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Functioning Of Mechanisms Of Hemocoagulation Restriction In Calves At Change Of Methods Of Nutrition.

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ABSTRACT

The great importance of clarifying the regularities of ontogenetic changes in the activity of the anticoagulant and fibrinolytic systems of blood plasma is recognized at the initial stages of individual development of calves. They are one of the most important physiological elements in providing homeostasis in the postnatal period. The necessary level of functional activity of anticoagulant and fibrinolytic systems largely ensures adaptation to the external environment of all body systems, controlling the liquid-bone properties of blood, maintaining the level of its fluidity along the vessels, contributing to the optimal deployment of the genetic program of calf development. At the same time, the ontogenetic dynamics of the activity of anticoagulant and fibrinolytic systems in healthy calves during a regular diet change during early ontogeny has not been studied enough. The combination of the dynamics of the activity of anticoagulation and fibrinolysis ensures, during the course of early ontogeny, the necessary level of liquid properties of blood and the optimal degree of perfusion of the internal organs, which greatly facilitates the metabolism in the calf tissues, contributing to its further growth and development, being an essential element of the final functional maturation of the body in conditions of preparation for consumption of only plant foods.

Keywords: phase of milk supply, phase of milk nutrition, phase of milk-calves, peroxide oxidation of lipids.

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INTRODUCTION

Stable, progressive development of the society is possible if there is sufficient quantity of quality food [1,2,3]. For many countries, its source is livestock, providing dairy and meat products, including for dietary nutrition in many diseases [4,5,6].

Further intensification of animal husbandry is possible with a firm reliance on a large number of physiological data, which should be obtained continuously [7]. It becomes clear that in this respect, the mechanisms that preserve blood in the liquid state and especially during the active growth and development of calves are very important [8]. In this regard, the great importance of clarifying the patterns of ontogenetic changes in the activity of anticoagulant and fibrinolytic systems of blood plasma is recognized at the initial stages of individual development of calves. They are one of the most important physiological elements in ensuring their homeostasis in the postnatal period [9]. The necessary level of functional activity of anticoagulant and fibrinolytic systems largely provides adaptation to the external environment of all body systems, controlling the liquid properties of blood, maintaining the level of its fluidity along the vessels, contributing to the optimal development of the genetic program of calf development [10]. At the same time, the ontogenetic dynamics of the activity of anticoagulant and fibrinolytic systems in healthy calves during a regular diet change during early ontogeny has not been studied enough.

In this regard, the goal of the study was formulated: to evaluate the ontogenetic dynamics of the physiological state of the anticoagulant and fibrinolytic systems of blood plasma in healthy calves in the phase of newborn, milk and dairy-vegetative 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 study included 29 physiologically mature, healthy newborn calves on the 1-2 day of life, 32 healthy calf dairy nutrition on the 11th day of life, 36 healthy calves of milk and vegetable nutrition, taken on the 31st day of life, black whip and se -menal breed with normal indicators of laboratory and instrumental research and subsequently not having deviations in the state of health. The complex of examinations consisted of the determination of the activity of peroxide oxidation of plasma lipids by the content of acyl hydroperoxides and thiobarbituric acid-active products by the Agat-Med company (Russia) with an evaluation of the antioxidant activity of the liquid part of the blood. Each calf under observation was evaluated for activity of the anticoagulant system of blood plasma by determining the activity of antithrombin III and protein C in plasma.

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To determine the activity of fibrinolytic ability of blood plasma in calves, a method for determining the time of spontaneous euglobulin lysis, the level of plasminogen, α_2 antiplasmin, and the content of fibrin degradation products with the phenanthroline method was used.

The calves included in the study were inspected and examined for the phase of milk supply: on 1-2 days, 3-4 days, 5-6 days, 7-8 days and 9-10 days. The observed calves of dairy nutrition were examined: on 11 days, 15 days, 20 days, 25 days. and 30 days. life. The indicators taken into account in healthy calves of milk and vegetable nutrition were determined: at 31, 45, 60, 75 and 90 days of life.

Statistical processing of the results was carried out using Student's t-test.

RESULTS OF THE STUDY

During the neonatal phase in healthy calves, the activity of peroxide oxidation of plasma lipids was found to be constant (Table 1). At the same time, the concentration of thiobarbituric acid-active products and the level of acyl hydroperoxides in their blood plasma did not experience significant dynamics during the whole phase of the neonatal period. The stability of lipid peroxidation was ensured by the constancy of the calves during the milk supply phase of the level of antioxidant protection of the body - the antioxidant potential of the plasma in them on the average was 33.7±0.14% for the newborn. During the phase of dairy nutrition in healthy calves, the level of antioxidant activity of plasma (an average of 32.6±0.21%) and the activity of lipid peroxidation was noted. Thus, the level of primary products of peroxide oxidation of lipid acyl hydroperoxides was 1.48±0.02 D_{233}/1ml with an average content of secondary products of free radical lipid oxidation-thiobarbituric acid-active compounds (average 3.29±0.02 µmol/l), authentically does not differ from research to research during the entire phase of dairy nutrition. In the phase of milk and vegetable nutrition in healthy calves, the attenuation of the antioxidant activity level of the plasma to 27.4±0.15% was registered for 45 days of life, with subsequent increase to 33.9±0.24% by 90 days of life. This led to the revealed dynamics of the level of the primary products of lipid peroxidation-acyl hydroperoxides: by 45 days, its peak (1.80±0.14 D_{233}/1ml) was observed with a subsequent decrease (1.41±0.11 D_{233}/1ml) to values below, than at the beginning of the phase. This was accompanied by a similar dynamics of the content of the secondary products of free radical lipid oxidation-thiobarbituric acid-active compounds (at 45 day 3.77±0.16 µmol/l, at 90 day 3.45±0.19 µmol/l), returning to the values, characteristic for thiobarbituric acid-products at the beginning of the phase of dairy-plant nutrition.

Thus, in healthy newborn calves, the stability of the antioxidant potential is noted, which ensures a constant level of lipid peroxidation in the liquid part of the blood, contributing to the maintenance of homeostasis, stabilizing the vascular structures, external membranes of blood elements, providing an optimal level of functioning of plasma proteins, including number of involved in clotting and fibrinolysis.

In the blood of healthy newborn calves, there is an easy tendency to increase the level of antithrombin III, an average of 99.3 ± 0.16%. At the same time, the level of protein C significantly increased in calves during all the first 10 days of life from 50.1±0.24% to 75.2±0.16% (Table 1).

During the neonatal phase, a small tendency of the plasminogen level to increase with a significant decrease in the inhibitor of its active form - α_2 antiplasmin by 27.5% was noted in healthy calves, which tended to slow the spontaneous euglobulin lysis time, the level of fibrin degradation products during the mammary feeding phase experienced the tendency to increase and being a marker of optimal adaptation of the organism to the external environment by maintaining the activity of fibrinolysis at the required level. A small but significant increase in the level of antithrombin III, an average of 105.2±0.13%, was established in the blood of healthy calves of dairy nutrition. Simultaneously, there was a significant increase during the milk feeding phase of protein C level in calves between 11 and 30 days of life from 76.0±0.10% to 83.5±0.08%. During the milk feeding phase, a significant increase in the level of plasminogen was observed in healthy calves at a downward decrease in the inhibitor of its active form, α_2-antiplasmin, by 5.1%. This provided a small but steady decrease in the time of spontaneous euglobulin lysis with a constant level of fibrin degradation products during the dairy phase.

The healthy calves of milk and vegetal nutrition tested had a significant increase in blood levels of antithrombin III to 45 days of life to 122.7±0.20%. At the same time, the activity peak of the protein C level was 98.0±0.08%. In the subsequent to 60 days of life, the activity of anticoagulants decreased, testing in the subsequent small significant increase.

During the phase of milk and vegetable nutrition in healthy calves, a similar dynamics of the level of plasminogen was noted with a marked decrease in the inhibitor of its active form-α2 antiplasmin by 5.1% to 45 days of life, followed by their restoration and a smooth dynamics of their activity. This provided a sharp acceleration at 45 days of life with a subsequent return to values close to the baseline and a peak at this age, and subsequently a constant level of fibrin degradation products during the phase of milk and vegetable nutrition.

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Table 1. Dynamics of calves in the change of methods of nutrition

Registered parameters	Newborn phase, n=29, M±m					Milk phase, n=32, M±m					Milk and vegetable nutrition phase, n=36, M±m				
	1-2 day of life	3-4 day of life	5-6 day of life	7-8 day of life	9-10 day of life	11 day of life	15 day of life	20 day of life	25 day of life	30 day of life	31 day of life	45 day of life	60 day of life	75 day of life	90 day of life
acyl hydroperoxides, D_{233} /1 ml	1.49±0.10	1.43±0.16	1.46±0.05	1.42±0.09	1.44±0.12	1.46±0.07	1.51±0.19	1.52±0.14	1.51±0.18	1.53±0.20	1.54±0.08	1.80±0.14 p<0.01	1.66±0.12 p<0.01	1.42±0.15 p<0.01	1.41±0.11
Thiobarbituric acid products, µmol / l	3.49±0.11	3.40±0.17	3.49±0.14	3.45±0.08	3.47±0.11	3.51±0.14	3.56±0.19	3.50±0.22	3.52±0.14	3.55±0.16	3.59±0.22	3.77±0.16 p<0.01	3.67±0.14 p<0.01	3.51±0.23 p<0.01	3.45±0.19
Antioxidant plasma potential, %	34.2±0.16	33.4±0.12	33.6±0.16	33.8±0.20	33.5±0.09	32.8±0.23	32.6±0.24	32.2±0.15	32.6±0.17	32.8±0.15	29.3±0.17	27.4±0.15 p<0.05	30.6±0.14 p<0.01	32.8±0.12 p<0.01	33.9±0.24
The activity of antithrombin III in plasma, %	96.2±0.11	98.9±0.22	99.6±0.16	99.9±0.15	102.1±0.19	101.7±0.07	103.6±0.14 p<0.05	105.4±0.12 p<0.05	106.9±0.10 p<0.05	108.2±0.16 p<0.05	109.1±0.04	122.7±0.20 p<0.01	114.6±0.06 p<0.01	116.8±0.08 p<0.05	119.9±0.10 p<0.05
Protein C, %	50.1±0.24	58.7±0.28 p<0.01	66.3±0.22 p<0.01	71.3±0.21 p<0.01	75.2±0.16 p<0.01	76.0±0.10	78.2±0.76 p<0.05	79.4±0.04 p<0.05	81.6±0.06 p<0.05	83.5±0.08 p<0.05	84.0±0.12	98.0±0.10 p<0.01	87.3±0.16 p<0.01	89.5±0.04 p<0.05	93.6±0.03 p<0.05
Time of spontaneous euglobulin lysis, minutes	186.3±0.52	183.6±0.38	181.6±0.49	179.6±0.46	178.9±0.42	178.2±0.34	175.4±0.15 p<0.05	173.0±0.22 p<0.05	172.0±0.18	170.3±0.15 p<0.05	170.0±0.26	152.3±0.10 p<0.01	167.7±0.14 p<0.01	165.0±0.13 p<0.05	162.1±0.09 p<0.05
Plasminogen, %	115.2±0.17	116.2±0.36	116.3±0.34	118.3±0.24	120.1±0.45	122.0±0.05	123.8±0.08 p<0.05	124.6±0.06 p<0.05	126.0±0.05 p<0.05	128.6±0.10 p<0.05	128.9±0.02	138.8±0.07 p<0.01	130.2±0.09 p<0.01	132.6±0.08 p<0.05	134.5±0.08 p<0.05
α2 antiplasmin, %	130.4±0.32	117.4±0.30 p<0.01	112.1±0.20 p<0.05	109.0±0.36 p<0.05	102.3±0.28 p<0.05	101.3±0.19	100.0±0.14	98.7±0.06 p<0.05	97.3±0.05 p<0.05	96.4±0.09 p<0.05	96.1±0.15	80.4±0.17 p<0.01	93.6±0.05 p<0.01	90.4±0.08 p<0.05	89.0±0.03 p<0.05
Fibrin degradation products, µg / ml	33.9±0.21	36.3±0.17	38.5±0.18	38.6±0.29	39.5±0.34	40.2±0.25	41.3±0.19	41.9±0.20	42.2±0.09	42.8±0.16	42.9±0.16	55.8±0.25 p<0.01	44.8±0.29 p<0.01	43.1±0.18	44.0±0.12

Legend: p - reliability of the dynamics of indicators from research to research.

Thus, during the change in dietary habits in early ontogenesis calves have a regular dynamics associated with a gradual and reliable increase in plasma levels of antithrombin III plasminogen, protein C activity and a decrease in α_2 antiplasmin with a jump in their activity by 45 days, followed by recovery at a level close to the values at the beginning of the phase of milk and vegetable nutrition, which is undoubtedly an important element in the adaptation of animals to new nutritional conditions, contributing to the transition of their hemostasis to the level required for a yes the most rapid growth and development of the body.

DISCUSSION

The study found that during the neonatal phase in healthy calves, the activity of the antioxidant potential of the plasma and the levels of the primary products of lipid peroxidation - acyl hydroperoxides and secondary - tiobarbituric acid-active compounds are stable, which is apparently necessary for this type of productive animals for the development of antistress mechanisms of it homeostasis at this stage of development [11,12]. A low level of plasma lipid peroxidation causes a weak alteration of endotheliocytes and components of the liquid part of the blood, contributing to weak stimulation of plasma hemostasis [13,14].

Very significant in the anticoagulant potential in healthy newborn calves is the activity of antithrombin III, plasminogen and protein C, ensuring the balance of anticoagulants and procoagulants [15,16]. This can be confirmed by the absence of signs of thrombosis and hemorrhage in a healthy newborn, largely due to active fibrinolysis and a sufficiently high anticoagulation activity that allows the calf organism to respond adequately to unfavorable environmental factors, which tend to have a procoagulant effect on haemostasis [17,18].

In calves in the phase of dairy nutrition, there is a lack of reliable fluctuations in the level of lipid peroxidation and antioxidant protection of the plazma with a certain dynamics of activity of anticoagulation and fibrinolysis, which undoubtedly allows the calf to adapt to the conditions of extrauterine existence, providing a normal rheological state of the blood, and thus adequate the inflow of nutrients and oxygen to the developing tissues of the animal's organism [19,20]. This is an important element of protection of calves against possible unfavorable environmental factors affecting their body in the phase of dairy nutrition. During the milk-feeding phase, the activity of coagulation inhibitors and the level of fibrinolytics significantly increase: antithrombin III, protein C and plasminogen increase and the activity of the fibrinolysis inhibitor - α_2 antiplasmin decreases [21,22]. Obviously, this is a physiological response to the adaptation of the organism, which, upon completion of the neonatal phase, requires an increase in fibrinolysis activity [23]. In view of the fact that the general inhibitor of the contact activation of plasma proteases, plasminogen gradually increases with the level of fibrin degradation products remaining in the blood, one can think about the optimal functioning of the mechanisms of hemostasis adaptation in these conditions without signs of hypocoagulant hemostasis direction at these times, providing optimal microcirculation conditions in hemodynamic adaptation in phase of milk nutrition [24].

The significant increase in the level of lipid peroxidation with the weakening of the antioxidant protection of the plasma at the beginning of the phase of milk and vegetable nutrition, with a pronounced increase in the activity of anticoagulation and fibrinolysis, allows the calf organism to adapt to new nutritional conditions, ensuring a normal rheological state of the blood, and thus an adequate inflow of nutrients and oxygen to the growing tissues of the animal's body [25,26]. Undoubtedly, this is an important element of the reaction of the calves' organism at the beginning of feeding with plant foods, which can be considered as a strong stimulus of the external environment that affects their organism at the beginning of the phase of milk and vegetable nutrition, and adaptation in the calf, which, apparently, [27,28]. The dynamics of the anticoagulation system that controls the aggregate state of blood and the fibrinolysis system that dissolves excess fibrin is largely ensured by the dynamics of lipid peroxidation, ensuring their adequate readiness to respond to environmental factors [29]. Thus, during the phase of milk-and-vegetable nutrition, the activity of inhibitors of coagulation and the level of fibrinolytic agents significantly increase: antithrombin III, protein C and plasminogen are increased and the activity of the fibrinolysis inhibitor - α_2 antiplasmin decreases with a sharp jump to 45 days and subsequent smooth change [30]. Obviously, this is a physiological response to the adaptation of the organism during the transition to plant nutrition with an increase in the activity of anticoagulation and fibrinolysis [31, 32]. In view of the fact that a slight increase in the total inhibitor of the contact activation of plasma proteases of plasminogen by the end of the phase is accompanied by the preservation of a stable level in the blood of fibrin degradation products, one can think of a stable adaptation during these periods of functioning of the mechanisms of hemostasis adaptation in these conditions without

signs of hypocoagulation of hemostasis [33], providing optimal conditions for microcirculation during hemodynamic adaptation at the end of feeding calf with milk.

CONCLUSION

The combination of the dynamics of the activity of anticoagulation and fibrinolysis ensures during the early ontogenesis the necessary level of fluid properties of blood and the optimal degree of perfusion of the internal organs, which greatly facilitates the metabolism in the calf tissues, contributing to its further growth and development, being an indispensable element of the final functional maturation of the organism in conditions of preparation for consumption of only plant foods.

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