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НАУКОВИЙ ВІСНИК ЛЬВІВСЬКОГО НАЦІОНАЛЬНОГО УНІВЕРСИТЕТУ ВЕТЕРИНАРНОЇ МЕДИЦИНИ ТА БІОТЕХНОЛОГІЙ ІМЕНІ С.З. ГЖИЦЬКОГО

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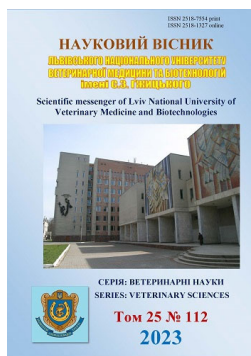
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The state of the body's immune system of beef cows with signs of endotoxiosis

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During the development of endotoxiosis of various etiologies, secondary immunodeficiency occurs in animals, which progresses due to exposure to toxic metabolites. The work aimed to study the influence of endotoxiosis on the dynamics of indicators of the immune system of beef cows. The research was conducted on the Ukrainian black and spotted dairy breed beef cows. In cows with signs of endotoxiosis, clinical signs such as swelling of the external genital organs and mammary gland, anemia of the mucous membranes, depressed state, impaired appetite, and functional disorders of the antrum and intestines were characteristic. The main immunological tests characterizing the state of the immune system of beef cows are indicators of humoral immunity (bactericidal and lysozyme activity of blood serum), cellular immunity (T- and B-lymphocytes), indicators of non-specific resistance of the organism (phagocytic activity and phagocytic index). In beef cows with clinical manifestations of endotoxiosis, inhibition of cellular, humoral, and non-specific links of the immune system was established, which led to the development of the so-called secondary immunodeficiency. This is evidenced by a decrease in the bactericidal and lysozyme activity of blood serum, a decrease in the number of T- and B-lymphocytes, and a decrease in the phagocytic index and phagocytic activity of the blood of sick cows. In particular, it was established that the lowest indicator of bactericidal and lysozyme activity was observed in the 9th month of pregnancy in the experimental group of cows, where it decreased by 15.9 and 4.91 %, respectively, compared to the control. In the above study periods, a decrease in the phagocytic activity of neutrophils by 5.04 % and the phagocytic index by 33.3 % was established compared to the indicators of the control group. During the analysis of cellular immunity, similar changes were found in determining the number of T- and B-lymphocytes; they were lower by 12.5 % and 4.19 %, respectively. Changes in the indicators of the immune system are significant and objective markers of the state of the cow's body during the development of endotoxiosis, regardless of its causes.

Key words: pregnant cows, endotoxiosis, immune system, bactericidal and lysozyme activity of blood serum, T- and B-lymphocytes, phagocytic activity, phagocytic index.

Introduction

One of the critical tasks in intensive livestock farming is to ensure healthy and viable young cattle. Non-infectious diseases of beef cows are often the cause of mortality of newborn calves. It is known that many diseases in pregnant animals are accompanied by intoxication. One of the reasons for the occurrence of internal intoxication is insufficient feeding of pregnant cows. It is important to note that beef cows' feeding and maintenance

conditions significantly affect their future offspring's health (Grymak et al., 2020; Bashchenko et al., 2021, 2023).

The term “endotoxin” is conditional since any normal metabolite exhibits toxic properties when excessively accumulated in the body of animals. According to the literature, endotoxins include tissue protein breakdown products, peroxides and other free radical oxidation products, low-molecular-weight, and microorganism toxins

(Mazurkevych Saulko, 2016; Gutyj et al., 2018; Mylostyvyi et al., 2021, 2023).

It is essential to consider that many diseases partly depend on the development of endogenous intoxication. Most of the clinical and immunological signs of this problem can be described as a discrepancy between the formation and elimination of products of normal and disturbed (in the case of pathology) metabolism (Mudrak et al., 2016; Saulko et al., 2017; Borshch et al., 2020, 2021).

The effect of endogenous intoxication factors on the body of pregnant cows is focused on three main directions. The first is the stoppage of exchange processes due to the delay in removing or removing the end products of exchange. The second is the transition of synthesis to the production of non-physiological compounds, which can lead to the “lethal synthesis” of toxic substances in the internal environment. The third direction is damage to cell membranes, which is the most harmful. According to modern ideas, the mechanisms of action of endotoxins on the body of pregnant cows are based on the determining role of the immune system (Broda et al., 2013; Denkovich et al., 2021; Slivinska et al., 2021).

All adverse effects of endotoxins on the organs and systems of the whole organism are revealed through a specific reaction to their primary negative effect. This reaction limits and expands the harmful effects of such substances and their penetration into the internal environment, which can be reflected as endotoxiosis (Grymak et al., 2020).

Modern ideas about the mechanism of action of endotoxins on the body of pregnant cows are based on the leading role of the immune system, which protects animals from infections and eliminates foreign agents of endogenous and exogenous nature. It has been established that toxic metabolites in the development of endotoxiosis in animals inhibit the activity of the body's immune system and reduce the body's resistance to the harmful effects of environmental factors (Vishchur et al., 2015).

The aim of the research

The work aimed to study the influence of the development of endotoxiosis on the dynamics of indicators of the immune system of pregnant cows.

Material and methods

The experiments were carried out in the laboratory of the Department of Hygiene, Sanitation, and General Veterinary Prevention, named after M. V. Demchuk, Stepan Gzhytskyi NUVMB, as well as FE “Mezhyrichia” (Zarichia village, Lviv region)

The research was conducted on the Ukrainian black and spotted dairy breed pregnant cows. We followed the norms and rules that are mandatory in zootechnical research, including the selection and maintenance of analog animals in groups, the procurement process, and the accounting of feed use. The cows' diet was carefully prepared and balanced regarding nutrients and minerals, ensuring that they needed the essential elements for healthy nutrition.

To achieve the goals of this series of studies, we formed two groups of 10 beef cows each: control and experimental. Cows of the control group had a physiological course of pregnancy. Clinical signs of endotoxiosis, such as swelling of external genital organs and mammary glands, anemia of mucous membranes, depressed state, impaired appetite, and functional disorders of pre-stomachs and intestines, were found in the animals of the experimental group.

Blood for biochemical studies was collected from animals from the jugular vein. The blood was stabilized with heparin. The blood serum was separated from the formed elements by centrifugation for 5–8 min at 3000 rpm.

The phagocytic activity of neutrophils (PhA) and phagocytosis (PhI) intensity was determined using generally accepted methods to modify Chumachenko V. E. et al. The total number of T-lymphocytes (E-RUK) - by the method of spontaneous rosette formation with ram erythrocytes according to M. Jondal et al. (Chumachenko V. E. et al., 1991), the total number of B-lymphocytes – according to N. F. Mendes et al. Bactericidal activity of blood serum (BABS) – according to the method by Yu. M. Markov; blood serum lysozyme activity (BSLA) – photonephelometrically, according to the method of V. G. Dorofeychuk (Vlizlo et al., 2012).

The obtained numerical values of the results of the biochemical blood indicators were expressed in international units of the SI system. The research results were subjected to biometric analysis by the method of mathematical statistics adopted in biology and medicine, using Microsoft Excel, “Statistica 5.0”.

The degree of probability, compared to the control group's data, was – $P < 0.05 - *$, $P < 0.01 - **$, $P < 0.001 - ***$.

Results and discussion

Immunity is a set of protective mechanisms of the body to preserve its genetic stability. Immunity helps the body fight against various external factors: bacteria, viruses, toxins, foreign bodies, and others (Martyshuk & Hutyi, 2021; Kuljaba et al., 2022).

Humoral immunity is known to be supported by specific macromolecules that function in the internal fluids of animal organisms. Blood plasma contains particular proteins capable of neutralizing microorganisms and toxins entering body fluids (Kisera et al., 2021; Gutyj et al., 2022).

The bactericidal activity of blood serum (BABS) is a critical factor in the body's natural resistance to the humoral immunity type, which confirms the blood's ability to self-purify. This is a property of blood serum due to the presence of a complex of substances, such as complement, antibodies, lysozyme, and properdin, which can neutralize or destroy the cells of microorganisms.

The data in Table 1 shows that the bactericidal activity of blood serum in pregnant cows in the control and experimental groups showed specific differences. For example, in the 8th month of pregnancy, in the control group of cows that had a physiological course of pregnancy, the bactericidal activity was $90.62 \pm 2.87 \%$, while in pregnant cows with signs of endotoxiosis, it was at the level

of 80.30 ± 2.64 %. The lowest rate of bactericidal activity was observed in the 9th month of pregnancy in the exper-

imental group of cows, where compared to the control group, it decreased by 15.9 %, respectively.

Table 1

Indicators of humoral immunity in cows with a physiological course of pregnancy and in cows with the development of endotoxycosis ($M \pm m$, $n = 10$)

Month of pregnancy	Group of animals	BSLA, %	BABS, %
8	C	28.84 ± 0.89	90.62 ± 2.87
	E	$22.44 \pm 0.71^{***}$	$80.30 \pm 2.64^*$
9	C	24.42 ± 0.85	85.21 ± 2.81
	E	$19.51 \pm 0.69^{***}$	$69.31 \pm 2.55^{***}$

Lysozyme activity of blood serum in fat cows with an ordinary course of calving in the 9th month of pregnancy was slightly lower than in the 8th month. In the heifers of the experimental group, a decrease in this activity was observed both in the 8th and 9th months, where compared to the control group, it decreased by 6.4 % and 4.91 %, respectively (Table 1).

Therefore, during the analysis of the indicators of humoral immunity, it was found that in pregnant cows suffering from endotoxycosis, a decrease in both bactericidal and lysozyme activity of blood serum is observed.

In the future, we investigated non-specific immunity, namely the immune system of animals, not related to antigens and antibodies. This system includes phagocytosis and general non-specific resistance. In addition to a decrease in the activity of humoral immunity in cows with indicators of endotoxycosis, we established suppression of a non-specific link of the immune system, revealed in a decrease in the phagocytic activity of neutrophils and a reduction in the phagocytic index.

Table 2

Indicators of non-specific immunity in cows with a physiological course of pregnancy and in cows with the development of endotoxycosis ($M \pm m$, $n = 10$)

Month of pregnancy	Group of animals	Phagocytic activity, %	Phagocytic index, units
8	C	63.89 ± 2.11	5.68 ± 0.14
	E	$59.32 \pm 2.14^*$	$4.22 \pm 0.17^{***}$
9	C	62.15 ± 2.27	7.80 ± 0.25
	E	$57.11 \pm 2.19^*$	$5.85 \pm 0.18^{***}$

It was established that on the 8th and 9th month of gestation in the blood of the control group of animals, the phagocytic activity of neutrophils and the phagocytic index were within the range of $63.89 \pm 2.11 - 62.15 \pm 2.27$ % and $5.68 \pm 0.14 - 4.22 \pm 0.17$ units. According to the development of endotoxycosis in the pregnant cows of the research group during the research periods indicated above, reduced phagocytic activity of neutrophils by 4.57 % and 5.04 %, and a phagocytic index by 25.7 % and 33.3 % were established compared to the indicators of the control group (table 2).

Therefore, the results obtained in the research indicate that in pregnant cows with clinical signs of endotoxycosis, a non-specific link of the immune system is suppressed.

Table 3 shows the indicators of cellular immunity in cows with a physiological course of pregnancy and those that developed endotoxycosis. It was found that in the 8th month of gestation, the number of T-lymphocytes in the blood of the control group of cows fluctuated within 47.53 ± 2.15 %. In the 9th month of pregnancy, the number of these lymphocytes in this group increased slightly, and compared to the previous values, it increased by 6.3 %.

Table 3

Indicators of cellular immunity in cows with a physiological course of pregnancy and in cows with the development of endotoxycosis ($M \pm m$, $n = 10$)

Month of pregnancy	Group of animals	T-lymphocytes, %	B-lymphocytes, %
8	C	47.53 ± 2.15	17.42 ± 0.91
	E	45.11 ± 2.31	16.91 ± 0.78
9	C	53.83 ± 2.47	22.02 ± 0.87
	E	$41.33 \pm 2.21^{**}$	$17.83 \pm 0.82^{**}$

A reduced number of T-lymphocytes with signs of endotoxycosis was found in pregnant cows during the entire study period. The minimum number of T-lymphocytes in

the blood of cows from the experimental group was observed in the 9th month of gestation, where the ratio of

these cells decreased by 12.5 % compared to the control group.

Similar changes were detected in determining the number of B-lymphocytes during the analysis of cellular immunity. In pregnant cows suffering from endotoxemia, the number of B-lymphocytes was lower by 0.51 % and 4.19 % at the 8th and 9th months of gestation, respectively.

We explain the decrease in the number of T- and B-lymphocytes in cows suffering from endotoxemia by the effect of toxins on the immune system of the sick cows of the experimental group. Thus, a decrease in the number of studied indicators indicates a weakening of the body's resistance to the development of endotoxemia.

Conclusions

1. In pregnant cows with clinical manifestations of endotoxemia, cellular, humoral, and non-specific links of the immune system are suppressed, which leads to the development of the so-called secondary immunodeficiency. This is evidenced by a decrease in the bactericidal and lysozyme activity of blood serum, a decrease in the number of T- and B-lymphocytes, and a reduction in the phagocytic index and phagocytic action of the blood of sick cows.

2. Changes in the immune system indicators are significant and objective markers of the state of the cow's body during the development of endotoxemia, regardless of its causes.

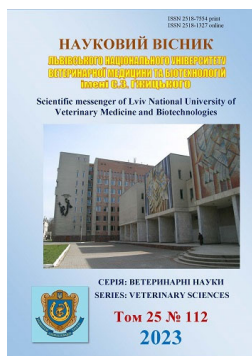
Conflict of interest

The authors declare no conflict of interest.

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Зміст

1. Чемеровська І. О., Рубленко І. О. Моніторинг мікрофлори за інфекційної патології у собак і котів	3
2. Медвідь О. О., Передера Ж. О., Щербакова Н. С., Передера С. Б. Аналіз ринку меду Італії	16
3. Головач П. І., Гутий Б. В., Коломієць І. А., Остапів Д. Д., Осередчук Р. С., Слобода О. М. Вплив вітамінів групи В (В ₁ , В ₂ , В ₅ , В ₆ , В ₁₀ , В ₁₂) на активність ферментів системи антиоксидантного захисту і вміст продуктів пероксидного окиснення ліпідів у крові бугайців на відгодівлі	22
4. Тишківська Н. В., Бартків Л. Г., Тишківський М. Я. Контамінація тушок курчат бройлерів <i>L. monocytogenes</i> і вплив дезінфікуючих засобів на органолептичні та фізико-хімічні показники м'яса	27
5. Лабунська О.-Л. І., Гунчак В. М., Гутий Б. В., Харів І. І., Солтис М. П. Дерматомікози в котів (поширення, діагностика, перебіг, лікування)	34
6. Бойчук Б. І., Карповський В. І., Гутий Б. В., Гришук І. А., Карповський В. В., Гришук А. В. Вегетативна регуляція жирнокислотного складу в плазмі крові кіз	42
7. Труханович Т. С., Кухтин М. Д., Перкій Ю. Б., Болтик Н. П., Климик В. Т., Рущинська Т. М., Тихонова Б. Є. Перспектива використання нізину для переддоїльної обробки вимені корів	47
8. Кремпа Н. Ю., Козенко О. В., Гутий Б. В., Двилюк І. В., Магрело Н. В., Сус Г. В., Вороняк В. В., Висоцький А. О., Вус У. М., Мартишук Т. В. Динаміка вмісту імуноглобулінів у сироватці крові поросят за дії імуностимулювальних засобів	52
9. Панікар І. І., Рудь В. О., Вартік Н. Гігієнічна оцінка якості і безпечності води відповідно до національних вимог	58
10. Koreyba L. V. Major diseases of pregnancy and abortion in cows	62
11. Борковський Р. О., Березовський А. В. Перспективи використання макро- та мікроелементів у годівлі птиці	67
12. Філіпенко О. В. Зміни показників периферійної крові ягнят за спонтанного еймеріозу	73
13. Lozynskiy I. R., Gutyj B. V., Ivashkiv R. M., Ichyshyn M. M., Martyshuk T. V., Todorciuk V. B., Dashkovskiy O. O., Magrelo N. V., Sus H. V., Voroniak V. V., Vus U. M. The state of the body's immune system of beef cows with signs of endotoxemia	78
14. Лавришин Ю. Ю., Гутий Б. В., Вєвега Б. М., Куцан О. Т., Гунчак В. М., Харів І. І., Кушнір В. І., Васів Р. О., Лєськів Х. Я., Гута З. А. Визначення параметрів гострої токсичності та кумулятивних властивостей препарату "Ліпоінтерсил"	83
15. Мазуркевич Т. А., Усенко С. І. Морфогенез імунних утворень кишечника качок	90