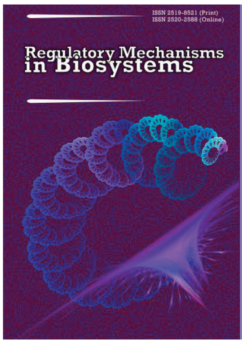

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst., 2020, 11(1), 133–138
doi: 10.15421/022020

Influence of zinc nanoaquacitrate on the immuno-physiological reactivity and productivity of the organism of rabbits

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Article info

Received 14.02.2020
Received in revised form 11.03.2020
Accepted 12.03.2020

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Boiko, O. V., Honchar, O. F., Lesyk, Y. V., Kovalchuk, I. I., & Gutyj, B. V. (2020). Influence of zinc nanoaquacitrate on the immuno-physiological reactivity and productivity of the organism of rabbits. Regulatory Mechanisms in Biosystems, 11(1), 133–138. doi:10.15421/022020

Zinc is necessary for maintaining the immune status, and its deficiency in the organisms of animals is usually accompanied by the condition of immune deficiency. The objective of the study was determining the effect of different amounts of zinc on the immune-biological reactivity and productivity of the organism of rabbits after their weaning on the 50th and 86th days of life. For the study, rabbits with the weight of 1.2–1.4 kg were selected and divided into four groups (control and three experimental). The rabbits of the control group were fed with unlimited balanced granulated compound feed, and had free access to water. The animals of the I, II and III experimental groups were watered with zinc nanoaquacitrate in the amounts of 0.25, 0.50 and 0.75 mg of Zn/kg of body weight. Compared with the control group, watering of the animals of the experimental groups with zinc nanoaquacitrate to a greater extent affected the content of phagocytic activity, lysozymic and bactericidal activities of the blood serum as integral factors of non-specific cellular and humoral resistance of the organism, which manifested in the increase in their content in blood on the 12th, 24th and 36th days of the experiment. Use of organic supplement in the diet of rabbits had a stimulating effect on the functioning of the immune system of their organism, which was seen in the higher content of total immunoglobulins, sialic acids and ceruloplasmin in the blood of animals watered with zinc nanoaquacitrate in the quantities of 0.50 and 0.75 mg of Zn/kg of body weight on the 24th and 36th days of the experiment. Use of organic compound of zinc in the diet caused high parameters of growth of the organism of rabbits during the period of 36 days, which manifested in the highest parameters of average-day increments and body weight on the 86th day of the life of the rabbits from the III experimental group, which received zinc nanoaquacitrate in the amounts of 0.75 mg of Zn/kg of body weight compared with the control group. Watering rabbits with zinc nanoaquacitrate during the study was accompanied by probable changes in the number of erythrocytes, concentration of hemoglobin and erythrocyte indices, which could indicate a positive effect of the employed additives on the hematopoietic function of the rabbits' organism. The data of the performed experiment suggest that watering with larger amounts of organic compound of zinc has a positive effect on the processes of formation of immuno-physiological reactivity of the rabbits' organism and increase in their productivity. The practical purpose is the study of the impact of watering with zinc nanoaquacitrate on the immuno-biological reactivity of the organism of rabbit dams during the period of lactation.

Keywords: immune system; phagocytic activity; lysozymic and bactericidal activities; blood serum; glycoproteins.

Introduction

Zinc in the organism of animals is a component of numerous enzymes and performs the function of co-factors of enzymes of non-protein nature and takes part in biosynthesis of nucleic acids and processes of cell division (Grosskopf et al., 2017). It also has a significant effect on physiological functions in the organism, such as regulation of acid-base homeostasis, activation of metabolism and resistance of the organism (Gaither & Eide, 2001). Most often Zn is used in the diets for swine and poultry to improve the parameters of growth and development of their organisms (Amen & Daraji, 2011). Research has shown that soybean grist and wheat brans are the main ingredients of granulated compound feed in rabbits' diet, enriched with phytates, which have an antagonistic effect on the alimentary zinc (Hendy et al., 2001). However, additional administration of zinc compensated the diets with phytic acid in compound feed. It has been reported that physiological amounts of zinc for rabbits range within 25–60 mg/kg (Mateos et al., 2010). Currently, the role of zinc in the diet of rabbits is a subject of special interest. The surveys demonstrated that it takes part in the synthesis of protein and the carbohydrate metabolism of the organism. Higher parameters of the body weight were determined after additional use of 90 mg/kg of zinc in the rabbits' diet (Wang et al., 2012). Also, higher body weight was determined in rabbits which additionally received zinc in the amount of 100 mg/kg within the diet (Nessrin et al., 2012). It has been determined that both organic and non-organic Zn could be used in the diets of animals for increasing the body weight. Furthermore, physiological levels of Zn are often included in the starter diet of young swine after weaning (Hahn & Baker, 1993; Case & Carlson, 2002). Zinc contributes to the absorption of vitamin E and maintenance of the normal concentration of this vitamin in the blood. It is necessary for the maintenance of the skin at the normal turgor pressure, growth of hair and nails, and also during the healing of wounds, because it plays an important role in the synthesis of proteins, activates the immune system of the organism and has a detoxifying effect – contributes to removal of carbon dioxide from the organism (Han et al., 2014).

The studies revealed that organic Zn is an alternative to the non-organic form in the diets of swine and broiler chickens due to better digestion in the organism and profitability in the production (Downs et al., 2000; Wang et al., 2010). Zinc in both organic and non-organic forms is generally considered to be allowable for use in the diets of animals for improvement of growth parameters. However, use of high levels of zinc has caused some ecological problems due to low bioavailability of non-organic zinc in the organism (Ferreira et al., 2002; Alikwe et al., 2011). Moreover, previous studies revealed that non-organic zinc differs by better bioavailability and efficiency in the organism of rabbits. Over the past decade, nanotechnologies have been actively developed, especially the use of macro- and microelements. This is supported by studies on obtaining positive stimulating effect of nanoaquachelates of separate biogenic microelements on biochemical processes in the organisms of animals, their productivity and quality of the obtained production (Borysevych et al., 2010; Chekman, 2011). Therefore, the objective of the study was to determine the effect of different amounts of nanoaquacitrate of zinc on the immuno-biological reactivity and productivity of the organism of rabbits after weaning from the 50th to 86th day of life.

Materials and methods

All the procedures with animals were performed according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Official Journal of the European Union L276/33, 2010). The research was performed on young rabbits of de Termonde breed in the vivarium conditions of the Institute of Biology of Animals of NAAS. Rabbits aged 40 days were selected for the study according to the pair analogues method, with the weight of 1.2–1.4 kg, divided into four groups (control and three experimental, each comprising of 4 animals (2 males and 2 females). Animals were kept in premises with regulated microclimate and light in grid cages of the size of 50 × 120 × 30 cm, according to the current veterinary-sanitary norms. The rabbits of the control group were fed without limitations with balanced granule compound feed and had free access to water. The animals of the I, II and III experimental groups were fed with the diet of the control group and in the course of the day were watered with nanoaquacitrate zinc in the amounts of respectively 0.25, 0.50 and 0.75 mg of Zn/kg of body weight. Solution of nanoaquacitrate zinc (0.5 g/dc^3, pH 1.35) was received from LLC Nanomaterials and Nanotechnologies, Kyiv. The experiment lasted 46 days, including a 10 days preparation period and 36 days experimental period. On the 10th day in the preparation period and 62nd, 74th and 86th days of life (12th, 24th and 36th days of watering with the supplements) in the experimental period, samples of blood were taken from marginal ear vein of 4 animals (2 males and 2 females) for the assay on phagocytic activity of neutrophils (PA) with addition of strain № 078 standardized to 2 b/mL of 24 h culture of *E. coli*, the smears were stained using the Romanowsky–Giemsa technique, and determined by number of active leukocytes of 100 counted (%). We took into consideration the phagocytic number (PN) and phagocytic index (PI) according to formulae: PI – number of phagocytic microorganisms / PA; PN – number of phagocytic microorganisms / 100. Blood serum bactericidal activity (BSBA) was determined using photoelectric colorimetry method (on PEC-56 [photoelectric colorimeter, Russian ФЕК-56], λ = 540 nm) in relation to microbial test culture *E. coli* (strain BKM-125). Lisozymic activity of blood serum (LABS) was determined in relation to microbial test culture *Micrococcus lisodeikticus* (strain BKM-109) on PEC-56 in cuvettes with operating length of 3 mm at the wavelength of λ = 540 nm. Transparency of the initial suspension was 20% (Vlislo et al., 2012). Nephelometric method of determining the content of immunoglobulins is based on the abilities of zinc to interact with immunoglobulin-containing biological fluids and change the structure of protein molecules Ig, as a result of which the solution becomes less transparent, and the intensity of opacification is proportionate to Ig concentration; the assay was performed on a photoelectric colorimeter at the wavelength of λ = 400 nm in the cuvette with working thickness of 5 mm. The control was the same amount of 18% solution of zinc sulfate. The content of immunoglobulins was determined according to the calibrating graph, the methods are described in the book (Vlislo et al., 2012). The assay on the content of sialic acids was performed using the resorcin method, based on the property of resorcin to form a blue-colored compound during interaction with sialic acids (acetylneuraminic) in absence of Cu^{2+} ions. In this reaction, glucose, mannose, galactose and fructose form yellow-coloured compounds which remain in the aqueous phase after the extraction with mixture of butanol and butyl acetate. The studies were performed on the photoelectric colorimeter with yellow optical filter (575–590 nm) in the cuvette with length of the optical path equaling 10 mm compared to the control sample. Results were expressed in the units of optical density multiplied by 1,000. We determined the content of hexoses' bond with proteins, the method is based on the property of 96% ethanol to settle the glycoproteins from blood serum. While interacting with orcinol reagent, hexoses which were released with sulfuric acid as a result of the subsequent hydrolysis give the solution a pink colour, the intensity of which is directly proportionate to the content of hexoses; the samples were photometered on the PEC with green optical filter (500–560 nm) in the cuvette with 10 mm thick optical layer. The content of ceruloplasmin was determined using the method based on the oxidation of p-phenylenediamine with involvement of ceruloplasmin. Enzymic reaction was stopped by adding sodium azide. Concentration of ceruloplasmin is directly proportionate to the optical density of the products of the reaction determined on the PEC with green optical filter (500–560 nm) in the cuvette with 10 mm thick working layer. The results are expressed in the units of optical density multiplied by 1,000 (Anasashvili, 1968). Blood for hematological assay was taken into test tubes which contained dipotassium salt of ethylenediamine - tetraacetic acid (EDTA-K^{2+}) which functioned as anticoagulant. In the blood, we determined total number of erythrocytes and erythrocyte indices (average volume of an erythrocyte, average content of hemoglobin in an erythrocyte, average concentration of hemoglobin in an erythrocyte, width of the distribution of erythrocytes), number of leukocytes and their forms – lymphocytes, monocytes, granulocytes and the quantity of thrombocytes and thrombocytic indices (average volume of thrombocyte, width of the distribution of thrombocytes by volume, platelet crit) on Mythic 18 hematologic analyzer (Vlislo et al., 2012).

The data were analyzed using Statistica 6.0 (StatSoft Inc., USA) program. The data are presented in Tables as x ± SD (x ± standard deviation). Differences between the values of the control and experimental groups were determined using ANOVA, where the differences were considered reliable at $P < 0.05$ (taking into account Bonferroni correction).

Results

Phagocytic activity of neutrophils in the blood of rabbits of the II and III groups which received 0.50 and 0.75 mg of Zn/kg of body weight was reliably higher ($P < 0.05$) on the 12th and 24th day of the study at a tendency towards higher level at the final stage of the study compared with the control (Table 1). The rabbits of the first experimental group, which received the lowest amount of nanoaquacitrate zinc, were observed to have a tendency towards increase in phagocytic activity during the study. These data indicate that watering with larger amounts of organic compound of zinc manifests a stimulating effect on cellular factors of the organism`s protection. The results of the study demonstrate that the values of phagocytic index and phagocytic number correlated with the parameters of phagocytic activity and were higher for the periods of the study in the blood of the rabbits of all the experimental groups, except rabbits of the I experimental group, the phagocytic index of which was reliably higher ($P < 0.05$) on the 12th day of the study compared with the control group.

The survey of humoral factors of non-specific protection of the organism and determination of the content of bactericidal activity in the blood serum of the rabbits of the II experimental group revealed increase in this parameter by 8.8% ($P < 0.05$) on the 12th day of using the supplement compared with the control group. Changes in the level of lysozymic activity of blood serum indicate the activating effect of separate amounts of compounds of zinc nanoaquacitrate. In particular, in the blood of rabbits of the I and II groups, on the 12th day of watering with the supplements, we observed higher activity by 4.8% and 7.2% respectively, and in the II and II groups its level was higher by 6.5% and 7.2% ($P < 0.05$) on the 36th day of the study compared with the control group. This indicates increase in the activity of the immune system of rabbits, especially the humoral link of non-specified protection of their organism during the influence of larger researched amounts of organic compound of zinc in the diet of the young rabbits after the weaning.

Table 1
Parameters of cellular and humoral factors of resistance of the organism of rabbits receiving different amounts of nanoaquacitrate zinc (M ± m; n = 4)

Parameters	Group of animals	Periods of the study: preparation, 50th day of the study	experimental (day of life/day of the study) 62/12	74/24	86/36
Phagocytic activity, %	C	40.8 ± 1.2	40.3 ± 1.5	41.3 ± 1.9	41.7 ± 0.9
	I	41.0 ± 1.2	43.0 ± 1.2	44.5 ± 1.0	42.7 ± 0.9
	II	40.3 ± 1.1	45.7 ± 0.8*	46.0 ± 0.2*	43.7 ± 0.9
	III	42.7 ± 1.4	45.0 ± 1.2*	47.0 ± 1.2*	42.7 ± 0.9
Phagocytic index, units	C	10.4 ± 0.2	10.1 ± 0.3	13.1 ± 0.6	13.3 ± 0.3
	I	11.2 ± 0.2	12.2 ± 0.7*	14.3 ± 0.5	14.1 ± 0.2
	II	10.1 ± 0.1	10.9 ± 0.3	14.3 ± 0.3	13.5 ± 0.4
	III	10.3 ± 0.1	11.3 ± 0.4	14.3 ± 0.2	14.2 ± 0.4
Number of phagocytes, units	C	4.11 ± 0.45	4.31 ± 0.31	5.43 ± 0.38	5.57 ± 0.17
	I	4.66 ± 0.12	5.23 ± 0.33	6.41 ± 0.25	6.05 ± 0.15
	II	4.08 ± 0.41	5.01 ± 0.16	6.31 ± 0.22	5.94 ± 0.29
	III	4.23 ± 0.13	5.15 ± 0.24	6.50 ± 0.24	6.14 ± 0.43
Bactericidal activity of blood serum, %	C	22.3 ± 1.2	24.5 ± 2.3	33.0 ± 1.1	28.8 ± 3.6
	I	23.2 ± 2.6	31.3 ± 3.3	34.0 ± 2.3	36.5 ± 2.3
	II	24.9 ± 2.2	33.3 ± 2.8*	34.1 ± 2.4	33.5 ± 3.7
	III	23.5 ± 1.3	31.7 ± 4.4	35.3 ± 1.3	34.1 ± 1.6
Lysozymic activity of blood serum, %	C	36.3 ± 2.2	37.5 ± 1.9	40.5 ± 1.6	38.5 ± 1.3
	I	38.1 ± 2.1	42.3 ± 2.6	42.7 ± 2.0	43.5 ± 1.3
	II	35.5 ± 1.2	44.7 ± 1.2*	44.0 ± 2.1	45.0 ± 1.7*
	III	37.4 ± 1.3	44.2 ± 1.8*	44.7 ± 2.2	45.7 ± 1.9*

Note: in this and the following tables the statistically probable differences are considered in comparison with the control group: * – P < 0.05; ** – P < 0.01; *** – P < 0.001; the selections were compared within one line (taking into account Bonferroni correction).

Provision of different amounts of zinc nanoaquacitrate in the drinking water was accompanied by changes in the contents of glycoproteins and their carbohydrate components in the blood of rabbits (Table 2).

Table 2
Content of immune complexes of glycoproteins and their carbohydrate components in blood of rabbits during intake of different amounts of zinc nanoaquacitrate (M ± m, n = 4)

Parameter	Group of animals	Periods of the study: preparation, 50th day of life	experimental (day of life/day of the study) 62/12	74/24	86/36
Immuno globulins, g/L	C	11.7 ± 0.9	12.7 ± 0.5	14.9 ± 0.4	15.3 ± 0.3
	I	13.8 ± 1.0	13.4 ± 0.4	14.8 ± 0.7	15.1 ± 0.6
	II	13.7 ± 0.2	12.9 ± 0.7	17.3 ± 0.5*	18.2 ± 0.6**
	III	14.3 ± 0.2	14.2 ± 0.5	17.6 ± 0.4**	18.9 ± 0.6**
Sialic acids, conventional units	C	124 ± 2	129 ± 3	131 ± 3	134 ± 3
	I	118 ± 1	121 ± 5	140 ± 4	142 ± 4
	II	127 ± 4	121 ± 4	153 ± 3**	151 ± 4*
	III	120 ± 2	123 ± 3	166 ± 4***	162 ± 4**
Bond of hexoses bond with proteins, conventional units	C	1.30 ± 0.03	1.32 ± 0.04	1.35 ± 0.06	1.36 ± 0.05
	I	1.33 ± 0.04	1.42 ± 0.05	1.23 ± 0.03	1.25 ± 0.04
	II	1.38 ± 0.04	1.30 ± 0.04	1.21 ± 0.03	1.23 ± 0.03
	III	1.35 ± 0.06	1.35 ± 0.05	1.25 ± 0.02	1.28 ± 0.03
Ceruloplasmin, conventional units	C	417 ± 2	432 ± 10	429 ± 9	416 ± 7
	I	413 ± 2	411 ± 12	448 ± 7	433 ± 9
	II	418 ± 2	433 ± 6	460 ± 5*	445 ± 7*
	III	413 ± 3	417 ± 7	458 ± 4*	451 ± 9*

Therefore, reaction of the immune system of animals, by content of immunoglobulins, significantly did not depend on the consumed amount of organic compound of zinc at the first stage of the survey in all the experimental groups and during the study on consumption of lower amount of zinc nanoaquacitrate compared to the control group. However, intake of higher quantities of the surveyed substances of the compound of zinc had a significant effect on the content of immunoglobulins in their blood. In particular, in blood of rabbits of the II and III experimental groups, the level of total immunoglobulins was higher by respectively 16.1% and 18.1% on the 12th day and by 18.9% and 23.5% (P < 0.01–0.05) on the 36th day of the study compared with the control group. In general the obtained data indicate dose-dependent stimulating effect of zinc nanoaquacitrate on the activity of cellular and humoral links of non-specific resistance of the organism of rabbits. To a larger extent this impact manifested in the organism of rabbits in the conditions of intake of larger amounts of the supplement in the II and III experimental groups.

Similar changes were noted in the content of glycoproteins and their carbohydrate components. Therefore, in blood of rabbits of the II and III experimental groups the content of sialic acids was higher by respectively 16.7% and 26.4% on the 24th day of the study and higher by 12.7% and 21.4% on the 36th day of the experiment compared with the control. It was characteristic that the content of the studied glycoproteins at the first stage of the study and the content of hexoses manifested no probable differences between the control and the experimental groups throughout the study, whereas the level of ceruloplasmin in the blood of rabbits of the II and III experimental groups was higher respectively by 7.2% and 6.7% on the 12th day of the study and exceeded the control group by 7.1% and 8.5% on the 36th day of the experiment. Thus, the obtained results of the increase in the level of non-specific resistance and the content of monosugars of carbohydrate components of glycoproteins in the blood of rabbits may indicate activation of immuno-physiological reactivity of the organism to larger amounts of organic compound of zinc.

The studies revealed that intake of different amounts of zinc nanoaquacitrates was accompanied by changes in the hematological parameters of the blood of rabbits (Table 3). Therefore, the number of leukocytes in the blood of animals of the II experimental group was lower by 24.0% and 32.6% (P < 0.05) on 12th and 24th days of the experiment compared with the control group. In the organism of animals, the function of lymphocytes is associated with the processes of immunogenesis, and monocytes and granulocytes are classified to active phagocytes of blood. The analysis of the absolute lymphocyte count by the periods of the study revealed increase (P < 0.05) of this parameter in the end of the experiment in the III experimental group compared with the control. The number of monocytes in the blood of rabbits significantly changed as a result of intake of different amounts of zinc nanoaquacitrate – decrease in the level (P < 0.01) in the I and II experimental groups on the 24th day of the study compared with the control group. The content of granulocytes in the blood of rabbits exhibited a tendency towards increase in their number in most of the experimental groups during the study, though these changes were not probable compared with the control. The results of the study may indicate more notable positive dose-dependent impact of zinc nanoaquacitrate on non-specific factors of the protection of the organism.

Table 3
Content of leukocytes in blood of rabbits during intake of different quantities of nanoaquacitrate of zinc (M ± m, n = 4)

Parameter	Group of animals	Periods of study: preparation, 50th day of life	experimental (day of life/day of the study) 62/12	74/24	86/36
Leukocytes, 10^9/L (WBC)	C	9.4 ± 0.5	10.4 ± 1.9	9.8 ± 0.6	9.4 ± 1.6
	I	8.2 ± 0.1	9.3 ± 1.5	8.6 ± 1.5	9.2 ± 1.6
	II	9.0 ± 1.1	7.9 ± 0.6*	6.6 ± 0.7*	11.7 ± 1.0
	III	10.0 ± 0.2	10.8 ± 2.7	9.1 ± 1.8	12.2 ± 1.7
Lymphocytes, 10^9/L (LYM)	C	2.6 ± 0.9	3.1 ± 0.3	2.8 ± 0.4	2.5 ± 0.3
	I	3.1 ± 0.2	2.8 ± 0.4	2.9 ± 0.6	2.4 ± 0.2
	II	2.8 ± 0.4	2.7 ± 0.2	2.0 ± 0.3	2.1 ± 0.3
	III	3.0 ± 0.2	3.1 ± 0.4	2.4 ± 0.4	4.2 ± 0.6**
Monocytes, 10^9/L (MON)	C	1.2 ± 0.5	2.6 ± 0.9	2.0 ± 0.1	1.3 ± 0.2
	I	1.6 ± 0.1	1.4 ± 0.3	1.1 ± 0.2**	1.2 ± 0.1
	II	1.8 ± 0.3	1.0 ± 0.1	1.0 ± 0.1**	1.3 ± 0.3
	III	1.4 ± 0.9	1.6 ± 0.4	1.6 ± 0.4	1.5 ± 0.3
Granulocytes, 10^9/L (GRA)	C	4.4 ± 0.5	3.4 ± 0.6	5.0 ± 0.7	4.8 ± 0.8
	I	5.1 ± 0.9	5.1 ± 0.8	4.4 ± 0.8	5.6 ± 1.0
	II	3.4 ± 0.8	4.2 ± 0.4	3.3 ± 0.3	8.4 ± 1.6
	III	4.7 ± 0.3	3.9 ± 0.3	5.7 ± 0.6	6.6 ± 1.4

Watering the rabbits with zinc nanoaquacitrate during the study was noted with insignificant changes in the number of erythrocytes, the concentration of hemoglobin and erythrocyte indices, except hematocrit, indicating proportion of the formed elements to liquid part of blood (Table 4). Therefore the changes in the hematocrit value in blood of rabbits during the study were more or less probable compared with the control, which may indicate the impact of the used supplements on the hematocrit function of the organism of rabbits. The number of formed elements in blood is an important parameter of physiological condition of animals and their provision with nutrients and mineral substances, because the blood is the main transport system of the organism which first reacts to their deficiency or excess in their diet.

Table 4
Number of erythrocytes and erythrocyte indices in blood of rabbits during the intake of different amounts of zinc nanoaquacitrate (M ± m, n = 4)

Parameter	Group of animals	Periods of the study			
		preparation, 50th day of life	experimental (day of life/day of the study) 62/12	62/12	62/12
Erythrocytes, 10^{12}/L (RBC)	C	5.4 ± 0.4	5.2 ± 0.2	5.4 ± 0.3	5.3 ± 0.2
	I	5.6 ± 0.1	4.8 ± 0.3	5.1 ± 0.2	5.2 ± 0.3
	II	4.9 ± 0.1	5.2 ± 0.2	5.3 ± 0.2	5.0 ± 0.2
	III	5.1 ± 0.9	5.1 ± 0.3	5.0 ± 0.3	5.8 ± 0.5
Hemoglobin, g/L (HGB)	C	107 ± 2	105 ± 6	110 ± 5	114 ± 5
	I	102 ± 3	94 ± 5	101 ± 4	108 ± 2
	II	100 ± 4	109 ± 4	111 ± 2	106 ± 5
	III	103 ± 5	98 ± 5	100 ± 4	113 ± 6
Hematocrit, L/L (HCT)	C	0.4 ± 0.09	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.02
	I	0.5 ± 0.03	0.4 ± 0.02*	0.4 ± 0.01*	0.5 ± 0.01
	II	0.4 ± 0.18	0.5 ± 0.01	0.5 ± 0.01	0.4 ± 0.02*
	III	0.4 ± 0.06	0.4 ± 0.02*	0.4 ± 0.01*	0.5 ± 0.04
Mean erythrocyte volume, fL (MCV)	C	90.5 ± 0.8	87.1 ± 4.6	87.1 ± 4.6	90.6 ± 1.5
	I	89.5 ± 0.1	89.2 ± 2.9	89.4 ± 2.2	88.6 ± 2.4
	II	91.0 ± 0.1	91.6 ± 2.1	90.2 ± 2.3	91.0 ± 2.6
	III	92.0 ± 0.1	84.4 ± 3.1	87.8 ± 3.9	87.9 ± 1.8
Mean content of hemoglobin in erythrocyte, pg/g (MCH)	C	21.6 ± 0.3	19.8 ± 0.9	20.3 ± 1.1	21.3 ± 0.4
	I	20.3 ± 0.3	19.5 ± 0.6	20.3 ± 0.6	20.8 ± 0.7
	II	19.8 ± 0.6	21.1 ± 0.6	20.8 ± 0.6	21.1 ± 0,5
	III	21.0 ± 0.1	19.2 ± 0.5	20.0 ± 0.5	20.7 ± 0.2
Mean concentration of hemoglobin in erythrocyte,%(RDW)	C	10.7 ± 0.2	11.1 ± 0.7	11.5 ± 0.8	10.3 ± 0.4
	I	10.1 ± 0.6	13.2 ± 0.7*	12.2 ± 1.0	11.8 ± 1.1
	II	11.5 ± 0.5	10.4 ± 0.6	11.9 ± 0.4	10.7 ± 0.4
	III	10.0 ± 0.3	11.0 ± 0.5	11.3 ± 0.7	11.5 ± 0.4

Despite the significant role of thrombocytes in the organism of rabbits, the research on their functional condition in the period of active growth and development is substantiated insufficiently. The watering with zinc and chromium citrate showed no significant differences between the control and experimental groups (Table 5). However, the detected probable changes and tendencies of the content of the studied parameters may suggest positive impact of the employed compounds of microelements both separately and in combination on the organism of young rabbits.

Table 5
Number of thrombocytes and thrombocyte indices in blood of rabbits over the intake of different quantities of zinc citrate and chromium citrate (M ± m, n = 4)

Parameter	Group of animals	Periods of the study			
		preparation, 50th day of the study	experimental (day of life/day of the study) 62/12	62/12	62/12
Thrombocytes, 10^9/L (PLT)	C	528 ± 38	610 ± 30	577 ± 61	535 ± 11
	I	588 ± 13	775 ± 51*	565 ± 81	514 ± 34
	II	591 ± 42	655 ± 118	615 ± 149	679 ± 207
	III	590 ± 60	578 ± 134	657 ± 100	588 ± 33
Mean volume of thrombocyte, fL (MPW)	C	5.00 ± 0.06	5.47 ± 0.30	5.63 ± 0.15	5.20 ± 0.13
	I	5.23 ± 0.10	5.32 ± 0.12	5.28 ± 0.21	5.10 ± 0.08
	II	4.23 ± 0.45	4.72 ± 0.26	5.12 ± 0.23	5.00 ± 0.15
	III	4.12 ± 0.09	5.20 ± 0.21	5.08 ± 0.24	5.35 ± 0.17
Thrombocrit, % (PWD)	C	13.5 ± 0.2	14.4 ± 0.1	14.2 ± 0.7	13.7 ± 0.6
	I	14.8 ± 0.7	14.6 ± 1.0	13.6 ± 0.4	14.1 ± 0.7
	II	13.5 ± 0.1	14.2 ± 0.2	13.8 ± 0.7	14.2 ± 0.3
	III	14.6 ± 0.3	13.3 ± 0.4	13.7 ± 0.5	15.1 ± 0.8
Thrombocyte distribution width in the volume, % (PCT)	C	0.29 ± 0.02	0.38 ± 0.01	0.33 ± 0.04	0.28 ± 0.01
	I	0.30 ± 0.05	0.36 ± 0.05	0.32 ± 0.01	0.26 ± 0.01
	II	0.31 ± 0.02	0.32 ± 0.06	0.36 ± 0.02	0.35 ± 0.11
	III	0.33 ± 0.08	0.35 ± 0.05	0.30 ± 0.02	0.31 ± 0.03

Despite the variability in hematological parameters in rabbits of modern industrial breeds depending on the breed and individual features, the indices of erythrocytes, leukocytes and thrombocytes were within the physiological parameters, indicating positive impact on the hematopoietic system of their organism.

Discussion

Factors of the natural resistance and specific immunity are known to underlie the protection of the organisms of humans and animals against pathogenic factors of biotic and abiotic nature (Boyko et al., 2016; Khariv et al., 2017; Kysera et al., 2018; Palchykov et al., 2019). Among the first factors, the main role belongs to the cellular mechanisms which have abilities to bind and absorb cellular or other foreign particles and digest them (Russell et al., 2016). The data in Table 1 demonstrate that in the animals of the control group the phagocytic activity of neutrophils in blood did not change throughout the experiment, indicating the development of this mechanism of natural protection on earlier stages of post-natal ontogenesis. This can be due to early inhabitation of the peripheral immunocompetent organs and tissues by cells with protective abilities and compensatory property of the immune system of rabbits to respond to the decrease in humoral factors of protection (Darmohray et al., 2019). At the same time, phagocytic index which characterizes the number of the microorganisms occupied by one active phagocyte and phagocytic number in rabbits of the control group with age had a tendency towards insignificant increase, compared with the preparation period. According to modern interpretations, phagocytosis is one of the most important factors of the structural and immune homeostasis, orientated to maintaining constant internal environment of the organism. This is an integral process which combines different cellular reactions in the direction of identification, neutralization and removal of the foreign agents from the organism (Wang et al., 2010). The results obtained from the research indicate that provision of rabbits with larger amounts of organic compound of zinc in their liquid diet positively affected the functional ability of phagocytic cells. To a larger extent, this effect manifested in rabbits of the II and III experimental groups on the 24th and 36th days of the study. This may suggest positive influence of the used quantities of zinc nanoaquacitrate on the immunobiological ability of the organism of rabbits, which is more notable during its prolonged use .

The conducted studies of the parameters of the humoral link of natural mechanisms of the protection of the organism revealed that in the rabbits of the control group the highest level of bactericidal and lysozymic activity of blood serum was recorded on the 74th day, indicating later development of humoral factors of natural resistence of the organism, compared to clinical mechanisms of the protection. Providing rabbits of the experimental groups with water with different amounts of zinc nanoaqucitrate had an effect on the activity of humoral factors of non-specific resistance of their organism. Therefore, on the 12th day of the study, there was seen a probably higher bactericidal activity of blood serum of rabbits of the II experimental group compared with the control. At the same time, a slightly higher effect was observed regarding lysozymic activity of blood serum, the level of which in the rabbits of the I and II experimental groups on the 12th day and II and III groups on the 36th day of the study probably exceeded the control. This could indicate dose-dependent impact of the additive on the peculiarities of the action of organic compound of zinc in the organism of rabbits at the first stage of the study and during long intake of zinc nanoaqucitrate. This is also indicated by probable differences of lysozymic activity of blood serum of rabbits of this group on the 62nd and 86th day of life.

Glycoproteins are an integral part of the immune system and their concentration in blood and ratio of separate monosugars changes in the course of the individual development of the organism (Borysevych et al., 2010). Analysis of the obtained results of the content of glycoproteins in blood of rabbits shows certain peculiarities of the impact of zinc nanoaquacitrate depending on its amount in the diet. Ingestion of different amounts of zinc nanoaquacitrate caused probable increase ($P < 0.05$) in the amount of total immunoglobulins, contents of sialic acids and ceruloplasmin in the blood of rabbits receiving 0.50 and 0.75 mg of Zn/kg of body weight on the 74th and 86th days of life compared with the control group. This indicates higher immunophysiological reaction – response of the organism to the action of organic compound of zinc in these concentrations by the fractions of globulins which contain monosugars in glycoproteins of blood. Sialic acids play an important role in the regulation of the immune response, functioning as markers of proteins of the organism, which makes these substances different from the foreign antibodies. They significantly contribute to the surface charge of molecules of glycoproteins and determine their resistance to the action of proteolytic enzymes, affect the immune-chemical properties, function as chemical mediators, regulating the functions of transmembrane receptors (Han et al., 2014). Ceruplasmin in the organism of animals is associated with the process of haematopoiesis and oxidative-response reactions, its high concentration in the blood of rabbits in the conditions of longer intake indicates active role of zinc nanoaquacitrate in the processes of haematopoiesis, more notable in the rabbits of the II and III experimental groups which received larger amounts of the supplement in the diet.

The provision of rabbits during the experiment with different amounts of the organic compound of zinc was accompanied by changes in hematological parameters in the animals of the experimental groups compared with the control, which, depending on the amount of the compound, was within upper or lower physiologic values. Tendencies towards changes in erythrocytes and erythrocyte indices can indicate more notable dose-dependent influence of the organic compound of zinc on hematopoietic function of the organism of rabbits during long term intake of the supplement. The same tendencies were seen in the changes of the content of hemoglobin, notable during watering with higher amounts of zinc.

In the organism of animals, the function of lymphocytes is known to be associated with the processes of immunogenesis, and monocytes and granulocytes are identified to active phagocytes of blood (Khariv et al., 2017; Kisera et al., 2018, 2019). Analysis of the absolute leukocyte count and monocytes in the blood of rabbits demonstrated the tendency towards change in their number in the experimental groups compared with the control. The results of the study may indicate more notable positive dose-dependent impact of zinc nanoaquacitrate on the factors of the organism`s protection. It should be noted that all the changes in the parameters of leukocytes of rabbits were within the physiologic values, suggesting stimulating impact of the organic compound of zinc on the main populations of leukocytes and haematopoiesis.

In the organism of mammals, thrombocytes play an important role at the physiological norm. They constantly circulate in blood and support the normal structure and the function of the vessels, participate in the coagulation processes. Impairment of one of these functions leads to changes in the system of hemostasis in the organism in general (Gutyj et al., 2018; Kovalenko et al., 2020). Thrombocytes play a significant part in the resistance, because they are the first to react to the infectious agents, forming specific antibodies which attach to the surface of antigen, forming a complex "antigen-antibody" which activates the response to inflammation. Thrombocytes have receptors which identify these complexes, i.e. particularly thrombocytes, and not leukocytes, react the first to infection, higher dose-dependent amounts of thrombocytes may indicate indirect influence of the organic compound of zinc depending on the amount employed.

Conclusion

Adding zinc nanoaquacitrate in the amounts of 0.50 and 0.75 mg of Zn/kg of body weight to the diet of rabbits led to physiological impact on the factors of cellular and humoral resistance in the blood of rabbits of the II and III experimental groups, manifesting in increase in the relative content of phagocytic activity and lyzocymic activity of blood serum during the experiment and bactericidial activity in the blood serum in the II experimental group on the 12th day of the study compared with the control group of animals.

Watering the young rabbits after the weaning with zinc nanoaquacitrate in the amounts of 0.50 and 0.75 mg of Zn/kg of body weight caused a reliably higher level of total immunoglobulins, contents of sialic acids, hexoses' bond to proteins and ceruloplasmin on the 24th and 36th days of the survey compared with the control.

Intake of different amounts of zinc nanoaquacitrate underlay the stimulating effect on hematological parameters of the organism of rabbits of the experimental groups, which was seen in the changes of erythrocytes and thrombocytes and their indices compared with the control.

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