



Hematopoiesis, biochemical and antioxidant parameters of blood of pigs consuming organic compounds of trace elements (Cu, Mn, Zn, Fe) in mineral feed supplement

V. Vlizlo*, D. Ostapiv**

**Institute of Agriculture of Carpathian Region the National Academy of Agrarian Sciences of Ukraine, Lviv Region, Ukraine*

***Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine, Lviv, Ukraine*

Article info

Received 10.08.2025

Received in revised form

17.09.2025

Accepted 14.10.2025

*Institute of agriculture
of Carpathian Region
the National Academy
of Agrarian Sciences
of Ukraine,
Hrushevs'koho st., 5,
Obroshyne, Lviv District,
Lviv Region, 81115,
Ukraine. Tel.: +38-093-401-
4633.
E-mail: vliizlovolodymyr
@gmail.com*

*Institute of Animal Biology
of the National Academy
of Agrarian Sciences
of Ukraine, V. Sus st., 38,
Lviv, 79034, Ukraine.
Tel.: +38-063-267-65-21.
E-mail: oddost@ukr.net*

Vlizlo, V., & Ostapiv, D. (2025). Hematopoiesis, biochemical and antioxidant parameters of blood of pigs consuming organic compounds of trace elements (Cu, Mn, Zn, Fe) in mineral feed supplement. *Regulatory Mechanisms in Biosystems*, 16(4), e25203. doi:10.15421/0225203

Development of mineral supplements containing necessary trace elements is essential for swine farming. Therefore, the objective of our study was to analyze the peculiarities of the influence of organic compounds of trace elements (copper, manganese, zinc, iron) on hematopoiesis, biochemical and antioxidant blood parameters of pigs. The experiment was conducted on three groups of pigs – one control and two experimental. The control group received a feed supplement with inorganic sulfate forms of trace elements (Cu, Mn, Zn, Fe). The pigs of the experimental groups – instead of inorganic salts of trace elements – were given a newly developed organic complex of trace elements (Cu, Mn, Zn, Fe) bonded using N-PEGylated glutamic acid as their feed supplement. The content of trace elements in the feed supplement of the experimental groups was lower than that in the control group. In particular, the concentrations of Cu, Mn, Zn, and Fe in Group 1 were lower by 51%, 42%, 20%, and 36%, respectively, whereas Cu, Mn, Zn, and Fe in Group 2 were lower by 48%, 39%, 17%, and 33%, respectively. Despite the low – compared with control group – contents of copper, manganese, zinc, and iron in organic form, the experimental animals were found to have improved hematopoiesis, metabolism, and antioxidant protection. The formation of erythrocytes and hemoglobin in the experimental pigs was stable, or even higher. The contents of total protein and its fraction, glucose, and total cholesterol in blood serum of the animals were at the physiological level throughout the intake of organic trace elements. The activities of the enzymes aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transferase in blood serum were within the normal range and remained largely consistent across the three groups. The activity of the antioxidant-defense enzymes (superoxide dismutase, glutathione peroxidase, and catalase) in blood of the pigs that received the trace elements in organic form were stable throughout the experiment and were consistent with the formation of lipid peroxidation products (malondialdehyde). In the future, it would be practical to determine the effects of the complex of trace elements with N-PEGylated glutamic acid on the state of metabolism in the muscular tissue, productive parameters, and release of the elements with feces.

Keywords: organic trace minerals; N-PEGylated glutamic acid; metabolism; pigs; mineral supplement.

Introduction

Copper (Cu), manganese (Mn), zinc (Zn), and iron (Fe) are vitally essential (biogenic) trace elements. They play an important role in the organism of animals, affect the hematopoiesis functions, endocrine glands, defensive reactions of the organism, microflora of the digestive tract, and partake in metabolism and other processes (Levchenko & Vlizlo, 2019; Shannon, & Hill, 2019; Corbett, 2023). The optimal contents and ratio of biogenic trace elements in the organism are the basis for the physiological course of metabolism and health of animals. In particular, copper and zinc are present in enzymes and proteins. Being components of antioxidant defense and immunity, they affect the growth and development of animals and enhance the organism's resistance to diseases (Maywald et al., 2017; Curnock, & Cullen, 2020). Manganese is important for bone growth and functioning of the reproductive organs. It is present in enzymes, in particular antioxidant-status enzymes, and is involved in metabolism of carbohydrates, lipids, and proteins (Broom et al., 2021). Iron is an important mineral compound necessary for synthesis of hemoglobin and myoglobin. Deficiency of this element in animals leads to iron deficiency anemia (Gozzelino, & Arosio, 2016). The animals' need in trace elements depends on age, growth and development intensity, productivity, metabolic state, and health (Goff, 2018; Palomares, 2022). Deficiency of several or even one of them can impair organ and system functions, leading to disease in animals (Silva et al., 2022; Daniel et al., 2023; Gul et al., 2024).

The main source of trace elements for animals is feeds. However, providing productive animals with trace elements using only fodders is insufficient. Therefore, additionally with the diets, they age given premixes, or feed supplements that contain various trace elements.

The biological availability of mineral compounds for animal organism varies because their absorption is closely associated with the chemical structure of the premixes and technological processes of their preparation. In animal farming, mineral feed additives are predominantly inorganic salts of macro- and microelements. Studies have confirmed a number of their disadvantages during storage and use, problems with their metabolism, and toxicity risks (Wang et al., 2019; Sun et al., 2020). Due to poor metabolism of inorganic trace element salts in animals, they are excreted in large amounts with feces and used as organic fertilizer, thereby contributing to environmental pollution (Liu et al., 2016; Qian et al., 2018; Ma et al., 2020).

As of now, various compounds that are able to form complexes with medical agents and biologically active compounds are created synthetically to improve the transport and biocompatibility in the animal organism (Iskra et al., 2017; Vlizlo et al., 2023; Kozak et al., 2025). In particular, there is a search for new forms and compounds of trace elements of organic origin, which can fulfill the animals' needs in small amounts (Shannon & Hill, 2019; Zhang et al., 2021). Organic forms of trace elements are more effective and are recommended as replacements of inorganic salts of trace elements. Organic compounds of minerals are created by binding them to amino acids, peptides, polysaccharides, and organic acids (Byrne, & Murphy, 2022; Xu et al., 2024). In particular, synthesized pseudo amino acids are non-toxic and bind effectively to the organism's biological transport systems as they reach cells in various organs (Varvarenko et al., 2015; Chekh et al., 2017; Stasiuk et al., 2022). Polymers created based on pseudo amino acids (polyoxyethylene derivatives of glutamic acid) can be successfully used as carriers for medical agents (Numata, 2015; Boddu et al., 2021; Fihurka et al., 2024), including mineral compounds. However, novel synthesized forms of organic compounds of

mineral elements require deep and multifaceted research of their chemical and physical properties, bioavailability for the organism, and also the effectiveness for animals in doses lower than those of inorganic salts of trace elements.

The objective of our study was to determine the peculiarities of the influence of organic compounds of trace elements (copper, manganese, zinc, and iron) in the feed supplement on hematopoiesis and biochemical and antioxidant parameters of blood of pigs during feeding by comparing them with inorganic salts of trace elements.

Materials and methods

Maintenance, feeding, monitoring, and all the procedures with the animals were carried out according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986) and the General Ethical Principles of Experiments on Animals, adopted by the First National Congress of Bioethics (Kyiv, 2001). The experiments were conducted adhering to the principles of humanity established in the Directive of the European community.

At the Lviv Polytechnic National University, we synthesized a transporter polymer using N-PEGylated glutamic acid (Glu), dextrin, and polyethylene glycol 400 (PEG400) – mPEG400Glu (Varvarenko et al., 2013). To bind the transporter polymer to mineral compounds, we prepared an aqueous solution of salts of trace elements (MEs) – $\text{CuSO}_4 \times 2\text{H}_2\text{O}$, $\text{MnSO}_4 \times 5\text{H}_2\text{O}$, $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$, and $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \times 6\text{H}_2\text{O}$. The concentration of solutions of trace elements was achieved within a range of 1–2% by adding a solution of sodium hydroxide in a 1:1.05 ratio. As a result, we obtained a sediment of metal hydroxides. This sediment was isolated by centrifuging, and the solution was decanted and rinsed in distilled water until pH of 7.

Then, we mixed a 15–25% solution of transporter polymer with aqueous solution of metal hydroxides at a molar ratio of 0.9 to 1 mol and mixed it for 1 hour at room temperature (Oleksa et al., 2018). Thus, we obtained a new complex preparation of organic forms of trace elements (Cu^{2+} , Mn^{2+} , Zn^{2+} , $\text{Fe}^{2+/3+}$) with transporter polymer based on N-PEGylated glutamic acid (MEMPEG400Glu). Using atomic absorption spectrophotometer, we determined that one gram of the new preparation contained 0.0222 mmol of copper (Cu^{2+}), 0.0359 mmol of manganese (Mn^{2+}), 0.0319 mmol of zinc (Zn^{2+}), and $0.03 \div 0.05$ mmol of iron ($\text{Fe}^{2+/3+}$). To prepare the microelement feed supplement, the MEMPEG400Glu preparation was applied to mixed feed.

To conduct the experiment at the farm, we formed three groups of hybrid F-4 pigs according to the analogue principle, one control and two experimental, each containing 10 animals. The pigs were being fattened and were 75 days old. The control and experimental animals received complete feed from a mixed-feed factory with addition of BMD (4542) Premix Maxcare Fattening X(Trouw Nutrition Ltd., Ukraine). Control Group was fed a diet supplemented with a mineral feed containing inorganic sulfate forms of trace elements. The contents of salts of trace elements in the diet of Control Group were as follows: Cu^{2+} – 17.6, Mn^{2+} – 47.1, Zn^{2+} – 100.1, and $\text{Fe}^{2+/3+}$ – 148.0 mg/kg. The pigs of the experimental groups received the same diet, but the mixed feed had been deprived of inorganic salts of trace elements (Cu, Mn, Zn, and Fe). In their stead, the diet was supplemented with the newly developed feed additive containing the complex of trace elements (Cu^{+2} , Mn^{+2} , Zn^{+2} , $\text{Fe}^{+2/+3}$) bond to N-PEGylated glutamic acid. The diet of Group 1 was supplemented with the trace elements in the following amounts: Cu^{2+} – 8.7, Mn^{2+} – 27.3, Zn^{2+} – 79.8, and $\text{Fe}^{2+/3+}$ – 95.4 mg/kg. The concentrations of the trace elements given to Group 1 were reduced compared with Control, in particular: Cu^{+2} to 49%, Mn^{+2} to 58%, Zn^{+2} to 80%, and $\text{Fe}^{+2/+3}$ to 64%. Group 2 received the trace elements in the following concentrations: Cu^{2+} – 9.1, Mn^{2+} – 28.7, Zn^{2+} – 83.6, and $\text{Fe}^{2+/3+}$ – 99.0 mg/kg of the new premix, or 52%, 61%, 83%, and 67%, respectively, of the amounts given to control group.

Blood samples for the laboratory studies were collected from the cranial vena cava of the pigs in the morning prior to feeding. These

samples were gathered prior to the experiment (pigs at 75 days of age), on day 30 (105 days of age), and day 90 (165 days of age) of intake of the trace-element feed supplements. To obtain whole blood and plasma, the blood was gathered into special test tubes with anticoagulant, and to obtain blood serum it was collected into test tubes without anticoagulant. The concentrations of red blood cells and hemoglobin in whole blood were determined on a Mindray BC-30 Vet hematological analyzer (Japan, 2020). The biochemical studies were conducted on a BS-120 analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China, 2017) using the PZ Cormay S.A. (Poland) reagents. In the blood serum, we measured the concentrations of total protein, glucose, total cholesterol, the activities of aspartate transaminase (AST, EC 2.6.1.1), alanine transaminase (ALT, EC 2.6.1.2), and gamma-glutamyltransferase (GGT; EC 2.3.2.2). Using electrophoresis in polyacrylic gel, we examined fractions of proteins in blood serum (albumin and α -globulins, β -globulins, and γ -globulins).

In the pigs' blood plasma, we measured the concentrations of malondialdehyde (TBA-active products) using reaction with thiobarbituric acid. In whole blood, we determined the activity of superoxide dismutase (SOD; EC 1.15.1.1) using the method based on the reduction of nitrotetrazolium by superoxide radicals; the activity of catalase (CAT; EC 1.11.1.6) was measured based on the reduction of color intensity of the complex formed between H_2O_2 and molybdenum salts; and the activity of glutathione peroxidase (GPx; EC 1.11.1.9) was identified according to the rate of GSH oxidation prior and after incubation with tert-butyl hydroperoxide (Vlizlo et al., 2012).

The blood parameters were statistically analyzed using Statistica 7 (StatSoft Inc., USA). The article presents the mean arithmetic values and standard deviation $\bar{x} \pm \text{SD}$ (mean \pm standard deviation), presented in the figures. To compare the differences between the mean parameters of the clinically healthy and sick animals, we used the Tukey Test, where the differences were considered statistically significant at $P < 0.05$.

Results

According to the analyses of the pigs' blood before the experiment, the red blood cell count and hemoglobin content exhibited no significant variations across the control and experimental groups (Table 1). On days 30 and 90 of intake of the traditional feed additive with inorganic forms of trace elements by Control Group and the newly prepared supplement with organic trace elements by the experimental groups, the RBC count in blood was increased. This was more pronounced in the experimental groups. In particular, in Group 1, the increase in the RBC count was 23.2% on day 30 and 46.4% ($P < 0.05$) on day 90, whereas in group 2 it was 27.1% ($P < 0.01$) on day 30 and 38.6% ($P < 0.01$) on day 90, compared with the values at the beginning of the experiment. Compared with control group, the RBC count in both experimental groups did not vary significantly.

Changes similar to those in the RBC count were observed in the hemoglobin content of the pigs' blood (Table 1). Thus, on days 30 and 90 of intake of the trace-element feed supplements, we observed increases in all the groups, measuring 9.2% and 13.2% in control group, 2.9% and 23.3% in Group 1, and 8.8% and 16.6% ($P < 0.05$) in Group 2, respectively. The differences in hemoglobin content in blood of the pigs between the control and experimental groups on day 30 showed only an upward tendency in the experimental animals, and on day 90 the changes were significant, with the increases measuring 19.6% ($P < 0.05$) in Group 1 and 13.8% ($P < 0.01$) in Group 2.

At the beginning of the studies, and also on days 30 and 90 of intake of the feed supplements, the concentrations of total protein in blood of the pigs of the control and experimental groups did not differ significantly (Table 2). Increases in the concentration of total protein in blood serum of the animals of Control and Group 1 and a decrease in Group 2, which we observed on day 30 of the experiment, were insignificant. On day 90, the concentration of total protein insignificantly increased in all the groups and was largely consistent across the control and experimental pigs.

Table 1Number of red blood cells and concentration of hemoglobin in blood of the pigs of the control and experimental groups ($x \pm SD$, $n = 10$)

Blood parameters	Periods of study	Groups of animals		
		control group (inorganic salts of trace elements)	group 1 (organic salts of trace elements in lower doses)	group 2 (organic salts of trace elements in higher doses)
Red blood cells, $\times 10^{12}/L$	before the experiment	7.50 \pm 1.28 ^a	6.91 \pm 0.68 ^a	7.04 \pm 0.33 ^a
	day 30 of intake of trace-element feed supplements	8.62 \pm 0.38 ^a	8.58 \pm 0.41 ^a	8.93 \pm 0.17 ^b
	day 90 of intake of trace-element feed supplements	9.95 \pm 0.12 ^a	10.13 \pm 0.31 ^b	9.75 \pm 0.40 ^b
Hemo-globin, g/L	before the experiment	124.7 \pm 6.0 ^a	136.8 \pm 16.0 ^a	137.6 \pm 4.3 ^a
	day 30 of intake of trace-element feed supplements	136.2 \pm 6.4 ^a	140.7 \pm 10.9 ^a	149.7 \pm 8.7 ^a
	day 90 of intake of trace-element feed supplements	141.2 \pm 2.5 ^a	168.8 \pm 6.7 ^c	160.6 \pm 2.8 ^{bc}

Note: different letters indicate values which reliably differ one from another within one line of the table according to the results of comparison using the Tukey Test with Bonferroni correction.

Table 2Concentrations of total protein and protein fractions in blood serum of the pigs of the control and experimental groups ($x \pm SD$, $n = 10$)

Blood parameters	Periods of study	Groups of animals		
		control group (inorganic salts of trace elements)	group 1 (organic salts of trace elements in lower doses)	group 2 (organic salts of trace elements in higher doses)
Total protein, g/L	before the experiment	63.1 \pm 1.3 ^a	62.1 \pm 2.8 ^a	68.6 \pm 7.6 ^a
	day 30 of intake of trace-element feed supplements	70.4 \pm 4.1 ^a	64.6 \pm 2.3 ^a	64.1 \pm 2.1 ^a
	day 90 of intake of trace-element feed supplements	75.3 \pm 1.4 ^a	78.0 \pm 1.2 ^a	76.1 \pm 2.7 ^a
γ -Globulin, %	before the experiment	25.9 \pm 1.1 ^a	27.3 \pm 0.3 ^a	27.2 \pm 1.8 ^a
	day 30 of intake of trace-element feed supplements	21.5 \pm 5.4 ^a	25.5 \pm 4.8 ^a	31.4 \pm 3.2 ^a
	day 90 of intake of trace-element feed supplements	29.5 \pm 4.1 ^a	31.2 \pm 2.4 ^a	33.2 \pm 0.6 ^a
β -Globulin, %	before the experiment	10.33 \pm 1.91 ^a	7.78 \pm 0.59 ^a	8.07 \pm 1.83 ^a
	day 30 of intake of trace-element feed supplements	10.02 \pm 1.84 ^a	8.67 \pm 1.28 ^a	27.2 \pm 1.82 ^a
	day 90 of intake of trace-element feed supplements	14.12 \pm 1.03 ^a	13.56 \pm 1.25 ^a	10.29 \pm 1.46 ^a
α -Globulin, %	before the experiment	5.23 \pm 0.72 ^a	4.66 \pm 0.87 ^a	3.58 \pm 0.59 ^a
	day 30 of intake of trace-element feed supplements	6.57 \pm 1.21 ^a	4.26 \pm 0.40 ^a	4.68 \pm 1.13 ^a
	day 90 of intake of trace-element feed supplements	5.04 \pm 0.73 ^a	4.88 \pm 0.61 ^a	5.33 \pm 0.87 ^a
Albumin, %	before the experiment	58.5 \pm 2.0 ^a	60.3 \pm 1.3 ^a	61.3 \pm 3.4 ^a
	day 30 of intake of trace-element feed supplements	61.6 \pm 5.1 ^a	61.7 \pm 2.3 ^a	55.3 \pm 2.3 ^a
	day 90 of intake of trace-element feed supplements	51.5 \pm 4.1 ^a	50.5 \pm 2.4 ^a	51.2 \pm 1.3 ^a

Note: see Table 1.

Prior to the studies, we determined that the concentration of γ -globulins in blood serum of the pigs of all groups was at the same level (Table 2). On day 30, the concentration of this fraction in blood of control and group 1 slightly declined, whereas in group 2 it insignificantly increased relative to the initial values. At the end of the experiment, the concentration of γ -globulins in blood serum of the pigs increased in all the groups, being the highest in group 2.

Before the experiment, the concentration of β -globulins in blood serum of the control pigs was somewhat higher than that in the experimental groups. During the intake of the microelement feed supplements, the parameters of β -globulins in blood serum of the control and experimental groups insignificantly increased, with the highest concentration observed in Control Group.

The concentration of α -globulins in blood serum at the beginning of the experiment was the highest in control group, whereas at the end of the experiment it was higher in the experimental groups. In particular, on day 90 of intake of organic trace elements, the increase in group 2 accounted for 53.4%.

At the beginning of the experiment, the concentration of albumin in blood serum did not vary significantly among the pigs of the control and experimental groups (Table 2) and no significant changes were observed on day 30. On day 90, i.e. at the end of the experiment, the concentration of albumin tended to decrease in all the groups, with no differences observed among the experimental and control pigs.

In blood of the pigs of the three groups, the glucose concentration at the beginning of the experiment also did not vary significantly (Table 3). During the experiment, we determined an insignificant increase in the glucose concentration in blood of the animals of all the groups. At the end of the experiment, the increase was 26.8% in control, 10.9% in Group 1, and 24.4% in Group 2. At the same time, during the studies, we found no differences between the values in blood of the pigs of the control and experimental groups.

The cholesterol concentration in blood serum of the pigs of the control and experimental group during the experiment exhibited no significant changes both according to the study periods and among

the groups. At the beginning of the experiment, the activity of AST in blood serum of the pigs of all groups was at the same level (Table 3). On days 30 and 90, we determined an insignificant increase in the enzyme activity, measuring 4.4 and 40.2% in Control, 2.9 and 29.1% in Group 1, and 15.6 and 46.7% in Group 2, respectively.

During the experiment, the activity of ALT in blood serum also showed no significant differences among the animals of the control and experimental groups. However, we found an insignificant increase in the ALT activity on day 30 of the experiment, measuring 4.3% in Control, 29.1% in Group 1, and 23.9% in Group 2. On day 90 of the experiment, the enzyme parameters in blood of the control animals unchanged from the initial values, while in the experimental groups they were elevated, by 39.3% in group 1 and 19.2% in group 2.

The activity of GGT at the beginning of the experiment in blood serum of the control pigs was the highest, but significantly did not differ from the experimental animals (Table 3). During the experiment, we determined an insignificant increase in the GGT activity in blood of all animals, with the differences among the groups being insignificant.

Prior to intake of the trace-element feed supplements, the content of malondialdehyde in blood plasma was somewhat higher in the control pigs. This tendency also persisted at 30 and 90 days of consumption of the trace elements. During the studies, the differences among the pig groups were insignificant, but the lowest values of malondialdehyde were determined in the experimental group that received higher doses of organic trace elements (Table 4).

Before the experiment, the SOD activity in blood of the pigs varied insignificantly among the groups. Thirty days later, the SOD activity in blood of the control and experimental groups was observed to be somewhat declined. On day 90 of intake of the trace-element supplements, compared with the previous analysis, we determined an increase in the SOD activity in blood of Control Group (by 39.2%) and a significant increase in group 2 (by 60.3%, $P < 0.01$), while, relative to the initial values, the parameter remained at the same level.

Table 3

Concentrations of glucose and cholesterol and the activities of AST, ALT, and GGT in blood of the pigs of the control and experimental groups ($\bar{x} \pm SD$, $n = 10$)

Blood parameters	Periods of study	Groups of animals		
		control group (inorganic salts of trace elements)	group 1 (organic salts of trace elements in lower doses)	group 2 (organic salts of trace elements in higher doses)
Glucose, mmol/L	before the experiment	4.17 ± 0.49 ^a	4.65 ± 0.31 ^a	4.13 ± 0.48 ^a
	day 30 of intake of trace-element feed supplements	5.12 ± 0.35 ^a	5.08 ± 0.28 ^a	4.59 ± 0.17 ^a
	day 90 of intake of trace-element feed supplements	5.23 ± 0.33 ^a	5.17 ± 0.35 ^a	5.18 ± 0.27 ^a
Cholesterol, mmol/L	before the experiment	3.71 ± 0.48 ^a	2.87 ± 0.37 ^a	2.28 ± 0.33 ^a
	day 30 of intake of trace-element feed supplements	2.80 ± 0.62 ^a	2.38 ± 0.29 ^a	2.29 ± 0.18 ^a
	day 90 of intake of trace-element feed supplements	2.05 ± 0.17 ^a	2.54 ± 0.19 ^a	2.47 ± 0.13 ^a
Aspartate aminotransferase, nkat/L	before the experiment	458 ± 50 ^a	474 ± 81 ^a	462 ± 87 ^a
	day 30 of intake of trace-element feed supplements	478 ± 85 ^a	492 ± 61 ^a	534 ± 68 ^a
	day 90 of intake of trace-element feed supplements	642 ± 68 ^a	612 ± 68 ^a	678 ± 45 ^a
Alanine aminotransferase, nkat/L	before the experiment	644 ± 92 ^a	432 ± 80 ^a	510 ± 53 ^a
	day 30 of intake of trace-element feed supplements	672 ± 56 ^a	558 ± 66 ^a	632 ± 47 ^a
	day 90 of intake of trace-element feed supplements	644 ± 74 ^a	602 ± 57 ^a	608 ± 54 ^a
Gamma-glutamyltransferase, nkat/L	before the experiment	162 ± 19 ^a	142 ± 17 ^a	154 ± 19 ^a
	day 30 of intake of trace-element feed supplements	166 ± 17 ^a	150 ± 20 ^a	162 ± 21 ^a
	day 90 of intake of trace-element feed supplements	180 ± 19 ^a	167 ± 19 ^a	177 ± 21 ^a

Note: see Table 1.

Table 4

Concentration of malondialdehyde and activities of antioxidant-defense enzymes in blood of the pigs ($\bar{x} \pm SD$, $n = 10$)

Blood parameters	Periods of study	Groups of animals		
		control group (inorganic salts of trace elements)	group 1 (organic salts of trace elements in lower doses)	group 2 (organic salts of trace elements in higher doses)
Malondialdehyde, $\mu\text{mol/L}$	before the experiment	1.104 ± 0.372 ^a	0.878 ± 0.257 ^a	0.837 ± 0.126 ^a
	day 30 of intake of trace-element feed supplements	0.958 ± 0.137 ^a	0.805 ± 0.096 ^a	0.860 ± 0.068 ^a
	day 90 of intake of trace-element feed supplements	1.276 ± 0.264 ^a	1.146 ± 0.277 ^a	1.088 ± 0.178 ^a
Superoxide dismutase, MO/mg Hb	before the experiment	2.627 ± 0.445 ^a	2.577 ± 0.537 ^a	2.812 ± 0.751 ^a
	day 30 of intake of trace-element feed supplements	1.979 ± 0.347 ^a	2.504 ± 0.336 ^a	1.749 ± 0.268 ^a
	day 90 of intake of trace-element feed supplements	2.756 ± 0.324 ^a	2.557 ± 0.194 ^a	2.803 ± 0.098 ^a
Glutathione peroxidase, $\mu\text{mol} \times \text{min/mg Hb}$	before the experiment	3.627 ± 0.439 ^a	3.327 ± 0.278 ^a	3.293 ± 0.372 ^a
	day 30 of intake of trace-element feed supplements	4.651 ± 0.617 ^a	4.576 ± 0.672 ^a	3.839 ± 0.393 ^a
	day 90 of intake of trace-element feed supplements	3.956 ± 0.197 ^a	3.574 ± 0.243 ^a	3.054 ± 0.272 ^a
Catalase, $\mu\text{mol} \times \text{min/mg Hb}$	before the experiment	2.127 ± 0.109 ^a	1.974 ± 0.234 ^a	1.981 ± 0.058 ^a
	day 30 of intake of trace-element feed supplements	2.446 ± 0.158 ^a	2.387 ± 0.163 ^a	2.274 ± 0.107 ^a
	day 90 of intake of trace-element feed supplements	1.802 ± 0.039 ^a	1.773 ± 0.056 ^a	1.923 ± 0.058 ^a

Note: see Table 1.

Before the experiment, the GPx activity in blood of the pigs of three groups was at the same level. During the first 30 days of the experiment, the GPx activity in blood increased 28.2% in control group, 37.5% in group 1, and 16.5% in group 2. The blood study on day 90 revealed a downward tendency in the enzyme's activity in the animals of all groups, compared with the analysis on day 30. In blood of the control and experimental pigs, the activity of GPx at the end of the experiment insignificantly differed from the values observed before the experiment. None of the measurements of the GPx activity in blood of the animals revealed any significant differences among the groups.

The CAT activity in blood of the pigs of all the groups changed similarly to GPx. After the first 30 days of the experiment, we determined increases, measuring 15.0% in control group, 20.9% in group 1, and 14.8% in group 2. Compared with day 30, on day 90 of intake of the trace-element feed supplements, the CAT activity declined in blood of all the groups, particularly by 26.4% in Control, 25.7% in group 1, and insignificantly, by 5.5%, in group 2. We determined no significant differences in the CAT activity in blood of the animals among the groups.

Discussion

Incorporating trace-element supplements into feeds for pigs is essential to ensure high productivity and support the animals' health. Commercially available feed supplements are different forms of inorganic salts of mineral compounds (oxides, carbonates, chlorides, and sulfates). However, they are poorly absorbed by the organism, and therefore for the purpose of intensive growth, the diets of fattened pigs are supplemented with high (sometimes excessive) doses of trace

elements (López-Alonso, 2012; Broom et al., 2021). Studies found that after consuming feed supplements with inorganic salts of mineral compounds, 70% to 95% of the elements are not absorbed and are released with feces (Zhang et al., 2021; Duplessis et al., 2023). Therefore, in recent years, inorganic forms of trace elements have been continuously developed to provide pigs with these nutrients (Pejsak et al., 2023; Xu et al., 2024; Xiong et al., 2025). Unlike inorganic salts, organic forms are capable of entering the intestines unaltered, retaining stability at different pH levels in the gastrointestinal tract, and are highly absorbable (Khatun et al., 2019; Venkata et al., 2021; Byrne & Murphy, 2022) and more available for the organism (Araújo et al., 2019; Sun et al., 2020), thus providing a positive effect on the animals' health (Pomport et al., 2021). Therefore, organic compounds of trace elements are effective and thus can be used in lower doses than inorganic forms (Xiong et al., 2023; Song et al., 2024). During the synthesis of organic forms of trace elements, it is important to search for biologically active compounds, binding which can produce high efficiency at lower concentrations. Positive effects were exerted by combinations of mineral compounds and amino acids (Delles et al., 2016; Byrne et al., 2021) or synthesized pseudo amino acids based on polyoxyethylene derivatives of glutamic acid (Sill et al., 2017). The compounds of copper, manganese, zinc, and iron that we created based on N-PEGylated glutamic acid allowed us to obtain an organic form of trace elements – MEmPEG400Glu. At the same time, the contents of trace elements in this pre-mix were lower compared with the pre-mix containing inorganic salts, with reductions of: Cu^{2+} by 51% and 48%, Mn^{2+} – by 42% and 39%, Zn^{2+} – by 20% and 17%, and $\text{Fe}^{2+/3+}$ – by 36% and 33%.

Our feed supplement with newly formed complex of organic forms of copper, manganese, zinc, and iron, and N-PEGylated glutamic acid exerted a positive effect on hematopoiesis, metabolism, and antioxidant defense of the pigs. The efficacy of the organic forms of trace elements for pigs was pointed out by other researchers (Ma et al., 2020; Zhang et al., 2021). At the same time, the pigs that received lower amounts of organic compounds of trace elements in fodder had better parameters of hemapoiesis than those who consumed the supplement with higher doses of inorganic salts of trace elements. This may suggest that $\text{Fe}^{2+/3+}$, Cu^{2+} , and Mn^{2+} in our premix were actively absorbed by the organism and had high functional properties, as iron is included in erythrocytes, whereas Cu and Mn partake in hematopoiesis (Duck, & Connor, 2016; Doguer et al., 2018).

A glutamic acid-based nanopolymer was found to be capable of binding to bovine serum albumin (Chekh et al., 2017). Therefore, such polymers can be potential transporters of proteins and their complexes with trace elements. Perhaps, this promoted the stability of the parameters of protein metabolism in blood of the pigs consuming the newly formed trace-element feed supplement. The content of total protein and its fractions in blood serum of the pigs was at the physiological level throughout the intake of organic forms of trace elements. It has to be noted that other parameters of metabolism of the pigs (glucose and total cholesterol in blood) were within the normal range. At the same time, the values of activities of the enzymes AST, ALT, and GGT were stable in all three groups. Thus, the main blood parameters that characterize protein, carbohydrate, and lipid metabolism, and also the integrity of the organism cell indicate positive effects of the developed organic trace-element feed supplement on the organism of pigs in lower doses compared with inorganic salts of trace elements.

The trace elements (Cu, Mn, Zn, and Fe) we studied are significant for the organism's antioxidant defense (Xiong et al., 2023; Gul et al., 2024). The activities of SOD, GPx, and CAT in blood of the pigs during the experiment were at the physiological level and corresponded to the formation of the products of lipid peroxidation, indicating a balanced course of antioxidant defense. The best parameters of the activities of antioxidant-defense enzymes were observed in the pigs that consumed organic forms of trace elements in doses lower than those of inorganic salts, particularly Cu^{2+} , Mn^{2+} , Zn^{2+} , and $\text{Fe}^{2+/3+}$ lower by 48%, 39%, 17% and 33%, respectively. Therefore, organic forms of trace elements are beneficial for the stability of the antioxidant system (Lin et al., 2020; Song et al., 2024), providing good immunity, high productivity, and supporting the animals' health (Mion et al., 2023; Xiong et al., 2025).

Conclusion

Binding of the trace elements (Cu^{2+} , Mn^{2+} , Zn^{2+} , $\text{Fe}^{2+/3+}$) to N-PEGylated glutamic acid allowed us creating an organic form of mineral complex that can be given to animals in the form of feed supplement. The fattened pigs that consumed the newly created feed supplement containing organic trace elements in lower amounts compared with the supplement of the animals that obtained inorganic sulfate forms of the trace elements (in particular 51% and 48% lower Cu^{2+} , 42% and 39% lower Mn^{2+} , 20% and 17% lower Zn^{2+} , and 36% and 33% lower $\text{Fe}^{2+/3+}$, respectively in group 1 and group 2) were observed to have improved hemapoiesis, metabolism, and antioxidant defense. The RBC count, concentration of hemoglobin, total protein and its fractions, glucose, and total cholesterol, and also the activities of the enzymes AST, ALT, and GGT in blood of the pigs that received the supplement with organic compounds of trace elements were at the physiological level. Despite the lower doses of organic compounds of trace elements, the activities of the antioxidant-defense enzymes (SOD, GPx, CAT) and the content of malondialdehyde in blood of the pigs during the experiment were stable. Moreover, consumption of the organic trace elements in lower doses (particularly 48%, 39%, 17%, and 33% lower Cu^{2+} , Mn^{2+} , Zn^{2+} , and $\text{Fe}^{2+/3+}$, respectively, compared with inorganic salts of the trace elements) provided the highest positive effect on the pigs' organism.

The authors declared no conflicting interests.

References

- Araújo, C. S. S., Hermes, R. G., Bittencourt, L. C., Silva, C. C., Araújo, L. F., Granghelli, C. A., Pelissari, P. H., Roque, F. A., & Leite, B. G. S. (2019). Different dietary trace mineral sources for broiler breeders and their progenies. *Poultry Science*, 98, 4716–4721.
- Boddu, S. H. S., Bhagav, P., Karla, P. K., Jacob, S., Adatiya, M. D., Dhameilya, T. M., Ranch, K. M., & Tiwari, A. K. (2021). Polyamide/poly(amino acid) polymers for drug delivery. *Journal of Functional Biomaterials*, 12(4), 58.
- Broom, L. J., Monteiro, A., & Piñon, A. (2021). Recent advances in understanding the influence of zinc, copper, and manganese on the gastrointestinal environment of pigs and poultry. *Animals*, 11(5), 1276.
- Byrne, L., & Murphy, R. A. (2022). Relative bioavailability of trace minerals in production animal nutrition: A review. *Animals*, 12(15), 1981.
- Byrne, L., Hynes, M. J., Connolly, C. D., & Murphy, R. A. (2021). Influence of the chelation process on the stability of organic trace mineral supplements used in animal nutrition. *Animals*, 11(6), 1730.
- Chekh, B. O., Ferens, M. V., Ostapiv, D. D., Samaryk, V. Y., Varvarenko, S. M., & Vlizlo, V. V. (2017). Characteristics of novel polymer based on pseudo-polyamino acids GluLa-DPG-PEG600: Binding of albumin, biocompatibility, biodistribution and potential crossing the blood-brain barrier in rats. *Ukrainian Biochemical Journal*, 89(4), 13–21.
- Corbett, R. B. (2023). Trace mineral nutrition in confinement dairy cattle. *Veterinary Clinics of North America: Food Animal Practice*, 39(3), 425–438.
- Curnock, R., & Cullen, P. J. (2020). Mammalian copper homeostasis requires retromer-dependent recycling of the high-affinity copper transporter 1. *Journal of Cell Science*, 133, jcs249201.
- Daniel, J.-B., Brugger, D., van der Drift, S., van der Merwe, D., Kendall, N., Windisch, W., Doelman, J., & Martín-Tereso, J. (2023). Zinc, copper, and manganese homeostasis and potential trace metal accumulation in dairy cows: Longitudinal study from late lactation to subsequent mid-lactation. *The Journal of Nutrition*, 153(4), 1008–1018.
- Delles, R. M., Naylor, A., Kocher, A., Dawson, K. A., & Samuel, R. S. (2016). Diets with organic trace minerals (Bioplex®) and yeast protein (NuPro®) improved the water-holding capacity of pork loin meat. *Journal of Animal Science*, 94(2), 66.
- Doguer, C., Ha, J.-H., & Collins, J. F. (2018). Intersection of iron and copper metabolism in the mammalian intestine and liver. *Comprehensive Physiology*, 8, 1433–1461.
- Duck, K. A., & Connor, J. R. (2016). Iron uptake and transport across physiological barriers. *Biometals*, 29, 573–591.
- Duplessis, M., & Royer, I. (2023). The importance of an integrated approach to assess trace mineral feeding practices in dairy cows. *Frontiers in Animal Science*, 4, 1155361.
- Fihurka, N., Tarnavchik, I., Nosova, N., Varvarenko, S., Dron, I., Ostapiv, D., Vlizlo, V., & Samaryk, V. (2024). Surface active polyesters based on N-substituted glutamic acid as promising materials for biomedical applications. *International Journal of Polymeric Materials and Polymeric Biomaterials*, 73(14), 1207–1215.
- Goff, J. P. (2018). Mineral absorption mechanisms, mineral interactions that affect acid-base and antioxidant status, and diet considerations to improve mineral status. *Journal of Dairy Science*, 101, 2763–2813.
- Gozzelino, R., & Arosio, P. (2016). Iron homeostasis in health and disease. *International Journal of Molecular Sciences*, 17(1), 130.
- Gul, F., Amin, H., Naz, S., Khan, M. T., Alhaidary, I. A., Khan, R. U., Pugliese, G., & Tufarelli, V. (2024). Evaluation of blood minerals and oxidative stress changing pattern in prepartum and postpartum Achai and Holstein Friesian dairy cows. *Reproduction in Domestic Animals*, 59, e14525.
- Iskra, R. Y., Vlizlo, V. V., & Fedoruk, R. S. (2017). Biologic efficiency of citrates of microelements in animal breeding. *Agricultural Science and Practice*, 4(3), 28–34.
- Khatun, A., Chowdhury, S. D., Roy, B. C., Dey, B., Haque, A., & Chandran, B. (2019). Comparative effects of inorganic and three forms of organic trace minerals on growth performance, carcass traits, immunity, and profitability of broilers. *Journal of Advanced Veterinary and Animal Research*, 6(1), 66–73.
- Kozak, M., Petruh, I., Kovalchuk, I., & Vlizlo, V. (2025). Toxicity analysis of amoxicillin, polyphosphate ester and its complex with amoxicillin on mice. *Scientific Reports*, 15, 18150.
- Levchenko, V. I., & Vlizlo, V. V. (2019). *Veterynarna klinichna biokhimiya [Veterinary clinical biochemistry]*. Bila Tserkva National Agrarian University, Bila Tserkva (in Ukrainian).
- Lin, G., Guo, Y., Liu, B., Wang, R., Su, X., Yu, D., & He, P. (2020). Optimal dietary copper requirements and relative bioavailability for weanling pigs fed either copper proteinate or tribasic copper chloride. *Journal of Animal Science and Biotechnology*, 11, 54.

- Liu, B., Xiong, P., Chen, N., He, J., Lin, G., Xue, Y., Li, W., & Yu, D. (2016). Effects of replacing of inorganic trace minerals by organically bound trace minerals on growth performance, tissue mineral status, and fecal mineral excretion in commercial grower-finisher pigs. *Biological Trace Element Research*, 173, 316–324.
- López-Alonso, M. (2012). Trace minerals and livestock: Not too much nor too little. *International Scholarly Research Notices*, 18, 704825.
- Ma, L., He, J., Lu, X., Qiu, J., Hou, Ch., Liu, B., Lin, G., & Yu, D. (2020). Effects of low-dose organic trace minerals on performance, mineral status, and fecal mineral excretion of sows. *Asian-Australasian Journal of Animal Sciences*, 33(1), 132–138.
- Maywald, M., Wessels, I., & Rink, L. (2017). Zinc signals and immunity. *International Journal of Molecular Sciences*, 18(10), 2222.
- Mion, B., Ogilvie, L., Van Winters, B., Spricigo, J. F W, Anan, S., Duplessis, M., McBride, B. W, LeBlanc, S. J, Steele, M. A. & Ribeiro, E. S. (2023). Effects of replacing inorganic salts of trace minerals with organic trace minerals in the pre- and postpartum diets on mineral status, antioxidant biomarkers, and health of dairy cows. *Journal of Animal Science*, 101, skad041.
- Numata, K. (2015). Poly(amino acid)s/polypeptides as potential functional and structural materials. *Polymer Journal*, 47, 537–545.
- Oleksa, V., Nagomiak, M., Chekh, B., Ostapiv, R., Vlizlo, V., Varvarenko, S., & Samaryk, V. (2018). Polymer complexes of microelements based on N-derivatives of L-glutamic acid for correction of metabolism. Ecology and human health, Czestochowa Publishing house of Polonia University in Czestochowa "Educator". Pp. 7–20.
- Palomares, R. A. (2022). Trace minerals supplementation with great impact on beef cattle immunity and health. *Animals*, 12(20), 2839.
- Pejsak, Z., Kaźmierczak, P., Butkiewicz, A. F., Wojciechowski, J., & Woźniakowski, G. (2023). Alternatives to zinc oxide in pig production. *Polish Journal of Veterinary Sciences*, 26(2), 319-330.
- Pomport, P. H., Warren, H., & Taylor-Pickard, J. (2021). Effect of total replacement of inorganic with organic sources of key trace minerals on performance and health of high producing dairy cows. *Journal of Applied Animal Nutrition*, 9, 1–8.
- Qian, X., Wang, Z., Shen, G., Chen, X., Tang, Z., & Guo, C. (2018). Heavy metals accumulation in soil after 4 years of continuous land application of swine manure: A field-scale monitoring and modeling estimation. *Chemosphere*, 210, 1029–1034.
- Qian, X., Wang, Z., Shen, G., Chen, X., Tang, Z., Guo, C., Gu, H., & Fu, K. (2018). Heavy metals accumulation in soil after 4 years of continuous land application of swine manure: A field-scale monitoring and modeling estimation. *Chemosphere*, 210, 1029–1034.
- Shannon, M. C., & Hill, G. M. (2019). Trace mineral supplementation for the intestinal health of young monogastric animals. *Frontiers in Veterinary Science*, 13(6), 73.
- Sill, K. N., Sullivan, B., Carie, A., & Semple, J. E. (2017). Synthesis and characterization of micelle-forming peg-poly(amino acid) copolymers with iron-hydroxamate cross-linkable blocks for encapsulation and release of hydrophobic drugs. *Biomacromolecules*, 18(6), 1874–1884.
- Silva, T. H., Guimaraes, I., Menta, P. R., Fernandes, L., Paiva, D., Ribeiro, T. L., Celestino, M. L., Netto A. S., Ballou M. A., & Machado V. S. (2022). Effect of injectable trace mineral supplementation on peripheral polymorphonuclear leukocyte function, antioxidant enzymes, health, and performance in dairy cows in semi-arid conditions. *Journal of Dairy Science*, 105(2), 1649–1660.
- Song, Y., Weng, Y., Liu, S., Usman, M., Loo, J. J, Lin, G., Hu, Q., Luo, J., & Wang, P. (2024). Effects of reduced levels of organic trace minerals in proteinate forms and selenium-yeast in the mineral mix on lactation performance, milk fatty acid composition, nutrient digestibility and antioxidant status in dairy goats. *Journal of Animal Science*, 102, skae187.
- Stasiuk, A., Fihurka, N., Vlizlo, V., Prychak, S., Ostapiv, D., Varvarenko, S., & Samaryk, V. (2022). Synthesis and properties of phosphorus-containing pseudo-poly(amino acid)s of polyester type based on n-derivatives of glutamic acid. *Chemistry and Chemical Technology*, 16(1), 51–58.
- Sun, X., Sarteshnizi, R. A., Boachie, R. T., Okagu, O. D., Abioye, R. O., Neves, R. P., Ohanenye, I. C., & Udenigwe, C. C. (2020). Peptide-mineral complexes: Understanding their chemical interactions, bioavailability, and potential application in mitigating micronutrient deficiency. *Foods*, 9(10), 1402.
- Taylor-Pickard, J. A., Nollet, L., & Geers, R. (2013). Performance, carcass characteristics and economic benefits of total replacement of inorganic minerals by organic forms in growing pig diets. *Journal of Applied Animal Nutrition*, 2, e3.
- Varvarenko, S. M., Figurka, N. V., Samaryk, V. Y., Voronov, A. S., Tarnachyk, I. T., Dron, I. A., Nosova, N. G., & Voronov, S. A. (2013). The new amphiphilic polyesters of a pseudoaminoacid based on natural two based aminoacids and diols, obtained by the Steglich esterification reaction. *Polymer Journal*, 35(3), 282–290.
- Varvarenko, S., Samaryk, V., Vlizlo, V., Ostapiv, D., Nosova, N., Tarnachyk, I., Fihurka, N., Ferens, M., Nagornyak, M., & Taras, R. (2015). Fluorescence-containing theranostics based on the pseudo-poly(amino acid)s for monitoring of drug delivery and release. *Polymer Journal*, 37, 193–199.
- Venkata, R. R. S., Bhukya, P., Raju, M. V. L. N., & Ullengala, R. (2021). Effect of dietary supplementation of organic trace minerals at reduced concentrations on performance, bone mineralization, and antioxidant variables in broiler chicken reared in two different seasons in a tropical region. *Biological Trace Element Research*, 199, 3817–3824.
- Vlizlo, V. V. (2012). Laboratorni metody doslidzen u biolohiyi, tvarynnytsvi ta veterynarniy medytsyni [Laboratory methods of investigation in biology, stock breeding and veterinary]. Spolom, Lviv (in Ukrainian).
- Vlizlo, V., Fedoruk, R., Kovalchuk, I., Tsap, M., Khrabko, M., Khomyn, M., & Ostapiv, D. (2023). Immunobiological reactivity of calf body during the compatible application of nanotechnological citrates of trace elements in the ration. *Studia Biologica*, 17(4), 63–72.
- Wang, G., Liu, L. J., Tao, W. J., Xiao, Z. P., Pei, X., Liu, B. J., Wang, M. Q., Lin, G., & Ao, T. Y. (2019). Effects of replacing inorganic trace minerals with organic trace minerals on the production performance, blood profiles, and antioxidant status of broiler breeders. *Poultry Science*, 98(7), 2888–2895.
- Xiong, Y., Cui, B., He, Z., Liu, S., Wu, Q., Yi, H., Zhao, F., Jiang, Z., Hu, S., & Wang, L. (2023). Dietary replacement of inorganic trace minerals with lower levels of organic trace minerals leads to enhanced antioxidant capacity, nutrient digestibility, and reduced fecal mineral excretion in growing-finishing pigs. *Frontiers in Veterinary Science*, 10, 1142054.
- Xiong, Y., Zhao, F., Li, Y., Wu, Q., Xiao, H., Cao, S., Yang, X., Gao, K., Jiang, Z., & Hu, S. (2025). Impact of low-dose amino acid-chelated trace minerals on performance, antioxidant capacity, and fecal excretion in growing-finishing pigs. *Animals*, 15(9), 1213.
- Xu, W., Zhou, M., Yang, Z., Zheng, M. & Chen, Q. (2024). Organic trace elements enhance growth performance, antioxidant capacity, and gut microbiota in finishing pigs. *Frontiers in Veterinary Science*, 11, 1517976.
- Zhang, W. F., Tian, M., Song, J. S., Chen, F., Lin, G., Zhang, S. H., & Guan, W. T. (2021). Effect of replacing inorganic trace minerals at lower organic levels on growth performance, blood parameters, antioxidant status, immune indexes, and fecal mineral excretion in weaned piglets. *Tropical Animal Health and Production*, 53, 121.