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ВЛИЯНИЕ СУБКЛИНИЧЕСКОГО КЕТОЗА КОРОВ НА ФОРМИРОВАНИЕ КОЛОСТРАЛЬНОГО ИММУНИТЕТА ТЕЛЯТ

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Резюме. Здоровье новорожденного всецело зависит от состояния организма матери на протяжении всей беременности. Обеспечение оптимальных условий содержания беременных животных базируется, в первую очередь, на полноценном кормлении и обеспечении санитарно-гигиенических условий окружающей среды. Большую нагрузку организм коровы претерпевает в транзитный период, который начинается за 3 недели до отела и продолжается в течение шести недель. При нарушении технологии кормления и содержания у коров в данный период часто возникают метаболические нарушения, которые проявляются повышенной выработкой кетонов.

Известно, что становление иммунитета в ранний постнатальный период у теленка во многом зависит от своевременной выпойки молозива. Материнские иммуноглобулины из молозива попадают непосредственно в системный кровоток новорожденного в тонком отделе кишечника по тубулярной системе эпителиальных клеток путем пиноцитоза. Задача исследования – изучить влияние субклинического кетоза у коров-матерей на формирование колострального иммунитета у родившихся от них телят. Для исследования были отобраны стельные коровы 3-6 лет за 3-7 дней до родов. У коров были взяты пробы мочи и крови. С целью выявления субклинического кетоза у коров было проведено исследование мочи на содержание кетонов. По результатам исследования были сформированы две группы по 10 голов – в первой группе (подопытной) уровень кетоновых тел в моче составил от 1,8 до 3,7 ммоль/л, во второй группе (контрольной) кетоны в моче не обнаружены. Сразу после отела у коров отбирали порции молозива, а у новорожденных телят брали кровь через сутки после первой выпойки молозива. В обезжиренном молозиве и в сыворотке крови новорожденных телят исследовали содержание иммуноглобулинов. В сыворотке крови суточных телят также определяли содержание общего белка биуретовым методом, альбуминов – фотометрическим методом с бромкрезоловым зеленым.

По результатам проведенного исследования было обнаружено снижение классов иммуноглобулинов G, M и A в сыворотке крови коров перед отелом на 19,1-23,5%, в молозиве – на 23,7-34,4% и в сыворотке крови суточных телят – на 21,7-27,6%. В наибольшей степени определялось снижение концентрации IgM. На содержание в крови телят альбуминов субклинический кетоз коров-матерей практически не оказывал влияние.

Ключевые слова: коровы, телята, иммуноглобулины, молозиво, колостральный иммунитет, кетоз

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INFLUENCE OF SUBCLINICAL KETOSIS IN COWS ON FORMATION OF COLOSTRAL IMMUNITY IN CALVES

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Abstract. The health of the newborn depends entirely on the state of the mother's body throughout the pregnancy. Ensuring optimal conditions for keeping pregnant animals is based, first of all, on adequate feeding and ensuring the sanitary and hygienic conditions of the environment. The cow's body undergoes a great load during the transition period, which begins 3 weeks before calving and lasts for six weeks. When the technology of feeding and housing is violated, during this period, metabolic disorders often occur in cows, which are manifested by increased production of ketones. It is known that the development of immunity in the early postnatal period in a calf largely depends on the timely feeding of colostrum. Maternal immunoglobulins from colostrum enter the systemic circulation of the newborn in the small intestine through the tubular system of epithelial cells by pinocytosis. The aim of the study is to study the effect of subclinical ketosis in mothers cows on the formation of colostral immunity in calves born from them.

For the study, pregnant cows 3-6 years old were selected 3-7 days before delivery. Urine and blood samples were taken from the cows. In order to identify subclinical ketosis in cows, urine was tested for ketones. According to the results of the study, two groups of 10 animals were formed – in the first group (experimental) the level of ketone bodies in the urine ranged from 1.8 to 3.7 mmol/l, in the second group (control) ketones were not found in the urine. Immediately after calving, portions of colostrum were taken from the cows, and blood was taken from newborn calves a day after the first colostrum was fed. The content of immunoglobulins was studied in skim colostrum and in the blood serum of newborn calves. In the blood serum of day-old calves, the content of total protein was also determined by the biuret method, albumin – by the photometric method with bromcresol green.

According to the results of the study, a decrease in the classes of immunoglobulins G, M and A was found in the blood serum of cows before calving by 19.1-23.5%, in colostrum – by 23.7-34.4%, and in the blood serum of day old calves – by 21.7-27.6%. The decrease in IgM concentration was determined to the greatest extent. Subclinical ketosis of mothers had practically no effect on the content of albumin in the blood of calves.

Keywords: cows, calves, immunoglobulins, colostrum, colostral immunity, ketosis

Introduction

The neonatal health depends entirely on the state of the mother's body throughout the pregnancy. Ensuring optimal conditions for keeping pregnant animals is based, first of all, on adequate feeding and ensuring the sanitary and hygienic environmental conditions. These factors are very broad: optimizing feeding means not only introducing all the necessary nutrients into the diet – proteins, lipids, carbohydrates, as well as vitamins and minerals in certain proportions, but also accurately tracking changes in the animal's need for nutrients and the energy value of the diet in connection with changing the phases of lactation [4, 14]. What matters is the frequency of feeding, the degree of feed crushing, moisture content, and taste. It should be also noted that it is important to check the quality of all feed, especially that of our own production, such as silage and haylage. An important component of an animal's optimal health is its habitat. The organization of the manure removal system, the choice of litter, milking technology, hierarchical relationships in the herd – all this is important for creating an optimal herd ecosystem. There is evidence that limitation of mobility in cows associated with lameness can lead to metabolic disorders [2, 4, 5]. The use of highly productive dairy cows in livestock farms in our country is unthinkable without adherence to livestock management technology and competent herd management. Errors made by livestock breeders will affect the health of cows and calves anyway. The consequences of violating conditions of feeding and keeping cows during the so-called transit, or transitional period, are especially pronounced [7, 9, 13].

The transit period is extremely important in the cow's production cycle, starting three weeks before calving and continuing for six weeks. This period is associated with a serious restructuring of metabolic processes, with a sharp change in the cow's need for nutrients after calving. Despite the intensive increase in fetal weight during deep pregnancy, energy and plastic costs before calving are not as high as during lactation. Breeding work in dairy farming has led to a significant increase in the milk production of modern cows. So, in the first month of lactation, they produce 40-45 liters of milk per day. With such milk yields, up to 50 kg of pure protein and fat, up to 60 kg of lactose, up to 2 kg of calcium per pure element are removed from the body in a month. Therefore, during the transit period, the cow's body is most prone to metabolic diseases [7, 13, 14]. A big problem is the temporary lack of feed energy immediately after calving. The digestive system of the cow plays a prominent role in the absorption of nutrients from the diet, the microflora of the proventriculus is especially important. In the proventriculus, chemical processing of feed nutrients – carbohydrates, fats, proteins – occurs under the influence of enzymes from bacteria, fungi, protozoa. The ecosystem of the ruminant proventriculus includes a community of a large number of microorganisms – bacteria (50-90%), protozoa (10-50%), fungi (5-10%), the density of which exceeds that of known natural ecosystems. The number of bacterial species in the rumen reaches 150 and up to 60 species of protozoa. In the contents of the cow rumen, the bacterial mass reaches up to 1.5-2.0 kg. The system of symbiosis macroorganism-microflora ensures the assimilation of both feed nutrients and microorganisms as necessary metabolites for the host animal [14]. The active participation of microorganisms as metabolites of nutrients in the digestion of cellulose and starch in the rumen of ruminants makes this process more effective in this species of animals, in comparison with one-hoofed herbivores.

The introduction of ruminant nutrients into the diet is normalized depending on the phase of productivity. The maximum introduction of nutrients in terms of metabolizable energy and dry matter is carried out at the peak of lactation. Milking of cows is forcibly stopped two months before calving, and during this period, which is called dry-period, the feeding of energy-intensive feed is minimized. Thus, during the dry period, the nutritional value of the rations is minimal. With a decrease in the nutritional value of diets at the end of pregnancy, the concentration of cicatricial microflora naturally declines. Therefore, the conversion of feed in the early postpartum period is physiologically reduced. Today it is believed that a temporary negative balance after calving is a completely normal physiological phenomenon that every cow goes through to one degree or another [7]. However, if the cow is not properly provided with easily digestible high-energy feed, then a cascade of events quickly develops, leading to the overproduction of ketone bodies – acetoacetic, beta-hydroxybutyric acids and acetone. Ketosis develops in response to carbohydrate starvation, its biological meaning is to compensate for glucose deficiency in brain cells. The onset of ketosis in late pregnancy is exacerbated by a significant increase in fetal glucose intake [11]. Mobilization of own fat reserves partially covers the energy deficit, but fatty acids are not able to pass through the blood-brain barrier, therefore they cannot be used for oxidative metabolism of brain cells. A significant disadvantage is the rate of fatty acid beta-oxidation, which is not high enough due to the hydrophobicity of substrates, and under conditions of energy starvation cannot quickly replenish ATP regeneration at the proper level. On the other hand, activation of fatty acid oxidation results in an excess of acetyl-CoA molecules, which are not only substrates of the tricarboxylic acid cycle, but also sources for ketogenesis. Cows with high fatness, which received an excessively high-calorie diet during the dry period, are especially prone to ketosis [13, 15]. In the period of preparation for calving, the mobilization of fats from the depot increases and the concentration of non-etherified fatty acids in the blood sharply increases, which has an inhibitory effect on appetite. As a result, a vicious circle occurs – cows with high fatness and large reserves of subcutaneous fat become more prone to metabolic disorders during the transition period. Thus, a complex of factors arises that lead to the development of subclinical or clinical ketosis, the first signs of which may appear during pre-calving period. It is known that the development of immunity in the early postnatal period in a calf largely depends on the timely feeding of colostrum [1, 10, 12]. Maternal immunoglobulins from colostrum enter the systemic bloodstream of the newborn in the small intestine through the tubular system of epithelial cells by pinocytosis, i.e., in an unsplit form [3, 10, 16]. The ability for effective pinocytosis of immunoglobulins is very limited in time and last only for few hours. It is recommended to drink the first colostrum within the first two hours after delivery. The rate and degree of absorption of immunoglobulins in the small intestine continuously decreases during the first days of the calf life. The possibility of absorption of uncleaved antibody molecules is explained by the absence of hydrochloric acid in the abomasum, the presence of a trypsin inhibitor in colostrum, and the immaturity of the small intestinal epithelial cells [10].

Colostrum secret is extremely enriched in immunoglobulins: according to diverse studies, from 50 to 100 g/l is found in the first portion of colostrum after calving, which is several times higher than their concentration in the blood serum.

It is known that transplacental transmission of maternal antibodies to the fetus in ruminants is practically impossible due to the syndesmochorial structure of the placenta in ruminants, which rules out the possibility of their transport across the placenta [11]. In contrast, in primates and rodents with a hemochorial type of placenta, it is quite possible to transfer of maternal immunoglobulins to the fetus.

During the period of deep pregnancy, the cow's body intensively prepares the udder for lactation [3]. Under the influence of hormones, mainly prolactin and cortisol, during this period, synthetic processes are activated in the alveolar epithelial cells. In addition to organizing the biosynthesis of nutritional components of milk secretion, intensive immunobiological processes take place in the mammary gland. So, there is a migration of various leukocyte and macrophage cells into the alveolar cavity. The transport of immunoglobulins from the bloodstream to the secretion of the mammary gland is also activated [16]. Since the concentration of immunoglobulins peaks in the first portion of colostrum after calving, the most intensive processes of globulin protein enrichment of milk secretion occurs several days before calving. Contribution to the formation of the colostrum immunoglobulin pool is provided not only by proteins exported from the bloodstream, but also by its own immunoglobulins, synthesized by immunocompetent cells directly in the mammary gland tissues.

The importance of timely feeding of colostrum lies not only in the ability of the newborn epithelium to absorb native immunoglobulins, but also in reducing their secretion by prolactin-influenced alveolar epithelial cells, an inhibitor of colostrogenesis. As a result, the secretory epithelium of the alveoli intensively synthesizes milk components and completely stops the immunoglobulin transfer [1, 12].

Harbingers of metabolic disorders in cows appear even before calving, while the intensive development of metabolic pathology, accompanied by ketonemia, decreased gluconeogenesis, and liver lipidosis, manifests itself already in the postpartum period. Considering the fact that the formation of the colostrum immunoglobulin pool occurs before calving, the question arises about the effect of ketonemia of pregnant cows on the protein composition of colostrum secretion and on formation of colostral immunity in calves.

Materials and methods

The studies were carried out in the livestock farm of the Leningrad region (Slantsevsky district). For the study, pregnant cows 3-6 years old were selected 3-7 days before delivery. Urine and blood samples were collected from the cows. In order to identify subclinical ketosis in cows, urine was tested for ketones. According to the study data, two groups of 10 animals were formed – in the first group (experimental) the level of ketone bodies in the urine ranged from 1.8 to 3.7 mmol/l, in the second group (control) ketones were not found in the urine. Immediately after calving, portions of colostrum were obtained from the cows, and blood was collected from newborn calves a day after the first colostrum was fed. Colostrum secretions were centrifuged, then frozen followed by separation of lipid fraction. The content of immunoglobulins was studied in skim colostrum and in the blood serum of newborn calves. Before laboratory examination, colostrum secretion was diluted with saline at 1: 5. The study of ketones in urine was carried out using a CL-50 semi-automatic optoelectronic urine analyzer. In the blood serum of cows, the content of immunoglobulin classes A, M and G was investigated by the method of discrete sedimentation.

The essence of the method implies salting out certain classes of immunoglobulins with solutions of different ionic strengths at certain pH values and subsequent nephelometric measurement of the turbidity degree. It is known that at a certain degree of heterogeneity, each class of immunoglobulins consists of a complex of proteins with similar physicochemical properties. In particular, each class shows common features in folding of the molecular tertiary and quaternary structure based on a certain set of light and heavy chains, they have a certain sedimentation constant, molecular weight. A certain class of immunoglobulins has a specific set of heavy chains, which unites them into several classes.

The method of discrete deposition of immunoglobulins according to Zh.Badin and F.Rousellet (1964) modified by E.G. Larsky and N.P. Kravchenko (1968) with additions by M.A. Kostyna (1983) [8] found its application in veterinary laboratory diagnostics as well as in research work.

For the analysis, working solutions were prepared under the conditions of a clinical and biochemical laboratory using analytical grade reagents and analytical balances VL-220S, in strict accordance with the procedure. Measuring the concentration of immunoglobulins was carried out by using a KFC-3 photoelectric colorimeter at a wavelength of 450 nm. In the blood serum of one-day-old calves, the content of total protein was also determined by the Biuret method, albumin – by the photometric method with bromocresol green. Statistical data processing was carried out by using the Microsoft Excel package. The significance of differences was assessed by the method of paired comparisons using the Student's t-test, and a significance was set at $p < 0.05$.

Results and discussion

Considering the data obtained, a significant difference can be noted in the content of all studied serum immunoglobulin classes in the cows with subclinical ketosis. Analyzing the data presented in table 1, it can be noted that the concentration of IgG, IgM and IgA in cows with ketonuria was lowered by 19.1, 23.5 and 22.3% ($p < 0.05$), respectively, in comparison with cows of the control group. Metabolic disturbances in the body of pregnant cows,

TABLE 1. CONCENTRATION OF IMMUNOGLOBULINS IN THE BLOOD SERUM OF COWS, CALVES AND COLOSTRUM SECRETIONS

Study groups	Investigated indicators	Immunoglobulin content:		
		in the blood serum of cows (before calving)	in colostrum secretion	in the blood serum of calves (one day after drinking colostrum)
Group 1	IgG, g/l	14.71±0.51	36.88±1.83	13.86±0.47
	IgM, g/l	2.51±0.19	7.16±0.34	2.02±0.16
	IgA, g/l	1.67±0.14	3.12±0.29	1.92±0.09
Group 2	IgG, g/l	18.19±0.61	48.32±2.44	17.70±0.47
	IgM, g/l	3.28±0.13	10.91±0.55	2.79±0.18
	IgA, g/l	2.15±0.12	4.15±0.29	2.60±0.14

TABLE 2. CONCENTRATION OF TOTAL PROTEIN, ALBUMIN AND GLOBULINS IN CALVES

Investigated indicators	Group 1	Group 2
Total protein, g/l	49.77±1.00	56.18±1.22
Albumins, g/l	25.48±0.79	26.71±1.35
Globulins, g/l	24.29±0.32	29.47±0.95

associated with the ketone hyperproduction had a negative effect on antibody production. As a result, the colostrum secretion of cows with subclinical ketosis showed a decrease in all studied immunoglobulin classes by 23.7-34.4% ($p < 0.05$). To the greatest extent, the difference was observed for serum and colostrum IgM of cows, because proteins of this class are characterized by a short half-life period. It can be assumed that under conditions of pathologically altered metabolism, the production of antibodies in response to natural antigenic stimuli from the external environment is impaired. A decrease in the concentration of immunoglobulins in colostrum secretions influenced their blood levels in calves. As our studies have shown, the blood serum of calves born to cows with subclinical ketosis had level of IgG, IgM and IgA lowered by 21.7, 27.6 and 26.2%, respectively, compared to calves of the control group ($p < 0.05$). It was found that ketosis has the least effect on the decrease in the serum and colostrum content of class G immunoglobulins of cows and in the blood serum of calves born to them. The unevenness of the degree of reduction for different immunoglobulin classes in the studied biological fluids may result from different half-life periods for these proteins [10].

While studying the concentration of total protein, albumin and globulins in the blood serum of newborn calves one day after drinking colostrum (Table 2), there may be noted quantitative and qualitative differences in the protein spectrum. Thus, the concentration of total protein in calves of the first group was significantly lowered by 11.4% compared to the control group. It might be due to the proteins of the globulin fraction (by 17.6%, $p < 0.05$), while the difference in the albumin concentration in calves was not significant ($p > 0.05$). Most of the serum albumin is synthesized directly in the liver of the calf, so that only a small amount is supplied with colostrum. Therefore, differences in the colostrum immunoglobulin profile do not affect the serum albumin content in newborn calves. The subclinical ketosis that develops at the end of pregnancy does not significantly affect the synthesis of albumin in the fetal liver.

Thus, subclinical ketosis that develops in cows before calving affects colostrogenesis in the mammary gland, suppressing the formation of immunoglobulins and their inclusion in colostrum. A decrease in the content of immunoglobulins in colostrum secretion leads to decreased colostral immunity in calves. Subclinical ketosis in mother cows has a significant effect on the serum content of globulins in calves, but virtually does not affect the albumin concentration, which level depends on their synthesis in the liver, but almost does not depend on the protein spectrum of colostrum secretion.

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