



Influence of Marmix premix on the state of lipid peroxidation and indices of non-specific resistance of the organism of pregnant mares with microelementosis

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We observed the processes of lipid peroxidation, characterized by increased content of intermediate and its end products (diene conjugates, lipid hydroperoxides, malonic dialdehyde), in the blood of the pregnant mares. The changes of the data of indicators in the organism of the mares at the 9–11 months of pregnancy were established. The level of diene conjugates in the blood of animals was $3.6 \pm 0.12 \mu\text{mol/l}$, 33.3 and 44.0%, which was higher compared to non-pregnant mares and mares at 4th month of pregnancy. The concentration of lipid hydroperoxides in the blood of mares increased by 100% and by 42.9% in the blood of non-pregnant mares and mares at 4th month of pregnancy. The concentration of malondialdehyde in the blood of mares increased by 75.0%, 51.2% and 25.0% compared to non-pregnant mares and mares at 4th and 7th month of pregnancy, respectively. The results of our research showed that the mineral-vitamin premix Marmix had a positive influence on the state of lipid peroxidation products, contributed to their lowering in the blood of pregnant mares, which demonstrated antioxidant action and stimulated the function of the immune system. It was established that the bactericidal and lysozyme activity of the blood serum and the concentration of circulating immune complexes increases in the mares with lack of trace elements. It was shown that feeding mares during the 9–11 months of pregnancy with the mineral-vitamin premix Marmix had a positive influence on the state of humoral factors of nonspecific resistance. In particular, the concentration of bactericidal activity of serum increased by 31.0%, lysozyme activity of blood serum – 45.4% and contributed to the decrease of the concentration of circulating immune complexes in serum by 3.8 times. The use of mineral-vitamin premix Marmix during 60 days in feeding pregnant mares caused a restoration of the clinical status, a decrease in the content of lipid peroxidation products.

Keywords: lipid hydroperoxides; malonic dialdehyde; immunity; bactericidal activity; blood serum; circulating immune complexes.

Introduction

Currently, there is a significant number of reports concerning the role of lipid peroxidation in the development of many diseases of non-infectious and infectious etiology (encephalomalacia and exudative diathesis, proteinuria, gastrointestinal, respiratory and metabolic diseases, in particular, microelementosis, kidney damage, liver, endometritis, mastitis, etc.) (Wingfield, 1984; Clarke et al., 1987; Holovakha et al., 2018). However, research on lipid peroxidation in relation to hypocalcemia and hypocalcemia in mares with the development of anemia has not been conducted (Droge, 2002; Gerber et al., 2014; Padalino et al., 2017).

The course of any pathological process in an organism depends on the intensity of lipid peroxidation (Esterbauer et al., 1992; Maksymovych & Slivinska, 2018). Most authors (Niki, 2009) consider the intensification of lipid peroxidation as one of the complex mechanisms of disorganization of the structural and functional integrity of various biological substances. The processes of lipid peroxidation can be considered as non-specific adaptive reaction of the organism. On the other hand, they lead to damage to the molecular structure of cell membranes, inhibition of the activity of enzymes (Bajpai et al., 2014). Thus, the processes of peroxidation of lipids are considered as one of the important mechanisms of cellular pathology, which is the basis of many negative effects. A certain role in the development of pathology is played by intermediate and finished products of peroxidation, which have cytotoxic and mutagenic effects (Gut'j et al., 2017). Indicators of lipid peroxidation are widely used in research on oxidative stress. Therefore, unsaturated

fatty acids are oxidized, which can cause the violation of the integrity and properties of biological membranes (Kuljaba et al., 2016). During this process, lipid radicals are formed from stable lipid molecules, which are subjected to gradual destruction. Since the lipid peroxidation occurs primarily in biomembranes, this leads to a violation of their functional properties. The most important marker of lipid peroxidation is malonic dialdehyde, the formation of which leads to hydrophilization of membranes, inhibition of protein biosynthesis and replication, etc. (Hutyi et al., 2016). In physiological conditions, the level of lipid peroxidation is maintained due to the balance of the system of anti- and prooxidants. The antioxidant defense system belongs to the key regulatory systems of an animal organism, since it counteracts the processes of the lipid peroxidation (Lavryshyn et al., 2016).

The activation of free radical oxidation of lipids' processes leads not only to the damage of hepatocytes, but also to changes in blood cells – the most mobile body system (Lavryshyn et al., 2016). However, some mechanisms of activation of the free radical oxidation processes for hypocalcemia and hypocalcemia are present in mares, which are accompanied by the development of anemia, their interconnection and interdependence with the state of the protective systems of the organism. In particular, the immune system remains unclear, which is closely linked to the system of antioxidant protection of the organism, since the decrease of the humoral and cellular links of the immune system, the activity of the antioxidant system is decreased and the intensity of peroxidation of lipids and the formation of free radicals that are harmful to the cells of the organism is increased.

It is well known that the predisposition of animals to diseases, the nature of their course and their consequences, are largely due to the state of an organism's resistance. The term resistance (resistibility) means the state of specific and nonspecific protective and adaptive mechanisms of the organism, able to counteract various unfavourable factors (Khariv et al., 2017; Slivinska et al., 2018). Humoral factors of non-specific resistance of the organism include bactericidal and lysozyme activity of the blood serum.

According to current views, the immune system is a branched structure of the integral nature and performs not only a unique function of immunity, but also plays the role of a unifying network, providing through the cytokines the connection between the nervous and endocrine systems of the organism. One of the important indicators of resistance is bactericidal activity of serum, which indicates the ability of blood to self-purify (Vishchur et al., 2015).

The research objective was to determine effectiveness of the mineral-vitamin premix Marmix in the system of complex measures for prevention of microelementosis, to analyze its influence on the state of peroxidation of lipids system and non-specific resistance of the organism of pregnant mares.

Material and methods

The research was conducted at the Scientific-Production Association "Plemekonecenter" (Transcarpathian region). The object of research was the blood serum of pregnant mares of the Hutsul breed, aged 4–18 years, with a weight of 400–450 kg. All mares were kept and fed in the same conditions. During the research, the rules were followed for conducting zootechnical experiments on the selection and maintenance of animal in groups, harvesting technology, forage given and counting of consumed food. The composition of the mineral-vitamin premix Marmix (Shherbatyj & Slivinska, 2013), its therapeutic and prophylactic effectiveness was substantiated experimentally based on the conducted research.

For this purpose, two groups of mares with signs of microelementosis were formed – experimental and control with 10 animals in each, who were in the 9th month of pregnancy. The research on the effectiveness of the mineral-vitamin premix Marmix for the mares was carried out in comparison with the indices of the mares with signs of a violation of mineral metabolism. The analysis of ration showed that provision to mares with cobalt was 54.7%, copper – 88.5%, zinc – 83.3%, ferum – 88.4%, manganese – 29.0% (Shherbatyj & Slivinska, 2013). Clinical examination established that 60% of the mares had dull hair cover, dry skin of low elasticity. In 40% of the examined mares, visible mucous membranes (conjunctiva, nose, mouth) were anemic. In the area of the mane, neck, trunk, on limbs and around the eyes alopecia was detected. There was a decrease in appetite and distortion of taste, which is a characteristic indicator of mineral deficiency (osteodystrophy, hypocalbaltosis and hypocuprosis).

In 20% of the mares, lameness during movement and incorrect position of the limbs was observed. In 40% of the mares damage to the hoofs was detected. In the blood serum of the mares a low level of cobalt (0.12–0.42 $\mu\text{mol/L}$) and copper (1.98–3.00 $\mu\text{mol/L}$) was established. We also established oligocythemia (3.7–5.9 T/L), oligochromemia (80–88 g/L) and hypochromia (14.2–16.7 pg) in the group which received the main ration (control group), which included (kg): hay of cultivated hayfields – 2.5, high altitude hay – 2, meadow hay – 2.5, wheat bran – 0.5, corn bran – 1, oat grain – 1, sunflower oil – 0.5, dry pulp, granulated – 1. Each mare drank about 30.3 ± 0.2 L of water per day, which was extracted from an artesian well.

In addition to the main ration, the experimental group received the mineral-vitamin premix Marmix (production of the "EGO" company) at a rate of 100 g per day for 60 days during the period of morning feeding. One kilogram of premix included: vitamin A – 250000 MO, D₃ – 25000 MO, E – 6000 mg, B₁ – 400, B₂ – 300, pantothenic acid – 300, niacin – 700, B12 – 0.75, biotin – 175, ascorbic acid – 600; mineral substances – zinc – 750 mg, ferum – 1100, copper – 180, manganese – 1200, iodine – 7.5, selenium – 2.5, cobalt – 25 mg; amino acids – lysine – 1.5%, threonine – 0.75%, methionine – 1.0%, filler (wheat bran – up to 1 kg). During the experiment, the animals were under clinical observation.

Before the start of the experiment, as well as 45 and 60 days after the beginning of feeding with the premix, blood samples from the jugular vein were taken for laboratory examination. In the blood, the content of malondialdehyde (Korabeinykova, 1989), hydroperoxides of lipids (Mironchik, 1984), diene conjugates (Stalnaia, 1977) were determined.

The state of non-specific resistance of horses was determined by the bactericidal and lysozyme blood serum activity by the method of photo-electrocolorimetry in the modification of the Department of Zoo IECVM. The state of humoral immunity was assessed by the content of circulating immune complexes, which were determined by the method of Frolov et al. (Vlizlo et al., 2012).

All manipulations with animals were carried out in accordance with the European Convention for the Protection of Vertebrate Animals, Used for Experimental and Scientific Purposes (Strasbourg, 1986). The mathematical processing of the research results was worked out statistically using a program package Statistica 6.0 software (Stat Soft, Tulsa, USA). Differences between the mean values were considered statistically significant at $P < 0.05$ (ANOVA).

Results

The accumulation of a significant amount of lipid peroxidation products in tissues of vital organs, plasma and erythrocytes were established. This provided the basis for blood testing with a diagnostic purpose to determine the intensity of free radical oxidation of lipids in tissues. The results of the research showed that the mineral-vitamin premix Marmix had a positive effect on the state of peroxidation of lipids, contributed to the reduction of its products in the blood of the pregnant mares, while the control group showed a gradual accumulation of them.

The content of diene conjugates in the blood of the mares of the experimental group at the 45th day was on average 2.67 ± 0.15 $\mu\text{mol/L}$ and was 25.6% ($P < 0.01$) lower than at the beginning of the experiment (Fig. 1). After completion of the experiment, the content of diene conjugates decreased significantly by 16.9 ($P < 0.05$), 38.2 ($P < 0.001$) and 40.0% ($P < 0.001$) compared with the 45th day, the start of the experiment and control, respectively. At the same time, the number of lipid hydroperoxides in the experimental mares decreased: on the 45th day – by 18.4% ($P < 0.05$) and on average it was 1.20 ± 0.07 $\text{unE}_{408}/\text{mL}$ (Fig. 2), at the end of the use of the premixes – by 20.4% and 25.5% ($P < 0.05$), compared with the beginning of the experiment and control, respectively.

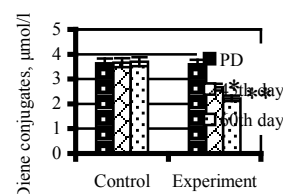


Fig. 1. Influence of mineral-vitamin premix Marmix on the concentration of diene conjugates in the blood of the pregnant mares ($\mu\text{mol/L}$, $n = 10$)

In the control group, the hydroperoxides of lipids content increased and at the 60th day it was 1.57 ± 0.15 $\text{unE}_{408}/\text{mL}$. Under the influence of the premixes, the amount of malonic dialdehyde, the final product of lipid peroxidation, in the blood decreased. On the 45th day, its amount was probably ($P < 0.01$) decreased by 20.9% and averaged 2.77 ± 0.18 $\text{unE}_{408}/\text{mL}$ (Fig. 3). It should be noted that the content of malondialdehyde in the blood of the mares of the control group at the 45th day was 3.43 ± 0.09 nmol/L . Subsequently, the content of malondialdehyde in the experimental group of mares continued to decrease and by the end of the experiment had decreased by 35.4% 48.9% and 47.2% ($P < 0.001$) compared with the 45th day, the start of the experiment and control, respectively. The following conclusions can be drawn according to the obtained results: the mineral-vitamin premix Marmix inhibits the processes of lipid peroxidation, namely, reduces the concentration of

oxidation products, thereby reducing the toxic influence on the cells of the body, which contributes to its recovery and the disappearance of symptoms of microelementosis, anemia, disturbance of liver and kidney function.

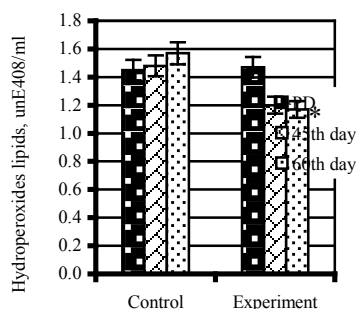


Fig. 2. Influence of mineral-vitamin premix Marmix on the content of lipid hydroperoxides in the blood of the pregnant mares (unE₄₀₈/mL, n = 10)

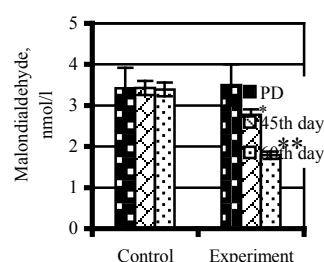


Fig. 3. Influence of mineral-vitamin premix Marmix on the content of malondialdehyde in the blood of the pregnant mares (nmol/L, n = 10)

Lysozyme activity of serum is an important factor in the natural resistance of the organism. It is included in the bactericidal activity of serum and, due to the specificity of directly affecting the cells of both microorganism and immune system, plays a significant role in humoral defense. The activity of serum lysozyme reflects the level of natural resistance of an organism (Vishchur et al., 2015). In blood of the pregnant mares at the beginning of the experiment, a low level of serum lysozyme activity in both groups of animals was established.

After feeding mares with Marmix, at the 45th day lysozyme activity of the blood serum tended to increase, compared to the beginning of the experiment (Fig. 4), and by its completion, the difference with the control animals was 16.5, with an initial rate of 16.8% ($P < 0.001$). It should be noted that the growth of lysozyme activity of the blood serum in the last 15 days of the experiment was more intense compared with the first 45-day period.

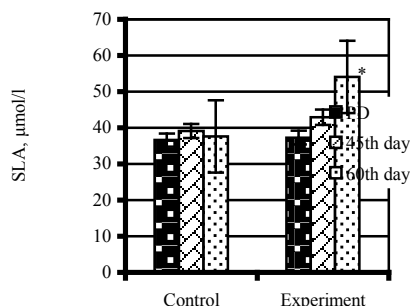


Fig. 4. Influence of mineral-vitamin premix Marmix on lysozyme activity of the blood serum of pregnant mares ($\mu\text{mol/L}$, n = 10)

Unlike lysozyme activity of the blood serum, another indicator of nonspecific resistance, which indicates the ability of blood to self-purify – bactericidal activity of serum, increased more intensively in the first 45 days (by 11.9%), and in the last 15 days changes were slight.

During the period of 45 days of premix feeding, bactericidal activity of serum ($P < 0.05$) increased by 11.2% and at 60th day by 12.5% ($P < 0.001$) (Fig. 5). Such increase indicates the restoration of nonspecific resistance due to a balanced ration of all nutrients and minerals contained in the mineral-vitamin premix Marmix.

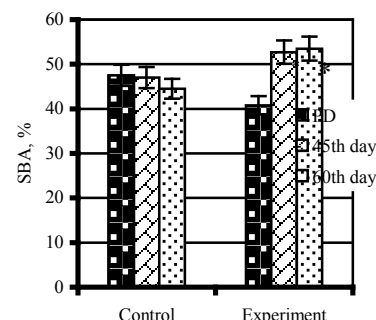


Fig. 5. Influence of mineral-vitamin premix Marmix on bactericidal activity of serum of pregnant mares (% , n = 10)

Determination of the contents and composition of circulating immune complexes is an important diagnostic test, which can be used to determine the degree of inflammation in the organism of animals. The formation of circulating immune complexes refers to the primary acts of the immune reaction of the organism. This physiological process, along with others, provides support for immunological homeostasis. Circulating immune complexes affect the function of lymphocytes, macrophages, and therefore participate in the regulation of the immune response. The concentration of the circulating immune complexes depends on the antigenic loading, the properties of the antibodies, the state of the complement system and phagocytic cells (Vishchur et al., 2015).

A probable decrease in circulating immune complexes was found on the 45th and 60th days of the experiment (Fig. 6). In particular, by the 45th day, the index had decreased by almost 2.65 times ($P < 0.001$) compared to the beginning of the experiment and averaged 28.2 ± 1.93 mg/ml, which may indicate a reduction in the influence of the pathological process in the organism. By the end of the experiment, the content of the circulating immune complexes had reduced by 3.83 and 4.30 times compared ($P < 0.001$) with the beginning of the experiment and control, respectively.

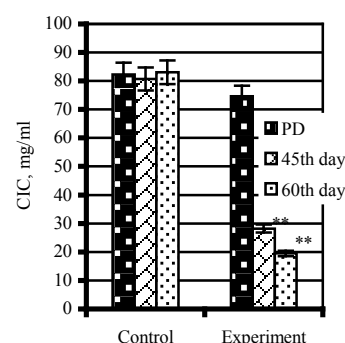


Fig. 6. Influence of mineral-vitamin premix on the content of circulating immune complexes in the blood of pregnant mares (mg/ml)

Thus, the mineral-vitamin premix Marmix positively affects the state of humoral factors of non-specific resistance of the pregnant mares, in particular, it increases the activity of bactericidal and lysozyme activity of the blood serum and reduces the content of the circulating immune complexes. A number of components of the preparation (vitamins A, C and E, copper, manganese, selenium) have pronounced antioxidant properties, since some of them (Cu, Mn and Se) are part of enzymes that have a major role in antioxidant defense. In this regard, in the process of using the preparation, the relationship between antioxidant defense system and the processes of the lipid peroxidation is restored, therefore the level of the latter in the blood of mares of the experimental group decreases.

Discussion

Pregnancy is a special physiological state of the organism of the pregnant mares, characterized by an intensive course of metabolic processes, aimed at the effective relationship between the organism of the mother and the fetus, ensuring its growth and development (Golovach et al., 2005). Mares, during the period of pregnancy, are very sensitive to malnutrition and conditions of retention. This especially applies to the mares during the last months of the pregnancy, which are characterized by intensive use of the mineral, energetic and plastic resources of the mother's body in the formation of fetal tissues (Shcherbatyy et al., 2017).

One of the main prerequisites for the high productivity of farm animals is their complete mineral nutrition. The lack of separate mineral elements, as well as violation of their ratios in rations, leads to a decrease in the efficiency of the use of nutrients in the feed. In animals, productivity, resistance, antioxidant status are reduced, the function of reproduction is worsened and susceptibility to disease is increased (Shcherbatyy et al., 2017). In recent years horses illness in Ukraine has been studied by many scientists, but insufficient attention has been paid to the biogeochemical pathology of horses. The biochemical province of Transcarpathia is characterized by specific features regarding the content of vital micro-elements. The consequences of their shortage, which are constantly registered in different regions of the Carpathians, require further research. In this regard, scientific and practical attention is paid to complex research, based on the determination of the content of trace elements in the soil, feed and blood of animals, the study of hematopoiesis and the state of metabolism, and effective measures to correct the violations discovered (Maksymovych, 2017).

By the actions of various unfavourable external factors in horses, diseases of different genesis, among them – microelementosis are diagnosed (Fielding et al., 2009). According to the existing views of the pathogenesis of microelementosis in animals, it is associated with free radical metabolites (Bomko et al., 2018; Sobolev et al., 2018). As a result of enhancement of peroxidation of lipid complexes of intracellular membranes, the activity of enzymes, a number of cell functions (protein synthesis, β -lipoprotein metabolism), degeneration of nucleotides occurs, etc. It is believed that the main place of formation of free radicals is endoplasmic net and cells microsomes (Lavryshyn et al., 2016). Results obtained by research indicate a significant imbalance in the prooxidant-antioxidant system of the liver and the predominance of mechanisms of damage to protective mechanisms. In order to combat the manifestations of toxic liver damage in recent years, antioxidants are used to correct antioxidant defense and neutralize free radical oxidation products. Finding active substances with antioxidant properties is a rather promising direction of research, although it requires consideration of the compatibility of natural and synthetic antioxidants. The determining factors of the antioxidant action of the preparation are the total number of antioxidant substances in its composition, a qualitative antioxidant spectrum (the presence of vitamins, vitamin-like substances, metal trace elements), as well as the total quantitative content of substances with antioxidant properties.

The results of our research showed that Marmix mineral-vitamin premix positively influenced the state of processes of peroxidation of lipids, contributed to their lowering in the blood of pregnant mares, which contributed to the prevention of oxidative stress in animals. Copper, zinc and manganese are involved in the functioning of the antioxidant system. Their reduction in the organism results in inhibition of the synthesis of mitochondrial superoxide dismutase and damage to membranes of mitochondria.

It was proven that Marmix contains a number of minerals and vitamins that have a positive effect on the antioxidant system. Retinol and tocopherol are considered powerful natural antioxidants. Therefore the content of products of lipid peroxidation was reduced by their use.

Thus, the introduction to mares' rations of the mineral vitamin premix Marmix in the biogeochemical province of Transcarpathian region during 60 days leads to the elimination of the symptoms of hypocalbaltosis and hypocuprosis, causes the restoration of indices of nonspecific resistance and reduces the content of blood products of lipid peroxidation.

Conclusions

The processes of peroxidation of lipids in mares is increased with hypocalbaltosis and hypocuprosis, which are characterized by an increase in the blood plasma of the levels of diene conjugates (compared to indices in non pregnant mares), the concentration of lipid hydroperoxides at 7 and 9–11 months and malonic dialdehyde for 9–11 months of pregnancy and compared to non pregnant and mares in the 4th and 7th months of the pregnancy. At the 4th month of pregnancy, a reverse correlation between the content of cobalt and malonic dialdehyde was established and lipid hydroperoxides, with vitamin A content and lipid hydroperoxides; for 7 months – between the level of copper and diene conjugates. The use of the mineral vitamin premix Marmix during 60 days for pregnant mares led to the restoration of clinical status, lowering the content of lipid peroxidation products. An inverse correlation between the content of malonic dialdehyde and copper, lipid hydroperoxides and vitamin A and tocopherol was established.

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