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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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 hypoco- baltosis and hypocuprosis 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 & Sliwinska, 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 (Gutyj 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 hypoco- baltosis and hypocuprosis 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 (Khuriv et al., 2017; Silivinska et al., 2018). Humoral factors of nonspecific 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 macroelementosis, to analyze its influence on the state of peroxidation of lipids system and nonspecific resistance of the organism of pregnant mares.

### Material and methods

The research was conducted at the Scientific-Production Association “Plenmekoncenter” (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, food given and counting of consumed food. The composition of the mineral-vitamin premix Marmix (Shherbatyi & Slivinska, 2013), its therapeutic and prophylactic effectiveness was substantiated experimentally 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 the animals of mineral-vitamin premix Marmix in the system of complex measures for prevention of macroelementosis, to analyze its influence on the state of peroxidation of lipids system and nonspecific resistance of the organism of pregnant mares.

### 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.

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 (Korbaen’ykova, 1989), hydroperoxides of lipids (Mi-ronchik, 1984), diene conjugates (Stalnaia, 1977) were determined. The state of nonspecific resistance of horses was determined by the bactericidal and lysozyme blood serum activity by the method of photoelectrocolorimetry in the modification of the Department of Zoo ECVM. 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).

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### Discussion

The state of nonspecific resistance of horses was determined by the bactericidal and lysozyme blood serum activity by the method of photoelectrocolorimetry in the modification of the Department of Zoo ECVM. 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).

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### Experimental group

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 μmol/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 μmol/μg/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.

### Control group

In the control group, the hydroperoxides of lipids content increased and at the 60th day it was 1.57 ± 0.15 μmol/μg/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 μmol/μg/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 μmol/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.

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.

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.

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.

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Discussion

Pregnancy is a special physiological state of the organism of the pregnant mare, 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). In recent years horses illness in Ukraine has been studied by many scientists, but insufficient attention has been paid to the biogeocoenic 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).

The processes of peroxidation of lipids in mares is increased with hypocholesterolemia 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 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.

Conclusions

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