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ПОКАЗАТЕЛИ ОБЩЕГО ГОМЕОСТАЗА У КОРОВ В РАЗНЫЕ ПЕРИОДЫ ЛАКТАЦИЙ

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Исследованы иммуноморфологические показатели коров крупного рогатого скота, находящегося в разных периодах лактации. В первые 3 мес лактации, исключая 7 дней молозивного периода, в сыворотке крови обнаружено 59,3 ед. циркулирующих иммунных комплексов, в середине лактации (4–7 мес) – 94,2 ($p < 0,05$), в конце (8–10 мес) – 94,1 ед. ($p < 0,05$). Достоверное различие между показателем в первые 3 мес и в последующие периоды лактации, связано с тем, что в начале лактации коровы еще не стельные. В молозивный период высокий показатель циркулирующих иммунных комплексов 116,1 ед. ($p < 0,05$) определен как последствие предродовой иммунной атаки плода на организм коровы, когда система мононуклеарных фагоцитов еще не справилась с элиминацией продуктов нейтрализации. В сухостойный период количество циркулирующих иммунных комплексов составляло 87,6 ед. ($p < 0,05$). Снижение показателя происходило в связи с увеличением активности мононуклеарных фагоцитов и отсутствием лактационной нагрузки на организм. Содержание сегментоядерных, функционально зрелых лейкоцитов в начале лактации составляло 39,4%, в середине лактации этот показатель снижался до 24,8% ($p < 0,05$), в конце ее составил 26,3% ($p < 0,05$). Установлено достоверное различие в относительном количестве сегментоядерных нейтрофилов в контроле и у нелактирующих коров в сухостойном периоде – 29,9% ($p < 0,05$). В молозивный период уровень содержания лимфоцитов в крови животных составил 62,0% ($p < 0,05$) и достоверно отличался от контроля – 43,6%. В середине и в конце лактации также прослеживалось достоверное отличие показателя от контроля до 58,9–59,4% ($p < 0,05$). Установлено достоверное различие с группой глубокостельных сухостойных коров – 53,9% ($p < 0,05$). В первые 1–3 мес лактации коровы или еще не стельные, или между матерью и плодом еще не сформирована тесная связь (плацента), поэтому низкая активность специфического иммунитета в это время вызвана отсутствием в крови коров чужеродных антигенов плода. Полученные данные свидетельствуют о возможности инициации родового процесса иммунной системой.

Ключевые слова: крупный рогатый скот, лактация, резистентность, иммунная система, гомеостаз, лимфоциты, циркулирующие иммунные комплексы, мононуклеарные фагоциты

INDICATORS OF TOTAL HOMEOSTASIS IN COWS IN DIFFERENT PERIODS OF LACTATIONS

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The immunomorphological parameters of cattle in different periods of lactation were studied. In the first 3 months of lactation, excluding 7 days of the colostrum period, 59.3 units of circulating immune complexes were found in the serum, in the middle of lactation (4–7 months) – 94.2 ($p < 0.05$), at the end (8–10 months) – 94.1 units ($p < 0.05$). The significant difference between the indicator in the first 3 months and in the subsequent periods of lactation is due to the fact that at the beginning of lactation the cows were not yet pregnant. In the colostrum period, a high rate of circulating immune complexes of 116.1 units ($p < 0.05$) was determined as a consequence of a fetal prenatal immune attack on the cow's body, when the system of mononuclear phagocytes had not yet coped with the elimination of neutralization products. During the dry period, the number of circulating immune complexes was 87.6 units ($p < 0.05$). The decrease in the indicator occurred due to an increase in the activity of mononuclear phagocytes and the absence of lactation effect on the body. The content of segmental, functionally mature leukocytes at the beginning of lactation was 39.4%, in the middle of lactation this indicator decreased to 24.8% ($p<0.05$), at the end it was 26.3% ($p<0.05$). A significant difference was found in the relative number of segmented neutrophils in the control group and in non-lactating cows in the dry period – 29.9% ($p < 0.05$). During the colostrum period, the level of lymphocytes in the blood of animals was 62.0% ($p < 0.05$) and it significantly differed from the control – 43.6%. In the middle and at the end of lactation, there was also a significant difference between the indicator and the control, up to 58.9–59.4% ($p<0.05$). A significant difference with the group of down-calving dry cows was established – 53.9% ($p < 0.05$). In the first 1–3 months of lactation, cows are either not yet pregnant, or a close bond (placenta) between the mother and the fetus has not yet been formed, therefore a low activity of specific immunity at this time is caused by the absence of foreign fetal antigens in the blood of cows. The findings suggest that the birth process may be initiated by the immune system.

Keywords: cattle, lactation, resistance, immune system, homeostasis, lymphocytes, circulating immune complexes, mononuclear phagocytes

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Conflict of interest
The authors declare no conflict of interest.

INTRODUCTION

Milk production technology assumes that within 7 months of lactation cows bear a fetus. The combination of the two such energy-consuming physiological processes as pregnancy and milk production depletes the body systems, causes a violation of compensatory mechanisms, and reduces the duration of operation and life of animals [1, 2]. To ensure the health of the mother and the fetus, the state of the immune system of cows is of particular importance [3, 4].

The aim of the work is to study the most informative homeostatic parameters of blood and blood serum of cattle in different periods of lactation.

MATERIALS AND METHODS

The studies were carried out on the basis of the Novosibirsk State Agrarian University and the Tomsk Agricultural Institute. The object of the study was the cows of Holstein hybrids of the Siberian offspring of the black-and-white breed with different proportions of blood between the first and seventh lactations with a

productivity of 3500–5500 kg. The spread in productivity is dictated by the need to increase the sample of animals. In previous studies, we did not find a significant difference when comparing immunological and hematological parameters in cows with a productivity of 3500 to 5500 kg.

To determine the indicators of general homeostasis in cows, depending on the lactation period, groups were formed with the number of animals from three to 32. Methods of statistical processing were used to compare them, which make it possible to reveal significant differences in groups with different numbers of animals. To assess the dynamics of homeostasis indicators, the obtained data were compared with the control, which was chosen as a group of cows at the beginning of lactation.

In all periods of the experiment, different animals were studied, which made it possible to avoid the possible influence of seasonality, changes in conditions of keeping and feeding on the results of the experiment.

In the blood of lactating cows, the cells were counted, the leukogram was determined according to generally accepted methods using microscopy in the Goryaev chamber and blood smears stained according to Romanovsky - Giemsa. The activity of serum lysozyme was investigated on a photoelectric calorimeter, evaluating the light transmission in control and experimental tubes. Phagocytic activity of neutrophils was determined by the method of opsonophagocytic reaction using a culture of Staphylococcus aureus (strain No. 209). Determination of circulating immune complexes in blood serum was carried out on a photoelectric calorimeter, preliminarily diluting the serum with borate buffer. To assess the blast transformation of lymphocytes, a stimulator phytohemagglutinin was used, the results were taken into account by the morphological method [5].

Statistical processing of the obtained digital data, including the calculation of the mean values and the calculation of the mathematically significant difference in the indicators of the results, was carried out using the statistical software package "Statistica for Windows 6.0".

RESULTS AND DISCUSSION

Analysis of changes in immunological parameters in different periods of lactation shows that the activity of serum lysozyme in the control was 31.6% of light transmission, in the dry period - 35.9% ($p < 0.05$), which indicates an increase in nonspecific immunity before calving (see. Table 1). With lactation lasting more than 12 months, the activity of lysozyme is significantly higher than the control group - 40.7% (p

Табл. 1. Иммунологические показатели крови коров в разные периоды лактации
Table 1. Immunological indicators of cows' blood in different lactation periods

Indicator	Lactation period				
	1–3 months (*n* = 16) control	4–7 months (*n* = 6)	8–10 months (*n* = 32)	Dry (*n* = 28)	Colostric (*n* = 7)
Lysozyme, % light transmission	31,6 ± 1,4	30,3 ± 2,0	32,3 ± 1,5	35,9 ± 1,1*	36,6 ± 3,2
Circulating immune complexes, units	59,3 ± 8,4	94,2 ± 14,5*	94,1 ± 6,4*	87,6 ± 7,0*	116,1 ± 21,0*
Blasts, %	54,8 ± 2,1	51,2 ± 5,1	53,3 ± 1,9	58,5 ± 2,2	53,1 ± 3,2
Average lymphocytes, %	22,6 ± 1,8	20,3 ± 3,2	19,8 ± 1,0	20,5 ± 1,1	23,3 ± 2,0
Small lymphocytes, %	22,7 ± 1,7	28,5 ± 3,3	27,1 ± 1,5	21,2 ± 1,7	23,1 ± 2,0
Active lymphocytes, %	77,3 ± 1,7	71,5 ± 3,3	73,2 ± 1,5	79,0 ± 1,8	76,6 ± 1,9
Spontaneous activity, %	25,4 ± 2,0	26,8 ± 3,7	23,6 ± 1,8	22,0 ± 1,5	24,9 ± 2,5
Active neutrophils, %	39,1 ± 2,0	40,0 ± 2,0	40,8 ± 1,6	37,1 ± 1,6	39,1 ± 3,8

*$p < 0,05$.

<0.05), which may be due to low milk productivity during prolonged lactation [6, 7].

In the first 3 months of lactation, excluding 7 days of the colostrum period, 59.3 units were found in the serum. circulating immune complexes, in the middle of lactation (4-7 months) - 94.2 units (p <0.05), at the end (8-10 months) - 94.1 units (p <0.05). The significant difference between the indicator in the first 3 months and in subsequent periods of lactation is probably due to the fact that at the beginning of lactation the cows are not yet pregnant[1] [8]. Of the 16 animals at the beginning of lactation, 15 were not pregnant, one with an undetermined pregnancy, as she was forced to kill due to an udder injury. The gestation period was determined after the birth of a healthy calf.

In the colostrum period, a high rate of circulating immune complexes is 116.1 units (p <0.05) - indicates a prenatal immune attack of the fetus on the cow's body, the system of mononuclear phagocytes has not yet coped with the elimination of antibodies neutralization products. During the dry period, when the number of circulating immune complexes was 87.6 units (p <0.05), there was a decrease in the indicator, probably due to an increase in the activity of mononuclear phagocytes [9–12].

When assessing the functional activity of lymphocytes, it was found that under the influence of phytohemagglutinin in the control group, 54.8% of lymphocytes were transformed into blasts, at 4–7 months of lactation the number of blasts decreased to 51.2, at 8–10 months the activity of lymphocytes increased to 53.3%.

Thus, the low activity of lymphocytes in the middle of lactation is a consequence of the high productivity and deep pregnancy of animals during this period. During the colostrum period, lymphocytes were transformed into blasts by 53.1%. During the dry period, the activity of lymphocytes increased to 58.5%, which is more than at the end of lactation. With a decrease or cessation of milk production, the specific reactivity and the ability of the animal body to withstand the antigenic load from the fetus increased.

The level of all active lymphocytes, including the average one, was the highest during the dry period - 79.0%, as well as during lactation lasting more than 12 months - 79.7%. The lowest (71.5%) indicator was observed in the middle of lactation, when milk production is highest. This indicates an inverse correlation between the amount of milk produced and the level of specific resistance [13, 14].

Spontaneous activity of lymphocytes was calculated without adding phytohemagglutinin in the control sample. At the beginning of lactation, the indicator was 25.4%, in the middle it increased to 26.8%.

Parameters of phagocytic activity of neutrophils in cows from the colostrum period to 10 months of lactation were stable and varied from 39.1 to 40.8% of active neutrophils.

Hematological blood parameters were studied (see table. 2). At the beginning of lactation, the content of stab neutrophils was 2.9%, in the middle - 2.0, at the end - 1.3%; with lactation lasting more than 12 months - 0.7% (p <0.05). During lactation, a gradual decrease in stab neutrophils was observed in cows, which indicates stabilization of the formation of leukocytes in the hematopoietic organs [15, 16].

The content of segmented, functionally mature leukocytes at the beginning of lactation was 39.4%, in the middle this indicator decreased to 24.8% (p <0.05), at the end of it - 26.3% (p <0.05). At the same time, a significant difference was established in the relative number of segmented neutrophils in the control and in non-lactating cows in the dry period - 29.9% (p <0.05). Thus, leukopoiesis in cows significantly decreased after the first 3 months of lactation; at the same time, the development of the glandular epithelium in the mammary gland of cows and an increase in milk production were established.

Eosinophilic granulocytes at the beginning of lactation were found to be 9.5%, in the middle - 12.0, at the end - 11.0, in the colostrum

[1]Yatsenko Yu.N., Mager S.N. Study of the level of circulating immune complexes in calves with different immune status in the early postnatal period // Actual problems of agro-industrial complex development in the works of young scientists of Siberia: materials of the XI regional scientific-practical conference of young scientists of the Siberian Federal District. Novosibirsk, 2015. P. 155–160.

Табл. 2. Гематологические показатели крови коров в разные периоды лактации
Table 2. Hematological blood counts of cows in different lactation periods

Indicator	Lactation period				
	1–3 months (*n* = 11) control	4–7 months (*n* = 6)	8–10 months (*n* = 25)	Dry (*n* = 26)	Colostric (*n* = 7)
Stab neutrophils, %	2,9 ± 0,9	2,0 ± 1,0	1,3 ± 0,2	1,9 ± 0,4	2,3 ± 1,1
Segmented neutrophils, %	39,4 ± 4,1	24,8 ± 4,6*	26,3 ± 2,2*	29,9 ± 2,6*	26,2 ± 5,2
Acidophiles, %	9,5 ± 2,5	12,0 ± 5,3	11,0 ± 1,3	9,8 ± 1,2	3,7 ± 1,7
Monocytes, %	3,8 ± 0,9	2,0 ± 2,0	2,5 ± 0,4	4,6 ± 0,6	5,8 ± 1,7
Lymphocytes, %	43,6 ± 2,7	59,4 ± 8,1*	58,9 ± 2,6*	53,9 ± 3,1*	62,0 ± 6,8*
Red blood cells, $\times 10^{12}$/l	5,2 ± 0,2	4,9 ± 0,5	5,2 ± 0,2	5,6 ± 0,2	6,1 ± 0,3*
White blood cells, $\times 10^{9}$/l	6,7 ± 0,8	7,7 ± 1,7	7,2 ± 0,5	6,6 ± 0,7	8,2 ± 1,7

*$p < 0,05$.

period - 3.7%. The low content of eosinophils in the colostrum period indicates a decrease in the level of nonspecific protection in favor of specific and the need for the synthesis of immunoglobulins to protect the newborn calf from infections [17].

The content of monocytes in the control group was 3.8%, in the colostrum period - 5.8%, in animals with lactation of 4–7 months - 2.0, in cows lactating for 8–10 months - 2.5%.

The level of lymphocytes in the blood of animals indicates a state of specific resistance [18]. In the colostrum period, this indicator was 62.0% (p <0.05) and significantly differed from the control - 43.6%. During this period, a large number of immunoglobulins appears in the secretion of the mammary gland in cows, which provide protection for the newborn calf. It can be assumed that the percentage of lymphocytes increased not due to a decrease in granulocytes. The increase in the absolute number of leukocytes - 8.2 × 109 / l - was due to the absolute increase in the number of lymphocytes. In the middle and at the end of lactation, there was also a significant difference between the indicator and the control, up to 58.9–59.4% (p <0.05). There was a significant difference with the group of deep-bodied dry cows - 53.9% (p <0.05). In the first 1–3 months of lactation, cows are either not yet pregnant, or the placenta has not yet formed between the mother and the fetus; therefore, the low activity of specific immunity at this time is explained by the absence of fetal antigens in the cows' organism [19–21].

In the first 3 months of lactation, the number of erythrocytes in the blood was 5.2 × 1012 / L, at 4–7 months - 4.9 × 1012 / L, at 8–10 months - 5.2 × 1012 / L. If the number of erythrocytes is considered as an indicator of the level of metabolism, then during lactation it changed insignificantly. A significant difference in the number of erythrocytes was found between the groups of cows at 1–3 months of lactation and the colostrum period - 6.1 × 1012 / l (p <0.05).

Thus, the highest metabolic rate during lactation was noted in the first 7 days after calving. In the dry period, the number of erythrocytes was higher than during lactation, due to the completion of fetal formation, preparation for childbirth and the upcoming lactation.

The dynamics of changes in the leukopoiesis index in lactating cows was revealed: the number of leukocytes at the beginning of lactation was 6.7 × 109 / L, in the middle this indicator

increased to 7.7 × 109, at the end of lactation it decreased to 7.2 × 109 / L. In the colostrum period, this indicator was 8.2 × 109 / l. During lactation lasting more than 12 months, the number of leukocytes decreased - 4.6 × 109 / l ($p < 0.05$), which indicates the stabilization of the immune system in cows with prolonged lactation [22, 23].

Changes in biochemical parameters in the blood serum of cows in different periods of lactation were recorded. Compared with the control (2.38 mmol / l), the calcium content in cows during the colostrum period was significantly different - 3.17 mmol / l ($p < 0.05$), which was due to a small volume of mammary gland secretion and a high level of element metabolism. A significant difference was also found with the group of dry cows - 2.90 mmol / l ($p < 0.05$), which is explained by the absence of calcium elimination with milk and intensive metabolism of the macronutrient associated with the formation of the fetal skeleton.

In the control group, the alkaline reserve was 42.0 vol.% CO^2, in the middle of lactation - 23.4 ($p < 0.05$), at the end - 36.10 vol.% CO^2. In the middle of lactation, there was a significant decrease in the alkaline reserve, which is due to the high productivity of cows during this period. The increase in the alkaline reserve (37.0 vol.% CO^2) at the end of lactation, in our opinion, is caused by a decrease in milk production during the dry period, an insignificant increase occurred after the cessation of feeding the animals with silage (see footnote 1).

At the beginning of lactation, the serum carotene content of cows was 0.92 × 10–2 μmol / L, in the middle it increased to 1.17 × 10–2 μmol / L, by the end of lactation it was 1.36 × 10–2 μmol / l ($p < 0.05$). In colostrum and dry periods, the index of carotene content was 0.89–1.08 × 10–2 μmol / l. During the lactation period, a gradual increase in this indicator was noted, which we associate with the growth, development of the fetus and a decrease in milk production.

The amount of sugar in the blood of animals in the first 1-3 months of lactation was 4.38 mmol / L, at 4-7 months - 2.0 ($p < 0.05$), at 8-10 - 1.91 mmol / L ($p < 0.05$). During lactation, there was a significant decrease in blood sugar. Probably, this process is influenced by an increase in the level of metabolism and an increase in milk production at the beginning of lactation. During the dry period, a significant difference was also found in comparison with the control - 2.16 mmol / l ($p < 0.05$), associated with a decrease in metabolism. A slight increase in glucose levels in dry conditions compared with the end of lactation can be explained by the exclusion of silage containing butyric acid from the diet.

CONCLUSIONS

1. Before calving, the activity of serum lysozyme increases from 31.6% of light transmission in the control to 35.9% ($p < 0.05$) during the dry period.

2. The significant difference between the serum content of circulating immune complexes in the first 3 months and in subsequent periods of lactation is due to the fact that at the beginning of lactation the cows are not yet pregnant.

3. In the colostrum period, a high rate of circulating immune complexes is 116.1 units. ($p < 0.05$) - was defined as a consequence of prenatal immune attack of the fetus on the cow's body, when the system of mononuclear phagocytes has not yet coped with the elimination of neutralization products. During the dry period, the number of circulating immune complexes was 87.6 units. ($p < 0.05$). The decrease in the indicator was probably due to an increase in the activity of mononuclear phagocytes and the absence of a lactational load on the body.

4. In the colostrum period, the level of lymphocytes in the blood of animals is 62.0% ($p < 0.05$) and significantly differs from the control - 43.6%. During this period, a large number of immunoglobulins appears in the secretion of the mammary gland in cows, which provide protection for the newborn calf. There was a significant difference with the group of deep-bodied dry cows - 53.9% ($p < 0.05$). In the first 1-3 months of lactation, cows are either not yet pregnant, or a close bond (placenta) has not yet been formed between the mother and the fetus, therefore, the low activity of specific immunity

at this time is explained by the absence of foreign fetal antigens in the blood of cows.

5. A significant difference in the number of erythrocytes was found between the groups of 1-3 months and the colostrum period - 6.1 × 1012 / l ($p < 0.05$). The highest metabolic rate during lactation was noted in the first 7 days after calving.

6. The immune conflict associated with the stressed state of specific immunity before childbirth and the high content of immune complexes in the blood during the colostrum period contributes to a decrease in the barrier function by the placenta, which, in our opinion, is the main reason for the expulsion of the fetus from the cow's body.

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