

The impact of endogenous intoxication on biochemical indicators of blood of pregnant cows

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Article info

Received 04.06.2017 Received in revised form 12.07.2017 Accepted 20.07.2017

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The notion "endotoxin" is conditional, for any normal metabolit has a toxic impact if it has an excessive accumulation in an organism. Endotoxins include products of breakdown of tissue proteins, peroxides and other products of free radical oxidation, low molecular weight toxins, toxins of microorganisms. Current knowledge on the mechanism of endotoxin action on the organism of pregnant cows is based on the key role of the immune system: it provides animals with protection against infections, eliminates extraneous elements of endogenous and exogenous origin. It is understood that under endogenous intoxication, toxic metabolites suppress the activity of an organism's immune system and decreases the resistance of an organism to negative effects of environmental factors. Animals under endogenous intoxication had the following clinical symptoms: swelling of mammary gland, edemas of external genitals, anemia of mucous membranes, functional disorders of proventriculuses and intestines, and the animals were stressed. It was found that under endogenous intoxication at 8-9 months of pregnancy, the blood of highly-productive cows had a tendency of decrease in the number of erythrocytes by 21.0% and decrease in the level of hemoglobin by 24.3%, and increase in the number of leucocytes by 7.6% compared to the value for the blood of cows with physiological pregnancy process. The development of endogenous intoxication among pregnant cows causes disorders in protein-synthesizing function of liver, which is indicated by decrease in the level of total protein and its fractions. We found that endogenous intoxication decreased the content of total protein in the blood by 18.4%. Over the period of study, among pregnant cows under the development of endogenous intoxication, heightened activity of amino transferases in the blood was observed. In the ninth month of pregnancy, the activity of amino transferases in blood serum of cows from the experimental group increased by 28.6%, and the activity of aspartate aminotransferase increased by 20.1% respectively. Increase in the activity of amino transferases and decrease in the level of total protein and its fractions in the blood serum is one of the first biochemical tests for the diagnosis of endotoxin manifestations and indicates destructive processes in the liver. We found that pregnant cows under the development of endogenous intoxication suffered disorders in detoxicating function of the liver and disorders in filtrating function of the kidneys. The developing endogenous intoxication of pregnant cows suppressed the activity of enzymes in their glutathione system of antioxidant protection, which is indicated by decrease in the activity of glutathione peroxidase by 27.5%, glutathione reductase by 42.9%, glucose-6-phosphate dehydrogenase by 11.2% in the blood serum. The lowest activity of enzymes of glutathione system in the blood of highly-productive cows under endogenous intoxication was observed in their ninth month of pregnancy, which is related to increase in activation of processes of lipid peroxidation and imbalance between the activity of antioxidant system and intensity of lipid peroxidation. A significant increase in the content of lipid hydroperoxides by 70.9% and in concentration of malondialdehide by 54.8% was observed. Imbalance in generation of active forms of oxygen and their metabolites, exhaustion of antioxidant system and disorders in its balance cause oxidative stress.

Keywords: toxicology; midwifery; endotoxin; enzymes; antioxidants; total protein; urine; creatinine

Introduction

It is known that conditions of feeding and maintaining pregnant cows significantly impact on their offspring (Sheldon et al., 2006; Mainardes and DeVries, 2016; Fabris et al., 2017; Fedorovych, 2017). Therefore, from the beginning of pregnancy, a cow should be provided with the necessary amount of nutrients, which would satisfy its own vital needs, and the needs of the fetus and lactation. Deficiency or excessive amount of energy, biologically active agents in diets causes disorders in the metabolism of pregnant cows (van der Drift et al., 2015; Macmillan et al., 2017; Slivinska et al., 2017; Vieira-Neto et al., 2017).

Many publications on the development of endogenous intoxication of different etiology among pregnant animals have given cause for concern (Gutyj et al., 2016; Rodríguez et al., 2017). Researchers distinguish several important biochemical mechanisms of development of endogenous intoxication of animals, particularly the activation of processes of free radical oxidation (Gutyj et al., 2016; Hariv and Gutyj, 2016; Khariv et al., 2016; Martyshuk et al., 2016; Khariv and Gutyj, 2017).

It has been shown that in the conditions of development of endogenous intoxication among pregnant cows, in the ninth month, the content of triiodthyronine and thyroxine decreases and the level of thyrotropic hormone of the pituitary gland increases. Therefore endogenous intoxication of pregnant cows causes suppression of thyroid gland function, which impacts on fat, protein and carbohydrate metabolism (Hrymak and Hunchak, 2014). The development of endogenous intoxication of pregnant cows causes decrease in arterial oxygen content and development of tissue hypoxia, which slows the processes of oxidation and recovery of NADP, a coenzyme of more than 250 dehydrogenases (Andersen, 2003; Hrymak et al., 2015; Panevnyk and Suprovych, 2016). We should also mention that uterus is especially vulnerable to hypoxia. The symptoms include decrease in macroergic compounds, particularly acid phosphatase and adenosine triphosphate, which causes general suppression of organisms of pregnant cows.

Veterinary medicine often focuses on studying indicators of animals' blood, for the values can indicate the extent of intensity of metabolic processes, development of intoxications, diseases, both of infectious and non-infectious etiology. Therefore this research aims at studying the impact of endogenous intoxication on hematological and biochemical indicators of the blood of pregnant cows.

Materials and methods

The research was conducted in "Barkom" PLC, Yampil' village of Pustomytovs'ky district of Lviv oblast. The research used pregnant cows of Ukrainian black and speckled dairy breed. All experimental studies were conducted in compliance with bioethical requirements, according to the Law of Ukraine "Protecting Animals from Abuse", 28.03.2006 and "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes", 13.11.1987.

The study used 20 selected cows, which were divided into two groups, each consisting of 10 individuals: the control and experimental group. The cows of the control group were healthy and had a physiological process of pregnancy. The cows of the experimental group had typical manifestations of endogenous intoxication. In particular the symptoms were: anemia of mucous membranes, functional disorders of proventriculuses and intestines, swelling of mammary glands and edemas of external genitals.

The blood of cows in 8th and 9th month of pregnancy was taken from the jugular vein for biochemical analysis. The number of leucocytes was calculated using a haemocytometer; the number of erythrocytes – using methodic of Havrylets et al.; the concentration of hemoglobin – using the method of Pimenova et al.; the content of total bilirubin, urea, creatinine – methods described in the handbook "Laboratory Methods of Researchn in Biology, Stock-breeding and Veterinary Medicine"; the level of total protein – method of Delektorskaya; fractional compound of proteins – method of electrophoresis in polyacrylamide gel; the level of malondialdehyde – method of Korobeynikov; the content of hydroperoxides of lipids – method described by Mironchuk; activity of amino transferases – Reitman Frankel method modified by Kapetanaki; activity of glutathione peroxidase (GPx) and glutathione reductase (GR) – method of Lemeshko; activity of glucose-6-phosphate dehydrogenase (G6PD) – method of Baquezetal (Vlizlo, 2012).

Mathematical analysis of the results was made in Statistica 6.0 (StatSoft Inc., USA). The differences between average values were considered statistically significant at P < 0.05 (ANOVA).

Results

We found that under the development of endogenous intoxication, the number of erythrocytes of cows in the 8th month of pregnancy from the experimental group decreased by 13.5%. Further into the experiment the number of erythrocytes continued to decrease, and in the 9th month of pregnancy it was 5.93 ± 0.32 T/l, respectively, i.e. it decreased by 21.0% compared to the control (Table 1). Similar changes were observed in the study of hemoglobin. The blood of cows from the control group with physiological process of pregnancy had the level of hemoglobin fluctuating within 110.0–119.1 g/l, whereas the hemoglobin content of cows with manifestations of endogenous intoxication was significantly lower: lower by 13.6% in the eighth month of pregnancy, and by 24.3% in the ninth month respectively.

In the control group, we observed an insignificant increase in the number of leukocytes among the cows in the ninth month of pregnancy compared to the cows in the eighth month of pregnancy. The number of leukocytes in the blood of the animals from this group fluctuated within 7.58–7.61 g/l. Over the progression of endogenous intoxication, the number of leukocytes in blood of cows from the experimental group began to increase, and in the eight month of pregnancy it increased by 4.7%, and in the ninth month – by 7.6%. The significant increase in the number of leukocytes in the blood of intoxicated animals is related to progressing inflammatory processes in the organism of pregnant cows as a result of the progression of endogenous intoxication.

The content of total protein and its fractions in the blood of cows from the control group was within the physiological values, whereas its content in the blood of cows from the experimental group was significantly decreasing already from the eight month of pregnancy (Table 2). The level of total protein in the blood of cows from the experimental group decreased by 12.8% in the eighth month of their pregnancy, and by 18.5% in the ninth month.

Table 1

Morphological indicators of the blood of pregnant cows with progressing endogenous intoxication ($x \pm SE$, n = 10)

Month of pregnancy	Group of animals	Erythrocytes T/l	Hemoglobin g/l	Leukocytes G/l
0	С	7.10 ± 0.24	110.01 ± 4.02	7.58 ± 0.32
8	Е	$6.14 \pm 0.28^{*}$	$95.09 \pm 3.18*$	7.94 ± 0.27
9	С	7.51 ± 0.25	119.11 ± 4.71	7.61 ± 0.28
	E	$5.93 \pm 0.32^{**}$	$90.19 \pm 2.82^{***}$	$8.19\pm0.17*$

Note: * - P < 0.05; ** - P < 0.01; *** - P < 0.001.

Table 2

The content of total protein and its fractions in the blood of pregnant cows with progressing endogenous intoxication ($x \pm SE$, n = 10)

Month	Crown		Protein fractions, %			
of pregnancy	of animals	Total protein, g/l	albumins	globulins		
	of animals			alpha-	beta-	gamma-
8	С	79.67 ± 1.68	44.11 ± 0.31	16.55 ± 0.21	14.24 ± 0.18	25.06 ± 0.45
	E	$69.44 \pm 1.87^{***}$	$40.61 \pm 0.25 **$	$19.14 \pm 0.13^{**}$	$16.36 \pm 0.19 **$	$23.85 \pm 0.40*$
9	С	78.72 ± 1.89	43.86 ± 0.52	16.85 ± 0.16	15.03 ± 0.15	24.22 ± 0.64
	Е	$64.19 \pm 1.92^{***}$	$38.94 \pm 0.40 **$	$21.54 \pm 0.11 **$	$16.89 \pm 0.24 **$	$22.59 \pm 0.38*$

Note: see Table 1.

The analysis of protein fractions in the blood of cows from the control group found that the content of albumin was 44.1% in the eighth month of pregnancy, but its content insignificantly decreased in the ninth month of pregnancy. A significant decrease in the level of albumins was observed in the blood of cows from the experimental group, which had typical clinical symptoms of endogenous intoxication. This indicator decreased by 7.9% on the eight month of their

pregnancy, and by 11.2% in the ninth month compared to the control. The decrease in the level of albumin in blood of pregnant cows with symptoms of endotoxicosis indicates deficiency of amino acid and protein in the cows' organism. Similar changes were observed in the analysis of the content of γ -globulins in the blood of cows from the experimental group, where it decreased by 4.8% in the eighth month of pregnancy and by 6.7% in the ninth month compared to blood

indicators of cows with physiological process of pregnancy. The analysis of α - and β -globulins in the blood of cows in the eighth month of pregnancy from the experimental group showed an increase of 19.1% and 16.4% respectively. In the ninth month of pregnancy, the level of α - and β -globulins in the blood of cows with manifestations of endogenous intoxication was high and increased by 27.8% and 12.4% respectively compared to the control.

We found that the activity of alanine- and aspartate aminotransferase in the blood serum of cows in the ninth month of pregnancy from the control group slightly increased compared to the values in the eighth month of pregnancy: the activity of alanine aminotransferases increased by 28.6%, and the activity of aspartate aminotransferase – by 20.0%. Such changes in the activity of aminotransferases in the blood of cows with physiological process of pregnancy is related to intensifying metabolic processes as a result of organism's preparation for birth.

Table 3

The activity of aminotransferases in the blood serum of pregnant cows with progressing endogenous intoxication ($x \pm SE$, n = 10)

Month of pregnancy	Group of animals	ALAT, IU/I	ASAT, IU/I	De Ritis ratio
8	С	26.9 ± 0.51	42.9 ± 0.69	1.59 ± 0.07
	Е	$32.0 \pm 0.81^{***}$	$50.4 \pm 0.71^{***}$	1.57 ± 0.05
9	С	29.6 ± 0.50	46.0 ± 0.64	1.55 ± 0.06
	E	$38.1 \pm 0.14 ***$	$55.2 \pm 0.57 ***$	1.45 ± 0.04

A significant increase in activity of alanine- and aspartate aminotransferases of cows from the experimental group, which had clinical symptoms of endogenous intoxication, was observed throughout the experiment. In the eighth month of their pregnancy, the activity of ALAT and ASAT in the blood serum of intoxicated cows increased by 19.0% and 17.5% respectively. In the ninth month of pregnancy, an increase in activity of the aminotransferases in the blood of cows from the experimental group was observed. This was a result of toxic impact of toxins, free radicals, active forms of oxygen on the liver cells. A significant increase of ALAT and ASAT in the blood serum of cows from the experimental group in the eighth and ninth months of pregnancy indicates disorders in the functional condition of the liver. This was proved by the decrease in De Ritis ratio (AST/ALT ratio).

In the blood of cows from the experimental group, we observed excessive content of total bilirubin. In the eighth month of cows' pregnancy it increased by 17.8%. In the ninth month, the content of total bilirubin in the blood of intoxicated cows reached $6.0 \pm 0.14 \,\mu$ mol/l.

The indicator for concentration of urea in the blood of animals the was within physiological values for the cows of the control group, whereas in the blood of cows from the experimental group, the urea concentration decreased by 14.9 and 31.3% in the eighth and ninth months of pregnancy respectively, compared to the control.

Table 4

Biochemical indicators for blood of pregnant cows with progressing endogenous intoxication ($x \pm SE$, n = 10)

Month	Group of	Urea,	Creatinine,	Total bilirubin,
of pregnancy	animals	μmol /l	µmol/l	μmol /l
8	С	4.6 ± 0.17	102.0 ± 3.51	4.4 ± 0.23
	Е	$3.9 \pm 0.12^{**}$	106.3 ± 4.07	$5.2 \pm 0.19^{*}$
9	С	4.7 ± 0.17	101.4 ± 3.03	4.5 ± 0.15
	Е	3.2 ± 0.14 ***	$112.3\pm3.02*$	$6.0 \pm 0.14^{***}$

The analysis of the level of creatinine in the blood of pregnant cows showed a significant increase in the blood of cows from the experimental group in the ninth month of pregnancy: it increased by 10.7% compared to the control.

Analysis of the results (Table 5) revealed a decrease in the activity of glutathione peroxidase in the blood serum of cows with typical clinical symptoms of endogenous intoxication. On the eighth month of pregnancy, the activity of the enzyme in the intoxicated cows fluctuated between 20.5 ± 1.02 nmol GSH/min mg of proteins, which was lower by 21.5% compared to the control. In the ninth month of pregnancy, a decrease in the activity of glutathione peroxidase was

observed: it became lower by 27.5% compared to the control. The analysis of activity of glutathione reductase determined that in the blood of cows from the control group in the ninth month of pregnancy this enzyme was slightly higher than values in the eighth month of pregnancy. The analysis of blood of animals from the experimental group (cows with manifestations of endogenous intoxication) showed a significant reliable decrease in the activity of glutathione reductase by 25.0% and 42.9% respectively compared to the control.

Table 5

Activity of glutathione system in the organism of cows with progressive endogenous intoxication ($x \pm SE$, n = 10)

Month of preg- nancy	Group of ani- mals	Glutathione peroxidase, nmol GSH/min·mg of proteins	Glutathione reductase, Глутатион-редуктаза, nmol NADPH/min·mg of proteins	Glucose-6-phosphate dehydrogenase, nmol NADPH/ min· mg of proteins
8	С	26.1 ± 1.25	3.2 ± 0.13	65.2 ± 2.01
0	Е	$20.5 \pm 1.02^{**}$	$2.4 \pm 0.16^{**}$	59.1 ± 2.62
0	С	26.9 ± 1.58	3.5 ± 0.07	65.9 ± 2.42
9	Е	$19.5 \pm 1.15^{**}$	$2.0 \pm 0.14^{***}$	$58.5 \pm 2.58*$

It is known that the activity of glutathione reductase in a cell decreases due to accumulation of oxidant form of NADP. Catalytic activity of the enzyme is related to regeneration of NADPH, one of the products of dehydrogenase reaction of pentose phosphate pathway.

Analysis of activity of glucose-6-phosphate dehydrogenase in the blood of cows from the experimental group showed a significant decrease in the activity of the enzyme in the ninth month of pregnancy: it decreased by 11.2% compared to the control.

Therefore, we can state that the processes of lipid peroxidation have a key role in the pathogenesis of progression of endogenous intoxication of cows: they activate and then cause imbalance in the complex "Antioxidant system \leftrightarrow lipid peroxidation".

Table 6 shows that the concentration of malondialdehyde and lipid peroxids, products of lipid peroxidation, in the plasma of blood of pregnant cows fluctuated within $235.4-240.2 \text{ E} \times 1000/\text{ml}$ (LOPs), 4.0–4.2 nmol/ml (MDA). The blood indicates the general condition of peroxidative processes in tissues, therefore the results given in Table 6 indicate that the organism of pregnant cows with progressing endogenous intoxication is affected by the processes typical for oxidative stress, which is manifested by increase in the products of lipid peroxidation in the blood of tested animals.

Table 6

The level of products of lipid peroxidation in the blood of cows with progressive endogenous intoxication ($x \pm SE$, n = 10)

Month of pregnancy	Group of animals	Lipid peroxides, E x 1000/ml	Malondialdehyde, nmol/ml
0	С	235.4 ± 9.81	4.0 ± 0.15
0	Е	389.5 ± 12.99***	$5.8 \pm 0.22^{***}$
9	С	240.2 ± 8.16	4.2 ± 0.17
	Е	$410.5 \pm 13.75^{***}$	$6.5 \pm 0.26^{***}$

Lipid peroxides in the blood of cows from the experimental group increased in the eighth and ninth month of pregnancy by 65.5% and 70.9% respectively. Concerning thiobarbituric acid active products: their level significantly increased in the blood of cows with clinical symptoms of endogenous intoxication by 45.0% and 54.8% in the eighth and ninth month of pregnancy relspectively.

Discussion

It is common knowledge that many animal diseases are followed by development of endogenous intoxication (Hrymak et al., 2015; Gutyj et al., 2017; Shcherbatyy et al., 2017). The destructive effect of endogenous intoxication on the organism of pregnant cows consists of the following main processes: a stoppage of metabolic processes; development of the so-called "lethal synthesis", which further results in accumulation of highly toxic agents in the interior milieu; is most dangerous as a form of damage to cell membranes (Katholm and Andersen, 1992; Eades, 1993; Andersen, 2003; Hrymak et al., 2015).

The notion "endotoxin" is conditional, for any normal metabolit has a toxic impact if it accumulates to excess in an organism (Culbertson and Osburn, 1980; Boosman et al., 1991; Clark et al., 1991; Luzhnikov et al., 2007). According to data from the literature , endotoxins include products of breakdown of tissue proteins, peroxides and other products of free radical oxidation, low molecular weight toxins, toxins of microorganisms (Clark et al., 1991; Luzhnikov et al., 2007; Hrymak et al., 2015). For studying development of endogenous intoxication in animals, hematological blood analysis is essential, for the blood performs a crucial role in vital activity of an organism. The content of blood reflects qualitative and quantitative changes in an organism affected by various pathological processes of intoxication (Gutyj et al., 2016).

We found that the pregnant cows with development of endogenous intoxication were affected by suppression of the hematopoiesis function of the bone marrow, which is identified by decrease in the number erythrocytes and hemoglobin in blood. Disproportional decrease in the amount of erythrocytes and hemoglobin is related to the toxic effect of toxins on the bone marrow and suppression of erythropoiesis. Such changes in the amount of erythrocytes and hemoglobin in the blood of animals with developing endogenous intoxication are mentioned by other authors (Clark et al., 1991; Andersen, 2003). Also decrease in erythrocytes is related to the fact that they are especially sensitive to the effect of oxidative stress, which develops together with the progression of endogenous intoxication of animals.

Apart from calculating the values of blood indicators, which provided the basis for analyzing the condition of hematopoiesis function of the bone marrow, we analyzed the indicators of leukocytes. Leukocytes in the organism of animals are responsible for a number of functions, particularly: protective, trophic and transport functions (Khariv et al., 2016). With development of endogenous intoxication, the number of leukocytes in the blood of pregnant cows increased in the 8th and 9th months of pregnancy. Leukocytosis is related to the progression of inflammatory processes caused by the impact of toxins on the organism of pregnant cows.

Analysis of the functional condition of the liver is considered of great value in clinical veterinary diagnostics and in the treatment of various intoxications. The liver is a barrier between toxic substances and homeostasis of animal organism. Therefore it is first to suffer the impact of toxins (Hariv and Gutyj, 2016; Kryshtalska et al., 2017).

Changes during pregnancy to a certain extent affect the organism and systems of mothers' organisms, and also affect metabolic processses. Progression of endogenous intoxication of pregnant cows results in liver disorders, which causes suppression of resynthesis of glucose and glycogen from lactic acid. In these conditions, the tissues of animal organisms suffer acidosis and disorders of proteins and nucleic acid, which causes development of progressive cell dystrophy.

Diagnostic tests for liver disorders are analysis of activity of aminotransferases, content of total protein and its fractions in blood serum. Blood proteins are functionally connected with proteins of different tissues and reflect the changes, which occur in the organisms of animals with metabolic disorders caused by endogenous intoxication (Martyshuk et al., 2016).

With development of endogenous intoxication, pregnant cows were observed to have a decrease in the level of total protein in the blood serum. Perhaps, the decrease in total protein is related to progression of hepatosis as a result of the negative impact of toxins. Hepatosis suppresses resynthesis of glucose and glycogen from lactic acid. These processes induce development of acidosis in the tissues of an animal's organism, which causes alterations of biochemical reaction. As a result, synthesis of proteins and nucleic acids is suppressed, resulting in progressive cell dystrophy.

Decrease in the content of albumins in the blood serum of pregnant cows is related to the effect of toxins on the liver and to weakening of its protein-synthesizing function. The liver synthesizes 80% of all albumins. Significant decrease in the level of albumin indicates deficiency of amino acid and proteins in the organism of pregnant cows. Compared to the control, the cows with endogenous intoxication were observed to have an increase in α - and β -globulins by 15.6% and 14.9% in the eighth month. Hyperglobulinemia of intoxicated animals is related to inflammatory processes in their organism under progressive endogenous intoxication. We found that the decrease in the level of total protein and its fractions in the blood serum is the consequence of suppression of the protein-synthesizing function of the liver of pregnant cows.

The study of the activity of alanine aminotransferase and aspartate aminotransferase is significant for defining the general physiological condition of an animal organism (Khariv et al., 2016). The pregnant cows with progressive endogenous intoxication were observed to have a significant increase in the activity of the abovementioned aminotransferases in their blood over the last months of pregnancy. High activity of aminotransferases in blood serum of pregnant cows was a result of their release from the cells into the blood serum, which heighted the activity of the enzymes. This occurs after endogenous toxin impacts on cell membranes, increasing their penetrability.

Therefore, establishing an increase in the activity of aminotransferases and decrease in the level of total protein in the blood serum is one of the first biochemical tests for diagnosing the manifestations of endogenous intoxication, which also indicates significant destructive processes in the liver.

Our experiments showed a decrease in the concentration of urea in the blood serum of cows with progressing endogenous intoxication, which is, perhaps, related to weakening of its synthesis in the liver. Low concentration of urea in the blood serum of cows was observed in the ninth month of their pregnancy. Such changes indicate decrease in the activity of metabolic processes in the cows' organism and suppression of protein-synthesizing function of the liver. High level of creatinine in the blood serum of pregnant cows with progressing endogenous intoxication is an indicator of not only the functional condition of the liver, but also the functional condition of the kidneys.

On the basis of our experimental studies, we found that pregnant cows with developing endogenous intoxication had intensified formation of free radicals and active forms of oxygen, which have a negative impact on cell membranes. Most authors (Lavryshyn et al., 2015; Chala and Rusak, 2016; Lavriv, 2016) consider intensification of formation of free radicals to be one of the universal mechanisms of disorganizing the structural-functional unity of an animal's organism. Therefore, it should be mentioned that increase in the activity of lipid peroxidation processes in physiological conditions is considered as an adaptive reaction of an organism to stress factors, including progression of endogenous intoxication (Hunchak et al., 2016; Martyshuk et al., 2016; Gutyj et al., 2017).

Initial activation of lipid peroxidation indicates a danger for the organism (Lavriv, 2016). We found that the organism of pregnant cows with progressing endogenous intoxication has intensifying lipid peroxidation in the blood, which is indicated by increase in the concentration of intermediate and final products of lipid peroxidation in the blood serum. Perhaps, such changes are related to the fact that a significant part of the mechanism of endogenous intoxication is intensified formation of active forms of oxygen, which later results in imbalance between the content of oxidants and antioxidants in the organism of pregnant cows. It is also worth mentioning significant changes in the level of lipid peroxides compared to the changes in the level of malondialdehyde. A less intensive, compared to the lipid peroxides, increase in the level of malondialdehyde is related to the fact that lipid peroxides are formed in the early stages of lipid peroxidation, whereas malondialdehyde is formed in the late stages (Lavryshyn et al., 2015). According to the data from the literature, changes in the level of malondialdehyde are related to its ability to form complexes with a number of compounds. Therefore, low concentration of malondialdehyde does not always reflect absence of intensified lipid peroxidation.

The results obtained coincide with the reports on intensification of lipid peroxidation processes under pathological process of any genesis (Chala and Rusak, 2016; Lavriv, 2016; Shcherbatyy et al., 2017). These reports are mentioned in the literature review. Free radical reactions of

lipid peroxidation, which occur in the lipid layer of biological membranes, are significant for the mechanism of progression of endogenous intoxication of animals. In such conditions, endotoxins cause negthe ative effect on functional condition of regulatory systems (Boosman et al., 1991; Hrymak and Hunchak, 2014; Gutyj et al., 2016).

Data from the literature (Lavryshyn et al., 2015; Martyshuk et al., 2016) and the preliminary results of our study (Hrymak et al., 2015) indicate that disorders in oxidative homeostasis, which is typical for increase in the products of lipid peroxidation as a result of decrease in the activity of the enzymatic and non-enzymatic part of antioxidant protection, is one of the pathogenetic factors of endogenous intoxication of pregnant cows.

The antioxidant protection system of an animal's organism is a powerful mechanism, which prevents development of the so-called oxidative stress and exponential free radical and oxidative reactions in the organism (Lavryshyn et al., 2015; Huberuk et al., 2017). A significant part of the antioxidant protection is the glutathione system of antioxidant protection. The components of this system consist of metabolite glutathione and enzymes, particularly: glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase.

The results of our studies on pregnant cows with clinical symptoms of endogenous intoxication showed possible changes in the system of antioxidant protection of their organism. The results of our studies indicate that the activity of enzymes of the glutathione system of antioxidant protection significantly increased in the eighth and ninth months of pregnancy. Lowered activity of enzymes-antioxydants is related to accumulation of lipid peroxidation products, which suppress the activity of enzymes.

The changes in the activity of enzymes of the glutathione system of antioxidant protection in the blood of pregnant cows discovered in our research show the additional aspects of toxic effect of endotoxins and could be used as a criterion for evaluating not only the condition of organisms, but also the efficiency of antidotal preparations for treating endogenous intoxication.

Therefore, the research conducted provides data on the dynamics of changes in main parameters of the glutathione system in the blood of cows with progressive endogenous intoxication during the last months of pregnancy, which indicate the significance of the glutathione system for the processes of detoxication. The research found a deficiency in the resources of the glutathione system of antioxidant protection of the organism of cows as a result of its exhaustion in the eighth and ninth months of pregnancy.

The study also determined that endogenous intoxication in an animal organism causes stress to the the animal. In stressed agricultural animals, two hormones form: adrenaline and cortisol. They contribute to the increase in the frequency of the heart rate and breathing, and also suppress the immune system of an organism. Stress also affects the reproductive function, decreasing secretion of sex hormones, and later results in cardiovascular diseases (Hrymak and Hunchak, 2014). During the stress, the organism of animals experiences the following processes: at the initial stage adrenaline, the hormones extracted in the adrenal glands flow into the blood. It causes tachycardia, at the same time increasing arterial pressure and blood sugar level, and reorganizing the blood flow: blood flow to the brain and limbs intensifies, and blood flow to the digestive system and skin reduces. Breathing increases and becomes shallow, and only the top of the lungs is active. Reserves of fat and sugar begin to be exhausted through the production of additional energy (Hrymak et al., 2015; Hunchak et al., 2016).

Therefore, after analyzing Ukrainian and foreign literature, a conclusion can be made that treating endogenous intoxication of pregnant cows requires preparations which should include antioxidants, iodine and magnesium.

Conclusions

Development of endogenous intoxication of pregnant cows in the eighth and ninth months of pregnancy is followed by decrease in the number of erythrocytes in the blood and decrease in hemoglobin and total protein. Also we observed an increase in the number of leukocytes, and increase in the activity of alanine- and aspartateaminotransferases compared to the animals of the control group. Progression of endogenous intoxication of pregnant cows causes disorders in condition of liver, disorders of protein-synthesis and disorders of the detoxicating function of hepatocytes. The progression of endogenous intoxication of pregnant cows suppresses the activity of enzymes of the glutathione system of antioxidant protection, which was indicated by the significantly low activity of glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase. Significant increase in the content of primary products of lipid peroxidation in the blood plasma of cows with symptoms of endogenous intoxication was followed by significant increase in the concentration of malondialdehyde. Imbalance in the generation of active forms of oxygen and their metabolites, exhaustion of antioxidant system and disorders in its balance cause oxidative stress.

References

- Andersen, P. H. (2003). Bovine Endotoxicosis Aspects of relevance to production diseases. A review. Acta Veterinaria Scandinavica, 44(1), 57.
- Boosman, R., Mutsaers, C. W., & Klarenbeek, A. (1991). The role of endotoxin in the pathogenesis of acute bovine laminitis. Veterinary Quarterly, 13, 155–162.
- Chala, I. V., & Rusak, V. S. (2016). Redoks-potencial ta stan perekysnogo okysnennja lipidiv krovi koriv, shho utrymujut'sja u ekologichno nespryjatlyvyh umovah [Redox-potential and the state of peroxide oxidation of blood lipids in cows kept under ecologically unfavorable conditions]. Scientific Messenger LNUVMBT named after S. Z. Gzhytskyj, 66, 197–201 (in Ukrainian).
- Clark, E. S., Gantley, B., & Moore, J. N. (1991). Effects of slow infusion of a low dosage of endotoxin on systemic haemodynamics in conscious horses. Equine Veterinary Journal, 23, 18–21.
- Culbertson, R., & Osburn, B. I. (1980). The biologic effects of bacterial endotoxin: A short review. Veterinary Research Communications, 4, 3–14.
- Eades, S. C. (1993). Endotoxemia in dairy cattle: Role of eicosanoids in reticulorumen stasis. Journal of Dairy Science, 76, 414–420.
- Fabris, T. F., Laporta, J., Corra, F. N., Torres, Y. M., Kirk, D. J., McLean, D. J., Chapman, J. D., & Dahl, G. E. (2017). Effect of nutritional immunomodulation and heat stress during the dry period on subsequent performance of cows. Journal of Dairy Science, 100(8), 6733–6742.
- Fedorovych, V. V. (2017). Vplyv pokaznykiv vidtvomoji zdatnosti na formuvannja molochnoji produktyvnosti koriv symental's'koji porody [The impact of reproductive capacity indicators of Simmental cattle on their milk productivity]. Scientific Messenger LNUVMBT named after S. Z. Gzhytskyj, 74, 52– 56 (in Ukrainian).
- Gutyj, B. V., Hufriy, D. F., Hunchak, V. M., Khariv, I. I., Levkivska, N. D., & Huberuk, V. O. (2016). The influence of metisevit and metifen on the intensity of lipid per oxidation in the blood of bulls on nitrate load. Scientific Messenger LNUVMBT named after S. Z. Gzhytskyj, 70, 67–70.
- Gutyj, B. V., Murs'ka, S. D., Gufrij, D. F., Hariv, I. I., Levkivs'ka, N. D., Nazaruk, N. V., Gajdjuk, M. B., Pryjma, O. B., Bilyk, O. J., & Guta, Z. A. (2016). Influence of cadmium loading on the state of the antioxidant system in the organism of bulls. Visnyk of Dnipropetrovsk University. Biology, Ecology, 24(1), 96–102.
- Gutyj, B., Khariv, I., Binkevych, V., Binkevych, O., Levkivska, N., Levkivskyj, D., & Vavrysevich, Y. (2017). Research on acute and chronic toxity of the experimental drug Amprolinsyl. Regulatory Mechanisms in Biosystems, 8(1), 41–45.
- Gutyj, B., Lavryshyn, Y., Binkevych, V., Binkevych, O., Paladischuk, O., Strons'kyj, J., & Hariv, I. (2016). Influence of Metisevit on the activity of enzyme and nonenzyme link of antioxidant protection under the bull's body cadmium loading. Scientific Messenger LNUVMBT named after S. Z. Gzhytskyj, 66, 52–58.
- Gutyj, B., Martyshchuk, T., Bushueva, I., Semeniv, B., Parchenko, V., Kaplaushenko, A., Magrelo, N., Hirkovyy, A., Musiy, L., & Murska, S. (2017). Morphological and biochemical indicators of blood of rats poisoned by carbon tetrachloride and subject to action of liposomal preparation. Regulatory Mechanisms in Biosystems, 8(2), 304–309.
- Gutyj, B., Paska, M., Levkivska, N., Pelenyo, R., Nazaruk, N., & Guta, Z. (2016). Study of acute and chronic toxicity of 'injectable mevesel' investigational drug. Biological Bulletin of Bogdan Chmelnitskiy Melitopol State Pedagogical University, 6(2), 174–180.
- Hariv, M. I., & Gutyj, B. V. (2016). Influence of the liposomal preparation Butaintervite on protein synthesis function in the livers of rats under the influence of carbon tetrachloride poisoning. Visnyk of Dnipropetrovsk University. Biology, Medicine, 7(2), 123–126.
- Hrymak, Y. I., & Hunchak, V. M. (2014). Yodna nedostatnist ta korektsija reproduktyvnoji funktsiji tvaryn preparatamy yodu [Iodine deficiency and correction of the cows' reproductive function using iodine Preparation]. Naukovyi

Visnyk Lvivskoho Natsionalnoho Universytetu Veterynamoi Medytsyny ta Biotekhnolohii imeni S. Z. Gzhytskoho, 59, 23–41 (in Ukrainian).

- Hrymak, Y. I., Hunchak, V. M., & Hutyi, B. V. (2015). Patent na korysnu model № 1026518. Sposib korektsiji fermentnoji systemy antyoksydantnoho zakhystu orhanizmu hlybokotilnykh koriv za endotoksykozu [Methods of correction of the enzyme system of antioxidant protection of the organism of cows in advanced pregnancy with endotoxicosis]; zaiavnyk ta patentovlasnyk Lvivskyi Natsionalnyi Universytet Veterynamoi Medytsyny ta Biotekhnolohii imeni S. Z. Gzhytskoho. zaiavl. 15.04.2015; opubl. 10.11.2015, Bull. 21 (in Ukrainian).
- Huberuk, V., Gutyj, B., Gufriy, D., Binkevych, V., Hariv, I., Binkevych, O., & Salata, R. (2017). Impact of antioxidants on enzyme activities of glutatione system of antioxidant defense in bodies of bulls with acute nitrate and nitrite toxicity. Scientific Messenger LNUVMBT named after S. Z. Gzhytskyj, 77, 220–224.
- Hunchak, A. V., Ratych, I. B., Gutyj, B. V., & Paskevych, H. A. (2016). Metabolichna diia yodu v orhanizmi ptytsi za yoho nestachi abo nadlyshku v ratsioni [Metabolic effects of iodine in poultry for its deficiency or excess in the diet]. Naukovyi visnyk Lvivskoho natsionalnoho universytetu veterynamoi medytsyny ta biotekhnolohii imeni S. Z. Gzhytskoho, 67, 70–76 (in Ukrainian).
- Katholm, J., & Andersen, P. H. (1992). Acute coliform mastitis in dairy cows: Endotoxin and biochemical changes in plasma and colony-forming units in milk. Veterinary Record, 131, 513–514.
- Khariv, M., & Gutyj, B. (2017). Dynamika fagocytarnoji aktyvnosti nejtrofiliv u shhuriv za umov oksydacijnogo stresu ta diji liposomal'nogo preparatu [Dynamics of phagocytic activity of neutrophils in rats under oxidative stress and the action of liposomal preparation]. Animal Biology, 19(1), 119–124 (in Ukrainian).
- Khariv, M., Gutyj, B., Butsyak, V., & Khariv, I. (2016). Hematological indices of rat organisms under conditions of oxidative stress and liposomal preparation action. Biological Bulletin of Bogdan Chmelnitskiy Melitopol State Pedagogical University, 6(1), 276–289.
- Kryshtalska, M., Hunchak, V., & Gutyj, B. (2017). Influence of the drug "Trifuzol" on the functional state of the liver in chickens for eymeriozic invasion. Scientific Messenger LNUVMBT named after S. Z. Gzbytskyi, 77, 76–79.
- Lavriv, P. Y. (2016). Antioxidant protection and peroxydation lipid of body cows of high calves for the prevention of salmonella under the influence of nanpreparation Germakap. Scientific Messenger LNUVMBT named after S. Z. Gzhytskyj, 66, 112–115.
- Lavryshyn, Y. Y., Varkholyak, I. S., Martyschuk, T. V., Guta, Z. A., Ivankiv, L. B., Paladischuk, O. R., Murska, S. D., Gutyj, B. V., & Gufriy, D. F. (2016). The biological significance of the antioxidant defense system of animals body. Scientific Messenger LNUVMBT named after S. Z. Gzhytskyj, 66, 100–111.
- Luzhnikov, Y. A., Goldfarb, Y. S., & Ilyashenko, K. K. (2007). Endotoxicosis as the contents of posresuscitative disease in acute poisoning. Obsaa Reanimatologia, 3(6), 48–54.

- Macmillan, K., López Helguera, I., Behrouzi, A., Gobikrushanth, M., Hoff, B., & Colazo, M. G. (2017). Accuracy of a cow-side test for the diagnosis of hyperketonemia and hypoglycemia in lactating dairy cows. Research in Veterinary Science, 115, 327–331.
- Mainardes, G. A., & DeVries, T. J. (2016). Effect of social feeding environment on the feeding behaviour of dairy cows and their willingness to consume a novel feed. Applied Animal Behaviour Science, 185, 23–29.
- Martyshuk, T. V., Gutyj, B. V., & Vishchur, O. I. (2016). Level of lipid peroxidation products in the blood of rats under the influence of oxidative stress and under the action of liposomal preparation of "Butaselmevit". Biological Bulletin of Bogdan Chmelnitskiy Melitopol State Pedagogical University, 6(2), 22–27.
- Panevnyk, V., & Suprovych, T. (2016). Etiologichni chynnyky mastytiv koriv ukrai'ns'koji chorno-rjaboji molochnoji porody [Etiological factors of mastitis of the Ukrainian black-pied dairy breed of cows]. Scientific Messenger LNUVMBT named after S. Z. Gzhytskyj, 70, 191–195 (in Ukrainian).
- Rodríguez, E. M., Arís, A., & Bach, A. (2017). Associations between subclinical hypocalcemia and postparturient diseases in dairy cows. Journal of Dairy Science, 2017.
- Shcherbatyy, A. G., Slivinska, L. G., Gutyj, B. V., Golovakha, V. I., Piddubnyak, A. V., & Fedorovuch, V. L. (2017). The influence of a mineral-vitamin premix on the metabolism of pregnant horses with microelemetosis. Regulatory Mechanisms in Biosystems, 8(2), 293–398.
- Sheldon, I. M., Wathes, D. C., & Dobson, H. (2006). The management of bovine reproduction in elite herds. Veterinary Journal, 171, 70–78.
- Slivinska, L., Demydjuk, S., Shcherbatyy, A., Fedorovich, V., & Tyndyk, I. (2017). Etiologija ta kliniko-biohimichni pokaznyky krovi za alimentarnoji osteodystrofiji koriv [Etiology and clinical biochemical parameters of blood for nutritional osteodystrophy of cows]. Scientific Messenger LNUVMBT named after S. Z. Gzhytskyj, 73, 79–83 (in Ukrainian).
- Smolynets', I. B., Gutyj, B. V., Khariv, I. I., Petryshak, O. Y., & Lytvyn, R. I. (2016). Pharmaceutical marketing: objectives and types. Scientific Messenger LNUVMBT named after S. Z. Gzhytskyj, 69, 151–154.
- van der Drift, S. G. A., Houweling, M., Bouman, M., Koets, A. P., Tielens, A. G. M., Nielen, M., & Jorritsma, R. (2015). Effects of a single glucocorticoid injection on propylene glycol-treated cows with clinical ketosis. Veterinary Journal, 204(2), 144–149.
- Vieira-Neto, A., Lima, I. R. P., Lopes, F., Lopera, C., Zimpel, R., Sinedino, L. D. P., Jeong, K. C., Galvão, K., Thatcher, W. W., Nelson, C. D., & Santos, J. E. P. (2017). Use of calcitriol to maintain postpartum blood calcium and improve immune function in dairy cows. Journal of Dairy Science, 100(7), 5805– 5823.
- Vlizlo, V. V. (2012). Laboratorni metody doslidzhen u biolohiyi, tvarynnytstvi ta veterynamiy medytsyni [Laboratory methods of research in biology, stockbreeding and veterinary medicine]. Spolom, Lviv (in Ukrainian).