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Functioning Of Platelets In Milk And Vegetable Nutrition Calves.

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ABSTRACT

The aim of the work is to evaluate the functional properties of platelets in calves in the phase of dairy-vegetative nutrition. We examined 36 healthy calves of milk and vegetal nutrition of black-motley and Simmental breeds, the assessment of the counted indicators was carried out 5 times: on 31 days, 45 days, 60 days, 75 days and 90 days. In calves of dairy-vegetative nutrition of black and motley breed at a normal content of platelets in the blood stream there is a small significant increase in the aggregation function of blood plates with individual inducers and their combinations. The level of platelets-discocytes in the blood for the phase of milk and vegetable nutrition was somewhat reduced. At the same time, the number of disco-echinocytes, spherocytes, sphero-echinocytes and bipolar forms of platelets in the bloodstream increased. The main mechanisms of increasing platelet aggregation activity in calves during the phase of milk and vegetable nutrition are the increase in their blood plates of the intensity of synthesis from endogenous arachidonic acid, thromboxane, an increase in the content of platelets of adenosine triphosphate, adenosine diphosphate, actin and myosin.

Keywords: calves, milk-and-vegetable phase, early ontogeny, platelets, aggregation, lipid peroxidation.

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INTRODUCTION

Increasing the population's demand for quality food is an important problem in almost all countries of the world [1,2,3]. Solve this problem is possible only with the support of modern livestock technologies, developed taking into account the latest achievements of physiological science [4,5]. Extremely physiologically significant in this respect are the data on blood [6,7], integrating the organism [8,9]. Thrombocytes [10] are of special functional importance for microcirculation, which can regulate their intensity of blood flow in capillaries [11,12].

In this regard, they are considered to be a very important component of the successful development of the homeostasis of a growing organism [13, 14]. Undoubtedly, their influence on the development of all body systems, largely controlling the fluid properties of blood, thereby determining micro-rheology in growing tissues and the sufficiency of oxygen and nutrient intake to them [15]. Great value for the organism of productive animals, incl. calves, has a phase of milk and vegetable nutrition, during which there is an adaptation to the reception of plant foods, which is marked by the further development of the functioning of internal organs [16] and the inevitable dynamics of the functional activity of platelet hemostasis [17]. It is from its adequate activity that microcirculation in calves' tissues during this period of life and the completeness of adaptation to changing feeding conditions depend [18]. In this connection, it is of great practical importance to evaluate the features of platelet activity in healthy calves that are physiologically mature and do not deviate from the homeostasis of the internal environment as a whole and the blood system in particular, which may later serve as age standards [19,20].

The aim of the study was to evaluate the functional properties of platelets in healthy calves during the phase of milk and vegetable nutrition.

MATERIALS AND METHODS

Research was conducted in strict accordance with ethical principles established by the European Convent on protection of the vertebrata used for experimental and other scientific purposes (adopted in Strasbourg March 18, 1986, and confirmed in Strasbourg June 15, 2006) and approved by the local ethic committee of Russian State Social University (Record №12 dated December 3, 2015).

Examined 36 healthy calves of dairy-vegetative nutrition of black-motley and Simmental breeds, evaluation of indicators which were performed 5 times: at 31 day, 45 day, 60 day, 75 day and 90 day. The ability of platelets to aggregation was evaluated visually with the use of inductors ADP ($0,5\times10^{-4}$ M), collagen (dilution 1:2 primary suspension), thrombin (0,125 U/ml), ristomycin (0,8 mg/ml), adrenaline (5.0×10^{-6} M), and combinations ADP+epinephrine, ADP+collagen, collagen+epinephrine, ADP+thrombin, ADP+collagen+epinephrine, ADP+thrombin+adrenaline ADP+collagen+thrombin+ epinephrine in similar concentrations in the plasma rich in platelets with a standardized number of blood platelets (thrombocytes $200x10^{9}$).

Indirect determination of the activity of the metabolism of arachidonic acid in platelets and the enzymes of its carrying out (cyclooxygenase and thromboxane synthetase) was carried out using three transport samples by recording platelet aggregation on a photoelectrocolorimeter.

The quantitative content in blood plates of ATP and ADP, the severity of their secretory ability under the action of collagen, the content of actin and myosin in them was evaluated traditionally. Intravascular platelet aggregation was recorded with a phase contrast microscope. The results are processed by Student's test (td).

RESULTS OF THE STUDY

Evaluation of platelet activity in healthy calves during the phase of milk and vegetable nutrition showed its significant peak gain by 45 days of life while maintaining the content of platelets in the bloodstream of animals within normal limits.

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Table. Aggregational activity of thrombocytes in healthy calves of dairy-vegetative food

Parameters of platelet aggregation	Milk and vegetable nutrition phase, n=36 M±m					Mean values, n=36 M±m
	31 day of life	45 day of life	60 day of life	75 day of life	90 day of life	
ADP, s	39.0±0.16	32.1±0.05 p<0.01	33.6±0.04 p<0.05	35.8±0.08 p<0.05	38.0±0.06 p<0.05	35.7±0.08
Collagen, s	28.7±0.12	22.4±0.07 p<0.01	24.5±0.09 p<0.05	26.8±0.11 p<0.05	28.2±0.05 p<0.05	26.1±0.09
Thrombin, s	53.0±0.07	46.3±0.03 p<0.01	48.1±0.10 p<0.05	49.6±0.05 p<0.05	51.9±0.12 p<0.05	49.8±0.07
Ristomycin, s	46.6±0.02	42.1±0.10 p<0.01	43.4±0.12 p<0.05	44.3±0.05 p<0.05	45.8±0.16 p<0.05	44.4±0.09
H_2O_2, s	40.1±0.03	35.0±0.05 p<0.01	36.8±0.06 p<0.05	38.0±0.12 p<0.05	39.5±0.14 p<0.05	37.9±0.08
Epinephrine, c	96.3±0.09	88.3±0.08 p<0.01	89.8±0.07 p<0.05	91.3±0.09 p<0.05	94.0±0.15 p<0.05	91.9±0.10
ADP+epinephrine, s	35.3±0.11	30.6±0.03 p<0.01	32.0±0.16 p<0.05	33.3±0.05 p<0.05	34.8±0.08 p<0.05	33.2±0.09
ADP+collagen, s	26.4±0.07	22.1±0.06 p<0.01	23.4±0.07 p<0.05	24.6±0.09 p<0.05	25.8±0.10 p<0.05	24.5±0.08
Epinephrine+ collagen, s	29.8±0.06	22.5±0.08 p<0.01	23.6±0.13 p<0.05	25.3±0.11 p<0.05	27.5±0.07 p<0.05	25.7±0.09
ADP+thrombin, s	26.2±0.05	20.3±0.03 p<0.01	22.1±0.11 p<0.05	23.6±0.15 p<0.05	25.2±0.06 p<0.05	23.5±0.08
ADP+ collagen+ epinephrine, s	21.4±0.08	17.1±0.07 p<0.01	18.4±0.04 p<0.05	19.5±0.03 p<0.05	20.6±0.09 p<0.05	19.4±0.06
ADP + thrombin + epinephrine, s	22.0±0.06	16.9±0.02 p<0.01	17.8±0.08 p<0.05	19.2±0.05 p<0.05	20.7±0.07 p<0.05	19.3±0.06
ADP+ collagen+ thrombin + epinephrine, s	18.5±0.03	14.1±0.04 p<0.01	15.3±0.06 p<0.05	16.5±0.01 p<0.05	17.7±0.04 p<0.05	16.4±0.04
The number of platelets in the aggregates,%	5.5±0.04	6.2±0.07 p<0.01	5.9±0.03 p<0.01	5.7±0.05 p<0.05	5.6±0.06 p<0.05	5.8±0.05
The number of small aggregates of 2-3 platelets per 100 freely lying platelets	4.2±0.03	6.5±0.04 p<0.01	6.0±0.08 p<0.01	5.4±0.04 p<0.01	4.9±0.02 p<0.01	5.4±0.04
The number of medium and large aggregates, 4 or more platelets, per 100 free-lying platelets	0.15±0.03	0.24±0.02 p<0.01	0.19±0.04 p<0.01	0.18±0.03 p<0.05	0.16±0.02 p<0.05	0.18±0.03

Legend: p - reliability of age dynamics of indicators.

Thus, in the calves for 45 days of life, the time of development of platelet aggregation under the influence of collagen decreased to 22.4±0.07 s, extending to the end of the phase to 28.2±0.05 s. A similar dynamics of platelet aggregation was observed under the influence of ADP and ristomycin. The thrombin and adrenaline aggregation of thrombocytes developed somewhat slowly, also extending to the end of the phase of milk and vegetable nutrition. Similar dynamics was revealed against the background of all tested combinations of inducers - platelet aggregation increased by 45% for ADP+epinephrine by 15.3%, for ADP+collagen by 19.4%, for epinephrine+collagen by 32.4%, for ADP + thrombin by 29.1%, for ADP+collagen+epinephrine by 25.1%, for ADP+thrombin+epinephrine by 30.2%, for ADP+collagen+thrombin+

epinephrine by 31.2%, then approaching time of development of platelet aggregation at the beginning of the phase.

During the phase of milk and vegetable nutrition in calves, an episode of a peak increase in the content of freely circulating small and large aggregates of platelets to the day of life was noted, which subsequently gradually decreased towards the end of the phase, almost to the level at its beginning. Similar dynamics was observed in calves of the given age and the number of platelets involved in the aggregation process [21,22].

The dynamics of the activity of arachidonic acid metabolism in them is a prominent mechanism of realizing the established dynamics of aggregation activity of platelets recorded in vitro and in vivo in calves during the phase of dairy-plant nutrition [23,24]. About it indirectly it was possible to judge by the aggregation of platelets in a simple transfer sample (for the 90th day of life 32.7 ± 0.08%). The revealed dynamics of the metabolism of arachidonic acid in the blood plates of healthy calves of milk and vegetable nutrition was possible as a result of a short-term increase in the activity of both enzymes of its transformation in platelets - cyclooxygenase and thromboxane synthetase to 45 days of life by 11.3% and 19.5%, respectively. At the end of the phase, the recovery of AT in the collagen-aspirin sample, which indirectly assesses the activity of cyclooxygenase in platelets, was already 81.5 ± 0.04%, and the restoration of platelet aggregation in the collagen-imidazole sample, which allows indirectly determining the thromboxane synthetase state in blood plates, also decreased to 42.3±0.05%, reaching the level characteristic for the beginning of the phase - 79.6±0.03% and 40.5±0.12%, respectively.

The quantitative content of actin and myosin on day 31 was 32.2±0.16 and 14.6±0.12% of the total protein in the platelet, increasing by 45 days to 37.2±0.05 and 16.7±0,07% to the total protein in the platelet, decreasing to 90 days to values close to the end.

DISCUSSION

The revealed dynamics of the activity of blood platelets in healthy calves of milk and vegetable nutrition is provided by age-related changes in the functional characteristics of their individual mechanisms, determining in many respects the necessary microrheological properties of platelets and, consequently, microcirculation in the tissues of a growing animal [25]. Taking into account these circumstances, evaluation of the activity of platelet hemostasis and the mechanisms of its realization in healthy calves of dairy-vegetative nutrition is of great urgency. As a result of the studies, the increase in the adhesive ability of platelets in healthy calves to the middle of dairy and plant nutrition was found, which is largely determined by the increase in these terms in the production of von Willebrand factor (WF) in their vessels, the cofactor of platelet adhesion, followed by its weakening toward the end of the phase [26]. The episode of WF production amplification in milk and vegetable nutrition calves is determined on the basis of a peak acceleration of the dynamics of platelet aggregation with ristomycin to the middle of the phase, which, in its ability to influence platelets, is similar to subendothelial vascular structures [27,28]. It is likely that an increase in the WF concentration in the blood is combined with an increase in the number of receptors on the surface of platelets, which makes it possible to form an "adhesion axis": Collagen-WF-GPIv, providing an increase in the response of blood plates to the inductor [29]. The peak increase in platelet aggregation with all tested inducers and their combinations to the middle of the phase was determined by the adaptive increase in the calves in this age of the number of receptors to them on the membranes of the blood plates [30, 31].

Evaluation of platelet aggregation with several aggregation inducers allowed us to consider that these animals have their mutual potential effect under physiological conditions enhancing platelet aggregation. The use of combinations of platelet aggregation inducers is able to repeat the intravascular conditions in growing calves to a certain extent, making it possible to evaluate the aggregation of platelets under conditions peculiar to real blood flow [33].

The number of free-circulating aggregates of various sizes in the blood of calves at the beginning of the phase was small, also peaking in its middle, which was combined with a similar dynamics of the number of platelets involved in the aggregates [34]. At the base of the recorded increase in the aggregative ability of the blood platelets in calves, in addition to the increase in the number of receptors on the membrane, the peak increase in the activity of the intra-platelet mechanisms of the realization of their haemostatic functions lies in

the mid-phase of the dairy-plant nutrition: short-term enhancement with subsequent thromboxane formation, actino- and myosinogenesis, and the severity of ATP and ADP secretion [35,36].

It can be assumed that the established patterns of platelet activity in healthy calves in the phase of milk and vegetable nutrition are determined by the body's response to the intake of plant foods, being an essential element of its adaptation [37-40].

CONCLUSION

In the blood of calves of milk and plant nutrition of black and motley breed there is a slight increase in the aggregation function of the blood platelets in response to individual inducers and their combinations. For this reason, the level of platelet discocytes in the blood of calves during the phase of milk and vegetable nutrition decreased slightly. This was accompanied by a small increase in the amount of disco-echinocytes, spherocytes, sphero-echinocytes and bipolar platelet forms in the blood. The main mechanisms for increasing platelet aggregation activity in calves during the phase of milk and plant nutrition should be considered an increase in the intensity of thromboxane synthesis in their blood plates, as well as an increase in the content of adenosine triphosphate, adenosine diphosphate, actin and myosin in platelets.

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