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

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Introduction

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 zinc/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-age-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.

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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; Alkove 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 80th 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 scientific purposes (Official Journal of the European Union L276/33, 2010). The research was performed on young rabbits of de Termede 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 groups, which received 0.50 and 0.75 mg of Zn/kg of body weight. Solution of nanoaquacitrate zinc 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 No 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 photometric colorimetry method (on PEC-56 photometric colorimeter, Russian ФЕК М-540 nm) in relation to microbial test culture E. coli (strain BKM-125). Lysosomal 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. Transparencies of the initial suspension was 20% (Vlisco 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 lg, as a result of which the solution becomes less transparent, and the intensity of opacification is proportional to lg 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 (Vlisco 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 Cu²⁺ ions. 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 (Anassashvili, 1968). Blood for hematologic assay was taken into test tubes which contained dipotassium salt of ethylenediamine - tetraacetic acid (EDTA-K²⁺) 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, 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 bacterial 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 to the control group. Changes in the level of lysosomal 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-specific 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 preparation, 50th day of life (animal) | Periods of the study | Periods of the study |
|------------|-----------------------------------------------|----------------------|----------------------|
|            | preperation, 50th day of life | 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 |
| Phagocytic index, units | C | 10.4 ± 0.2 | 10.1 ± 0.3 | 13.1 ± 0.6 | 13.3 ± 0.3 |

**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).

The studies revealed that intake of different amounts of zinc nanoaquacitrate was accompanied by changes in the hematological parameters of the blood of rabbits (Table 3). Therefore, the number of leucocytes 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) in 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 leucocytes in blood of rabbits during intake of different quantities of nanoaquacitrate of zinc (M ± m, n = 4)

| Parameter | Group of preparation, 50th day of life | Periods of the study | Periods of the study |
|-----------|-----------------------------------------------|----------------------|----------------------|
|           | preperation, 50th day of life | experimental (day of life/day of the study) | 62/12 | 74/24 | 86/36 |
| Lymphocytes, 10^9/L (WBC) | C | 8.2 ± 0.1 | 9.3 ± 1.5 | 8.6 ± 1.5 | 9.2 ± 1.6 |
| Leukocytes, 10^7/L (LYM) | C | 3.1 ± 0.2 | 2.8 ± 0.4 | 29.6 ± 0.6 | 24.2 ± 0.4 |
| Monocytes, 10^7/L (MON) | C | 1.6 ± 0.1 | 1.4 ± 0.3 | 1.1 ± 0.2** | 1.2 ± 0.1 |
| Granulocytes, 10^7/L (GRA) | C | 4.4 ± 0.5 | 3.4 ± 0.6 | 50.0 ± 0.7 | 48.0 ± 0.8 |

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.

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...
rabbis 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.

Despite the variability in hematological parameters in rabbits of modern industrial breeds depending on the breed and individual features, the indices of erythrocytes, leucocytes 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; Khairiv et al., 2017; Kysens et al., 2018; Palchikov 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 (Russel et al., 2010). 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 activities and compensatory property of the immune system of rabbits to respond to the decrease in humoral factors of protection (Darmohry 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 resistance 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 life of zinc nanoaquacitrate. 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 ceruloplasm 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

### Table 4

| Parameter | Group of animals | Preparation, 50th day of life | Experimental, 50th day of life | Periods of the study |
|-----------|------------------|-------------------------------|-------------------------------|---------------------|
| Hemoglobin, g/L (HGB) | C | 107 ± 0.1 | 105 ± 0.1 | 116 ± 0.5 |
| | I | 102 ± 0.3 | 94 ± 0.5 | 101 ± 0.4 |
| | II | 100 ± 4.3 | 106 ± 1.1 | 102 ± 0.5 |
| | III | 100 ± 5 | 105 ± 5 | 113 ± 3 |

**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; Khairiv et al., 2017; Kysens et al., 2018; Palchikov 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., 2010). 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 activities and compensatory property of the immune system of rabbits to respond to the decrease in humoral factors of protection (Darmohry 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 resistance of the organism, compared to clinical mechanisms of the protection. Providing rabbits of the experimental groups with water with different amounts of zinc nanoaquacitrate 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 life of zinc nanoaquacitrate. 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 ceruloplasm 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 to 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). Ceruloplasmin in the organism of animals is associated with the process of hematopoiesis and oxidative-responses 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 hematopoiesis, 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 hematopoietic 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 time 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; Kisersa et al., 2018, 2019). Analysis of the absolute leukocyte count and monocytes in the blood of rabbits demonstrated the tendency towards change in 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 hemapoiesis.

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 (Gutj 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 bactericidal 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 hematopoietic 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.

References

Alkawe, P. C. N., Ojiozeh, T. I., & Olagbeye, S. A. (2011). Effects of zinc supplement on rabbits performance and growth rate. Journal of Agriculture and Social Research, 11(2), 46–50.

Amen, M. H. M., & Daraji, H. J. A. (2011). Influence of dietary supplementation with zinc on sex hormones concentrations of broiler breeder chickens. Pakistan Journal of Nutrition, 10(11), 1089–1093.

Ametaasvili, A. C. (1968). Glikoproteidy syvorotki krovi i mochi [Serum and urinary glycoproteins]. Medicina, Moscow (in Russian).

Borysevych, V. B., Kaplanenko, V. G., & Kosinov, M. V. (2010). Nanomaterialy v biokhimi. Osnovy nanoveterinarii [Nanostructures in biology. Fundamentals of nanoveterinary]. Avienniu, Kyiv (in Ukrainian).

Boyko, O. O., Zazhynska, N. M., & Bryadytenko, V. V. (2016). The influence of the extent of infestation by helminths upon changes in body weight of sheep in Ukraine. Visnyk of Dniproprovsk University, Biology, 24(1), 3–7.

Case, C. L., & Carlson, M. S. (2002). Effect of feeding organic and inorganic sources of additional zinc on growth performance and zinc balance in nursery pigs. Journal of Animal Science, 80(7), 1917–1924.

Chekman, I. S. (2011). Nanofarmacology [Nanopharmacology]. Zadruga, Kyiv (in Ukrainian).

Darmohry, L. M., Luchyn, I. S., Gutj, B. V., Golovakh, P. I., Zhelevska, M. S., Paskevych, G. A., & Vishchur, V. Y. (2019). Trace elements transformation in young rabbit muscles. Ukrainian Journal of Ecology, 9(4), 616–621.

Downs, K. M., Hess, B. J., Macdlin, K. S., & Norton, R. A. (2000). Dietary zinc complexes and vitamin E for reducing celluctis incidence in broilers. Journal of Applied Poultry Research, 9(3), 319–323.

Ferreira, W. M., Cavalcante, S. G., Naranjo, A. P., & Santiago, G. S. (2002). Bioavailability of different zinc sources for rabbits. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia, 54(3), 636–642.

Gaither, L. A., & Eid, D. J. (2001). Eukaryotic zinc transporters and their regulation. BioMetals, 14(1), 251–270.

Grosskopf, H. M., Schwartz, C. I., Machado, G., Bottari, N. B., da Silva, E. S., Gabriele, M. E., Lucca, N. J., Alves, M. S., Schettinger, M. R. C., Morsch, V. M., Mendes, R. E., & da Silva, A. S. (2017). Cattle naturally infected by Eurytrema coomum: Relation between adenosine deaminase activity and zinc levels. Research in Veterinary Science, 110, 79–84.

Gutj, B., Grymak, Y., Hunchak, V., Mysyk, A., Nazaren, N., Brezyn, O., Hariv, I., Shecherbutyk, A., Semeniv, B., Buzheva, I., Parchenko, V., & Kaplunchenko, A. (2018). Preclinical searches of the preparation Tamirognalne. Ukrainian Journal of Ecology, 8(1), 688–695.

Hahn, J. D., & Baker, D. H. (1993). Growth and plasma zinc responses of young pigs fed pharmacologic levels of zinc. Journal of Animal Science, 71(11), 3020–3024.

Han, X. Y., Ma, Y. F., Lv, M. Y., Wu, Z. P., & Qian, L. C. (2014). Chitosan-zinc chelate improves intestinal structure and mucosal function and decreases apoptosis in ileal mucosal epithelial cells in weaned pigs. British Journal of Nutrition, 111(8), 1405–1411.

Hendry, H. A. E., Yousaf, M. I., & El-Naga, N. I. A. (2001). Effect of dietary zinc deficiency on hematological and biochemical parameters and concentrations of zinc, copper, and iron in growing rats. Toxicology, 167(2), 163–170.

Khariv, M., Gutj, B., Ohorodnyk, N., Vishchur, O., Khariv, I., Solovodzinska, I., Mudrak, D., Grymak, C., & Bodnar, P. (2017). Activity of the T- and B-system of the cell immunity of animals under conditions of oxidation stress and effects of the liposomal drug. Ukrainian Journal of Ecology, 7(4), 536–541.

Khariv, M., Gutj, B., Ohorodnyk, N., Vishchur, O., Khariv, I., Solovodzinska, I., Mudrak, D., Grymak, C., & Bodnar, P. (2017). Activity of the T- and B-system of the cell immunity of animals under conditions of oxidation stress and effects of the liposomal drug. Ukrainian Journal of Ecology, 7(4), 536–541.

Kisersa, Y. V., Storechuk, Y. V., Gutj, B. V., Bouthyk, L. Y., Mageilo, N., Sai, Y., Dashkovsky, O., Bouthyk, L. Y., Vas, U., Kh, L., & Sachuk, R. (2019). Structural and functional features of the vermiciform appendix at the tissue and cellular levels in rabbits after the introduction of immunobiological drugs. Ukrainian Journal of Ecology, 9(2), 217–226.

Kovalenko, A. M., Tkach, A. V., Tkacheva, O. L., Gutj, B. V., Prystoga, O. I., Kukhtyn, M. D., Dutka, V. R., Veres, V. M., Dashkovsky, O. G., Smelchan, V. V., Ryz, M. B., & Kotelevych, V. A. (2020). Analgetic effectiveness of new nanosilver drug. Ukrainian Journal of Ecology, 10(1), 300–306.

Kysers, Y. V., Storechuk, Y. V., & Gutj, B. V. (2018). Experimental study of immunoprophylactic anti-pneumococcal medicine and its immunogenic properties. Ukrainian Journal of Ecology, 8(1), 307–316.

Lesyk, Y. V. (2013). Resistance of the rabbit dams’ organism at drinking supplemented with Chlorella suspension, sodium sulfate, chromium chloride and citrate. The Animal Biology, 15(2), 90–96.
Mateos, G. G., Rebollar, P. G., & de Blas, C. (2010). Minerals, vitamins and additives. The nutrition of the rabbit. CABI Publishing, Wallingford. Pp. 119–150.

Nessrin, S., Abdel-Khalek, A. M., & Gad, S. M. (2012). Effect of supplemental zinc, magnesium or iron on performance and some physiological traits of growing rabbits. Asian Journal of Poultry Science, 6(1), 23–30.

Palchykov, V. A., Zazharskiy, V. V., Brygadyrenko, V. V., Davydenko, P. O., Kulishenko, O. M., Borovik, I. V., Chumak, V., Kryvaya, A., & Boyko, O. O. (2019). Bactericidal, protistocidal, nematodicidal properties and chemical composition of ethanol extract of *Punica granatum* peel. Biosystems Diversity, 27(3), 300–306.

Russell, R. F., McDonald, J. U., Lambert, L., & Tregoning, J. S. (2016). Use of the microparticlenanoscale silicon dioxide as an adjuvant to boost vaccine immune responses against influenza virus in neonatal mice. Journal of Virology, 90(9), 4735–4744.

Vlislo, V. V. (2012). Laboratorni metody doslidzhen u biolohii, tvarynnytstvi ta veterynarnyi medytsyni [Laboratory methods of research in biology, animal husbandry and veterinary medicine]. Spolom, Lviv (in Ukrainian).

Wang, K. K., Cui, H. W., Sun, J. Y., Qian, L. C., & Weng, X. (2012). Effects of zinc on growth performance and biochemical parameters of piglets. Turkish Journal of Veterinary and Animal Sciences, 36(5), 519–526.

Wang, Y., Tang, J. W., Ma, W. Q., Feng, J., & Feng, J. (2010). Dietary zinc glycine chelate on growth performance, tissue mineral concentrations, and serum enzyme activity in weanling piglets. Biological Trace Element Research, 133(3), 325–334.