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Functional Antiaggregatory Properties Of Blood Vessels In Calves During Transition From Dairy To Plant Type Of Nutrition.

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ABSTRACT

One of the most physiologically important mechanisms to ensure the vital activity of the body are the hemostatic properties of the vessels. They largely provide liquid properties of blood and trophic tissue. The processes of platelet aggregation and control from the side of the vascular wall in 36 calves during the transition from dairy to plant nutrition were studied. The index of the anti-aggregation capacity of the vascular wall was determined at the age of 31, 45, 60, 75 and 90 days, measuring the platelet aggregation in blood during a blood vessel occlusion test, in the presence of one, two or three agonists (ADP, collagen, thrombin, ristomycin, adrenalin). Up to the age of 45 days, the values of the index of the antiaggregatory capacity of the vascular wall increased, indicating a compensatory reaction of the vascular wall to increased platelet aggregation and lipid peroxidation. In later terms, the values of the index of the antiaggregative capacity of the vascular wall gradually returned to the initial level.

Keywords: calves, transitional period of nutrition, hemostasis, lipid peroxidation, aggregation, platelets.

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INTRODUCTION

Modern practical biology poses one of its main tasks to provide a basis for increasing food production [1,2,3]. This process should largely ensure the solution of the food problem through the intensification of animal husbandry [4]. To realize it is possible using modern knowledge on the physiology of cattle at different ages, especially during its active growth and development [5].

It becomes clear that a very important moment in providing vital activity of the body are hemostatically significant functions of the vessels [6, 7], which ensure fluid properties of blood and trophic tissues [8,9].

The walls of blood vessels are actively involved in the regulation of blood supply to tissues. The processes of synthesis of haemostatically significant substances occurring in the vascular wall largely control the aggregation properties of the blood constituents, supporting the liquid properties of blood and optimal microcirculation in the tissues of the growing animal [10, 11]. The antiaggregative capacity of vascular walls in calves during dairy plant nutrition plays an important role in adapting the calf's organism to switching to feeding plant foods, laying the foundation of the productive qualities of the animal [12,13,14]. However, the degree of participation of the vascular wall in the regulation of platelet aggregation processes in calves has not yet been sufficiently elucidated. The purpose of this work is to study the antiaggregatory activity of the vascular wall in healthy calves in the phase of milk and vegetable nutrition (at the age of 31-90 days).

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 study was performed on 36 healthy calves of black-motley and Simmental breeds during the dairy-vegetative diet. Inspection and examination of animals were carried out at the age of 31, 45, 60, 75 and 90 days. All the calves used blood samples in the morning before feeding without temporal venous occlusion of jugular vein punctures to determine the biochemical parameters and platelet aggregation taken into account. In a sample with a temporary venous occlusion, which makes it possible to evaluate the antiaggregation capacity of the vessel wall, blood was taken from the popliteal vein in 3 min. after putting on the thigh of the cuff of the tonometer with a pressure in it of pressure of 10 mm Hg higher than systolic.

Plasma blood lipid peroxidation activity and acetic hydroperoxides and thiobarbituric acid-active products (products determined by boiling with thiobarbituric acid, Agat-Med kit (Russia), as well as antioxidant activity of plasma, were determined in blood plasma. 1.79 ml of isotonic sodium phosphate buffer (pH = 7.4), 0.2 ml of rat brain homogenate and 0.01 ml of the plasma to be investigated, followed by incubation under air access conditions at 37°C for 60 minutes and eduyuschim addition of 28% trichloroacetic acid and 0.1% ethylene-diamine-tetraacetic acid. In parallel, a control sample was evaluated oxidizability used brain homogenate rats studied in the absence of plasma (instead of in a test tube were added 0.01 ml of sodium phosphate buffer).

Calculation of the antioxidant activity was carried out according to the formula: antioxidant activity = 100% x [1- (E0; 60-E0; 0) / (Vk; 60-Ek; 0)], where Ek; 60 and Ek; 0 - 60 minutes of incubation and at the "zero" time point for samples containing the plasma under study, and E0; 60 and E0; 0 are analogous extinction values for samples that do not contain the plasma under study. The level of endotheliocythemia was determined in the blood.

The state of the antiaggregative ability of the vessel walls was determined in a sample with a temporary ischemia of the vascular wall. The method is based on calculating the degree of inhibition of platelet aggregation in plasma obtained from blood after temporary venous occlusion as compared to aggregation without venous occlusion.

Blood was taken with sodium citrate 3.8% in a ratio of 9: 1, centrifuged for 5 minutes. at 1000 rpm to produce a platelet rich platelet. A portion of the plasma was withdrawn and the remaining centrifuged at 3000 rpm for 20 minutes, obtaining a platelet-poor plasma. Platelet-rich plasma was standardized by the number of platelets by diluting the original platelet-rich plasma with an autologous sample of poor platelets of plasma (obtained before or after venous stasis - 1st test or 2nd sample) to 200×10^9/l. The concentration of blood platelets in the original platelet-rich plasma was counted in the Goriaev chamber (in 50 large squares). The volumes of plasma-rich plasma mixed with platelet-poor plasma were determined by the formula: platelet-poor plasma = volume of platelet-rich plasma × [(counting platelet count in the original plasma-rich platelet / 200000) -1].

From the selected volume of standardized plasma, 0.02 ml of plasma and another 0.02 ml pipet were applied to the slide solution inductor, mixing them with a glass rod. With the appearance of distinct aggregates, the clarification of the solution, and the adhesion of some aggregates to the glass, the platelet aggregation time was fixed. Evaluation of platelet aggregation was carried out on a standard slide of 7.5x2.5 cm with ADP (0.5×10^{-4} M), collagen (1:2 dilution of the main suspension), thrombin (0.125 U/ml), ristomycin (0.8 mg/ml) and epinephrine (5.0×10^{-6} moll/l), as well as with combinations of these agents - ADP + adrenaline, ADP + collagen, collagen + adrenaline, ADP + thrombin, ADP + collagen + adrenaline, ADP + thrombin + adrenaline, ADP + collagen + thrombin + adrenaline at the same concentrations with a standardized platelet count in the investigated plasma volume (200 × 109 cells per liter) before and after temporary venous occlusion. The index of antiaggregatory activity of the vascular wall was calculated by dividing the time of onset of aggregation platelets against the background of temporary venous occlusion for the time of onset of platelet aggregation without it. The results were treated with Student's test ($p < 0.05$).

RESULTS OF THE STUDY AND DISCUSSION

During observation during the whole period of milk and vegetal nutrition, a peak increase in the activity of lipid peroxidation with an increase in the amount of acyl hydroperoxides and thiobarbituric acid-active compounds in blood plasma to 1.80±0.14 D_{233}/ml and 3.77±0.14 D_{233}/ml was observed at the 450-day with a gradual decrease to the age of 90 days to 1.41 ± 0.11 D_{233}/ml and 3.45±0.19 µmol/l, respectively. The registered dynamics of peroxidation was due to a decrease in antioxidant activity from 27.4±0.15%, followed by an increase to 33.9±0.24% at the end of the period.

For calves in dairy and plant nutrition, the high integrity of the endothelial lining was characteristic, as indicated by the low level of endotheliocythemia (1.7±0.05 for the 31st day of life, 2.1±0.03 for the 45th day and 1.8±0.04 cells/µl for 90 days).

All the calves registered an increasing dynamics of the index of antiaggregatory activity of the vascular wall with all tested inductors and their combinations to the 45-day age (table). The greatest increase in the index of antiaggregatory activity of the vascular wall was noted when used as an agonist ADP, due to the maximum retardation of platelet aggregation with this agent in a sample with temporary venous occlusion. A somewhat lower level was the index of antiaggregatory activity of the vascular wall with adrenaline and collagen. Even lower were the values of the index of antiaggregatory activity of the vascular wall in the sample with thrombin (at the age of 45 days, 1.63±0.08) and ristomycin (at the age of 45 days 1.64±0.12); in the subsequent they also decreased until the end of the phase of milk and vegetable nutrition. The values of the index of antiaggregatory activity of the vascular wall with the simultaneous use of several inductors turned out to be lower, but their dynamics had the same pattern as the index of antiaggregatory activity of the vascular wall with individual agonists - they increased by 45 days, followed by a decrease to 90 days, the intensity of production in the vessel wall of antiaggregants in calves of milk and vegetable nutrition returned to the values inherent in the beginning of the transition period.

Thus, in healthy calves, a short-term increase in the antiaggregatory activity of the vascular wall is observed toward the middle phase of the dairy-vegetative nutrition, with a subsequent return to the level peculiar to its onset.

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Table. Antiaggregatory ability of blood vessels in calves during dairy plant nutrition (M±m, n=36)

The index of antiaggregatory activity of the vascular wall with used agonists of platelet aggregation	Age, days					Mean values
	31	45	60	75	90	
with ADP	1,79±0,12	1,85±0,24**	1,82±0,09*	1,78±0,02*	1,78±0,07	1,80±0,11
with collagen	1,63±0,05	1,71±0,24**	1,68±0,08*	1,65±0,09*	1,64±0,03	1,66±0,10
with thrombin	1,54±0,05	1,63±0,08**	1,60±0,12*	1,56±0,06*	1,53±0,05	1,57±0,07
with ristomycin	1,54±0,07	1,64±0,12**	1,60±0,04*	1,56±0,05*	1,53±0,08	1,57±0,07
with epinephrine	1,68±0,12	1,76±0,09**	1,72±0,05*	1,68±0,09*	1,68±0,02	1,70±0,07
with ADP + epinephrine	1,46±0,04	1,57±0,08**	1,54±0,05*	1,49±0,09*	1,45±0,08	1,50±0,07
with ADP +collagen	1,38±0,02	1,47±0,08**	1,43±0,07*	1,39±0,03*	1,39±0,08	1,41±0,06
with epinephrine + collagen	1,50±0,04	1,60±0,11**	1,57±0,04*	1,52±0,05*	1,49±0,08	1,53±0,06
with ADP + thrombin	1,35±0,06	1,46±0,21**	1,43±0,18*	1,38±0,16*	1,36±0,15	1,39±0,15
with ADP+ collagen+ epinephrine	1,33±0,08	1,41±0,22**	1,39±0,16*	1,35±0,13*	1,33±0,08	1,36±0,13
with ADP+ thrombin + epinephrine	1,31±0,09	1,39±0,17**	1,36±0,15*	1,32±0,09*	1,31±0,11	1,34±0,12
with ADP+ collagen+ thrombin+ epinephrine	1,28±0,10	1,36±0,23**	1,33±0,17*	1,30±0,14*	1,29±0,13	1,31±0,15

Note: * p <0.05, ** p <0.01 on the t-test when compared with the 31-day age.

The phase of milk and vegetable nutrition in calves is a stage of ontogenesis, which determines the adaptation of animals to nutrition with plant foods with a gradual decrease in the intake of milk into the gastrointestinal tract [15,16]. An important role in this process is played by the functional activity of the vascular wall, supporting hemostasis in the organism of a growing animal [16,17]. Being multifunctional in terms of hemostasis control, the vascular wall largely determines the liquid properties of blood as a result of the synthesis of substances [18, 19], limiting platelet aggregation and stimulating fibrinolysis [20,21,22].

The peak increase in the activity of lipid peroxidation in plasma in calves by the 45-day age enhances the alteration of endotheliocytes during these periods, which is accompanied by compensatory enhancement in the vessels of haemostatically significant substances [23], followed by the restoration to the end of the phase of these parameters at a level close to the initial one.

In healthy calves, during the period of milk and vegetable nutrition, a regularity is established that the adhesive ability of the blood plates increasing by the age of 45 days is compensated by the increasing control of the vascular wall, apparently due to the depressive effect of prostacyclin and nitric oxide of the vascular wall on the amount of collagen receptor-glycoprotein Ia-IIa and VI on the platelet membrane [24,25,26]. This is indicated by the lengthening of the onset of aggregation of platelets with collagen in conditions of temporary venous occlusion [27, 28].

As a result of the increase in the vascular wall of natural antiplatelet agents (prostacyclin and nitric oxide) by the vascular wall by the 45th day of age, it seems that a temporary weakening of the fixation of strong aggregation agonists (collagen and thrombin) with their receptors on the platelet membrane, increases. This is a deterrent to the activity of phospholipase C of the blood platelets, leading to increased control of the vascular wall over aggregation of platelets by inhibiting the phosphoinositol pathway of activation of the blood platelets [29,30] and weakening of the phosphorylation of the proteins of the contractile system [31]. Obviously, the short-term increase in the formation of prostacyclin [32,33] and nitric oxide [34] in the calves during the 45 days of age causes an increase in body control over aggregation of platelets with weak aggregation inducers (ADP and epinephrine) noted at this age [35,36]. This can be realized by reducing the expression of fibrinogen receptors (GPIIv-IIIa) and weakening the functionality of phospholipase A_2 releasing arachidonic acid from the phospholipids of the platelet membranes. Weakening of platelet aggregation capacity by 90 days (due to adaptation of the calves organism to plant food) is accompanied by a parallel decrease in the antiaggregative capacity of the vascular wall with respect to individual inducers and especially their combinations, which indicates the elimination of tension in the primary hemostasis system, while maintaining the balance of pro- and antiaggregational influences [37-40].

CONCLUSION

Thus, in calves in the phase of dairy-vegetative nutrition, there is a short-term increase in the antiaggregatory activity of the vascular wall to the 45-day age, which contributes to limiting the physiological growth of platelet aggregation during these periods, providing an effective adaptation of the organism at this stage of ontogenesis.

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