on a Mythic 18 hematologic analyzer. Study of biochemical parameters: activity of alkaline phosphatase, cholesterol, triacylglycerols, overall calcium and inorganic phosphorus, total protein, activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) in the blood plasma of the rabbits was carried out using a Humalazer – 2000 biochemical analyzer and a standard toolkit LACHEMA (Czech Republic). In the blood, we determined phagocyte activity of neutrophils (PA) using the method of completed phagocytosis with microbial test culture, phagocyte index (PI), phagocyte number (PN), lysozyme activity of blood serum (LABS), bacteri-

cidal activity of blood serum (BABS), and also the content of immunoglobulin (Ig) using nephelometric method, mean molecular weight (MMW), circulating immune complexes (CIMC), protein-associated hexoses, sialic acids and ceruloplasmin using methods approved in biology and described in the guide (Vlisko, 2012).

Mathematical analysis of the results of the studies was carried out statistically using software pack Statistica 7.0 (Stat Soft, Tulsa, USA). Differences between the parameters of the control and experimental groups were determined using ANOVA, where the differences were considered significant at $P < 0.05$ (taking into account Bonferroni's correction).

Results

In the experiment with mother rabbits consuming sulfur citrate in the dose of 8 µg of S/kg of body weight (experimental group I) and sodium sulfate in the dose of 40 mg of S/kg of body weight (II experimental group), changes in hematological parameters in animals of the experimental group were observed throughout the study, compared with the control group, where the parameters were within the upper and lower physiological norms (Table 1). Specifically, the number of erythrocytes and hemoglobin concentration in the blood of mother rabbits of experimental group I, which received sulfur citrate, were respectively higher by 19.5% ($P < 0.05$) and 21.0% ($P < 0.05$) on day 20 of lactation (65th day of consumption), compared with the control.

Organic and inorganic sulfur compounds acted positively through dynamic changes in red blood indices. Specifically, mean corpuscular hemoglobin in the blood of mother rabbits of experimental groups I and II became higher respectively by 15.6% ($P < 0.05$) and 20.0% ($P < 0.05$) on day 65 of consuming supplements compared with the control group. At the same time, compared with the control, red cell distribution width in the blood of mother rabbits significantly changed only in animals of experimental group I, its percentage being higher by 14.7% during tendency changes in group II.

Table 1

Number of red blood cells, hemoglobin concentration and red blood cell indices in mother rabbits that had been consuming sulfur citrate and sodium citrate ($x \pm SE$, $n = 5$)

Parameter	Group	Periods of the study	
		preparation	experimental
RBC, $10^{12}/L$	C	4.58 ± 0.35	4.71 ± 0.17
	I	4.35 ± 0.49	5.20 ± 0.18*
	II	5.35 ± 0.51	4.59 ± 0.15
HGB, g/L	C	115.2 ± 14.2	107.8 ± 8.2
	I	113.0 ± 4.9	136.8 ± 8.3*
	II	130.4 ± 5.8	108.6 ± 1.9
HCT, L/L	C	0.377 ± 0.041	0.397 ± 0.053
	I	0.369 ± 0.030	0.404 ± 0.058
	II	0.448 ± 0.043	0.393 ± 0.020
MCV, fL	C	86.2 ± 1.8	86.1 ± 1.1
	I	85.4 ± 1.3	87.5 ± 0.5
	II	83.9 ± 1.2	83.5 ± 1.7
MCH, p/g	C	22.0 ± 0.4	22.4 ± 1.8
	I	23.4 ± 0.2	25.9 ± 1.5
	II	21.3 ± 0.4	26.9 ± 0.3
MCHC, g/L	C	281.6 ± 18.8	255.2 ± 2.3
	I	303.0 ± 18.9	258.7 ± 2.9
	II	319.6 ± 2.6	255.0 ± 1.6
RDW, %	C	10.0 ± 0.2	10.2 ± 0.3
	I	10.6 ± 0.4	11.7 ± 0.5*
	II	10.3 ± 0.2	11.3 ± 0.5

Note: C – control group, I – sulfur citrate in the amount of 8 µg of S/kg of body weight, II – sodium sulfate in the dose of 40 mg S/kg of body weight; statistically significant changes were taken into account compared with the control: * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$; the selections were compared within one row (taking into account Bonferroni correction).

The obtained results of studying the number of white blood cells and their indices in blood indicate an insufficient effect of the inorganic supplement, though they may suggest activation of protective functions of the organisms of lactating animals (Table 2). Specifically, the number of white blood cells in animals of experimental group I was 37.5% higher on

the 65th day of consuming sulfur citrate compared with the control group; it has to be noted that those values were within physiological parameters and may indicate higher tolerance of the animals' organisms. Granulocyte concentration in the blood of mother rabbits that had been consuming sulfur citrate was 38.3% higher compared with the control group, while tending toward a higher level in experimental group II.

Table 2

Number of white blood cells and their functional forms in the blood of mother rabbits that had been consuming sulfur citrate and sodium citrate ($x \pm SE$, $n = 5$)

Parameters	Group	Periods of study	
		preparation	experimental
WBC, $10^9/L$	C	8.2 ± 1.3	8.0 ± 0.4
	I	9.0 ± 1.3	11.0 ± 1.0*
	II	9.2 ± 1.1	9.4 ± 1.4
LYM, $10^9/L$	C	2.72 ± 0.71	3.20 ± 0.14
	I	3.18 ± 0.71	2.54 ± 0.22*
	II	3.46 ± 0.82	3.00 ± 0.37
MON, $10^9/L$	C	1.62 ± 0.24	1.28 ± 0.12
	I	1.64 ± 0.30	1.08 ± 0.17
	II	1.80 ± 0.13	1.30 ± 0.16
GRA, $10^9/L$	C	3.41 ± 0.45	3.78 ± 0.35
	I	4.11 ± 0.56	5.23 ± 0.22**
	II	4.54 ± 0.66	4.55 ± 1.12

Note: C – control group, I – sulfur citrate in the amount of 8 µg of S/kg of body weight, II – sodium sulfate in the dose of 40 mg S/kg of body weight; statistically significant changes were taken into account compared with the control: * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$; the selections were compared within one line (taking into account Bonferroni correction).

The conducted studies on effect of adding organic and inorganic sulfur compounds to the diet of mother rabbits in the period before impregnation and during lactation revealed no significant differences between the control and experimental groups (Table 3). However, the determined changes in the content of examined parameters may indicate a positive influence of sulfur compounds on the organisms of mother rabbits in the period of physiological load.

Table 3

Number of platelets and platelet indices in the blood of mother rabbits that had been consuming sulfur citrate and sodium citrate ($x \pm SE$, $n = 5$)

Parameter	Group	Study periods	
		preparation	experimental
PLT, $10^9/L$	C	323.2 ± 81.1	499.0 ± 19.4
	I	390.8 ± 55.9	528.8 ± 15.6
	II	414.0 ± 53.9	592.4 ± 29.6
MPV, fL	C	5.91 ± 0.20	4.56 ± 0.18
	I	4.15 ± 0.19***	5.43 ± 0.12**
	II	5.11 ± 0.10**	4.20 ± 0.44
PWD, %	C	30.1 ± 0.4	37.7 ± 0.8
	I	27.8 ± 0.2**	30.4 ± 0.2***
	II	33.1 ± 0.2**	30.6 ± 0.1***
PCT, %	C	12.3 ± 0.5	13.9 ± 0.6
	I	11.1 ± 0.5	12.7 ± 0.1
	II	13.5 ± 0.5	12.5 ± 0.3

Note: C – control group, I – sulfur citrate in the amount of 8 µg of S/kg of body weight, II – sodium sulfate in the dose of 40 mg S/kg of body weight; statistically significant changes were taken into account compared with the control: * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$; the selections were compared within one row (taking into account Bonferroni correction).

Intake of sulfur citrate and sodium citrate positively influenced the content of the total protein and activity of enzymes of transamination in the blood of mother rabbits of the experimental groups compared with the control, which were within the physiological parameters throughout the study period (Table 4). Therefore, content of total protein in the blood of mother rabbits of experimental group I was 8.5% higher ($P < 0.05$) on the 65th day of the study compared with the control group.

Consumption of organic and inorganic sulfur compounds in the diet by mother rabbits was accompanied by changes in the activity of aminotransferases in blood compared with the control. This is reflected in significant heightened activity of aspartate aminotransferase and alanine amino-

transferase in the blood of animals of experimental group I, respectively by 12.9% ($P < 0.05$) and 29.6% ($P < 0.01$) on day 20 of mother rabbits' lactation compared with the control group. The studies determined a 19.8% increase in activity of alkaline phosphatase in the blood of mother rabbits that had been consuming sulfur citrate compared with the control. It has to be noted that the level of albumin increased throughout the study and manifested in higher parameters after consumption of organic compounds of sulfur, though the results were insignificant.

Table 4
Biochemical parameters in the blood of mother rabbits that had been consuming sulfur citrate and sodium sulfate ($\bar{x} \pm SE$, $n = 5$)

Parameter	Group	Study period	
		preparation	experimental
Total protein, g/L	C	68.2 ± 2.0	65.4 ± 2.0
	I	68.8 ± 2.6	71.0 ± 0.9*
	II	61.4 ± 2.3	65.7 ± 1.8
Aspartate aminotransferase, U/L	C	19.7 ± 1.0	23.1 ± 0.8
	I	21.4 ± 1.0	26.1 ± 0.9*
	II	20.1 ± 1.7	22.5 ± 1.8
Alanine aminotransferase, U/L	C	35.3 ± 2.0	31.0 ± 1.4
	I	37.3 ± 1.6	40.2 ± 1.6**
	II	37.7 ± 1.9	35.0 ± 1.7
Alkaline phosphatase, U/L	C	101.4 ± 5.5	93.4 ± 2.2
	I	99.8 ± 4.9	111.9 ± 2.8
	II	107.0 ± 4.3	91.7 ± 1.9
Albumin, g/L	C	34.0 ± 1.6	45.2 ± 1.2
	I	33.7 ± 1.4	47.1 ± 1.6
	II	35.2 ± 1.7	47.9 ± 2.4
Triacylglycerols, Mmol/L	C	0.82 ± 0.10	1.06 ± 0.10
	I	0.84 ± 0.18	0.55 ± 0.18*
	II	0.93 ± 0.13	0.80 ± 0.08
Cholesterol, Mmol/L	C	2.7 ± 0.3	1.4 ± 0.1
	I	2.1 ± 0.5	1.2 ± 0.1
	II	3.0 ± 0.7	1.1 ± 0.1
Total calcium, mmol/L	C	3.5 ± 0.1	2.3 ± 0.1
	I	3.6 ± 0.1	2.0 ± 0.1
	II	3.7 ± 0.1	2.2 ± 0.1
Inorganic phosphorus, mmol/L	C	1.9 ± 0.3	1.5 ± 0.2
	I	1.7 ± 0.1	1.6 ± 0.1
	II	1.7 ± 0.1	1.5 ± 0.2
Calcium : Phosphorus	C	1.86:1	1.51:1
	I	2.11:1	1.26:1
	II	2.22:1	1.46:1
Protein-associated hexoses, g/L	C	2.4 ± 0.2	1.7 ± 0.1
	I	2.8 ± 0.5	2.4 ± 0.3
	II	2.7 ± 0.2	2.1 ± 0.2
Sialic acids, conditional units	C	98.4 ± 2.5	102.1 ± 4.0
	I	95.8 ± 2.5	122.1 ± 3.4**
	II	97.1 ± 2.7	112.4 ± 2.3
Ceruloplasmin, conditional units	C	344.9 ± 17.3	590.9 ± 10.5
	I	352.5 ± 8.8	711.0 ± 7.3***
	II	360.6 ± 12.3	670.6 ± 12.2**

Note: C – control group, I – sulfur citrate in the amount of 8 µg of S/kg of body weight, II – sodium sulfate in the dose of 40 mg S/kg of body weight; statistically significant changes were taken into account compared with the control: * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$; the selections were compared within one row (taking into account Bonferroni correction).

The studies revealed that the content of triacylglycerols in the blood plasma of mother rabbits of experimental group I was significantly lower by 51.8% on day 65 of the studies compared with the control. The obtained results may suggest activation of processes of metabolic pressure on plastic components of cellular membranes and energy expenditures of the organism's tissues, which was more expressed in the action of organic sulfur compound.

Intake of organic sulfur compound decreased the content of cholesterol in animals of the experimental groups compared with the control, though the results were insignificant, indicating greater use of cholesterol in their organisms under the influence of physiologically substantiated amounts of sulfur compounds.

In young animals under increased physiological load (pregnancy and lactation), calcium and phosphorus were absorbed more intensely. Studying the influence of consumption of sulfur citrate and sodium sulfate

revealed changes in the levels of calcium and phosphorus in blood plasma in animals of the experimental groups compared with the control, though the results were insignificant (Table 6). However, the conducted analysis of the results indicates the normalization of parameters, phosphorus and its high levels during intake of organic compound of sulfur. It is important that the ratio of calcium to phosphorus was higher in experimental group I compared with the control and other experimental group of mother rabbits.

Consumption of sulfur compounds stimulated the resistance of the organism of mother rabbits, indicating increase in the content of glycoproteins and their carbohydrate components in blood (Table 4).

In particular, content of protein-associated hexoses in blood of animals of experimental groups I and II was higher respectively by 43.7% and 25.4% ($P < 0.05$) on day 65 of the study compared with the control group. A less expressed effect on the content of sialic acids in blood was displayed by sodium sulfate, though animals of experiment group I were seen to have 19.5% higher ($P < 0.05$) concentration of this parameter compared with the control. The content of ceruloplasmin in mother rabbits of experimental groups I and II was significantly higher by 20.3 ($P < 0.001$) and 13.4% ($P < 0.01$) on the 65th day of the experiment compared with the control group.

The conducted studies resulted in determining a stimulating effect of sulfur compounds on the cellular link of non-specific resistance of the organisms of mother rabbits (Table 5). Specifically, in the blood of animals of group I, which drank sulfur citrate in the dose of 8 µg of S/kg of body weight, and animals of experimental group II, which consumed sodium sulfate in the amount of 40 mg of S/kg of body weight during the study, the level of phagocytic activity of neutrophils was higher correspondingly by 24.6% ($P < 0.001$) and 17.8% ($P < 0.01$) on the 65th day of the intake of supplements compared with the control. At the same time, phagocytic index and phagocytic value were higher in animals of the experimental groups compared with the control, specifically the phagocytic number was higher by 22.1% ($P < 0.01$) and 12.4% ($P < 0.05$) in groups I and II respectively.

Table 5
Parameters of non specific link of immunity and the content of immune complex in the blood of mother rabbits that had been drinking sulfur citrate and sodium sulfate ($\bar{x} \pm SE$, $n = 5$)

Parameters	Group	Study period	
		preparation	experimental
Phagocytic activity, neutrophils, %	C	33.1 ± 0.5	32.5 ± 0.88
	I	34.2 ± 0.6	40.5 ± 1.09***
	II	33.8 ± 0.7	38.3 ± 1.41**
Phagocytic index, units	C	8.8 ± 0.3	8.1 ± 0.6
	I	8.4 ± 0.7	8.9 ± 0.3
	II	8.3 ± 0.8	8.5 ± 0.1
Phagocytic value, units	C	3.05 ± 0.22	2.89 ± 0.09
	I	2.89 ± 0.73	3.53 ± 0.18*
	II	3.20 ± 0.28	3.25 ± 0.15
Bactericidal activity of blood serum, %	C	35.4 ± 1.4	39.7 ± 0.3
	I	34.4 ± 1.2	49.1 ± 0.5***
	II	34.2 ± 1.4	43.6 ± 0.7***
Lysozymic activity of blood serum, %	C	34.1 ± 1.2	34.5 ± 0.6
	I	34.8 ± 1.5	44.8 ± 0.5***
	II	33.2 ± 1.5	39.9 ± 0.5**
Immune globulins, g/L	C	10.2 ± 0.3	4.1 ± 0.3
	I	10.1 ± 0.6	6.6 ± 0.4**
	II	10.7 ± 0.1	5.1 ± 0.2*
Circulating immune complexes, mmol/L	C	34.5 ± 2.5	42.1 ± 1.7
	I	32.1 ± 3.9	50.1 ± 1.2**
	II	35.3 ± 2.3	51.9 ± 2.1**
Average-weight molecules, conditional units	C	0.23 ± 0.03	0.23 ± 0.03
	I	0.25 ± 0.07	0.26 ± 0.02
	II	0.23 ± 0.01	0.24 ± 0.05

Analysis of bactericidal and lysozymic activity of blood serum of mother rabbits indicates significant influence of compounds of sulfur on cellular and humoral immunities in their organism. Therefore, percentage of bactericidal activity of blood serum of animals of experimental groups I and II was higher respectively by 23.8% and 9.8% ($P < 0.001$) on day 65 of the study compared with the control group. Relative content of lysozymic activity of blood serum of mother rabbits of experimental groups I

and II was higher by 29.8% and 15.6% on the 20th day of lactation compared with the control.

The results of the study on the content of glycoproteins and their carbohydrate components in the blood were confirmed by higher concentration of immune complexes in the blood of mother rabbits (Table 5). Therefore, consumption of sulfur citrate caused significant increase ($P < 0.05$) in the concentration of immunoglobulins in the blood of mother rabbits of experimental group I on day 65 of the study compared with the control.

Thus, consumption of sulfur citrate in the dose of 8 μg of S/kg of body weight and sodium sulfate in the dose of 40 mg of S/kg of body weight by mother rabbits for 65 days caused significant effect on cellular and humoral immunity of non-specific resistance of their organism.

Discussion

The organism of mother rabbits in the period of physiological load requires additional sources of nutrients, their bioavailability rather than number being especially important. A special need occurs for micro- and macroelements in their organism, which are the most important factors of providing complete nutrition. It has to be noted that consumption of sulfur compounds led to varying effect on blood parameters, more expressed on red and white blood and insignificant on platelets. Similarly, (Syrvatka et al., 2014) reported that all the studied blood parameters were insignificantly altered by nano-argentum compound in New Zealand white mother rabbits. Other studies (Raheem, 2018) revealed increase in the number of erythrocytes, platelets and hematocrit in rabbits that had been consuming nanocompounds of biogenic elements compared with regular salts of mineral compounds. In our opinion, the obtained results may indicate more manifested influence of organic compound of sulfur on hematopoietic function of organism of mother rabbits for a long period of time prior to impregnation and during physiological load – lactation. Evidences of positive effect of organic compound of sulfur were significant changes expressed in higher contents of the studied indices of red blood compared with the control. The amount of formed elements in the blood is an important parameter of physiological condition of animals and their supply of nutrients and mineral substances, because blood is the main transport system of the organism, which is the first to react to deficiency or their excess in diet. Analysis of changes in parameters of red blood of mother rabbits that had been consuming sulfur compounds indicates stable physiological status of their organism in the period of lactation, though sulfate citrate exerted higher effect on parameters and indices of red blood of mother rabbits compared with inorganic compound of sodium sulfate. The results of the study may suggest more manifested positive effect of organic compound of sulfur on non-specific link of the protection of the organism and phagocytic activity of the blood of female rabbits, as confirmed by our earlier study on immune-specific reactivity of the organisms of young rabbits (Lesyk et al., 2020). It should be noted that all changes in parameters of white blood of mother rabbits were within the physiological parameters, which may indicate a stimulating effect of the organic compound of sulfur on the main populations of white blood cells and hematopoiesis.

Despite the significant role of platelets in organism of rabbits, studies of their functional condition in the period of lactation are scarce. Our studies revealed no significant changes in number of platelets and their indices, though these parameters significantly increased under the action of sulfur compounds, which may suggest an indirect effect on the processes of development of these blood cells. In the organism of mammals, platelets constantly circulate in the blood and support normal structure and function of vessels and take part in the processes of coagulation (Gary et al., 2013; Borshch et al., 2020; Bashchenko et al., 2021; Mylostyvyi et al., 2021; Vasylyev et al., 2021). Platelets play significant role in resistance, for they are the first to react to infectious agents, resulting in formation of specific antibodies that attach to the surface of antigens, forming an antigen-antibody complex that activates response to inflammation. Platelets have receptors that recognize those complexes, i.e. platelets rather than leukocytes, are those that react to an infection first (Harkness et al., 2013; Martyshuk et al., 2020; Khalak et al., 2021; Brezvyin et al., 2021). The obtained results of the study of number of platelets may indicate advantages of additional use of sulfur compounds in the period of technological pressure in industrial rabbit breeding.

Despite variable changes in the studied hematological parameters in rabbits depending on breed and individual peculiarities, parameters of red, white blood and platelets, their indices were within the physiological norms (Syrvatka et al., 2014; Bojko et al., 2020). The following studies reported higher concentrations of red blood cells, hemoglobin and hematocrit, depending on adapted breed of rabbits and rabbits brought from other regions which were given new nanosupplements (Fathi et al., 2017).

The results of analysis of formed elements in the blood indicate that consumption of sulfur citrate resulted in greater positive effect on hematopoietic function of their organism than the inorganic compound of sodium sulfate, which may be physiologically significant for functioning of organisms of mother rabbits and fetal development of rabbits. As is known, in the period of physiological load, pregnancy or lactation, and especially when giving birth and lactation combine, the organisms of mammals experience physiological anemia, which was seen to be more expressed in animals that gave birth many times (Lesyk et al., 2020). Intake of organic compounds of sulfur promoted activation of metabolism in the organisms of lactating animals and removal of this unfavourable effect, whereas the inorganic compound was characterized by weak tendency toward increases in number of red blood cells, white blood cells and hemoglobin concentration compared with the control group.

Consumption of sulfur citrate increased the content of total protein in the blood of mother rabbits on day 20 of lactation. The study results may indicate higher biological value of organic compound of sulfur for the organism of rabbits compared with non-organic. The literature sources report that the diets balanced in macro- and microelements, without taking into account their availability, provide no favourable effect on biochemical and productive parameters of the organisms of animals, especially rabbits, which have highest parameters of growth among mammals. The studies of broiler chickens that had been consuming nanocompounds of argentum led to no changes in total protein and aminotransferases (Saleh et al., 2018), whereas we observed a different effect of sulfur citrate. Higher concentrations of protein in the blood confirm the activity of aminotransferases in the blood of experimental groups I and II, and also the research revealed increase in the activity of alkaline phosphatase on day 20 of lactation compared with the control. We should note that the level of albumin increased during the study and was higher during intake of the organic compound of sulfur, though the results were insignificant.

The activity of aspartate aminotransferase in the blood of rabbits was insignificant and less expressed compared with alanine aminotransferase, unlike in carnivorous animals due to the specifics of functioning of their organism. Our studies revealed somewhat other changes in the activity of aminotransferases. In particular, activity of aminotransferases in the blood of rabbits of groups I and II was higher during the action of organic sulfur compound, which caused one-direction influence on the activities of those enzymes in the organisms of mother rabbits in the period of physiological load. The blood parameters of animals are known to reflect the intensity of course of metabolic processes occurring in their organisms and characterize physiological status of animals. The obtained data indicate activation of protein metabolism in the blood during consumption of sulfate citrate, which surely does positively influence the course of metabolic processes of the organisms of lactating mother rabbits.

The studies revealed significantly lower content of triacylglycerols and tendency toward lower level of cholesterol in the blood plasma of mother rabbits of experimental group I on day 65 of the study compared with the control. The obtained results suggest activation of the processes of metabolic pressure of flexible components of cellular membranes and energy needs of the tissues of the organism, which was more expressed during the action of organic compound of sulfur. Those results demonstrated that variation of lipids of serum of rabbits corresponded to biological variability of those animals.

Cholesterol is the main lipid component of the plasmatic membrane of cells of mammals, measuring up to 45% in relation to other lipids. However, even a limited increase in the level of cholesterol in blood or the tissues, which 2–3 times exceeds the physiological level, is cytotoxic and related to the development of cardiovascular diseases. The main source of influence of cholesterol on the cellular functions is its ability to alter the function of multiple membrane proteins, including ion channels (Saleh & El-Magd, 2018). Therefore, the results reveal that the tendency to decrease

in the content of cholesterol in the blood of mother rabbits is a positive change in their organism.

The studies found no significant changes in the content of total calcium and inorganic phosphorus in the blood of mother rabbits, though during the action of sulfur compounds, we observed better parameters of phosphorus level, which was more expressed during the influence of sulfur compound. The determined changes indicate a more expressed effect of sulfur citrate and sodium citrate which activated metabolic processes of absorption of phosphorus in the organism of rabbits. The organic compound of sulfur obviously had created an optimal environment for vital activities of the microflora of the large intestine of rabbits, which increased enzymic activity, resulting in activation of digestion and absorption of food and mineral compounds in the digestive tract, especially in experimental group I, and less so in the mother rabbits of group II, compared with the control.

The above mentioned changes in calcium and phosphorus in the organism of mother rabbits are a important parameter of influence of sulfur compounds, but the most important is their ratio. Therefore, the ratio of calcium and phosphorus was within 1.86–2.22 : 1 at the beginning of the experiment. On day 65 of the experiment, the ratio between the indicated elements in animals of experimental groups I and II became within 1.26–1.46 : 1, indicating a more expressed influence of sulfur compounds, nonetheless more sulfur citrate influenced the metabolism of phosphorus during a long period of consuming supplements.

The research conducted indicated the stimulating effect of sulfur compounds on the cellular link of non-specific resistance of the organism of mother rabbits, which manifested in a significantly higher level of phagocytic activity of neutrophils and phagocytic value on day 65 of consuming supplements compared with the control. Increase in the functional activity of neutrophils in the blood of mother rabbits of experimental groups may suggest increase in protective ability of their organism in the period of lactation during the action of organic and inorganic compounds of sulfur on the natural mechanism of detoxification.

In the blood of mother rabbits of experimental groups I and II, the level of bactericidal and lysozymic activity of blood serum was higher on the 20th day of lactation compared with the control. An important parameter of humoral link of natural immunity is the bactericidal activity of blood serum. It destroys microbial cells. A high level of bactericidal activity of blood serum is associated with the content of lysozyme, which has a cytolytic effect on microorganisms. Lysozyme has dual properties as an enzyme, it ruins the relationships between N-acetylmuramic acid and N-acetylglucosamine and is able to activate other non-specific factors of an organism's defense. As is known, one of the factors of activation of resistance is complete influx of all necessary microelements in the complex with nutrients to the organism. Heightened demand of mother rabbits for vitamins and microelements in the period of lactation is to a high degree caused by intensified metabolism for producing milk, nutritive value of which exceeds milk of other mammals, and decrease in the natural defenses of the organism. Consumption of sulfur compounds by mother rabbits significantly influenced the development of cellular and humoral mechanisms of non-specific resistance of their organism, which is likely associated with the activating effect of sulfur on mineral compounds and metabolic processes in animals during the period of increased physiological load.

The following studies determined that the content of protein-associated-hexoses and ceruloplasmin in the blood of mother rabbits of experimental groups was higher on day 65 of the study compared with the control. Those changes in blood may promote activation of the system of immune-physiological defense in the period of heightened physiological load. Ceruloplasmin is cuprum-containing glycoprotein which influences the protection of the organism from free radicals and reactive oxygen species at the level of cell in the organism. Obviously, sulfur compound – to a greater degree sulfur citrate – promoted biosynthetic processes of formation of this glycoprotein in the organism of mother rabbits in the period of lactation. Content of sialic acids was significantly different compared with the control only while consuming sulfur citrate. The literature sources report that sialic acids in the organism have a dual function. They are camouflaging factors of certain sites of recognition and counter-receptors that recognize extracellular markers in the processes of molecular-cellular interactions, specifically proteins of the complement system or ga-

lactose-specific receptors of cells of the immune system. It was determined that there is a direct dependence between the content of sialic acids in blood and immune-physiological reactivity of the organism.

Functional activity of glycoproteins significantly depends on the structure of their carbohydrate component. In most cases, glycans are carbohydrate structures, covalently bound to poly-peptide chain, which promote biological effect of glycoprotein and take part in regulating metabolic processes. One of the mechanisms of regulation of functional activity of proteins is attachment of N-acetylglucosamine to radicals serine or threonine. This mechanism regulates the activity of many factors of transcription, enzymes, neuronal and muscular proteins. Obviously, silicon – as a stimulating microelement in the organisms of animals and humans – took effect on the activation of processes of glycoprotein status and resistance of the organisms of lactating mother rabbits.

Intake of sulfur citrate promoted significant increase in the concentration of immune-globulins in blood of mother rabbits of experimental group I on day 65 of the experiment compared with the control. This may indicate stimulating effect of organic compound of sulfur on synthesis of some classes of immune globulins in the lymphatic system that regulates the immune mechanisms.

The results of the study of the content of circulating immune complexes in the blood of animals of the experimental groups – compared with the control – revealed no significant changes, which may suggest absence of negative effect of supplements in the organisms of lactating mother rabbits.

Concentration of MCM in blood of mother rabbits of the control and experimental groups in the preparation and experimental periods underwent no significant changes. Average-weight molecules are products of catabolism of endo- and exogenic proteins. Some fractions of mean molecular peptides are neurotoxic, alter penetrability of membranes, and impair sodium-potassium balance. The results of MCM content in the blood of animals show absence of toxic effect of consumed supplements on their organism.

Therefore, intake of sulfur citrate in the dose of 8 µg of S/kg of body weight and sodium sulfate in the dose of 40 mg of S/kg of body weight by mother rabbits for 65 days caused a significant effect on blood parameters, cellular and humoral link of non-specific resistance of their organism.

Conclusion

Including zinc citrate in the dose of 8 µg of S/kg of body weight in the diet of mother rabbits 14 days prior to insemination and until the 20th day of lactation increased the number of red blood cells, white blood cells and granulocytes, hemoglobin concentration, mean corpuscular hemoglobin, red cell distribution width, activated protein metabolism, which was followed by an increase in the content of protein and activation of aspartate and alanine aminotransferase, alkaline phosphatase and decrease in the content of triacylglycerols in the blood on day 20 of lactation. Consumption of sulfur citrate heightened the parameters of immunobiological reactivity of the organism of mother rabbits on day 65 of the experiment with significantly higher level of phagocytic number, phagocytic activity, lysozymic and bactericidal activities of blood serum, protein-associated hexoses, sialic acids and ceruloplasmin and immune globulins compared with the control. Intake of sodium sulfate in the amount of 40 mg/kg of body weight for 65 days caused less manifested changes in the blood with increased mean corpuscular hemoglobin, higher phagocytic value, phagocytic activity, lysozymic and bactericidal activities in blood serum, protein-associated hexoses, and ceruloplasmin. The results we obtained indicate the possibility of additional use of sulfur citrate supplement in the amount of 8 µg of S/kg in the diet of mother rabbits to increase metabolism, reproductive ability and immunobiological resistance during the period of heightened physiological load.

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