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EFFECT OF ENDOTOXICOSIS ON THE MORPHOLOGICAL AND BIOCHEMICAL BLOOD INDICES OF PREGNANT COWS

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The research findings about the influence of endotoxicosis in cows on morphological and biochemical blood indicators are showed in the article. It is found that the development of endotoxicosis in pregnant cows is accompanied by decreased red blood cell count, hemoglobin level, total protein, increased activity of aminotransferases and increased number of white blood cells in blood compared with the value of these indicators in the blood of cows with a physiological course of pregnancy. The development of endotoxicosis in pregnant cows also contributes to the violation of protein and bilirubin synthesiz, detoxifying functions of hepatocytes and the disruption of a kidney filtration function.

The cows with the signs of endotoxicosis showed lower content of total protein compared with the control group of cows, and also were reported to show decreased content of albumin during the 8th and 9th months of pregnancy. The study of globulin fractions in the blood of the cows of the experimental group during the 8th month of pregnancy showed the increase of α — and β-globulins by 16 and 15 % in comparison to the values of the control group. A low level of urea in blood of the cows of the experimental group, the values of which decreased by 15 and 31 % during the 8th and 9th months of pregnancy respectively, compared to the control animals, indicates the decrease of detoxification function of the liver.

Key words: COWS, ENDOTOXICOSIS, BLOOD, PREGNANCY, PROTEIN, HEMOGLOBIN, AMINOTRANSFERASES.

МОРФОЛОГІЧНІ ТА БІОХІМІЧНІ ПОКАЗНИКИ КРОВІ КОРІВ З ФІЗІОЛОГІЧНИМ ПЕРЕБІГОМ ТІЛЬНОСТІ ТА У КОРІВ З РОЗВИТКОМ ЕНДОТОКСИКОЗУ

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У статті наведено результати досліджень впливу ендотоксикозу у корів на морфологічні і біохімічні показники їх крові. Встановлено, що розвиток ендотоксикозу у тільних корів супроводжується зниженням в крові кількості еритроцитів, рівня гемоглобіну, загального протеїну, підвищення активності амінотрансфераз та збільшення кількості лейкоцитів порівняно з величиною цих показників у крові корів із фізіологічним перебігом вагітності. Також розвиток ендотоксикозу у тільних корів сприяє порушенню білоксинтезувальної, білірубіносинтезувальної та детоксикаційної функції гепатоцитів та порушенню фільтраційної функції нирок.

У корів з ознаками ендотоксикозу спостерігали нижчий вміст загального протеїну порівняно з контрольною групою корів, також встановлено зниження вмісту альбумінів уже на 8 і 9 місяці тільності. При дослідженні глобулінових фракцій у крові корів дослідної групи на 8 місять тільності встановлено підвищення α- і β-глобулінів на 16 і 15 % відносно величин контрольної групи корів. Низький рівень сечовини в крові корів дослідної групи, де відповідно на 8 і 9 місяць тільності він знизився на 15 і 31 % відносно контрольної групи тварин вказує на зниження дезінтоксикаційної функції печінки.

Ключові слова: КОРОВИ, ЕНДОТОКСИКОЗ, КРОВ, ТІЛЬНІСТЬ, ПРОТЕЇН, ГЕМОГЛОБІН, АМІНОТРАНСФЕРАЗИ

МОРФОЛОГИЧЕСКИЕ И БИОХИМИЧЕСКИЕ ПОКАЗАТЕЛИ КРОВИ КОРОВ С ФИЗИОЛОГИЧЕСКИМ ТЕЧЕНИЕМ СТЕЛЬНОСТИ И У КОРОВ С РАЗВИТИЕМ ЭНДОТОКСИКОЗА

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В статье приведены результаты исследований влияния эндотоксикоза у коров на морфологические и биохимические показатели их крови. Установлено, что развитие эндотоксикоза в стельных коров сопровождается снижением в крови количества эритроцитов, уровня гемоглобина, общего белка, повышение активности аминотрансфераз и увеличение количества лейкоцитов по сравнению с величиной этих показателей в крови коров с физиологическим течением беременности. Также развитие эндотоксикоза в стельных коров способствует нарушению белоксинтезирующей, билирубиносинтезирующей и дезинтоксикационной функции гепатоцитов и нарушению фильтрационной функции почек.

У коров с признаками эндотоксикоза наблюдали снижение уровня общего белка по сравнению с контрольной группой коров, также установлено снижение содержания альбуминов уже на 8 и 9 месяце стельности. При исследовании глобулиновых фракций в крови коров опытной группы на 8 месяц стельности установлено повышение α- и β-глобулинов на 16 и 15 % относительно величин контрольной группы коров. Низкий уровень мочевины в крови коров опытной группы, где соответственно на 8 и 9 месяц стельности он снизился на 15 и 31 % относительно контрольной.

Ключевые слова: КОРОВЫ, ЭНДОТОКСИКОЗ, КРОВЬ, СТЕЛЬНОСТЬ, ПРОТЕИН, ГЕМОГЛОБИН, АМИНОТРАНСФЕРАЗЫ

In veterinary medicine, pathology is considered as a dynamic process of adaptation that characterizes the changes (temporary, permanent) of biochemical processes, functions and morphology of cells, organs and systems [1]. Therefore, it is important to diagnose the beginning of the disease, which is particularly relevant to veterinary obstetrics and gynecology [2, 3].

During pregnancy, the cow's organism experiences a series of changes close to pathology. Symptoms of intoxication usually accompany the disease and its complications, associated with increased tissue destruction, enhanced catabolic processes, liver and kidneys insufficiency, violation of the microcirculation [4–7].

It is believed, that all forms of a malicious effect of endotoxins on organs and organism systems are realized in the form of a specific response to the primary action of these substances. Such a body's response does not only limit, but also extends both, the harmful effects of such substances and their entry into the internal environment which may be defined as endotoxicosis [8–10].

The aim of our work was to elucidate the influence of endotoxicosis on the morphological and biochemical blood indicators of pregnant cows

Materials and methods

Ukrainian dairy black and white breed cows were used for the research. To solve the set tasks, two groups of animals with 10 pregnant cows in each: control and experimental ones were formed. The animals of the experimental group showed clinical signs of endotoxicosis: congestive edemas of external genitals, swollen udder, anaemia of mucous membranes, depression, dysorexia, functional disorders of proventriculus and intestine. The signs of endotoxicosis in pregnant cows developed when the conditions of keeping and the level of pregnant cows feeding did not meet the

physiological needs of the fetus for nutrients, resulting in disturbance of the physiological course of pregnancy.

Cows of the control group were healthy, with physiological course of pregnancy

The studies followed the rules, which were mandatory when performing zootechnical experiments on the selection and keeping animals-analogues in groups, techniques of preparation, use and accounting of consumed fodder. The diet of the animals was balanced for nutrients and mineral substances, which provided their need for essential nutrients.

Blood for analysis was taken from the jugular vein on 8 and 9 months of pregnancy.

The blood was tested for the following indicators: the number of white blood cells (WBC) — with Goryaev's graticule in the counting chamber; the number of red blood cells (RBC) — photocolorimetry by the method of E. S. Gavrilets et al.; the concentration of total hemoglobin according to the method of L. M. Pimenova et al.

The protein-synthesizing function of liver was determined by the content of total protein and its fractions (albumins and globulins) in serum. The level of total protein was determined with biuret reagent according to the method of N. L. Delektorskij, fractional composition of proteins in serum by electrophoresis method in polyacrylamide gel.

The functional state of liver was determined by biochemical parameters of blood: the concentration of total bilirubin, urea, creatinine were determined by the methods described in the handbook "Studying the blood system in clinical practice".

The serum was examined for the activity of aspartate-aminotransferase (AST) (EC 2.6.1.1.) and alanine aminotransferase (ALT) (EC 2.6.1.2.) — according to the method of Frenkel and Reitman in the modification of K. G. Kapetanaki.[11]

Research and discussion

The blood in the organism of animals is a relatively constant and mobile environment, which performs a significant number of vital functions in order to maintain a stable physiological status of the organism.

In veterinary medicine, more attention is paid to the study of hematological parameters, since it is possible toevaluate the overall health of the animal specifically judge about the intensity of metabolic processes, the development of infectious and noninfectious diseases basing on their determining.

Table 1 shows the morphological blood indicators of the cows with a physiological course of pregnancy and the cows with the development of endotoxicosis. It is established that the number of RBC in blood of the control group of 8 and 9 months pregnant cows ranged within physiological values. The number of RBC in the blood of cows of the experimental group was slightly lower, namely during the 8th month of pregnancy — by 14 %, while during the 9th month of pregnancy — by 21 % compared to the parameters of the control group.

Table 1

Morphological parameters of blood of the cows with a physiological course of pregnancy and the cows with the development of endotoxicosis

Months of pregnancy	Groups of animals	RBC, T/L	Total Hemoglobin, g/L	WBC, $\times 10^9$/L
8	C	7.11±0.25	110.02±4.03	7.59±0.33
	E	6.15±0.29*	95.10±3.19*	7.95±0.28
9	C	7.52±0.26	119.12±4.72	7.62±0.29
	E	5.94±0.33**	90.20±2.83***	8.20±0.18*

Note: variance compared to the control group in this and the following tables are: * — P<0.05; ** —P<0.01; *** — P<0.001

It is known that hemoglobin is a transport protein responsible for carrying oxygen from lungs to organs and tissues of an animal organism, thereby providing a tissue breathing. The results of studies of hemoglobin show that the blood of cows with a physiological course of pregnancy during the studied months of pregnancy contained hemoglobin within physiological values, whereas in the blood of the cows with signs of endotoxicosis, its content during the 8th month of pregnancy decreased by 14 % and during the 9th month of pregnancy — by 24 %.Increased content of hemoglobin in the blood of the cows with a physiological course of pregnancy indicates a high level of metabolic processes in the organism of these animals.

The number of WBC in the blood of the cows reflects the state of protective forces of their organism. In case of the development of endotoxicosis, the cows of the experimental group experienced an increase in the studied parameters by 5 % and 8 % during the 8th and the 9th month of pregnancy respectively. In our opinion this is caused by the development of inflammatory processes in the organism of the cows due to the development of endotoxicosis.

Biochemical blood composition depends on various factors, in particular on the conditions of keeping, feeding, age and a physiological state of the animals, as well as diseases of various etiologies. Our research found that the dynamics of total protein and its fraction in blood of the cows of the control group is within physiological values (*Table 2*). The cows with the signs of endotoxicosis showed a slightly lower content of total protein compared with the control group with the content of the studied parameters during the 8th month of pregnancy decreased by 13 %.

Table 2

Total protein content and its fraction in blood of the cows with a physiological course of pregnancy and the cows with the development of endotoxicosis,M±m, n = 10

Months of pregnancy	Groups of animals	Total Protein, g/L	Protein fractions, %			
			albumins	globulins		
				α -	β -	γ -
8	C	79.68±1.69	44.12±0.32	16.56±0.22	14.25±0.19	25.07±0.46
	E	69.45±1.88***	40.62±0.26 ***	19.15±0.14 ***	16.37±0.20 ***	23.86±0.41*
9	C	78.73±1.90	43.87±0.53	16.86±0.17	15.04±0.15	24.23±0.65
	E	64.20±1.93***	38.95±0.41 ***	21.55±0.12 ***	16.90±0.25 ***	22.60±0.39*

The cows with the signs of endotoxicosis were reported to show a decreased content of albumin during the 8th and 9th months of pregnancy, which decreased by 8 and 11 % compared to the control group. Albumins are considered amino acid reserves of the body, therefore, a significant reduction in their level indicates the amino acid and protein deficiency in the organism of the cows.

The study of globulin fractions in the blood of the cows of the experimental group during the 8th month of pregnancy showed the increase in α — and β-globulins by 16 and 15 % relative to the values of the control group cows. During the 9th month of pregnancy, the blood of the cows with the signs of endotoxicosis also showed an significant increase in the content of α- and β-globulins.

The study of γ-globulins in the blood of the experimental group cows showed a decrease to 22.60 %. Thus, the proportion of γ-globulins in the composition of protein fractions in the blood of the cows with the signs of endotoxicosis during the whole experiment was lower.

The study of the activity of ALT and AST is essential for the determination of the overall physiological condition of the organism

of cows, with a number of biosynthetic processes depending basically on the speed of biochemical reactions which are regulated by the activity of enzyme systems.

The dynamics of the activity of aminotransferases in blood serum of the control cows and the cows with the development of endotoxicosis are shown in *Table 3*. The activity of ALT and AST in blood serum in the control group of cows during the 8th month of pregnancy ranged within physiological values. During the 9th month of pregnancy in the serum of the cows of the control group, the activity of aminotransferases increased slightly, with ALT by 7 %, AST by 9 %. Accordingly, the intensity of metabolism when approaching calfbirth, of course, affects the functional state of animals, which apparently is one of the causes of an increased activity of aminotransferases, which are central in protein metabolism. The cows with the signs of endotoxicosis showed an increased activity of the studied enzymes in their blood throughout the experiment. Thus, during the 8th month of pregnancy, the activity of ALT in the serum of the experimental group cows rose by 20 %, whereas during the 9th month of pregnancy — by 28 % relative to the values of the control group cows.

Table 3

Dynamics of the activity of aminotransferases in serum of the cows with a physiological course of pregnancy and the cows with the development of endotoxicosis, M±m, n = 10

Months of pregnancy	Groups of animals	ALT, U/L	AST, U/L	De Rytis coefficient (AST/ALT ratio)
8	C	27.0±0.52	43.0±0.70	1.59±0.07
	E	32.1±0.82***	50.5±0.72***	1.57±0.05
9	C	29.7±0.51	46.1±0.65	1.55±0.06
	E	38.2±0,15***	55.3±0.58***	1.45±0.04

The study of the activity of AST in the blood serum of the cows with the signs of endotoxicosis showed an increase in this enzyme during the 8th and 9th month of pregnancy to 17 % and 20 % respectively. Such a rise in the enzymes is due to toxic effects of toxins on the liver, followed by an increase in aminotransferases. The increased activity of aminotransferases in the blood of sick cows indicates a violation of the functional state of liver.

To evaluate the activity of aminotransferases in veterinary practice, de Rytis coefficient (the ratio AST to ALT activity) is determined. According to literature data, it is known that with pathology of muscle tissue (mainly of the myocardium), it increases, while with hepatopathy, on the contrary, it decreases. On average, de Rytis coefficient of the sick cows was significantly decreased which focuses on toxic effects of endoto[icosis on the liver.

The cows with endotoxicosis show disrupted metabolism of bile pigments, which is proved by a high content of total bilirubin in serum. The average level of pigment of the experimental group of cows during the 8th month of pregnancy was higher than a corresponding value of the control group cows by 18 %. During the 9th month of pregnancy, this indicator in the blood of the experimental group cows increased by 33 % (*Table 4*)

Endotoxicosis of cows leads to a decreased detoxification function of the liver, indicated by a low level of urea in blood of the cows of the experimental group, the values of which decreased by 15 and 31 % during the 8th and 9th months of pregnancy respectively compared to the control animals.

Unlike urea, another indicator of residual nitrogen — creatinine in sick cows of the experimental group during the 8th month of pregnancy did not differ significantly from

Table 4

Biochemical parameters of blood of the cows with a physiological course of pregnancy and the cows with the development of endotoxicosis, M±m, n = 10

Months of pregnancy	Groups of animals	Urea, mmol/L	Creatinine, μmol/L	Total bilirubine, μmol/L
8	C	4.7±0.18	102.1±3.52	4.5±0.24
	E	4.0±0.13**	106.4±4.08	5.3±0.20*
9	C	4.8±0.18	101.5±3.04	4.6±0.16
	E	3.3±0.15***	112.4±3.03*	6.1±0.15***

the values of clinically healthy cows. However, during the 9th month of pregnancy, the experimental group of cows with clinical signs of endotoxicosis showed hypercreatinemia, which means the violation of a filtration function of kidneys.

Therefore, the obtained results enable us to suggest that the development of endotoxicosis in pregnant cows, caused by the inadequate sugar-protein diet, leads to probable changes in the morphological and biochemical blood composition.

Conclusions

The cows with the signs of endotoxicosis during 8-9th months of pregnancy show a decreased number of RBC, level of hemoglobin, total protein, an increased activity of aminotransferases and an increased number of WBC compared with the value of these indicators in the blood of the cows with a physiological course of pregnancy. Hence the development of endotoxicosis in pregnant cows contributes to the violation of protein-synthesizing, bilirubin-synthesizing and detoxifying functions of hepatocytes and the disruption of a kidney filtration function.

Perspectives of further research. With the purpose to comprehensively study the pathogenesis of endotoxicosis in pregnant cows, it is advisable in the future to conduct research of the immune system of pregnant cows with the aim of developing the effective treatment and prevention of their of endotoxicosis.

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