The severity of platelet activity in young cattle of the Kholmogory breed at the end of early ontogenesis

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Abstract. Of great scientific interest is the assessment of the functionality of platelets in dairy calves at the end of early ontogenesis. 44 healthy calves of the Kholmogory breed were observed, obtained from completely healthy cows after their 2nd-3rd pregnancy. During the observation period, the young animals developed an acceleration of the process of platelet aggregation in vitro. This was accompanied in calves by an increase in the number of activated platelets in the blood and the number of their aggregates of different sizes. An increase in the functionality of platelets occurred in calves during the phase of plant nutrition due to an increase in the amount of all adenosine phosphates, an increase in the synthesis of thromboxane in them, an increase in the content of actin and myosin in them, and an intensification of their self-assembly in the case of the participation of platelets in hemostasis. The increase in hemostatic capabilities noted in calves of the Kholmogory breed is of great importance for the success of their growth and development processes during the phase of plant nutrition.

1 Introduction

The optimum blood flow in the small vessels of the internal organs is of great importance for ensuring the normal functional status of the animal [1, 2]. A serious role in this is played by the level of platelet activity [3]. Their activity significantly affects the state of microcirculation, the level of anabolism, and the degree of development of the productive characteristics of reared animals [4, 5]. The functional parameters of platelets can change at any stage of ontogenesis in response to various environmental factors [6], regulating the course of metabolic processes [7].

With the understanding of the serious physiological role of the hemostatic properties of platelets, their level in productive animals of different ages is of increasing interest. Unfortunately, there are still few fragmentary observations on cattle with an indication of the breed, devoted to the study of platelet activity [8]. The data found in the literature do not provide grounds for the formation of a clear understanding of the age-related dynamics

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of platelet activity during ontogenesis in cattle with different genetic characteristics. this to maintain the optimum functional status of animals [9]. The existing differences in the level of productive qualities between individual breeds of cattle [10] indicate the need for further studies of interbreed differences in platelet properties in calves at the end of early ontogenesis.

Purpose - to find out the dynamics of platelet activity in calves of the Kholmogory breed during the phase of plant nutrition.

2 Materials and methods

The study was carried out in strict adherence to the ethical principles defined in the European Convention for the Protection of Vertebrate Animals used for any scientific purposes (approved in Strasbourg on March 18, 1986 and fully confirmed for practical application in Strasbourg on June 15, 2006).

The work was carried out on 44 calves at the beginning of the vegetable feeding phase. All calves were purebred of the Kholmogory breed. The observed calves were obtained after the second or third pregnancy from completely healthy cows. Animals were examined four times during the plant feeding phase: at the age of 91 days, at the age of 6 months, at the age of 9 months and at the age of 12 months.

In platelets, the intensity of thromboxane synthesis was recorded and the enzymatic capabilities of platelet enzymes cyclooxygenase and thromboxane synthetase were determined. For this purpose, three portable samples were used according to standard methods. In platelets in animals, the levels of adenosine triphosphate and adenosine diphosphate deposited in granules were determined with the determination of the degree of their secretion in the case of influence on collagen platelet receptors. In the composition of inactive platelets, the amount of actin and myosin was recorded and the amounts of these proteins in platelets were determined after exposure to adenosine diphosphate.

The severity of platelet aggregation (AP) under in vitro conditions was assessed by a visual micromethod. For this purpose, a number of well-known AP stimulants were used: adenosine diphosphate (at a concentration of $0.5\times10x10^{-6}$ M) and ristomycin (at a concentration of 0.8 mg/ml). Assessment of the state of AP was performed in platelet-rich plasma after its standardization by the number of platelets in it to a value of 200×10^{9} platelets per liter. The state of intravascular platelet aggregation was determined by phase contrast microscopy. The processing of the data obtained using the mathematical method was carried out using the “Statistics for Windows v. 6.0” “Microsoft Excel” software package. The significance of differences in the compared parameters was considered significant in the case of $p<0.05$.

3 Research results

In the blood of calves, the level of inactive platelets with a normal discoid form decreased slightly during the entire study (by 7.5%), amounting to 65.1 ± 0.18% in the final study. In the observed calves in the blood, during the observation period, the number of activated platelets increased by 16.3%, amounting to 34.9±0.17% at its end. The number of platelet aggregates in their blood having a small size and aggregates having a medium size or a large size during the entire observation increased by 20.4% and 23.8%, respectively (Table 1).

In the calves of the Kholmogory breed taken into work, during the observation period, an increase in the physiological characteristics of platelets takes place. Collagen AP, they accelerated by 14.2% during the work carried out. This was accompanied in calves by a

gradual reduction in AP time under the action of all aggregation inducers tested in the work: with adenosine diphosphate - by 13.9%, with ristomycin by 10.1%, with thrombin by 10.1% and with adrenaline by 10.4%.

Table 1. Intravascular platelet aggregation in calves of the Kholmogory breed during the phase of plant nutrition.

Platelet indicators	Age of calves, n=44, M±m			
	91 day	6 months	9 months	12 months
Number of medium and large platelet aggregates, per 100 free platelets	0.21±0.012	0.23±0.010 p<0.05	0.25±0.013 p<0.01	0.26±0.016 p<0.01
Number of small platelet aggregates, per 100 free platelets	5.4±0.16	5.7±0.15	6.2±0.11 p<0.05	6.5±0.17 p<0.01
The amount of activated forms of platelets, %	30.0±0.16	31.9±0.23	32.5±0.19	34.9±0.17 p<0.05
The level of platelets-discocytes, %	70.0±0.10	69.1±0.27	67.5±0.22	65.1±0.18

Note: p - the reliability of the dynamics of indicators in comparison with their state on the 91st day of life. The rest of the tables have similar designations.

Of great importance for the acceleration of AP during the phase of plant nutrition in young animals of the Kholmogory breed is an increase in platelet thromboxane generation. This was indicated by a 16.5% increase in AT occurring during a simple portable test that could reveal the intensity of thromboxane synthesis. This effect in calves was due to activation during the entire observation of the platelet enzyme cyclooxygenase (by 9.5%) and the platelet enzyme thromboxane synthetase (by 14.7%). The presence of their enhancement was indicated by an increase in the level of AP during the phase of plant nutrition that occurs in the collagen-aspirin test (up to 89.6±0.16%) and an increase in AP that develops during the collagen-imidazole test (up to 56.8±0.11%) (Table 2).

Table 2. The ability of platelets to aggregate in vitro in calves of the Kholmogory breed during the phase of plant nutrition.

Platelet indicators	Age of calves, n=44, M±m			
	91 day	6 months	9 months	12 months
Time of development of AP with adrenaline, s	85.9±0.15	84.3±0.14	82.6±0.13	77.8±0.17 p<0.05
Time of development of AP with ristomycin, s	43.5±0.10	42.6±0.12	41.3±0.14	39.5±0.17 p<0.05
Time of development of AP with thrombin, s	44.5±0.19	42.5±0.15	41.9±0.16	40.4±0.19 p<0.05
Time of development of AP with collagen, s	28.2±0.18	27.5±0.16	26.3±0.12	24.7±0.15 p<0.05
Time of development of AP with ADP, s	32.8±0.12	32.2±0.20	30.6±0.17	28.8±0.22 p<0.05

During the phase of plant nutrition in platelets of calves, there was an increase in the content of adenosine triphosphate by 10.2% and adenosine diphosphate by 10.7%. At the same time, the secretion of both substances from their platelets increased by 11.7% and 11.8%, respectively.

In calves, in inactive platelets at the end of the observation, an increase in actin protein by 12.0% and an increase in the amount of myosin protein by 14.1% were found, providing their output to levels of 42.8±0.16% and 21.9±0.14% of total protein in platelet, respectively. Throughout the study, during platelet aggregation, an increase in the generation of actin molecules by 11.0% and myosin molecules by 13.2% developed (Table 3).

Table 3. Hemostatically important intraplatelet parameters in calves of the Kholmogory breed during the phase of plant nutrition.

Platelet indicators	Age of calves, n=44, M±m			
	91 day	6 months	9 months	12 months
The amount of myosin in platelets during ADP aggregation,% of the total protein in platelets	36.3±0.10	37.4±0.12	38.8±0.15	41.1±0.09 p<0.05
The amount of myosin in inactive platelets,% of the total protein in platelets	19.2±0.07	19.7±0.12	20.4±0.10	21.9±0.14 p<0.05
The amount of actin in platelets during ADP aggregation,% of the total protein in platelets	47.2±0.10	48.5±0.18	49.8±0.15	52.4±0.19 p<0.05
The amount of actin in inactive platelets,% of the total protein in platelets	38.2±0.18	39.4±0.22	40.6±0.17	42.8±0.16 p<0.05
AP level in a simple transfer sample, %	37.5±0.04	38.3±0.15	40.4±0.12	43.7±0.13 p<0.05
The level of AP recovery during the collagen-imidazole test, %	49.5±0.08	50.9±0.14	53.5±0.12	56.8±0.11 p<0.05
The level of AP recovery during the collagen-aspirin test, %	81.8±0.05	84.0±0.12	85.7±0.07	89.6±0.16 p<0.05
Expression of ADP secretion,%	49.1±0.09	50.7±0.18	52.4±0.12	54.9±0.10 p<0.05
Expression of ATP secretion,%	41.6±0.10	43.2±0.17	44.3±0.15	46.5±0.14 p<0.05
The amount of ADP in platelets before the onset of secretion, μmol/10^9 platelets	3.82±0.008	3.91±0.017	4.09±0.011	4.23±0.010 p<0.05
The amount of ATP in platelets before secretion, μmol/10^9 platelets	5.80±0.017	6.08±0.017	6.19±0.012	6.39±0.014 p<0.05

4 Discussion

Continuously developing physiology has come to the conclusion that blood parameters are of high importance throughout ontogeny [11]. Hematological parameters are now considered important markers of the status of a living organism [12]. In view of their serious biological significance, to maintain homeostasis, it is necessary to continue their detailed study in different species of productive animals throughout ontogeny [13].

Modern researchers recognize the great importance of hematological parameters, including different characteristics of platelets [14]. At the same time, changes in their activity during ontogenesis in cattle of high-milk breeds have not yet been established. Many researchers are of the opinion that monitoring the main functional manifestations of platelets can help in understanding the mechanisms of formation of their milk production and in developing approaches to its additional increase [1]. It is understood that a detailed study of the functional parameters of platelets in dairy calves makes it possible not only to assess their dynamics, but also the possible level of their impact on microcirculation, which is significant for milk production. Awareness of the serious physiological importance of this issue served as the basis for performing this observation on the young of the Kholmogory breed at the end of its early ontogenesis.

Determining the time of AP in response to collagen and ristomycin in young animals of the Kholmogory breed during the phase of plant nutrition made it possible to establish a gradual acceleration of this process. These changes proved the activation of platelet adhesion in the observed animals at the end of early ontogenesis. This was possible due to the work of at least two hemostatic mechanisms in calves at once. One mechanism has been associated with a reduction in the time it takes calves to develop platelet collagen aggregation. This mechanism was based on an increase in the content of type Ia–IIa and

type VI glycoproteins capable of interacting with collagen on the platelet membranes of the Kholmogory breed calves. As the second mechanism of activation of platelet adhesion in the observed calves, one should consider an increase in the number of type Ib glycoprotein molecules on the surface of platelets, which can bind to the von Willebrand factor dissolved in plasma. In the implementation of this process, the increase in the level of this substance in the blood of animals is of great importance. The existence of another mechanism for enhancing the adhesive process is indicated by the revealed acceleration in the observed animals during the entire phase of plant nutrition of the AP process after contact with ristomycin platelets.

The acceleration of the development of platelet aggregation provided high protection of the body from blood loss in calves of the Kholmogory breed at the end of early ontogenesis. This is due to the gradual strengthening of a number of regulatory systems in this young animal. During the entire observation period, calves showed a gradual reduction in AP time in response to strong inducers (collagen and thrombin). This phenomenon was based on an increase in the number of receptor molecules for them on platelet membranes, an increase in the function of platelet phospholipase C, and the activation of enzymes that implement the phosphoinositol pathway and enzymes that phosphorylate proteins, including the platelet contractile system. The increase in AP under the action of strong inducers on platelets is associated with an increase in the formation of certain amounts of inositol triphosphate in them and an increase in the release of dense calcium into the cytoplasm. These changes should be assessed as a factor that increases the content of actin and myosin in platelets and intensifies their contraction as part of the actomyosin complex. All these changes can be considered very important for accelerating platelet aggregation in calves of the Kholmogory breed during the plant nutrition phase in response to strong agonists and increasing the severity of secretion processes from platelets.

The acceleration of AP observed in the observed calves with the use of weak agonists - adenosine diphosphate and adrenaline, indicated the activation in animals of the mechanisms that ensure the participation of their platelets in the process of hemostasis. The upcoming reduction in AP time with these inducers was associated in calves with an increase in the number of molecules on the surface of their platelets, which are receptors that could bind to fibrinogen (GPIIb-IIIa) with an increase in the enzymatic properties of platelet phospholipase A_2. The resulting increase in the amount of arachidonate released from platelet membranes created a supply of raw materials for the formation of thromboxane A_2 in platelets [6]. The activation of this process found in calves using portable samples was associated with an increase in platelet enzymes, cyclooxygenase and thromboxane synthetase, during the plant nutrition phase. Of particular importance for accelerating the onset of AP under the action of weak agonists in calves during the entire observation is also an increase in the content of actin and myosin, adenyl nucleotides in their platelets and an increase in their release from platelets during hemostasis.

The degree of platelet activation in the blood of animals throughout the phase of plant nutrition was assessed using phase contrast microscopy. A slight increase in different types of activated platelets noted in young animals during the entire observation period indicated an increase in the sensitivity of their platelets to the aggregation stimulator in their plasma. This also indicated an increase in the degree of expression of collagen in the blood from the vascular subendothelium, which is capable of powerfully activating platelets. An increase in the intravascular activity of platelets noted in the Kholmogory breed calves was also associated with an increase in their plasma during the fourth phase of early ontogenesis in the amount of soluble platelet aggregation stimulators capable of dissolving [12]. All these moments contributed to an increase in the blood of calves of the Kholmogory breed of the level of platelet aggregates of various sizes capable of circulating.

The found changes in platelet activity should be associated with the completion of growth processes and the maturation of calves. The obtained changes minimized the risk of bleeding without the risk of blockade of small vessels in animal tissues by platelet aggregates. Strengthening at the end of early ontogenesis of intravascular platelet aggregation can be considered a breed feature of young animals of the Kholmogory breed. Preserving the health of young animals in these conditions is associated in these calves with a balance in their prothrombotic and antithrombotic processes in the blood due to the increase with age of the hemostatic capabilities of their vessels. Apparently, the normal functioning of primary hemostasis in the observed calves at the end of early ontogenesis, based on the balance of hemostatic capabilities of platelets and vascular endothelium, is associated with a high functional tension between them, which is a feature of the Kholmogory breed.

5 Conclusion

During the phase of plant nutrition in calves of the Kholmogory breed, there is a slight increase in platelet activity. The growth of their hemostatic parameters is manifested by an increase during the fourth phase of early ontogenesis of the activity of mechanisms that implement platelet adhesion, aggregation and secretion. Developing changes in platelet activity in calves of the Kholmogory breed are important in maintaining hemostasis at the end of early ontogenesis and contribute to the formation of conditions in their body for the manifestation of the existing productive potential.

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