Physiological features of platelet hemostasis in calves during the phase of plant nutrition, kept in the ecological conditions of central Russia

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Abstract. During the final phase of early ontogenesis in calves, there is a low activity of lipid peroxidation, pronounced antioxidant protection of platelets, an increase in the intensity of the actin-myosin complex, a significant content of adenosine phosphates in their granules and their intensive secretion under conditions of platelet activation. In calves during the phase of plant nutrition, there was a tendency to increase the hemostatic capacity of platelets, assessed in vitro and in vivo. A certain increase in platelet aggregation and secretion found in calves during the phase of plant nutrition is apparently caused by regular age-related changes in the implementation of post-receptor processes in their platelets, which ensure an increase in their hemostatic characteristics in the conditions of completion of growth and development of their body.

1 Introduction

The process of ontogeny in different species of productive animals is associated with gradual changes in the morphological and functional parameters of their organism in the existing environmental conditions [1, 2]. Researchers have formed an opinion about the high biological significance of hemostasis for maintaining the constancy of the internal environment of the body and ensuring the adaptive characteristics of the animal throughout life [3]. A very significant role in the formation of physiological conditions for the implementation of microcirculation and tissue trophism in ensuring the productive potential of animals is played by the hemostatic parameters of platelets and especially the level of their aggregation [4].

The phase of plant nutrition is considered to be the final stage of the early ontogenesis of cattle [5]. At this time, the maturation of all internal organs and their systems ends in calves. This is true for the blood system as well. However, platelet activity in calves at this stage of their development under different environmental conditions is still poorly understood. In plant-based calves kept in the conditions of central Russia, the features of intravascular platelet aggregation and their aggregation readiness, manifested in vitro in response to individual agonists, have not been fully established. Also, the state of antioxidant enzymes and the level of lipid peroxidation in platelets, which can affect their

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hemostatic characteristics, have not been fully elucidated. In this regard, the aim of the work is to trace the dynamics of the functional characteristics of platelets during the phase of plant nutrition in calves of optimal functional status kept in the ecological conditions of central Russia.

2 Materials and methods

The study was conducted on healthy plant-based calves in the amount of 39 heads kept in the ecological conditions of central Russia. Their examination was carried out on the 91st day, at the age of 6 months, 9 months and 12 months of their ontogeny. After taking blood from calves, platelets were isolated from it by washing and resuspension. Subsequently, the amount of malondialdehyde in platelets was determined during the reduction reaction with thiobarbituric acid and the amount of acyl hydroperoxides was recorded. The biological activity of intraplatelet antioxidant enzymes, catalase and superoxide dismutase, was determined. In the cytoplasm of animal platelets, the amount of adenosine triphosphate (ATP) and the content of adenosine diphosphate (ADP) were recorded, and the severity of their secretory release was assessed in the case of exposure to collagen platelets. Actin and myosin components of the contractile platelet cytoskeleton were quantified in intact platelets, as well as under the action of ADP and thrombin on them. The content of platelets in the blood of calves was determined using a Goryaev camera. The state of platelet aggregation (AP) was determined using a visual micromethod using the following as process stimulators: ADP (0.5×10^{-4} M), collagen (1:2 dilution of the main suspension), thrombin (0.125 units/ml), ristomycin (0.8 mg/ml), H_2O_2 (7.3×10^{-3} M), epinephrine (5×10^{-6} M). The severity of intravascular platelet aggregation was assessed using a phase contrast microscope. Mathematical processing of the research results obtained in the work was associated with the calculation of the Student's t-test.

3 Research results

All calves observed in the work were under continuous control of their general condition. Prior to each blood draw in this study, animals were monitored for key physiological parameters and assessed for major hematological parameters.

At the beginning of the observation, the content of acyl hydroperoxides in platelets of calves was not high - 2.92±0.12 $D_{233}/10^9$ platelets. Their number slightly decreased during the entire observation period to the level of 2.63±0.13 $D_{233}/10^9$ platelets. The level of malondialdehyde in platelets was initially 0.90±0.11 nmol/10^9 platelets in calves. Subsequently, during the observation period, it decreased and amounted to 0.73±0.14 nmol/10^9 platelets by its end.

The biological capabilities of platelet catalase and superoxide dismutase in the examined calves increased during the phase of plant nutrition from 9720.1±10.12 IU/10^9 platelets to 10196.3±14.34 IU/109 platelets and from 1680.3±3.65 IU/10^9 platelets up to 1950.3±3.32 IU/10^9 platelets, respectively.

During the entire observation period, the amount of ATP and ADP in the composition of platelets in the examined calves gradually increased from 5.72±0.16 μmol/10^9 platelets to 5.81±0.18 μmol/10^9 platelets and from 3.56±0.10 μmol/10^9 platelets to 3.64±0.17 μmol/10^9 platelets, respectively). The intensity of the secretion of ATP and ADP from platelets in the case of collagen entering the plasma during the observation period increased by 7.6% and 9.2%, respectively.

The content of actin in inactive platelets in calves at the age of 91 days reached 34.0 ± 0.18% of the total protein in the platelet, increased by the age of one to 37.0 ± 0.14% of the total protein in the platelet (table 1). The level of additional self-assembly of actin in the observed young animals in the case of activation of platelets by an inductor of any strength and in the case of platelet aggregation under their influence also increased throughout the entire observation period.

In the calves included in the study, platelets showed similar dynamics of myosin levels. In inactive platelets of animals during the first examination, the content of myosin was 15.3±0.14% of the total protein content in the platelet. Subsequently, it gradually increased and by the end of the plant nutrition phase of early ontogenesis reached a value of 17.5±0.11% of the total protein content in the platelet. In calf platelets, in the case of their activation and their aggregation with the help of a strong or weak inducer, a gradual increase during the plant nutrition phase of additional self-assembly of the myosin protein was found (table 1).

Table 1. The number of contractile elements in platelets of calves during the phase of plant nutrition**.**

Platelet parameters	**Age of examination of calves, n=39 M±m**			
	91 days of life	**6 months of life**	**9 months of life**	**12 months of life**
Actin level in platelets				
in an inactive state, % of the total protein content	34.0±0.18	35.2±0.12	36.3±0.09	37.0±0.14
in the case of ADP activation, % of the total protein content	34.2±0.16	36.1±0.14	37.4±0.19	38.3±0.22
in the case of ADP aggregation, % of the total protein content	42.9±0.24	43.5±0.19	44.2±0.09	45.6±0.20
in case of thrombin activation, % to total protein content	37.1±0.23	37.9±0.25	39.2±0.20	40.3±0.17
in the case of thrombin aggregation, % of the total protein content	31.4±0.12	33.5±0.16	35.7±0.17	37.6±0.22
Myosin level in platelets				
in an inactive state, % of the total protein content	15.3±0.14	16.1±0.18	16.9±0.09 $p<0.05$	17.5±0.11 $p<0.05$
in the case of ADP activation, % of the total protein content	21.4±0.16	23.0±0.18	24.7±0.20 $p<0.05$	25.6±0.15 $p<0.05$
in the case of ADP aggregation, % of the total protein content	29.9±0.15	30.8±0.12	31.7±0.09	33.6±0.18 $p<0.05$
in case of thrombin activation, % to total protein content	36.1±0.14	37.9±0.15	38.5±0.23	39.7±0.19 $p<0.05$
in the case of thrombin aggregation, % of the total protein content	44.8±0.23	45.6±0.20	47.3±0.16	49.0±0.18 $p<0.05$

Note: significance of differences with the level at 91 days of life. In the following table, the designations are similar.

During the survey, the calves accelerated the onset of AT in response to all the inductors used in the work (Table 2).

In the examined calves, AP under the action of collagen occurred at the beginning of the observation for 26.7±0.14 s, accelerating to a small extent throughout its duration. A pronounced acceleration of the onset of AT in the examined calves was found during the fourth phase of early ontogenesis under the action of ADP and ristomycin. A little later, AP attacked in response to H_2O_2, thrombin, and adrenaline. The time of onset of AP under their action also tended to decrease during the phase of plant nutrition. The acceleration of the development of AP in vitro that occurred in calves during the observation was consistent with the data from the assessment of intravascular aggregation. At the same time, the number of discoid platelets in the blood of calves on the 91st day of ontogenesis turned out to be 74.0±0.25%, subsequently having a downward trend. At the end of the observation, this parameter reached 71.1±0.23%. The total level of active varieties of platelets during the observation period increased by 15.0%. At the same time, in the blood of animals during the fourth phase of early ontogenesis, the number of small and large platelet aggregates increased from 5.4±0.05 and 0.17±0.06 per 100 free-lying platelets to 5.9±0.12 and 0, 23±0.09 per 100 free-lying platelets at the end of the observation. The level of involvement of platelets in aggregates in calves during the observation increased by 8.6%. The changes found indicated that in calves during the phase of plant nutrition, the hemostatic capabilities of platelets are enhanced, which accompanies the implementation of growth, development and adaptation processes in their body to environmental conditions.

Table 2. Aggregation function of calf platelets during the phase of plant nutrition.

Aggregation Options	**Age of examination of calves, n=39 M±m**			
	91 days of life	**6 months of life**	**9 months of life**	**12 months of life**
Platelet aggregation in vitro				
Aggregation in response to ADP, s.	35.9±0.19	34.8±0.21	33.5±0.09 $p<0.05$	32.8±0.15 $p<0.05$
Aggregation in response to collagen, s.	26.7±0.14	26.0±0.12	25.2±0.17	23.6±0.19 $p<0.05$
Aggregation in response to thrombin, s.	49.4±0.09	48.3±0.08	46.9±0.20	45.5±0.15 $p<0.05$
Aggregation in response to ristomycin, s.	44.2±0.11	42.9±0.16	41.3±0.23	40.4±0.14 $p<0.05$
Aggregation in response to H_2O_2, s.	38.2±0.14	36.8±0.12	35.2±0.09	34.1±0.18 $p<0.05$
Aggregation in response to adrenaline, s.	92.0±0.23	90.2±0.17	89.1±0.26	87.8±0.19
Platelet aggregation in vivo				
The amount of active platelets in the blood, %	26.0±0.12	26.9±0.15	27.8±0.19	29.9±0.13 $p<0.05$
The number of platelets in aggregates, %	5.8±0.09	6.0±0.06	6.1±0.11	6.3±0.08
The number of small aggregates of 2-3 platelets, per 100 free platelets	5.4±0.05	5.5±0.10	5.8±0.19	5.9±0.12 $p<0.05$
The number of medium and large aggregates of 4 or more platelets, per 100 free platelets	0.17±0.06	0.19±0.08 $p<0.05$	0.21±0.05 $p<0.01$	0.23±0.09 $p<0.01$

4 Discussion

Sufficiently high activity of all vital processes in the body is provided by a mass of strictly interconnected processes implemented at different levels of the body organization [6]. The central place in this range of processes is occupied by the effect of self-regulation of the body achieved against their background [7]. In recent years, it has become more and more obvious that a particularly significant place in this belongs to the optimum parameters of the blood, which ensures the connection of the whole organism into a single whole. It implements humoral self-regulation, delivery of nutrients to cells, heat transfer, and many other vital processes [8].

Only under conditions of optimal implementation of processes in the blood is it possible to preserve the health of the body. This is achieved in the case of normal rheological and hemostasiological blood parameters that can fluctuate during the entire ontogenesis [9]. Of great importance in ensuring these blood properties in all productive animals is the functional state of platelets, which can experience age-related dynamics [10].

The work found that healthy calves are characterized by the dynamics of platelet parameters during the phase of plant nutrition. They were characterized by an increase in the antioxidant characteristics of platelets, which inhibit the processes of lipid peroxidation in them. Achieved low intensity of free radical phenomena during the phase of plant nutrition in calves at this age contributes to the optimum operation of all hemostatic mechanisms of platelets. This concerns the severity of self-assembly of the contractile complex of the actin-myosin composition, the content of ADP and ATP in platelets, and the degree of their secretion [11].

In calves, during the phase of plant nutrition, a small AP was noted, which showed some tendency to accelerate the onset as their chronological age increases. Apparently, this is caused by the reaction of their body to anabolic processes in it and the existing environmental conditions associated with an increase in the level of von Willebrand factor in the plasma, which acts as a platelet adhesion cofactor [12]. All this is accompanied during the phase of plant nutrition by an increase in the level of GPIb receptors on their surface, which are able to combine with it [13]. A certain increase in the activity of platelet receptors is undoubtedly caused by the activation of a number of adaptive and biosynthetic reactions in the body under conditions of an increase in the mass of environmental influences [14].

In calves, during the phase of plant nutrition, an increase in platelet aggregative function was noted. An increase in the time of observation of AP in relation to strong inducers of aggregation indicated the activation of phospholipase C in them, which implements the phosphoinositol mechanism of platelet activation. On an increase in the level of diacylglycerol and the activity of protein kinase C with an increase in the number of actin and myosin molecules in the cytoplasm of platelets. The acceleration of the onset of antibodies in response to weak inducers of aggregation, including ADP and adrenaline, was regarded as a consequence of an increase in the availability of specific receptors for them and/or a consequence of an increase in their density on platelet membranes. This was also caused by an increase in the amount of GPIIb-IIIa, which are fibrinogen receptors, on the surface of platelets and an increase in the functional activity of phospholipase A_2, which is capable of releasing arachidonic acid from platelet structures to form thromboxane A_2 from it.

The development of an increase in intravascular platelet aggregation indicated an age-related increase in all of the indicated mechanisms of platelet participation in hemostasis in calves. Under in vitro conditions, an increase in platelet aggregation capabilities should also be associated with a certain functional preponderance of proaggregants over deaggregants in the blood of calves during the phase of plant nutrition, as well as with an increase in the expression of receptors for fibrinogen and all physiological inducers of aggregation on platelet membranes. In addition, for this effect in calves, an increase in intraplatelet processes with age, providing aggregation, was required.

5 Conclusion

During the phase of plant nutrition of early ontogenesis in the body of calves kept in the ecological conditions of central Russia, a lot of functional changes occur associated with the completion of their growth and development. This is true for all systems of their body, including the blood system and the hemostasis system. In the study, in calves located in central Russia, during the phase of plant nutrition, an increase in the ability of platelets to aggregate under in vitro conditions and under conditions of blood flow in the body was noted. These changes should be considered as the result of the development of a hereditary program in the animal.

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