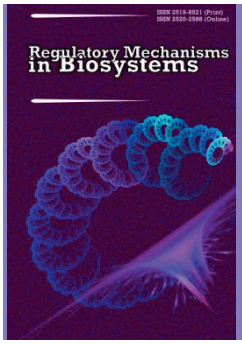

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2023, 14(1), 106–111
doi: 10.15421/022316

Antioxidant system of the body of young Ukrainian beef cattle under the action of microelements

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Article info

Received 29.01.2023
Received in revised form 24.02.2023
Accepted 25.02.2023

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Mylostyva, D. F., Farafonov, S. J., Puzniak, O. M., Stakhiv, V. I., Borshchenko, V. V., Tsisinska, S. V., & Voloshin, S. V. (2023). Antioxidant system of the body of young Ukrainian beef cattle under the action of microelements. Regulatory Mechanisms in Biosystems, 14(1), 106–111. doi:10.15421/022316

Active forms of oxygen are formed in the course of the organism's vital activity in biochemical reactions. These forms, when the pro/antioxidant balance is disturbed, trigger a cascade of lipid peroxidation, which can be the cause of the development of various pathological conditions. To prevent the negative influence of lipid peroxidation products in the body, a powerful antioxidant system is activated. This system consists of an enzymatic and a non-enzymatic link. An important aspect of the normal functioning of this system is the provision of the body with important trace elements. A number of minerals are included in the active center of antioxidant enzymes or have an effect on the reactions of non-enzymatic antioxidants. Research was conducted on fattening bulls of the Ukrainian meat breed. During the monitoring of microelements in feed, it was found that the vast majority of farm feed was deficient in copper, selenium and manganese and for this reason the animals consumed an insufficient amount of these minerals. These data were confirmed by the low content of these trace elements in blood serum. The addition of inorganic salts of microelements to the basic diet led to an increase in the concentration of copper, manganese and selenium in the blood serum by 20.5%, 37.3% and 23.9%. The study of the content of lipid peroxidation products showed that during the 30 days of the experiment, the level of lipid hydroperoxide increased by 25.5%, diene conjugates by 22.8%, and malonic dialdehyde by 22.0%. This indicates that against the background of increased age-related metabolism in the body of young animals, the oxidation-reduction reactions that are a predictor of the start of peroxidation processes increase. It was also noted that with a deficiency of certain trace elements, the activity of both the enzymatic and non-enzymatic links of the antioxidant system was reduced. Thus, in 30 days, the level of catalase, superoxide dismutase, and glutathione peroxidase decreased by 9.4%, 15.3%, and 13.0%, respectively. During this time, the content of tocopherol and ceruloplasmin decreased by 16.8% and 9.8%. Additives also had a positive effect on the activity of the antioxidant system by increasing its components. Additives of trace elements had different effects on the activity of antioxidant enzymes. The greatest effect on the level of catalase and superoxide dismutase was observed when copper salts were added, when the increase of these enzymes was noted by 1.11 and 1.23 times, respectively. Accordingly, the level of glutathione peroxidase was the highest in animals that received additional selenium – 1.21 times. The addition of copper also had the greatest biological effect on the important non-enzymatic component of antioxidant protection – ceruloplasmin. Its level increased by 1.24 times under the action of copper sulfate. The level of tocopherol was higher under the action of manganese, when its concentration was 1.11 times higher than the control. Against this background, there was a decrease in the products of lipid peroxidation: lipid hydroperoxides – 1.19 times under the action of selenium; diene conjugates – by 1.22 times and malonic dialdehyde – by 1.11 times under the influence of copper and manganese compounds, respectively.

Keywords: mineral additive; young beef cattle; catalase; superoxide dismutase; glutathione peroxidase; ceruloplasmin; tocopherol; lipid peroxidation, lipid hydroperoxides; diene conjugates; malonic dialdehyde.

Introduction

In all aerobic organisms, in the process of their vital activity, aggressive free radicals are constantly formed, which are necessary metabolites that ensure the course of many physiological reactions (Sreekumar et al., 2022). To maintain the oxidative potential, most tissues require a constant supply of molecular oxygen. However, even under normal conditions, molecular oxygen is a source of toxic substances, thanks to which a small amount of its active forms is produced. These active forms of oxygen are strong oxidants or reactive free radicals, which are molecular particles with unpaired electrons. Free radicals with their pro-oxidant effect are necessary for such processes as cellular signaling, growth and differentiation of normal cells, destruction of infected and malignant cells, and death of disease-causing organisms (Miyazawa et al., 2019). In physiological conditions, the process of formation of free radicals is balanced with their utilization (recovery), as well as the content of activating factors (prooxidants) and suppressing factors (antioxidants) in this process, since both an excess of free radicals and their deficiency lead to a violation of the struc-

ture and function of cells. The oxygen molecule by its nature is a biradical, since it contains a pair of unpaired electrons. However, they are arranged in such a way that the molecular oxygen molecule remains relatively stable (Dresen et al., 2022). Metabolic stress, as a consequence of excessive formation of reactive oxygen species, is one of the key problems arising from physiological changes associated with excessive mobilization of lipids with further development of oxidative stress and immune dysfunction (Azeez et al., 2020; Song et al., 2021; Xiao et al., 2021). This leads to the fact that oxidative stress reduces the functional capabilities of immune cell populations and increases the body's susceptibility to diseases (Abuelo et al., 2019). Oxidative stress is an active field of research in veterinary medicine and is associated with numerous pathological processes, including sepsis, mastitis, acidosis, ketosis, enteritis, pneumonia, diseases of the respiratory and joint organs, and parasite infestation (Sinan Aktas et al., 2018; Ayemele et al., 2021). In addition to endogenous factors, environmental factors contribute to the occurrence and development of oxidative stress: housing conditions (Mylostyvyi et al., 2020), elevated air temperature (heat stress) (Mylostyva et al., 2022). Also, unbalanced feeding contributes to the increase in oxidative processes. According to separate studies, the content of lipid peroxidation products (an increase in the concentration of primary and secondary products of peroxidation) decreased, and the activity of such antioxidant enzymes as superoxide dismutase and catalase increased in cows receiving feed with a high content of antioxidant compounds (Celi, 2011; Sun et al., 2019). It should also be noted that it is the young organism that is very sensitive to excessive formation of peroxidation products due to unstable protective systems and enhanced metabolic reactions of the organism (Haser et al., 2015; Rubio et al., 2021).

Microelements have traditionally been added to animal diets to meet their needs. But the source of mineral additives can affect the productivity of individual animals. The importance of optimal mineral nutrition for improving immune function and health has been recognized in previous decades (Kegley et al., 2016). Calf rearing systems depend on feed as the main source of nutrients, which may vary in quality and may be nutritionally deficient. Fodder should contain minerals vital for almost all processes in the body. Many factors affect the mineral content of feed, including soil, plant species, stage of maturity, yield, climate and feed production methods. In general, beef cattle are raised primarily on feed that can be deficient in nutrients for optimal health, especially trace elements such as copper (Cu), manganese (Se), zinc (Zn), and selenium (Se) (Kegley et al., 2016; Michalczyk et al., 2020; Palomares, 2022). Clinical deficiency of these nutrients causes classic symptoms common to the deficiency of these substances (for example, slow growth, low productivity, decreased reproductive performance). But subclinical deficits are more common and more difficult to detect. They can lead to larger economic losses. It was found that the micronutrient status of cattle is the result of a balance between their feed intake and animal needs. Concentrations of minerals in the diet, which are often considered sufficient for maximum development, reproductive function or optimal immune function, are insufficient during physiological stress (weaning, transfer to another system of cultivation and maintenance, transfer to pasture, transportation, mating) with a decrease in feed consumption (Harvey et al., 2021; Pandey et al., 2022). Therefore, when growing and keeping cattle, mineral premixes are sometimes used to cover the needs of animals in deficient trace elements.

The goal of our research was to study the effect of deficient trace elements on the activity of the antioxidant system and the content of lipid peroxidation products in young Ukrainian meat breeds.

Materials and methods

During the research on young cattle, the requirements of the international and national bioethical position on animal experiments were adhered to ; the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123, Strasbourg, 1986), the Law of Ukraine "On the Protection of Animals from Cruel Treatment" No. 3447-IV dated 21.06.2006 with amendments dated 04.08.2017. Ethical norms regarding animals in the experiment could be violated when taking blood, since the animals were restricted in their movements, which could lead to their anxiety and a stressful state. The research was conducted on calves of the Ukrainian beef breed at the age of 6 months. The animals were kept in the experimental farm "Polyvanovka" in Magdalinovsky district, Dnipropetrovsk region. The care and maintenance of the animals were the same, according to the mandatory norms of DSTU 7823:2015 Livestock Farms. The requirements for the parameters of the microclimate in the livestock premises (temperature, humidity, light regime and gas content in the air in the premises) were within the limits of zoohygienic standards. The animals received a ration balanced according to feeding standards that corresponded to their age, live weight and average daily growth. In order to determine the micronutrient deficiency of the ration, a survey of the farm's fodder was conducted. The content of trace elements in the feed was determined by an atomic absorption spectrophotometer AAS-30 (Prais, 1976). Also, to study trace element deficiency, blood serum of calves was analyzed by the same method. When selecting animals, age, sex and body weight were taken into account. Male animals – young bulls – were selected for the experiment. 4 groups of animals with 9 animals in each group were formed. During the experiment, the diet of the animals was supplemented with supplements of deficient trace elements in the form of inorganic salts. Additives were provided together with concentrates: the control group received only the main ration of the farm, and the first group received the micronutrient supplement copper sulfate along with the main ration, the second group received the sodium-selenite, and the third group of animals received manganese sulfate. The experiment lasted 30 days, i.e. the animals were 6 to 7 months old. Based on the needs of beef cattle for micronutrients, 5 mg/kg of copper sulfate, 5 mg/kg of manganese sulfate, and 3 mg/kg of sodium selenite were added to concentrated feed for young cattle. For laboratory studies, blood was taken from the jugular vein on an empty stomach before feeding, taking into account all the rules of veterinary sepsis and antiseptics. In order to investigate the antioxidant capacity of the body, the content of primary and secondary peroxidation products (hydrogen peroxide, diene conjugates, and malondialdehyde) was studied; the activity of the antioxidant system was determined by the content of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase) and nonenzymatic antioxidants (ceruloplasmin and tocopherol).

In laboratory studies, we used a spectrophotometer UNICO (United Products & Instruments, USA), a photoelectrocolorimeter Solar PV 1251 C (ZAO Solar, Minsk, Belarus), a LOIP LB-140 water bath, and a C 2204 centrifuge. The concentration of malonic dialdehyde was determined by the reaction with thiobarbituric acid ("Sphera Sym", Lviv, Ukraine) (Vlizlo et al., 2012). The content of lipid hydroperoxides was determined by the principle of protein precipitation with a solution of trichloroacetic acid followed by the introduction of ammonium rhonide (Vlizlo et al., 2012). Determination of diene conjugates was carried out according to the method of I. D. Stalnaya (Vlizlo et al., 2012). Glutathione peroxidase activity (GPx, EC 1.11.1.9) was determined according to the scheme of reduced glutathione (Vlizlo et al., 2012). Catalase activity was determined in blood hemolysates based on the ability of hydrogen peroxide to form a coloured complex with ammonium molybdate ("Sphera Sym", Lviv, Ukraine) Vlizlo et al., 2012). Superoxide dismutase (SOD, EC 1.1.15.1) was studied using the nitrotrazole reduction method ("Sphera Sym", Lviv, Ukraine) (Vlizlo et al., 2012). Tocopherol content was determined in lipid extracts from blood by colour reaction with dipyridyl (Reagent, Dnipro, Ukraine) and ferric chloride (Reagent, Dnipro, Ukraine) (Vlizlo et al., 2012). Determination of ceruloplasmin in blood serum was performed on the oxidation of p-phenylenediamine with the participation of ceruloplasmin (Vlizlo et al., 2012).

Samples were checked for belonging to normally distributed general populations according to the Shapiro-Wilko criterion. Parametric one-way analysis of variance was used to determine reliable differences between sample means; post-test comparison was performed using Tukey's test with Bonferroni's correction. In all cases, the results were considered significant when P was less than 5% ($P < 0.05$). Results were presented as mean ± standard error of the mean ($x \pm SE$).

Results

At the final blood serum test of the calves, low levels of copper selenium and manganese were detected. This indicates a deficiency of trace

elements in the body, which, in turn, can lead to a negative effect on the vital activity of the animals. During the period of feeding the mineral compound, the concentration of microelements in the blood serum of the animals increased: thus, copper on average increased by 20.5% ($P < 0.001$), selenium – by 23.9% ($P < 0.001$) and manganese – by 37.3% ($P < 0.001$, Table 1).

Table 1

Concentration of microelements (μg/mL) in blood serum of calves before and after feeding microelement compounds (x ± SE, n = 14)

Indicator	At the beginning of research	At the end of the study
Copper	1.877 ± 0.029	2.261 ± 0.011***
Selenium	0.3429 ± 0.0056	0.4250 ± 0.0059***
Manganese	0.2664 ± 0.0086	0.3657 ± 0.0075***

Note: * – P < 0.05, ** – P < 0.01, *** – P < 0.001.

The intensification of oxidative processes leading to an increase in lipid peroxidation products in young cattle is based on metabolic processes associated with increased growth and development of ontogenesis. The results of studies of the antioxidant status of calves indicated the accumulation of lipid peroxidation products in the blood plasma in the dynamics observed for 30 days against the background of trace element deficiency in the control group of animals: the content of lipid hydroperoxide significantly increased by 25.5% ($P < 0.001$), diene conjugates − by 22.8% ($P < 0.001$), malonic dialdehyde − by 22.0% ($P < 0.001$), which indicates an increase in the activity of peroxidation processes as a result of accelerated metabolic processes during the period of intensive growth of young animals. Within 30 days, a decrease in the activity of the enzymatic antioxidant system was noted (Table 2). Thus, the level of superoxide dismutase decreased by 15.3% ($P < 0.01$), catalase – by 9.4% ($P < 0.001$) and glutathione peroxidase – by 13.0%. The observed decrease in indicators of antioxidant protection could be caused by damage to any of its links caused by the increase and excessive formation of under-oxidized and free-radical substrates. This can create conditions for the toxicity of hydrogen peroxide and the initiation of chain reactions of lipid peroxidation.

Analysis of the results of microelement correlation revealed that the use of microelement deficiency leads to the process of peroxidation and reduces the level of accumulation of products of active forms of acid. In all the research groups, a decrease in lipid peroxidation products was observed, while oxalic minerals had a small greater antioxidant effect. Thus, a more significant reduction in hydroperoxides was found in the animals after sodium selenite infusion – by 18.8% ($P < 0.001$); diene conjugates – under the influence of copper by 15.2% ($P < 0.01$), and malonic dialdehyde under the influence of manganese by 15.8% ($P < 0.001$) respectively (Table 3).

Table 2

Concentration (μmol/ mL) of lipid peroxidation products and antioxidant enzymes in blood serum of young cattle (x ± SE, n = 14)

Indicator	At the beginning of research	At the end of research
Lipid hydroperoxide	41.74 ± 0.37	52.38 ± 0.40***
Diene conjugates	44.69 ± 0.64	54.89 ± 0.71***
Malonic dialdehyde	6.10 ± 0.11	7.44 ± 0.15***
Superoxide dismutase	14.47 ± 0.33	12.55 ± 0.30**
Catalase	67.37 ± 0.12	61.59 ± 0.46***
Glutathione peroxidase	13.79 ± 0.56	12.20 ± 0.56

Note: see Table 1.

Feeding the animals compounds of trace elements led to an increase in the concentration of trace elements in their blood serum (Table 4). Under the influence of trace elements, the activity of antioxidant enzymes increased. The highest value of the superoxide dismutase activity indicator was under the action of copper sulfate. The value of this enzyme increased by 22.1% ($P < 0.01$). At the same time, under the action of manganese and selenium, the activity of superoxide dismutase increased by 14.6% ($P < 0.01$) and 13.3% ($P < 0.01$), respectively. In relation to other enzymes of the antioxidant system, an increase in their concentration was also noted. Thus, catalase also had the highest values in animals receiving the copper compound, it was 1.11 times greater ($P < 0.001$); under the influence of manganese sulfate – by 1.09 ($P < 0.001$) and under the influence of sodium selenite – by 1.07 ($P < 0.01$). Accordingly, the level of glutathione peroxidase was the highest in animals that received additional selenium – by 1.21 times ($P < 0.05$); under the action of copper and manganese, the indicators of this enzyme increased by 1.12 ($P < 0.05$) and 1.10 times. The positive influence of selenium on the activity of glutathione peroxidase is explained by the strengthening of the activity of the protein part of the enzyme molecule as a result of the effect of this trace element on the state of sulfhydryl groups of proteins.

Table 3

The effect of deficient microelements on the level of lipid peroxidation products (μmol/ mL) in blood serum of young cattle (x ± SE, n = 14)

Indicator	Control	Correction (basic ration+$CuSO_4$)	Correction (basic ration+ Na_2SeO_3)	Correction (basic ration+$MnSO_4$)
Lipid hydroperoxide	41.74 ± 0.37^a	36.24 ± 0.59^b	35.14 ± 0.53^b	36.06 ± 0.32^b
Diene conjugates	44.69 ± 0.64^a	36.56 ± 0.38^b	40.33 ± 0.63^b	38.19 ± 0.53^b
Malonic dialdehyde	6.10 ± 0.11^a	5.56 ± 0.08^b	5.69 ± 0.11^b	5.50 ± 0.12^b

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

Table 4

The concentration of antioxidant enzymes (μmol/ mL) in the blood of animals under the action of deficient trace elements (x ± SE, n = 14)

Indicator	Control	Correction (basic ration+$CuSO_4$)	Correction (basic ration+ Na_2SeO_3)	Correction (basic ration+$MnSO_4$)
Superoxide dismutase	14.47 ± 0.33^a	17.74 ± 0.29^b	16.39 ± 0.34^b	16.58 ± 0.32^b
Catalase	67.37 ± 0.12^a	74.70 ± 0.35^b	72.06 ± 0.62^b	73.63 ± 0.36^b
Glutathione peroxidase	13.79 ± 0.56^a	15.44 ± 0.40^b	16.67 ± 0.47^b	15.15 ± 0.52

Note: see Table 3.

With similar dynamics of the concentration of products of lipoperoxidation, the study of the non-enzymatic link of the body's antioxidant system is advised. In this regard, the concentrations of ceruloplasmin and tocopherol were used as markers to assess the state of the nonenzymatic antioxidant system. Ceruloplasmin is a non-enzymatic antioxidant capable of neutralizing molecular oxygen radicals. By its very nature, it is a metal-containing protein, as it contains copper ions in its composition. A decrease in the concentration of ceruloplasmin can be caused by oxidative damage due to insufficient copper content in the protein or a decrease in redox enzymes due to a lack of copper in the body, which leads to an increase in oxidative stress. The imbalance of the prooxidant and antioxidant system indicates a decrease in the activity of the main components of the antioxidant defense system – ceruloplasmin and tocopherol by 9.8% ($P < 0.001$) and 16/8% ($P < 0.001$), respectively, from day 1 to day 30 of the study (Fig. 1).

When micronutrient deficiency was eliminated, the content of ceruloplasmin increased: its concentration was 1.24 times ($P < 0.01$) higher with an additional source of copper; 1.14 times – under the influence of sodium selenite ($P < 0.001$) and 1.13 ($P < 0.001$) more under the influence of a manganese additive. An increase in ceruloplasmin may be a direct result of a decrease in the concentration of hydrogen peroxide radicals caused by the reduction of superoxide radicals mediated by superoxide dismutase. The antioxidant property of ceruloplasmin is that it is a member of the family of copper-containing oxidases that have the ability to bind to molecular oxygen and reduce it to water. In addition, ceruloplasmin exerts an amine oxidase effect on other organic substrates (phenyle-

nediamine), which also leads to the formation of water and hydrogen peroxide. The level of tocopherol was also higher: by 7.2% ($P < 0.01$), 10.2% ($P < 0.001$) and 11.3% ($P < 0.001$), respectively, due to the effects of copper, selenium, and manganese. The increased concentration of tocopherol is associated with its role in the work of the antioxidant system, which protects cell membranes from oxidative damage.

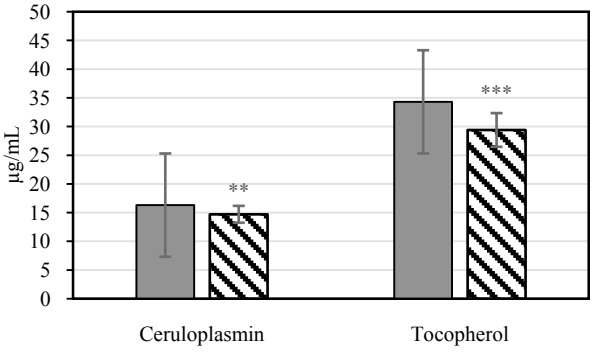

Fig. 1. Non-enzymatic components (µg/mL) of the antioxidant system in the blood of calves on the 1st and 30th day of studies (x ± SE, n = 14)

Discussion

Separate studies have established the influence of trace elements on the metabolic status of the body, including changes in blood composition and the flow of biochemical reactions (Barcelos et al., 2022; Silva et al., 2022). This is explained by the fact that, in most cases, metal ions enter the active center of a number of enzymes that catalyze biochemical processes. In addition, certain trace elements participate in the processes of oxidative stress, acting both as catalysts of oxidative reactions (Michalczyk et al., 2020) and as inhibitors of peroxidation processes. For example, divalent iron ions can cause autoxidation and lead to the formation of molecular oxygen, while interaction with hydrogen peroxide can generate hydroperoxides in the Fenton and Haber-Weiss reactions (Lowe et al., 2017; Mandil et al., 2020). Dysregulation of oxidative stress destroys cellular DNA repair mechanisms due to the transformation of reactive oxygen species and is an important mechanism for the development of various pathological conditions (Tang et al., 2019). Enzymatic mechanisms of antioxidant protection include superoxide dismutase, which converts the superoxide radical anion into molecular oxygen or hydrogen peroxide, which is less capable of reacting, and glutathione peroxidase. This enzyme catalyzes the transformation of hydrogen peroxide into water by converting reduced glutathione into oxidized glutathione. (Mantzarlis et al., 2017). Manganese (Mn), copper (Cu), selenium (Se) and a number of minerals are essential trace elements involved in cellular metabolism, are part of various enzyme systems and regulate the activity of enzymes. Micronutrient homeostasis is tightly regulated by several compensatory mechanisms that balance their concentration, including transporters, importers, and metallothioneins (Giménez et al., 2021). Altered consumption of only one of these trace elements can cause a violation of their concentrations and lead to their competition during assimilation (Bordignon et al., 2019). But it should be taken into account that the levels of trace elements can change under the influence of many environmental factors.

To overcome the deficiency of trace elements in all areas of animal husbandry, compounds of trace elements are used. Copper sulfate is the most common form used as a feed additive as a source of copper in feed for agricultural animals and poultry (Wen et al., 2019). Dietary supplementation of various antioxidant compounds, such as vitamin C, vitamin E, and selenium, is effectively practiced to reduce oxidative stress (Leskovec et al., 2018; Amer et al., 2021; Ibrahim et al., 2021). The biological activity of copper, iron, manganese and selenium is largely associated with the presence of unpaired electrons, which allows them to participate in oxidation-reduction reactions. Copper affects the activity of certain enzymes important for cellular respiration, protection against free radicals, melanin synthesis and the formation of connective tissue (Król et al., 2020). Changes in copper levels can also affect iron metabolism. Examples of these enzymes include Cu/Zn superoxide dismutase (Cu/Zn-SOD), cytochrome oxidase, tyrosinase, dopamine hydroxylase, and lysine oxidase. Copper can also act as their cofactor and as their allosteric component. Copper deficiency can cause a decrease in energy levels, cause abnormal glucose and cholesterol metabolism, increase oxidative damage, and change the functions and structure of circulating blood cells and immune cells. In the case of copper deficiency, the oxidation system can be disturbed due to the fact that copper is a catalytic cofactor of superoxide dismutase (Cu/Zn-SOD). Copper is also a component of ceruloplasmin, which is involved in the body's defense systems, especially in animals under the influence of stress factors. This enzyme is synthesized in hepatocytes and secreted into blood plasma after inclusion of copper atoms (Arredondo et al., 2008). In multicellular organisms, the metabolism of copper consists of absorption, distribution, sequestration and excretion both at the cellular and systemic levels. Mammalian enterocytes absorb bioavailable media ions from food depending on Ctr1. After switching on, copper ions are delivered to the ATP7A transport system, which pumps copper ions from enterocytes into the blood. Inside the cells, copper ions can be transported with the help of COX17 chaperones in mitochondria and with the help of Cu-chaperone antioxidant-1 (ATOX1) to the ATPase that transports divalent copper ions, alpha-polypeptide (ATP7A) or ATPase AT. Copper ions enter the liver through the portal vein and are incorporated into hepatocytes by the copper transporter protein Ctr1 (Zheng et al., 2014). Then copper can be secreted into bile or blood by Atox1/ATP7B/ceruloplasmin. In the bloodstream, this trace element can reach peripheral tissues and Ctr1 is activated again. In the cells of peripheral tissues, copper ions are either sequestered by molecules such as metallothioneins, or directed to the utilization pathway with the help of chaperones such as Atox1, Cox17, and CCS (Chen et al., 2020).

Selenium is one of the most effective substances with a powerful antioxidant effect (Kiełczykowska et al., 2018). Selenium compounds in the body can stimulate the synthesis of reduced glutathione in vivo and play a functional role in increasing the antioxidant capacity and the body's immune response to increased oxidative stress (Barcelos et al., 2022). For example, the antioxidant properties of selenium include the activation of glutathione peroxidase, one of the key cytosolic enzymes of the glutathione defense link, capable of reducing the bactericidal activity of neutrophils (Bai et al., 2017; Giménez et al., 2021). Selenium deficiency leads to a decrease in the activity of antioxidant enzymes and the transformation of glutathione peroxidase into glutathione-S-transferase, which, in turn, is accompanied by significant disturbances in the functioning of the entire antioxidant enzymatic glutathione system (Dresen et al., 2022). Manganese is also one of the most important trace elements. Like copper, it is included in the active center of the isoform of a powerful antioxidant enzyme – manganese-containing superoxide dismutase (MnSOD). In addition, manganese participates in the catalysis of the splitting of hydrogen peroxide (H_2O_2), a key radical obtained from molecular oxygen, one of the main reactive oxidizing species produced by chondrocytes (Bizeau et al., 2017). Manganese ions also neutralize free radicals and cytologically protect pancreatic islets of Langerhans *in vitro* (Tootoonchi et al., 2017).

Ceruloplasmin is a universal extracellular antioxidant (prevents lipid peroxidation of cell membranes). It is a blood plasma protein that performs a number of important biological functions in the body: it increases the stability of cell membranes, participates in immunological reactions (in the formation of the body's defense forces), ion exchange, and stimulates hematopoiesis (blood formation). In addition, ATP7B transports excess copper across tubular membranes and mediates copper excretion with bile (Gulec et al., 2014). Lack of copper can be the reason for the development of anemia, since copper activates ferroxidase and participates in the transportation of iron and its inclusion in the hemin ring during the synthesis of hemoglobin (Broderius et al., 2010; Mostad et al., 2011). Ceruloplasmin has superoxide dismutase activity: it restores superoxide radicals in the blood to oxygen and water and protects against damage to the lipid structure of membranes. It can convert divalent iron into less toxic trivalent iron without releasing active forms of oxygen (Vasilyev, 2019). One of the main functions of ceruloplasmin is the neutralization of free radicals released externally by macrophages and neutrophils during phagocytosis, as well as during the intensification of free radical oxidation in

foci of inflammation. It oxidizes various substrates: serotonin, catecholamines, polyamines, polyphenols, turns divalent iron into trivalent iron (Liu et al., 2022). Also, ceruloplasmin acts as a transport protein – it transports copper from the liver to organs and tissues, where it functions as cytochrome-C reductase and superoxide dismutase. Tocopherol is characterized by many biological functions. By its nature, it is a powerful non-enzymatic antioxidant. The antioxidant effect of tocopherol isomers depends on the nature of the structure, namely, the number of methyl groups in the chroman ring. Among the reactions where tocopherol has antioxidant properties, inhibition of lipid peroxidation is widely known. This is due to the fact that tocopherol is located in the cell membrane and probably participates in the reaction of lipid peroxidation of the cell membrane (Miyazawa et al., 2019). Therefore, the study of tocopherol content in blood serum supplements information about the state of the body's antioxidant activity in response to the accumulation of peroxidation products (Dai et al., 2018; Ognik et al., 2018). It was suggested that the active form of tocopherol has the ability to enhance the activity of catalase in the liver of rats (Bahri et al., 2019).

We have expanded the data on the influence of deficient trace elements on the level of production of lipid peroxidation, the state of the enzymatic and non-enzymatic antioxidant system of young cattle. This confirms the antioxidant properties of individual trace elements and can be used as a preventive measure to correct oxidative stress in the field of animal husbandry.

Conclusions

When the appropriate balance of pro-oxidant and antioxidant factors in the body is disturbed, excessive formation of active forms of oxygen occurs, leading to the occurrence of oxidative stress. During the study, it was found that under the influence of certain factors, the calves during the 30-day period of the experiment were characterized by an increased content of peroxidation products: malonic dialdehyde increased by 1.21 times ($P < 0.001$), lipid hydroperoxides by 1.25 times ($P < 0.001$), and diene conjugates by 1.23 times ($P < 0.001$). The level of antioxidant enzymes, on the contrary, decreased: the level of catalase decreased by 1.09 times ($P < 0.001$), supercosid dismutase and glutathione peroxidase – by 1.15 ($P < 0.01$) and 1.13 times, respectively. The concentration of ceruloplasmin and tocopherol also decreased by 1.10 ($P < 0.001$) and 1.17 times ($P < 0.001$), respectively, against the background of low power of the body's antioxidant defense. Under the action of micronutrient supplements, there was a redistribution of the antioxidant balance. The dynamics of changes in the activity of antioxidant enzymes were directly proportional to changes in the content of peroxidation products under the action of trace elements. Various trace elements had different effects on the antioxidant system of the calves. The highest index of catalase and superoxide dismutase was under the action of the copper additive, when the level of these enzymes was higher by 10.9% ($P < 0.001$) and 22.6% ($P < 0.01$), respectively. Glutathione peroxidase was the largest when sodium selenite was added – by 20.9% ($P < 0.05$). The level of tocopherol was the highest under the influence of manganese – by 11.3% ($P < 0.05$), and the level of ceruloplasmin – under the influence of copper – by 23.9% ($P < 0.05$). Microelement additives have a positive biological effect on the antioxidant defense system of the body due to the inclusion of metal ions in the active centers of antioxidant enzymes. As for the non-enzymatic antioxidant link, the corresponding minerals also influenced their concentration due to a general increase in the trace element status of the calves' body. Accordingly, the level of peroxidation products decreased noticeably.

The research was carried out on the complex theme of the Department of Physiology and Biochemistry of Agricultural Animals of the Dnipropetrovsk State Agrarian and Economic University "Adaptation of animals to the conditions of the central steppe zone of Ukraine under the influence of various conditions of maintenance and biologically active substances", state registration number 0101U004234. The grant-giving organization is the Ministry of Education and Science of Ukraine.

The authors declare that there is no conflict of interest in the preparation of this article.

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