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Functional Features Of Hemostasis In Calves Of Dairy And Vegetable Nutrition.

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ABSTRACT

The transition to the nutrition of plant foods in calves is an important stage in their individual development and requires adequate activity of adaptive mechanisms, including all elements of the hemostasis system. The onset of plant feed into the gastrointestinal tract against the background of a gradual decreasing calf consumption of milk is normally accompanied by adequate changes in all body systems, ensuring their adaptation to the existing nutritional conditions. The study took 36 healthy calves black-and-white breed of dairy and vegetable nutrition. In calves, by the 45th day of life, peak amplification of platelet aggregation activity was noted. This was accompanied by an increase in their control of the vascular wall over the aggregation of platelets in the bloodstream during these periods. At the same time, an increase in the production of antithrombin III by endotheliocytes and tissue plasminogen activators was detected in animals by 45 days. In their blood, an increase in the level of fibrinogen, factors II, VII, IX, X, XI, and XII was noted in the blood at this age with stability of factors V and VIII, followed by a weakening of the activated factors by the end of the third phase of early ontogenesis. An assessment of the state of the hemostasis system in calves during the dairy-plant nutrition phase indicates the presence of a regular change in the activity of its individual components as the age of the animal increases. After a peak change in activity by 45 days of platelet, vascular and plasma hemostasis in the calf, they are rapidly returning to the outcome, which ensures the optimum liquid properties of their blood and, thus, their normal adaptation to environmental conditions.

Keywords: calves, milk-plant nutritional phase, platelets, vascular wall, blood coagulation system.

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INTRODUCTION

The effective functioning of the hemostasis system largely determines in many respects the fluid properties of blood and the adequacy of its flow to the organs and tissues of the developing organism during the entire early ontogenesis [1-5].

The transition to the nutrition of plant foods in calves is an important stage in their individual development [6,7] and requires adequate activity of adaptive mechanisms [8,9], including all elements of the hemostasis system [10,11]. The onset of plant feed into the gastrointestinal tract against the background of a gradual decreasing calf milk consumption is accompanied by normal adequate changes in all body systems [12,13], ensuring their adaptation to the current nutritional conditions [14,15].

Blood plates, the vascular wall and the coagulation system are closely functionally connected [16,17], determining the aggregative state of the blood in young cattle [18,19]. However, the age-related aspects of the development of hemostasis activity in calves of dairy-plant nutrition are poorly studied, which requires the simultaneous assessment of their state of platelet and vascular hemostasis and the blood coagulation system.

Considering the above, in the present work the goal was set: to establish the physiological features of platelet, vascular and coagulative hemostasis in healthy calves of dairy and vegetable nutrition.

MATERIALS AND METHODS

Research was conducted in strict accordance with ethical principles established by the European Convent 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) and approved by the local ethic committee of Russian State Social University (Record №12 dated December 3, 2015).

The 36 healthy calves under observation were examined and examined during the milk-plant nutrition phase 5 times: on day 31, day 45, day 60, day 75, and day 90.

The activity of platelet hemostasis in animals was assessed by platelet aggregation (AP) using as inducers 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 ($7.3\text{x}10^{-3}$ M), adrenaline (5.0×10^{-6} M) and combinations of inductors: ADP and adrenaline; ADP and collagen; collagen and adrenaline; ADP and thrombin; ADP, collagen and adrenaline; ADP, thrombin and adrenaline; ADP, collagen, thrombin and adrenaline in similar concentrations with a standardized platelet count in the plasma of $200\text{x}10^9$ platelets.

The severity of endogenous arachidonic acid metabolism in platelets and the functional activity of their cyclooxygenase and thromboxane synthetase were determined in three transfer samples with the registration of platelet aggregation on a photoelectrocolorimeter.

A quantitative determination of the ATP and ADP content in platelets was carried out, the severity of their secretion was assessed under the action of an inducer (collagen) with the identification of the state of the protein composition of the cytoskeleton of blood plates (actin and myosin).

The hemostatic ability of the vascular wall was determined by its antiaggregation activity recorded by AP before and after temporary venous occlusion with all inductors and their combinations by calculating the antiaggregation index of the vessel wall (IAAVW) during the division of the duration of AP against the background of venous stagnation by the time when AP appeared without it.

The level of antithrombin III (AT III) in the observed animals was detected before and after temporary venous occlusion with the calculation of the vascular wall anticoagulant activity index (IACAVW) by dividing the activity of AT III against the background of venous occlusion by its activity without it.

The degree of control of the vascular wall over the fibrinolytic ability of blood plasma was estimated by the dynamics of spontaneous euglobulin lysis before and after temporary venous occlusion using the index of

fibrinolytic activity of the vascular wall (IFAVW), calculated by dividing the time of euglobulin lysis before occlusion by the time of lysis after it.

The coagulation hemostasis activity was recorded by the functional ability of coagulation factors (I, II, V, VII, VIII, IX, X, XI, XII), the duration of activated partial thromboplastin time, prothrombin time and thrombin time.

The digital data obtained during the study were processed using Student's criterion (td).

RESULTS

In the blood of healthy calves dairy plant nutrition is recorded normal platelet count. On the 31st day of life in calves, the time of AP development under the influence of collagen was 28.7 ± 0.12 s., Significantly shortening by the 45th day of life and subsequently lengthening by the end of the milk-vegetable diet to the level close to the outcome (28.2±0.05s). Similar AP dynamics in the observed animals was observed under the influence of ADP (on 45 days 32.1 ± 0.05 s) and ristomycin (on 45 days 42.1±0.10s), thrombin developed somewhat slowly (on 45 days 46.3±0.03s) and adrenaline (at 45 days 88.3±0.08s) AP, also returning to the end of the phase of milk-vegetable nutrition to the level of 31 days of life for calves. Platelet aggregation with all tested physiological combinations of inductors in the observed animals also experienced a short-term acceleration to 45 days of life.

An important mechanism to accelerate the process of platelet aggregation in young cattle of dairy and vegetable nutrition is a pronounced increase in the intensity of arachidonic acid exchange in blood plates with a peak intensification of thromboxane formation found, which could be indirectly judged by AP in a simple transfer sample 45 days 37.5 ± 0.08%). This dynamics was provided by an episode of enhancing their activity of both enzymes of its transformation in platelets - cyclooxygenase and thromboxane synthetase, returning to the end by the end of the phase of milk-plant nutrition. The degree of AP recovery in the collagen-aspirin test, which indirectly evaluates the activity of cyclooxygenase in platelets, increased briefly by 45 days to 88.6±0.06%. The intensity of AP recovery in a collagen-imidazole sample, which indirectly determines the functional activity of thromboxane synthetase in the blood platelets, increased in calves by 45 days (37.5 ± 0.08%), subsequently rapidly decreasing to the level typical for the beginning of this phase of early ontogenesis.

The initially low content of ATP and ADP in the platelets of healthy calves significantly increased by 45 days to 5.89±0.05 μmol/10^9 platelets and 3.67±0.04 μmol/10^9 platelets, respectively, gradually decreasing by 90 days. At the same time, their level of secretion from platelets experienced a similar dynamics, amounting to 36.0±0.05% and 45.0±0.03% by the end of the phase, respectively.

The content of actin and myosin in intact platelets in animals on 31 days of life reached 32.2±0.16% and 14.6±0.12% of the total protein in the platelet, significantly increasing by 45 days of life to 37.2±0.05% and 16.7±0.07% of the total protein in the platelet and returning to the initial level by the end of the milk-plant nutrition phase.

In healthy calves of milk-plant nutrition, a peak enhancement of vascular wall control over AP was observed, coinciding with its enhancement. The highest IAAVW registered with ADP. A slightly lower level of IAAVW is detected with collagen and adrenaline. IAAVW for thrombin and ristomycin in absolute values were slightly lower, but also increased to 45 days of life. IAAVW with a combination of inductors also proved to be quite high and experienced compensatory gain in the same time frame.

In the milk-plant calves, a peak increase by the middle of the phase of endotheliocyte production of one of the main anticoagulants, antithrombin III, was established (IACAVW by 45 days was 1.39±0.12). In addition, a short-term increase in the intensity of secretion of tissue plasminogen activators was detected in animals at these times, which was detected during the creation of a temporary ischemia of the venous wall at 45 days of life.

A stable pattern in the dynamics of the activity of coagulation factors was noted in calves during the milk-plant nutrition phase. Thus, on the 31st day of life in calves, a low activity of coagulation factors was

recorded. On the 45th day of life, the animal showed a significant increase in the content of fibrinogen and factors II, VII, IX, X, XI, XII against the background of stability of factors V and VIII. Already on the 60th day of life in calves, a significant decrease in the increased factors was observed, which lasted up to 75 days to a level close to the initial one and changed to 90 days by a slight increase in the activity of factors I, II, VII and XII.

Evaluation of coagulation tests in healthy calves during the dairy-plant nutrition phase revealed their regular dynamics reflecting changes in the content of individual coagulation factors in their plasma. So, by 45 days, the acceleration of the activated partial thromboplastin time was established to 34.2 ± 0.18 s., the prothrombin time to 12.8±0.12 s and thrombin time, accelerated by 15.4%, followed by inhibition by the end of the phase.

DISCUSSION

An important integrative system of the calf is the hemostatic system [20,21]. It is on its optimal activity during the entire early ontogenesis [22] that the rheological properties of the blood and, thus, the homeostasis of the growing organism [23,24] largely depend. At the same time, analysis of the level of activity of platelet, vascular and coagulation hemostasis and the subtle mechanisms of its implementation in healthy calves in the dairy-vegetable diet [25,26], marked by the beginning of a change in the diet of the animal with a gradual increase in the consumption of vegetable feed by them [27, 28].

Evaluation of platelet aggregation under the action of a number of inductors and a large number of their physiological combinations allowed to establish from 31 to 90 days of life in calves a peak increase in the level of sensitivity of platelets to them to 45 days of life, returning to the level of outcome by the end of the phase. It has been found that the adhesive ability of platelets is experiencing a similar dynamic, probably due to an increase in the concentration in their blood of von Willebrand factor, a platelet adhesion cofactor, apparently combined with an increase in the number of receptors for it - (GPIb) on the surface of platelets [29,30]. A short-term increase in the level of von Willebrand factor in calves to 45 days of life can be assumed in animals on the basis of the acceleration of platelet aggregation with ristomycin in these periods [31], which in its ability to influence platelets is identical to the subendothelial structures of the vessels [32,33]. Connecting one end of the molecule with collagen, and the other with the platelet via the glycoprotein Ib, von Willebrand factor forms the “adhesion axis”: collagen – von Willebrand factor – GPIb [34-37]. This makes it possible to suspect an increase in the number of these receptors on platelet membranes in calves at the beginning of the milk-plant nutrition phase [38,39].

The increase in sensitivity of platelets to various agonists of aggregation and their combinations in calves of 45 days old is probably associated with a peak increase in the expression of fibrinogen receptors (GPIIb-IIIa), the level of stimulation of phospholipases A_2 and C [40,41], the intensity of thromboxane formation, actin-myosin formation and secretion of adenosine phosphates from blood platelets at the beginning of feeding on plant foods [42,43].

The established compensatory enhancement of the antiaggregation activity of the vascular wall in calves by 45 days of life is explained by a short-term increase in the synthesis of prostacyclin [44] and NO in it, providing the necessary level of microcirculation in the tissues of a growing animal [45,46].

A prominent role in ensuring the atrombogenic properties of the vascular wall in calves during the dairy-plant nutrition phase belongs to the severity of its anticoagulant and fibrinolytic properties [47-50]. The first ones are caused by peak intensification of active production in the endothelium and subendothelium of one of the most powerful physiological anticoagulants - antithrombin III [51-53]. Another antithrombogenic mechanism of the vascular wall is the short-term increase by 45 days of the formation of plasminogen activators in it, which eliminates the overgrowth of fibrin [50,51].Unexpressed coagulation plasma activity in calves at the beginning of the phase was replaced by its short-term enhancement by 45 days of life and was associated with increased activity of factors I, II, VII, IX, XI, and XII, realizing both coagulation pathways activated partial thromboplastin time, prothrombin and thrombin time.

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CONCLUSION

An assessment of the state of the hemostasis system in calves of the milk-plant nutrition indicates the presence of a regular dynamics in their activity of its individual components as the age of the animal increases. With a peak change in activity by 45 days of platelet, vascular and plasma hemostasis in the calf, their rapid return to the state of outcome is observed, providing the necessary fluid properties of the blood and, thereby, promoting the adaptation of the animal to environmental conditions.

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