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Physiological Properties of Platelets in Dutch Calves during the Dairy Phase

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Abstract

Platelets' functionality is important for the activity of hemostasis and metabolism in mammals such as cattle. They are of particular importance in the growth of livestock offsprings. 43 healthy calves belonging to the Dutch breed were observed. The evaluation of blood parameters was carried out in these calves at the age of 11, 15, 20, 25, and 30 days. During this time, calves showed some tendency to inhibition of platelet aggregation and a tendency to increase discoid platelets in their blood. This was accompanied in calves by a decrease in the blood of platelets that showed their hemostatic properties and the number of their dynamic aggregates of any size. These processes were associated in calves with a decrease in the synthesis of platelet thromboxane, a decrease in the amount of phosphated adenosine in their platelets. During the milk feeding phase, the number of contractile proteins in platelets of calves tended to decrease, with a tendency to a decrease in the severity of their additional synthesis under conditions of platelet aggregation. In purebred Dutch calves, during the phase of milk nutrition, a functional optimum of platelet activity is noted, which provides the basis for the most active microcirculation, providing all the conditions for the intensive development of their organs and tissues.

Keywords: Platelets, Aggregation, Secretion, Calves, Dutch breed

Introduction

It is now recognized that blood is an important integrative medium of the whole organism (Abreu *et al*., 2016; Oshurkova & Medvedev, 2018a). The degree of functional perfection of its various regulatory systems largely ensures the success of maintaining mammalian homeostasis (Zavalishina, 2018b; Azevedo *et al*., 2021). Among them, a very important place is occupied by the homeostasis system. It includes a lot of components, but platelets have a very significant place in it. Their functional state strongly determines the implementation of hemocirculation processes in all organs (Glamazdin *et al*., 2021a; Godoy *et al*., 2022). It is known that many parameters of platelet functioning are very sensitive to various influences from the external environment. This is true for humans, unproductive and productive animals. The study of any aspects of platelet hemostasis in cattle is of great practical importance since this point can clarify its connection with the productivity of these animals. Now, certain moments of platelet activity in these animals have been elucidated (Kulikov *et al*., 2020; Tkacheva & Medvedev, 2020c; Karpov *et al*., 2021). Due to the importance of the level of platelet activity for capillary hemodynamics and the state of tissue trophism, there is reason to consider platelets highly significant in the implementation of the growth and maturation of body structures, and, consequently, in the development of animal productivity (Korepanova *et al*., 2015; Zavalishina, 2018c; Manzanares *et al*., 2018). In view of the presence of many morpho-functional differences between animals, individual breeds of cattle, it becomes clear that it is necessary to search for the physiological characteristics of platelets in young dairy breeds, including the Dutch breed.

Purpose - to assess the level of hemostatic properties of platelets in purebred calves of the Dutch breed during the phase of milk feeding.

Materials and Methods

The work was carried out in close connection with the ethical standards outlined by the European Committee for the Protection of Vertebrate Animals that are taken into experiments (adopted in Strasbourg on March 18, 1986 and confirmed in Strasbourg on June 15, 2006). This study was supported by the local ethics committee established at the All-Russian Research Institute of Physiology, Biochemistry and Nutrition of Animals - a branch of the Federal Scientific Center for Animal Husbandry - VIZH named after academician L. K. Ernst (protocol No. 4 dated April 04, 2017).

The work was carried out with the involvement of 43 purebred calves of the Dutch breed, which are at the age of 11 days. All young animals were obtained from Dutch cows of optimal physical status after an uneventful second or third pregnancy. Examination and examinations of all young animals were performed on the 11th day, 15th day, 20th day, 25th day and 30th day of their ontogeny.

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In the examined animals, the level of thromboxane generation in platelet structures was recorded, indirectly assessing the functional properties of cyclooxygenase and thromboxane synthetase in platelets using a photoelectrocolorimeter device. In calf platelets, the concentrations of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) involved in hemostasis were determined with an assessment of the level of their secretory release process in response to the appearance of collagen molecules in the plasma. The content of actin and myosin in intact and active platelets was determined (Ermolaeva *et al*., 1992).

The ability of platelets to aggregate (AP) was recorded using a standard micromethod, using ADP (at a concentration of 0.5 x 10^{-4} M), collagen (at a doubling of the main suspension), thrombin (at a concentration of 0.125 U/ml), and adrenaline as stimulators of this process. (at a concentration of $5.0x10^{-6}$ M) and ristomycin (at a concentration of 0.8 mg/ml). The study was conducted in plasma enriched with platelets and standardized in their number to a concentration of $200x10^9$ platelets contained in one liter of blood (Medvedev *et al*., 2009). Platelet activity inside the vessels was monitored during phase contrast microscopy (Medvedev *et al*., 2009). Mathematical processing of the obtained indicators was performed by the computer program "Statistics for Windows v. 6.0". Reliability of differences between the compared indicators was noted in the case of $p<0.05$.

Results and Discussion

During the observation period, calves belonging to the Dutch breed showed a slight decrease in platelet activity. In the observed animals, platelet collagen aggregation was inhibited during the study by 5.2%, reaching 10.5±0.19 s by the 30th day of age. A comparable tendency to inhibition of the AP process was observed in ADP and ristomycin calves, which slowed down by 10.1% and 9.7% during the second phase of ontogenesis, respectively. A tendency to delay AP in response to thrombin (by 7.8%) and adrenaline (by 7.4%) was revealed **(Table 1)**.

Table 1. Platelet values in Dutch breed calves during the lactation phase

Indicators	Age of calves, n=43, M±m				
	11 day	15 day	20 day	25 day	30 day
The value of the collagen-aspirin test, %	73.1±0.12	72.4±0.10	71.9±0.14	71.0±0.12	70.1±0.08
The value of the collagen-imidazole test, %	34.5±0.10	34.0±0.09	33.4±0.11	32.7±0.15	31.9±0.16
Simple transfer sample value, %	24.5±0.15	24.0±0.16	23.4±0.15	22.9±0.14	22.1±0.20 p<0.05
The amount of ATP in inactive platelets, µmol/10^9 platelets	5.21±0.19	5.15±0.20	5.07±0.014	4.95±0.018	4.86±0.010
The amount of ADP in inactive platelets, µmol/10^9 platelets	3.09±0.004	3.00±0.008	2.95±0.006	2.89±0.005	2.81±0.009 p<0.05
The severity of the secretory release of ATP, %	24.4±0.11	2.40±0.11	23.5±0.13	23.1±0.08	22.4±0.08
The severity of the secretory release of ADP,%	31.6±0.05	31.0±0.14	30.6±0.05	30.0±0.10	29.3±0.16
The content of actin protein in discoid platelets,% of the total protein level in platelets	19.3±0.18	18.9±0.15	18.5±0.06	18.0±0.09	17.4±0.08 p<0.05
The content of actin protein in aggregating platelets,% of the total protein level in platelets	31.2±0.14	30.6±0.11	29.8±0.15	28.4±0.17	27.6±0.19 p<0.05
The content of myosin protein in discoid platelets,% of the total protein level in platelets	9.0±0.12	8.7±0.09	8.2±0.16 p<0.05	7.7±0.14 p<0.05	7.4±0.19 p<0.01
The content of myosin protein in aggregating platelets,% of the total protein level in platelets	21.3±0.12	21.0±0.14	20.7±0.08	19.2±0.16 p<0.05	18.2±0.18 p<0.05
Time of development of platelet aggregation under the action of ADP, s	47.7±0.21	47.9±0.16	48.3±0.19	48.8±0.21	52.5±0.24 p<0.05
The period of development of platelet aggregation under the influence of collagen, s	38.5±0.17	38.8±0.18	39.2±0.15	39.7±0.13	42.8±0.19 p<0.05
Time of development of platelet aggregation under the action of thrombin, s	60.0±0.16	60.5±0.10	60.9±0.14	62.6±0.23	64.7±0.17
The period of development of platelet aggregation under the action of ristomycin, s	56.7±0.16	57.1±0.19	57.7±0.23	59.4±0.16	62.2±0.22

The period of development of platelet aggregation under the action of adrenaline, s	108.4±0.27	108.9±0.24	110.7±0.19	112.6±0.25	110.4±0.30
Number platelet count discocytes, %	85.7±0.30	86.2±0.23	86.9±0.16	87.5±0.25	88.8±0.28
Total active platelet count, %	14.3±0.16	13.8±0.15	13.1±0.14 p<0.05	12.5±0.12 p<0.05	11.2±0.18 p<0.01
Platelet aggregates of small size, per 100 free platelets	2.4±0.07	2.2±0.02 p<0.05	2.0±0.04 p<0.01	1.9±0.06 p<0.01	1.8±0.08 p<0.01
Platelet aggregates of medium and large size, per 100 free platelets	0.06±0.012	0.05±0.014 p<0.01	0.04±0.018 p<0.01	0.03±0.012 p<0.01	0.03±0.012 p<0.01

Note: p - statistically significant changes in values compared with the value at 11 days of age.

The number of inactive platelets in the blood of calves during the period of the milk feeding phase showed an upward trend. The number of activated platelet variants in them underwent a decrease, in general, amounting to 27.7%. The small and large platelet aggregates in the blood of the examined animals during the observation gradually decreased by a total of 33.3% and 2 times, respectively.

In Dutch breed calves, between the 11th and 30th days of life, thromboxane formation in platelets was inhibited by 10.8%, reaching 22.1 ± 0.20% on the 30th day of life. The result obtained was ensured during the observation of the animals taken in the study by the tendency to weaken the enzymatic functions of thromboxane synthetase and cyclooxygenase in platelets.

The low content of ATP and ADP in the platelets of the observed young animals during the study underwent a downward trend, amounting to 4.86±0.010 and 2.87±0.009 μmol/10^9 platelets in the last study. During the study, the degree of their release from platelet granules decreased by the end of the observation to 22.4±0.08% and 29.3±0.16%, respectively.

In the examined calves, in inactive platelets, the levels of myosin and actin during the observation period decreased by 11.2% and by 21.6%, amounting to 17.4±0.08 and 7.4±0.19% of the total at the end of the observation of calves. the level of protein in platelets during the phase of milk nutrition. Additional formation of contractile protein molecules during the induction of platelet aggregation in purebred Dutch calves taken into the study slightly decreased for actin by 13.0%, for myosin by 17.0%.

The inhibition of AP found in the observed young animals under the influence of collagen and ristomycin indicated a gradual weakening of manifestations of platelet adhesion in their blood (Glagoleva & Medvedev, 2020; Glamazdin *et al*., 2021b). This phenomenon in calves was provided by a tendency to reduce the number of receptors on platelets that can interact with collagen - glycoproteins Ia - IIa and VI (Sharkayeva & Sharkayev, 2016). In addition, in this process, a decrease in platelet sensitivity to ristomycin was very significant in the young of the Dutch breed (Zavalishina, 2018m). This was possible due to the simultaneous minimization of von Willebrand factor in their blood and a decrease in the density on the surface of their platelets of receptor molecules capable of binding to it (GPI b) (Chinarov, 2018; Skoryatina & Medvedev, 2019).

It has been established that during the second phase of early ontogenesis for calves of the Dutch breed, a slight decrease in the severity of platelet aggregation in the blood is characteristic. These changes optimize blood circulation in the capillaries (Zavalishina, 2018l; Mikhaylova *et al*., 2021). A weakening of platelet sensitivity to aggregation stimulators of any strength developed. The slowdown in the development of platelet aggregation in response to its strong stimulants - collagen and thrombin, was realized due to a decrease in the functionality of the platelet enzyme phospholipase C and the severity of phosphorylation of all components of the actin-myosin contractile complex (Zavalishina, 2018h). This contributed to a functionally beneficial depression of the synthesis of platelet inositol triphosphate, inhibiting the entry of Ca^{2+} into platelets, which restrained the intensity of actomyosin self-assembly, and, consequently, its participation in the secretion process (Zavalishina, 2018k).

Weak inducers of platelet aggregation - ADP and adrenaline caused the aggregation process to be more and more delayed in young purebreds of the Dutch breed between 11 and 30 days of life (Zavalishina, 2018e). Apparently, these changes were based on a decrease in the density of molecules that act as receptors on the surface of platelets, on a weakening of the expression of glycoproteins capable of interacting with fibrinogen (GPIIb-IIIa) and on a depression of the biological properties of phospholipase A_2 under the action of ADP and adrenaline on platelets. Under these conditions, a functional minimum was created for the release of arachidonic acid from platelet phospholipids and, as a result, the synthesis of thromboxane A_2 was inhibited (Zuev & Osadchaya, 2006; Zavalishina, 2018f). In addition, the Dutch breed calves were characterized by a small biosynthetic activity of both enzymes that ensure the synthesis of thromboxane in platelets (Paiva *et al*., 2016; Zavalishina, 2018i). Low AP in young Dutch breeds, recorded between 11 and 30 days of their life under the influence of all inducers, was also associated with a decrease in basal levels of actin and myosin in platelets (Zavalishina, 2018n; Tkacheva & Medvedev, 2020a), with a weakening of their additional assembly under conditions of platelet activation, as well as with a weakening of the process of release from platelets adenoin phosphate granules (Zavalishina, 2018j; Zavalishina *et al*., 2022).

The low blood level of activated platelets taken under observation of animals additionally confirmed the low functional

characteristics of platelets in relation to stimulating hemostasis effects (Zavalishina, 2018o; Tkacheva & Medvedev, 2020b). The low intravascular activity of platelets revealed in them indicated a low degree of contact of collagen molecules, which is part of the subendothelium due to the high preservation of vascular endothelial cells (Zavalishina, 2018d). This was ensured by a low amount of activated platelets in the blood of the examined calves, including those that entered the process of aggregation (Santa-Rosa *et al*., 2018; Zavalishina, 2018g). This moment confirmed the decrease in the sensitivity of platelet receptors in young purebreds of the Dutch breed to small amounts of substances in their blood - stimulators of platelet aggregation (Oshurkova & Medvedev, 2018b; Zavalishina *et al*., 2021). Initially found in calves, the low ability of platelets to aggregate provided their very moderate intravascular activity, which further declined during the observation period. This was accompanied by a decrease in the number of platelets in the blood, which began to participate in hemostasis and were not associated with the walls of blood vessels of platelet aggregates of any size (Zavalishina, 2018a). The low activity of platelets in young purebreds of the Dutch breed proved a small degree of participation of their platelets in hemostasis under blood flow conditions. Apparently, this is due in the Dutch breed of young animals with a high ability of their platelets to disaggregate between 11 and 30 days of life. This phenomenon is obviously based on a gradual increase in their receptors for antiaggregant substances in the blood.

Conclusion

In young purebreds of the Dutch breed, optimally low platelet activity is noted during the milk feeding phase. This circumstance provides them with optimum perfusion of all capillaries. This is due to the low activity of hemostatic platelets in these calves. The small platelet activity formed in Dutch breed calves is one of the factors that ensure the optimum tissue vitality during their growth and development during the second phase of early ontogenesis.

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