

Conference Paper

Physiological Characteristics of Platelet Activity in Calves of the Dairy and Plant Nutrition of the Holstein Breed

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The hemostatic properties of platelets to a large degree determine the activity of metabolic processes that have great biological significance especially in early ontogeny. This study was conducted on 43 calves of the Holstein breed during the phase of milk-vegetable diet. It was found during the observation period that the indicators were stable in the calves between 31 and 60 days of life, and the platelet aggregation weakened. In the blood of the calves of the Holstein breed there was a slight increase in the number of discocytes. The total number of active thrombocytes in calves observed after stability between 31-60 days of life decreased during the follow-up observation. The levels of circulating platelet aggregates of small and large sizes decreased between 60 and 90 days by 66.7% and 2.5 times, respectively. This contributed to a weakening of the calves' platelet synthesis of thromboxane, a decrease in the content of adenosinofatom, and to inhibition of their secretion. During the observation period, the number of actin and myosin in the platelets of the animals also decreased, which reduced the overall platelet activity. In the second part of the phase of lacto-vegetarian nutrition, the synthesis of actin and myosin in the exposed aggregate platelets weakened in the calves. The stability of the hemostatic platelet counts of the Holstein calves at the age of 31-60 days was typical, which changed their physiologically acceptable weakening by the end of observation.

Keywords: calves, dairy plant phase, Holstein breed, platelets, aggregation, secretion.

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1. Introduction

Morphofunctional characteristics of the organism are largely determined by the current processes of microcirculation realized in the blood vessels of the least caliber [1]. A huge role in the implementation of this process in different species of mammals belongs to blood cells including platelets [2, 3]. The level of functional activity of platelets tends to ontogenesis against the background of growth and development [4], aging [5] during the development of various dysfunctions [6] with the appearance of disease symptoms

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[7] and on the background of the implementation of the health impacts [8]. However, many aspects of platelet activity in cattle remain poorly understood.

This primarily refers to the clarification of the level of activity of platelets in calves and cows of normal functional status [9, 10]. For this reason, there is no possibility to form a clear picture; there is a need for further research in this direction. Based on known facts, we can assume that the level of functional activity platelets strongly influences the activity of blood flow through the capillaries, and thus the supply of nutrients and oxygen necessary for the functioning of the morphological organism [11, 12]. Given there is an availability of genetic and physiological characteristics in different breeds of cattle, it is of great scientific interest to elucidate the activity of platelets in calves of high-milk Holstein breed during the individual phases of early ontogenesis.

The goal is to assess the state of functionality of the platelets in healthy calves of Holstein breed during the phase of milk-vegetable diet.

2. Materials and Methods

The research was conducted in strict accordance with ethical principles established by the European Convention 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).

The work was performed on 43 purebred calves of Holstein breed, which were obtained from completely healthy cows after an optimal course of 2-3 styles. The animals were examined 5 times: on the 31st, 45th, 60th, 75th and 90th days of ontogenesis.

In calves, an indirect assessment was made at the level of thromboxane synthesis in platelets with an indirect detection of the level of enzymatic activity in them of cyclooxygenase and thromboxane synthetase. For this, 3 portable samples were performed on a photoelectrocolorimeter [13]. In calves platelets, the amount of adenosine triphosphate and adenosine diphosphate, the severity of their secretion in response to the effect of collagen, the levels of actin and myosin in them in an intact state and in the case of platelet activation with adenosine diphosphate were determined [13].

The time of onset of the platelet aggregation in the examined calves was taken into account using a visual micromethod using a number of agonists: adenosine diphosphate (0.5×10^{-4} M), collagen (dilution 1:2 of the main suspension), thrombin (0.125 units/ml), adrenaline (5.0×10^{-6} M) and ristomycin (0.8 mg/ml) in plasma after its standardization by the content of platelets in it to the level of 200×10^9 platelets in 1 liter [13]. Platelet activity in the bloodstream was determined using a phase-contrast nozzle using a light

microscope [13]. Statistical processing of the received information was made with the help of a programme packet “Statistics for Windows v. 6.0”, “Microsoft Excel”. Differences in the data were considered reliable in case $p < 0.05$.

3. Results

The calves of the Holstein breed taken in the study showed a stable low platelet activity in the first half of the phase of dairy and plant nutrition, followed by its weakening. In the examined animals, the platelet aggregation under the action of collagen between 31 and 60 days of life did not change its activity, developing on day 60 in 42.4 ± 0.17 s, and subsequently inhibited by 90 days of life to 57.6 ± 0.16 s. Comparable changes in the platelet aggregation between 60 and 90 days of life are also noted for the rest of the tested inducers. In response to adenosine diphosphate and ristomycin after an episode of stability, an increase in the time of development of the platelet aggregation to 66.5 ± 0.15 s and 76.0 ± 0.29 s, respectively, was noted. A similar attenuation between 60 and 90 days of life was noted for the platelet aggregation with thrombin (the platelet aggregation development time increased to 79.2 ± 0.19 s, for the platelet aggregation with adrenaline (the platelet aggregation time increased to 132.8 ± 0.28 s). These changes also occurred after a period of stability of the platelet aggregation with these agonists (table 1).

The content of discocytes in the blood of calves observed in the study in the second half of the phase of milk and vegetable nutrition increased by 13.5%. This was accompanied during these periods of observation by a decrease of almost 6 times in the total number of active platelet species after the stability of their level between 31 and 60 days of life. In the blood of animals the amounts of free aggregates of small sizes, as well as aggregates of medium and large sizes, remained stable for up to 60 days, and then gradually decreased until the end of the phase of milk-vegetable nutrition.

TABLE 1: Platelet function in calves of the Holstein breed of dairy and plant nutrition

Registered parameters	Holstein calves, n=43, M±m				
	31st day	45th day	60th day	75th day	90th day
Restoring platelet aggregation during a collagen-aspirin test, %	67.2±0.22	67.0±0.19	67.3±0.14	61.6±0.23 p<0.05	58.3±0.17 p<0.01
Restoring platelet aggregation during a collagen-imidazole test, %	31.0±0.16	30.9±0.11	31.2±0.09	28.1±0.12 p<0.05	26.2±0.08 p<0.01
Platelet Aggregation in a Simple Transfer Sample, %	21.8±0.08	21.5±0.09	21.7±0.15	18.4±0.15 p<0.01	17.0±0.14 p<0.01

Registered parameters	Holstein calves, n=43, M±m				
	31st day	45th day	60th day	75th day	90th day
The content of adenosine triphosphate in platelets before the onset of secretion, $\mu\text{mol}/10^9$ platelets	4.96±0.022	5.00±0.016	4.98±0.018	4.12±0.012 p<0.05	4.06±0.019 p<0.01
The content of adenosine diphosphate in platelets before secretion, $\mu\text{mol} / 10^9$ platelets	2.83±0.008	2.80±0.011	2.84±0.010	2.32±0.009 p<0.01	2.10±0.014 p<0.01
Adenosine triphosphate secretion level, %	21.5±0.24	21.2±0.20	21.0 ±0.16	18.0±0.18 p<0.01	16.4±0.21 p<0.01
Adenosine diphosphate secretion level, %	29.0±0.17	28.7±0.18	28.6±0.10	25.1±0.12 p<0.05	23.8±0.23 p<0.05
The amount of actin in inactive platelets, % of the total protein in platelets	17.8±0.19	17.9±0.17	17.6±0.12	15.2±0.22 p<0.01	14.8±0.14 p<0.01
The amount of actin in platelets with adenosine diphosphate aggregation, % of the total protein in platelets	28.9±0.16	28.6±0.11	28.5±0.18	25.1±0.21 p<0.05	23.2±0.17 p<0.01
The amount of myosin in inactive platelets, % of the total protein in platelets	7.9±0.12	7.8±0.08	7.8±0.09	6.1±0.07 p<0.01	5.6±0.10 p<0.01
The amount of myosin in platelets with adenosine diphosphate aggregation, % of the total protein in platelets	19.3±0.12	19.4±0.14	19.0±0.08	16.5±0.07 p<0.05	15.6±0.10 p<0.01
Platelet aggregation with adenosine diphosphate, s	51.0±0.12	51.6±0.10	51.2±0.15	61.3±0.16 p<0.01	66.5±0.15 p<0.01
Platelet aggregation with collagen, s	42.1±0.19	42.0±0.23	42.4±0.17	50.1±0.15 p<0.01	57.6±0.16 p<0.01
Platelet aggregation with thrombin, s	63.4±0.18	63.0±0.22	63.2±0.15	71.6±0.18 p<0.05	79.2±0.19 p<0.01
Platelet aggregation with ristomycin, s	59.6±0.12	59.4±0.23	59.7±0.26	68.0±0.31 p<0.05	76.0±0.29 p<0.01
Platelet aggregation with adrenaline, s	108.9±0.27	109.4±0.22	109.7±0.31	120.6±0.25 p<0.05	132.8±0.28 p<0.01
Discocyte count, %	85.8±0.16	85.2±0.20	86.0±0.24	95.0±0.30 p<0.05	97.6±0.18 p<0.05
The sum of the active platelet forms, %	14.2±0.15	14.1±0.18	14.0±0.14	5.0±0.19 p<0.01	2.4±0.20 p<0.01
The number of small platelet aggregates, per 100 free platelets	2.0±0.05	2.1±0.08	2.0±0.05	1.5±0.07 p<0.01	1.2±0.08 p<0.01
The number of medium and large platelet aggregates, per 100 free platelets	0.05±0,012	0.05±0.019	0.05±0.021	0.03±0.013 p<0.01	0.02±0.017 p<0.01

Note: p – the reliability of the dynamics of hematological parameters in relation to the level of 31 days of age.

Apparently, the prolongation of the onset of the platelet aggregation in calves of the Holstein breed in the second half of the phase of dairy and plant nutrition is associated with the development of weakening of thromboxanan synthesis during these periods. This was indicated by a decrease in the platelet aggregation found in calves between 60 and 90 days of ontogenesis in a simple transfer request by 27.6%. These changes were provided in calves by a decrease in the enzymatic activity of cyclooxygenase and thromboxane synthetase in their platelets. The dynamics of the functionality of the first enzyme between 60 and 90 days of ontogenesis was judged by a decrease in the degree of restoration of the platelet aggregation in a collagen-aspirin sample (at the end of the observation, $58.3 \pm 0.17\%$). At the same time, a decrease in the platelet aggregation in a collagen-imidazole test was noted, indicating a weakening of the activity of thrombocytic synthetase by 18.7% (by the end of the observation, $26.2 \pm 0.08\%$).

Initially, a small amount of adenosine triphostat and adenosine diphosphate of calves in platelets remained stable between 31 and 60 days of life, decreasing thereafter and reaching 4.06 ± 0.019 and 2.10 ± 0.014 $\mu\text{mol}/10^9$ platelets at the age of 90 days. The activity of their secretion from platelets of calves at the beginning of the phase of milk and vegetable nutrition remained stable. Over the age of 60 days, its level in the observed animals gradually decreased by 31.1% and 21.8%, amounting to 16.4 ± 0.21 and $23.8 \pm 0.23\%$ by the age of 90 days, respectively.

Being stable between 31 and 60 days of life in intact platelets of calves, a stable small amount of actin and myosin subsequently decreased, reaching by the end of the observation 17.5 ± 0.08 and $7.8 \pm 0.14\%$ of the total protein in the platelet. Similar dynamics was tested in the examined animals with indicators of their additional self-assembly. After a month of stability, they decreased between 60 and 90 days of life for actin by 24.5%, for myosin – by 23.7%.

4. Discussion

Modern science recognizes the great importance of any aspects of hematological parameters in animals and especially in productive ones [13]. The results of studies in this direction provide a further understanding of the mechanisms of self-regulation in mammals [14]. Researchers attach particular importance to the study of hemostasis. However, the features of the activity of an important component of hemostasis - platelets in young cattle, primarily in highly productive breeds, are still poorly understood [15]. The study for the first time allowed us to determine the dynamics of the functional

activity of platelets in calves of the Holstein breed during the phase of dairy and plant nutrition.

Given there is a dynamics in calves in the duration of onset of the platelet aggregation in response to the addition to plasma of collagen, ristomycin, we can talk about the development of them over the age of 60 days the weakening of the adhesion ability of platelets, occurring after a month of stability. Apparently, to ensure early permanence, and then the weakening of this property of platelets was lying immutability, and then depression, as a minimum, implement two adhesion mechanisms [16, 17]. The first mechanism can be judged by stability in start, and then by the reduction of the platelet aggregation in response to collagen [18]. It pointed to the original stable reduction in the number of glycoproteins Ia-VI, which are receptors, bound to collagen [19]. The second mechanism is the weakening of the adhesive properties of platelets from Holstein calves older than 60 days which was associated with a slowdown in response to ristomycin [20]. This was possible in consequence of the lowering of their blood content factor of Villebranda in combination with a decrease of the platelets number of receptors thereto (GPIIb) [21, 22].

It is shown that for Holstein calves in the second half of phase lacto-vegetarian diet is a weakening of the platelet aggregation that highly positively can affect the activity of the microcirculation in all organs [23]. This happened after the original stable low platelet activity against all tested inducers aggregations in the observed calves. One might think that the onset of functional changes of platelets is largely due to the dynamics of activity of receptors on the platelets in calves older than 60 days [24]. When applying strong inducers were able to establish in these animals, there was the weakening of the activity of platelet phospholipase C, the decrease in activity phosphoinositol way, lowering the intensity of phosphorylation of the proteins which implement the processes of reduction in platelets [25]. Development in calves between 60 and 90 days of life, a slight weakening of the synthesis in platelets of inositol phosphate weakened the flow of Ca^{2+} from the depot in their cytoplasm. It weakened calves at this age of the activity of the Assembly and the intensity of the actomyosin contractile [26].

Weak inducers of adenosine diphosphate and adrenaline in the first month of observation caused the consistently low platelet aggregation, which for the second month was somewhat abated. The basis of this dynamic apparently was first stability quantity and then decreasing the number of receptors able to contact them on the surface of platelets and physiologically acceptable changes in the expression of fibrinogenic receptors (GPIIb-IIIa). This was accompanied by a source of stability, successive weakening of

the activity of phospholipase A_2 , involved in the implementation of the process of the platelet aggregation under the action of weak inducers [27].

This mechanism ensures that at the beginning of the constant there was a decrease in the release from platelet membranes of servings of arachidonic acid, which inhibited the synthesis of thromboxane A_2 . The detected in Holstein calves gradual decline in the second half of phase lacto-vegetarian food activity in platelets cyclooxygenase and thromboxane synthetase was another reason for the weakening in this period the synthesis of thromboxane A_2 . In the performed study it was possible to identify, following the dynamics of the results of trial transfer, the ability to assess the level of synthesis of thromboxane in platelets and the activity of both enzymes that implement this process in platelets. It is very important to ensure the dynamics of the platelet aggregation from Holstein calves during the phase of milk-vegetable diet which is growing they have the observation of lowering actin formation and myosin formation and the weakening of the intensity of the secretion of their adenosinediphosphate platelets and adenosine diphosphate in response to the action of the inducers of the aggregation [28].

Observation detected in calves older than 60 days a decrease of blood amounts of active forms of thrombocytes noted as a decrease in the degree of their sensitivity to external stimulation [5]. Minimal intravascular activity of platelets of Holstein calves to 90 days of life is an undoubted proof of the weak availability to them of the blood collagen of the subendothelial vascular wall. The basis of this, apparently, was a decrease in the second half of phase lacto-vegetarian food quantity in the blood of animals activated platelets and their aggregates of any size. In addition, the weakening of these terms of Holstein calves intravascular platelet activity was possible due to the reduction in their blood plasma concentrations of the inducers of the aggregation, capable of dissolution (epinephrine, adenosine diphosphate, thrombin) [12].

Identification in calves of the weakening of the ability of platelets to the aggregate at the age of 60-90 days can be considered as the physiological basis of the lowering in their blood levels of the active platelets and any size of platelet aggregates. There was the stability of platelet activity in the early phase of milk-vegetable food considered as a feature of the reaction of platelet hemostasis in calves of the Holstein breed at the beginning of the consumption of vegetable feed. The subsequent easing, excluding the possibility of even a partial blockade of aggregates of capillaries in growing tissues should be considered as a result of the successful conclusion of the adaptation of animals to change the composition of the feed [23]. Revealing a reduction of intravascular activity of platelets of Holstein calves between 60 and 90 days of life points to decrease in the severity of the adhesion and aggregation properties of platelets and

is the result of increase in the ability to disaggregate. We guess it could be related to the simultaneous weakening and strengthening preaggregation of disaggregative mechanisms in the platelets of these animals.

5. Conclusion

In calves of the Holstein breed, functional perfection of platelets is recorded during the phase of dairy and plant nutrition. The state of their activity forms physiologically favorable conditions for hemocirculation in the capillaries. This is ensured by the stability of the activity of platelet adhesion, aggregation and secretion processes between 31 and 60 days of life and their subsequent weakening. A decrease in intravascular platelet activity in Holstein calves between 60 and 90 days of ontogenesis creates the best conditions for capillary perfusion and efficient metabolism in muscles and in all internal organs. This circumstance is very physiologically beneficial for the intensive growth and realization of the genetic potential of their high productivity.

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Conflict of Interest

The authors have no conflict of interest to declare.

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