Platelet functions in tethered heifers in the process of growing them

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Abstract. In the course of growing heifers on tethered content, there is a tendency to some decrease in the severity of platelet lipid peroxidation due to an increase in their antioxidant protection. In platelets, this is accompanied by activation of the actin-myosin system, a high level of adenosine phosphates, and their intense secretion in the case of platelet participation in hemostasis. The observed heifers showed a tendency to increase the hemostatic functions of platelets, manifested in the blood and in vitro. The revealed slight increase in platelet activity in tethered heifers is caused by maturation during rearing of surface and intracellular mechanisms of platelet participation in hemostasis and microcirculation processes.

1 Introduction

The development of any species of productive animals is associated with regular hereditary changes in various parameters of their organism [1, 2]. It becomes clear that during the maturation of the organism and the formation of its adaptive qualities, the blood system has a great biological significance [3, 4]. Of particular importance in ensuring normal microcirculation conditions in all internal organs, which are required for the growth, development and realization of the productive potential in animals, is the state of hemostasis, and especially platelets. Their hemostatic properties are manifested by aggregation, adhesion and secretion, affecting the blood rheology and the level of tissue perfusion [5].

Nursing is a very important stage in the life of heifers, as it comprehensively prepares their body for insemination and pregnancy. During it, the maturation of all organ systems is completed in the conditions of continued animal growth processes [6]. Despite the great importance of this stage in the life of cattle, it was not possible to find scientific studies of the activity of platelet hemostasis in heifers with the conditions of their maintenance taken into account. In this regard, in the work carried out, the goal of the study was set - to establish the features of the level of platelet activity in heifers of optimal functional status, which are tethered during their rearing.

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2 Materials and methods

46 heifers were examined, which were tethered during their rearing. Animals were assessed at about 12 months old, about 13 months old, about 14 months old, about 15 months old, and about 16 months old. In the examined heifers, after taking blood, platelets were washed and resuspended with further registration of the amount of malondialdehyde and acyl hydroperoxides in them. The level of activity of antioxidant intraplatelet enzymes superoxide dismutase and catalase was determined. In the composition of platelet granules, the amount of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) was determined, taking into account the degree of their secretory release after collagen was added to the plasma with platelets. As part of the cytoskeleton of platelets, the level of actin and myosin was determined in their inactive state, under conditions of their activation, and during platelet aggregation in response to ADP and thrombin. The ability of platelets to aggregate was determined by a visual micromethod in response to ADP (0.5×10^{-4} M), collagen (1:2 dilution of the main suspension), thrombin (0.125 units/ml), ristomycin (0.8 mg/ml), on H_2O_2 (7.3×10^{-3} M), on adrenaline (5×10^{-6} M) and on a number of their combinations (on ADP and adrenaline; on ADP and collagen; on adrenaline and collagen; on ADP and thrombin; to ADP, collagen and adrenaline; to ADP, thrombin and adrenaline; to ADP, collagen, thrombin and adrenaline). The severity of intravascular activity of platelets was detected during phase-contrast microscopy. Mathematical processing of the obtained results consisted in the calculation of Student's t-test.

3 Research results

Throughout the study, all heifers had an optimal functional status and were completely healthy.

Table 1. Biochemical parameters of platelets in tethered heifers during rearing**.**

Indicators taken into account	Growing period, n=46, M±m				
	about 12 months life	about 13 months life	about 14 months life	about 15 months life	about 16 months life
platelet acyl hydroperoxide, $D_{233}/10^9$ platelets	2.78±0.19	2.62±0.11	2.57±0.14	2.49±0.16	2.40±0.13
Platelet malondialdehyde, nmol/10^9 platelets	0.84±0.012	0.72±0.010	0.68±0.014	0.64±0.009	0.60±0.008
Platelet catalase, IU/10^9 platelets	10110.6±8.75	10280.0±9.23	10600.0±8.11	11160.0±11.15	11310.0±10.51
platelet superoxide dismatase, IU/10^9 platelets	1920.4±4.16	1996.3±2.27	2071.0±3.62	2131.6±2.91	2206.0±2.46
Content before ATP secretion, μmol/10^9 platelets	5.71±0.17	5.86±0.22	5.96±0.12	6.11±0.18	6.23±0.22
Content before secretion of ADP, μmol/10^9 platelets	3.45±0.10	3.61±0.07	3.72±0.12	3.88±0.09	3.94±0.12
ATP secretion level,%	39.8 ±0.12	41.2±0.15	43.9±0.16	45.1±0.19	46.8±0.14
ADP secretion level,%	48.7±0.21	50.6±0.19	52.8±0.26	54.9±0.17	58.8±0.19

In platelets of heifers, the number of acyl hydroperoxides at the age of about 12 months reached 2.78±0.19 $D_{233}/10^9$ platelets. During subsequent observations, their level in

platelets somewhat decreased and reached 2.40±0.13 $D_{233}/10^9$ platelets by the age of 16 slightly decreasing during the observation period to the level of 0.60±0.008 nmol/10^9 platelets at 16 months of age (Table 1).

During the observation period, the functionality of catalase and superoxide dismutase in platelets in tethered heifers increased slightly, reaching 11310.0±10.51 IU/10^9 platelets and 2206.0±2.46 IU/10^9 platelets, respectively, by the end of the observation period.

During the entire observation, the amount of ATP and ADP in the platelets of the examined heifers slightly increased from 5.71±0.17 μmol/10^9 platelets to 6.23±0.22 μmol/10^9 platelets and from 3.45±0.10 μmol/10^9 platelets to 3.94±0.12 μmol/10^9 platelets, respectively). The degree of secretory release of ATP and ADP from platelets of heifers in the case of the influence of collagen on them increased to a small extent.

The level of actin in inactive platelets in the observed 12-month-old heifers was 35.2±0.18% of the total protein in the platelet, while at 16 months of age it reached 41.2±0.14% of the total protein in the platelet (Table 2).

Table 2. The content of actin in platelets of heifers on a tethered content in the process of rearing**.**

Accountable indicators	Growing period, n=46, M±m				
	about 12 months life	about 13 months life	about 14 months life	about 15 months life	about 16 months life
Actin content in intact platelets,% of the total protein content in the cell	35.2±0.18	36.3±0.14	38.2±0.21	39.9±0.15	41.2±0.14
The content of actin in platelets against the background of ADP-activation,% of the total protein content in the cell	36.2±0.11	37.5±0.05	39.2±0.12	41.1±0.08	42.4±0.09
The content of actin in platelets against the background of ADP aggregation,% of the total protein content in the cell	41.8±0.11	43.6±0.14	45.0±0.13	46.9±0.10	48.5±0.12
The content of actin in platelets against the background of thrombin activation,% of the total protein content in the cell	38.1±0.15	39.8±0.08	41.6±0.07	43.6±0.16	44.3±0.09
The content of actin in platelets against the background of thrombin aggregation,% of the total protein content in the cell	40.3±0.12	41.8±0.10	43.7±0.08	45.1±0.14	46.8±0.13

A gradual increase in the intensity of additional generation of actin in the platelets of the observed heifers was found in the case of activation of platelets by an inductor of any strength and under conditions of their aggregation.

Similar changes in platelet activity in growing heifers occurred in relation to the myosin mechanism (Table 3). It was found that in the intact platelets of the observed heifers at the age of about 12 months, the content of myosin was 16.2±0.14% of the total protein content in the platelet. Subsequently, its level increased and amounted to 20.7 ± 0.08% of the total protein content in the platelet at the age of 16 months. Under the conditions of platelet activation and aggregation under the influence of a strong or weak inducer, the observed heifers in the process of rearing had an increase in the level of additional myosin generation.

Table 3. The content of myosin in platelets of heifers on a tethered content in the process of rearing**.**

Accountable indicators	Growing period, n=46, M±m				
	about 12 months life	about 13 months life	about 14 months life	about 15 months life	about 16 months life
The content of myosin in intact platelets,% of the total protein content in the cell	16.3±0.14	17.1±0.12	18.3±0.09	19.5±0.10	20.7±0.08
The content of myosin in platelets against the background of ADP-activation,% of the total protein content in the cell	22.7±0.12	23.6±0.10	25.3±0.16	27.1±0.08	28.3±0.11
The content of myosin in platelets against the background of ADP aggregation,% of the total protein content in the cell	30.5±0.11	32.0±0.17	34.7±0.09	35.8±0.13	38.7±0.10
The content of myosin in platelets against the background of thrombin activation,% of the total protein content in the cell	37.2±0.14	38.6±0.12	40.1±0.15	42.3±0.10	44.1±0.12
The content of myosin in platelets against the background of thrombin aggregation,% of the total protein content in the cell	45.2±0.21	46.5±0.17	47.9±0.16	49.8±0.20	51.2±0.18

In the heifers taken into the study, already from the age of 12 months, a gradual acceleration of the onset of platelet aggregation in response to all platelet aggregation inducers and their combinations used in the study was traced (Table 4).

Table 4. Platelet aggregation activity in tethered heifers during rearing**.**

Accountable indicators	Growing period, n=46, M±m				
	about 12 months life	about 13 months life	about 14 months life	about 15 months life	about 16 months life
Platelet aggregation with ADP, s.	34.6±0.14	33.5±0.12	32.7±0.15	32.1±0.11	31.5±0.13
Platelet aggregation with collagen, s.	25.6±0.12	25.0±0.10	24.2±0.09	23.4±0.16	22.7±0.12
Platelet aggregation with thrombin, s.	48.5±0.21	47.8±0.19	46.3±0.16	44.5±0.25	43.8±0.23
Platelet aggregation with ristomycin, s.	43.6±0.19	42.5±0.15	41.4±0.14	40.6±0.23	39.5±0.19
Platelet aggregation with H_2O_2, s.	35.1±0.11	34.7±0.12	33.2±0.09	32.6±0.17	31.8±0.24
Platelet aggregation with adrenaline, s.	93.6±0.27	91.5±0.30	89.7±0.25	87.6±0.33	86.1±0.39
Platelet aggregation with ADP and adrenaline, s.	32.6±0.08	31.9±0.12	31.0±0.10	30.4±0.16	29.5±0.12
Platelet aggregation with ADP and collagen, s.	23.9±0.07	23.0±0.19	22.4±0.16	21.5±0.13	20.7±0.20
Platelet aggregation with adrenaline and collagen, s.	23.5±0.12	22.9±0.09	22.0±0.14	21.3±0.08	20.5±0.21
Platelet aggregation with ADP and thrombin, s.	23.7±0.11	22.9±0.16	21.7±0.18	20.5±0.14	19.6±0.10
Platelet aggregation with ADP, collagen and adrenaline, s.	19.8±0.07	18.9±0.05	18.0±0.06	17.4±0.11	17.0±0.09
Platelet aggregation with ADP, thrombin and adrenaline, s.	18.5±0.10	17.9±0.12	17.2±0.08	16.5±0.11	16.1±0.12
Platelet aggregation with ADP, collagen, thrombin and adrenaline, s.	16.5±0.07	16.1±0.06	15.3±0.11	14.7±0.14	14.2±0.09

The onset of platelet aggregation under the action of collagen was 25.6 ± 0.12 s at the beginning of the observation, gradually accelerating throughout it. The observed heifers had a reduction in the time of development of platelet aggregation under the influence of ADP and ristomycin. Slightly slower platelet aggregation occurred in response to H2O2, thrombin, and adrenaline. Her onset time with these inducers in heifers also decreased during the growing period. The revealed acceleration of platelet aggregation in response to

one inducer in tethered young animals was consistent with a reduced time of platelet aggregation during rearing, caused by the simultaneous effect of two, three and four agonists of the aggregation process.

The age-related changes in platelet activity revealed during in vitro registration were confirmed by the data of determination of intravascular platelet activity (Table 5). At the age of 12 months, the number of discocytes in the blood of the observed heifers was 73.4±0.12%. Subsequently, their level gradually decreased and amounted to 69.1±0.23% by the end of the study. The amount of active varieties of platelets in the blood of heifers increased by 13.0% during the growing period. In the observed animals, the number of free small and large platelet aggregates increased from 5.5±0.08 and 0.18±0.005 per 100 free-lying platelets in the outcome to levels of 6.6±0.10 and 0.28±0.012 per 100 free platelets. lying platelets at the end of the study. The degree of involvement of platelets in the composition of aggregates in the observed heifers during the growing period increased by 20.0%.

Table 5. Intravascular activity of platelets in heifers on tethered content in the process of rearing**.**

Accountable indicators	**Growing period, n=46, M±m**				
	about 12 months life	**about 13 months life**	**about 14 months life**	**about 15 months life**	**about 16 months life**
Platelets - discocytes, %	73.4±0.12	72.2±0.16	71.6±0.20	70.2±0.19	69.1±0.23
Platelets-disco-echinocytes, %	17.1±0.15	17.7±0.17	17.9±0.22	18.9±0.14	19.2±0.16
Platelets - spherocytes, %	5.0±0.08	5.2±0.12	5.4±0.04	5.7±0.09	6.2±0.05
Platelets-sphero-echinocytes, %	3.7±0.06	3.9±0.07	4.0±0.05	4.2±0.08	4.4±0.09
Platelets - bipolar forms, %	0.8±0.03	1.0±0.02	1.1±0.04	1.0±0.01	1.1±0.03
The amount of active forms of platelets, %	26.6±0.17	27.8±0.23	28.4±0.16	29.8±0.20	30.9±0.18
The number of platelets in the aggregates, %	5.9±0.15	6.2±0.12	6.4±0.11	6.7±0.09	6.9±0.12
The number of small platelet aggregates of 2-3 platelets, per 100 free-lying platelets	5.5±0.08	5.7±0.10	6.1±0.12	6.3±0.14	6.6±0.10
The number of medium and large platelet aggregates of 4 or more platelets, per 100 free-lying platelets	0.18±0.005	0.20±0.009	0.23±0.011	0.25±0.010	0.28±0.012

4 Discussion

Maintaining the normal viability of the organism is possible only in the case of continuous delivery of nutrients and oxygen to all cells. The success of this process strongly depends on many characteristics of blood cells that can change during ontogeny [7]. A serious contribution to the state of blood rheology in productive animals is made by platelet activity, which can also change during ontogenesis [8].

In the course of the observation, it was noted that in the body of healthy heifers that are tethered during their rearing, there is an increase in platelet antioxidant enzymes. They are able to sufficiently restrain the severity of lipid peroxidation and reduce the amount of products of this process in the composition of the membranes of these cells. The low severity of free radical phenomena in the platelets of growing heifers on a tethered content significantly ensures their low hemostatic manifestations between 12 and 16 months of their life. These include an unexpressed increase in self-assembly of the components of the

actin-myosin system, a slight increase in the content of ADP and ATP in platelet granules with an increase in their secretory release from activated platelets [9].

The found activation during the observation of the process of platelet aggregation in response to strong inducers of platelet aggregation - collagen and thrombin, was possible as a result of an increase in platelets of the observed heifers of phospholipase C due to an increase in the level of diacylglycerol in them, activation of protein kinase C and an increase in the degree of self-assembly in platelets of actin and myosin [10].

The revealed reduction in the time of platelet aggregation in response to a weak inducer of aggregation, ADP or adrenaline, was associated with an increase in a number of mechanisms [11]. These included an increase in the availability of the number of receptors for ADP and adrenaline on the surface of heifer platelets, an increase in the number of fibrinogen receptors (GPIIb-IIIa) on them, and an increase in platelet phospholipase A_2, which releases arachidonic acid from membrane lipids for its conversion into thromboxane A_2 [12]. Found some acceleration in the development of platelet aggregation in the case of the simultaneous use of two or three stimulators of this process, said that heifers that were tethered during their rearing had an age-related increase in their mutually reinforcing effects on platelets [13]. The tendency to increase the intravascular activity of platelets, which was revealed in heifers kept in tether during rearing, confirmed the activation of all these mechanisms under blood flow conditions. This is true in relation to the activation of membrane receptors to stimulators of aggregation that are constantly present in the blood of animals and in relation to an increase in the functionality of intraplatelet mechanisms for implementing adhesion, aggregation, and secretion [14].

5 Conclusion

Growing is an important stage in the life of heifers associated with their preparation for the realization of productive opportunities. It is of great interest to elucidate the effect of housing conditions on the hemostatic parameters of platelets, which are very significant for microcirculation and trophic tissues of heifers during their rearing. In this regard, in the study, platelet activity was considered in heifers that are tethered under standard conditions of the livestock complex. In the course of rearing, these heifers showed some increase in the hemostatic properties of platelets, realized under conditions of adhesion, aggregation, and secretion. This article is part of a large ongoing study. It will be possible to finally answer the question about the effect of housing conditions on platelet activity after completing the assessment of its condition in free-range heifers. This will allow us to accurately state that the found features of platelet activity are associated not only with the age of the animals, but also with the conditions of their maintenance.

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