ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Physiological Features Of The Rheological Properties Of Erythrocytes In Calves During The Dairy-Plant Nutrition Phase.

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ABSTRACT

The rheological properties of blood have great physiological significance for the realization of all manifestations of the viability of the organism. This has a great practical interest in productive animals, which are a source of food for humans. All this is quite true for cattle at the stages of its active growth and development. Objective: to find out the functional features of red blood cells in healthy calves in the phase of milk-vegetable nutrition. The object of observation was 34 healthy calves of dairy and vegetable nutrition, which do not have deviations in the objective status and the results of instrumental and laboratory research methods. In healthy calves of dairy-plant nutrition, regular fluctuations in the surface geometry and aggregation properties of erythrocytes occur in response to changes in the dietary pattern, providing the rheology of blood necessary at this stage of development, largely ensuring the growth and development of the animal. The low aggregation activity of the erythrocytes helps the calf to adapt in the middle of early ontogenesis to further existence.

Keywords: healthy calves, milk-plant nutrition phase, rheological properties of erythrocytes.

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ISSN: 0975-8585

INTRODUCTION

The rheological properties of blood have a great physiological significance for the realization of all manifestations of the viability of the organism [1-5]. This is of great practical interest in productive animals that are a source of food for humans [6, 7]. All this is quite true for cattle at the stages of its active growth and development [8,9].

The circulatory properties of blood are largely provided by the functional characteristics of red blood cells, their surface membrane properties and the ability to aggregate [10,11]. It is these properties of erythrocytes that largely determine hemodynamics in the microvasculature [12], causing the influx of the necessary amount of oxygen to the tissues [13]. The functional activity of erythrocytes in calves in early ontogenesis is very important, as it can either affect the emergence and development of deviations from homeostasis and the formation of pathological states during active growth [14,15], or ensure adaptation to the external environment of all body systems [16,17], contributing to the optimal growth and development of the animal [18-21]. At the same time, the age-related dynamics of the activity of the functional properties of erythrocytes in healthy calves in the dairy-vegetable nutrition phase remains not fully elucidated. In this regard, the goal of the study is formulated: to clarify the functional characteristics of erythrocytes in healthy calves in the dairy-vegetable nutrition phase.

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).

34 healthy calves of dairy and vegetable nutrition, not having deviations in the objective status and results of instrumental and laboratory research methods, were under observation.

The level of plasma lipid peroxidation activity was assessed by the content of thiobarbituric acid-active products using the Agat-Med kit (Russia) and acylhydroperoxides [22]. To assess the antioxidant potential of the liquid part of the blood, its antioxidant activity was determined [23].

In washed and resuspended erythrocytes, cholesterol levels were quantified by an enzymatic colorimetric method using Vital Diagnosticum and total phospholipids based on their phosphorus content [24], followed by calculating the ratio of cholesterol / total phospholipids in red blood cells.

The intensity of intraerythrocytic lipid peroxidation was determined by the concentration of malondialdehyde in the reduction reaction of thiobarbituric acid in washed and resuspended erythrocytes and the content of acylhydroperoxides [22]. The activity of intraerythrocyte antioxidant enzymes was established for catalase and superoxide dismutase [25].

To assess the structural and functional properties of the erythrocyte membrane, their cytoarchitecture was studied. Information on the surface geometry of erythrocytes was obtained using light-phase-contrast microscopy of cells [26].

In a quantitative assessment of the ratio of pathological and normal forms of erythrocytes, the transformation index was calculated, taking into account the percentage of discocytes, the percentage of reversibly deformed erythrocytes, and the percentage of irreversibly deformed erythrocytes. The reversible transformation index, the irreversible transformation index, and the reversibility index were also calculated.

Erythrocyte aggregation was determined using a light microscope [26]. At the same time, in the Goryaev chamber, the number of erythrocyte aggregates, aggregated and non-aggregated erythrocytes in suspension of washed erythrocytes in blood plasma with the calculation of the average size of the aggregate taking into account the sum of all erythrocytes in the aggregate and the number of aggregates. In the work, the aggregation index was calculated taking into account the number of free red blood cells. The percentage

of non-aggregated red blood cells was also determined. Statistical processing of the results was carried out by t-student criterion.

RESULTS

It is established that in the blood of calves, dairy and vegetable nutrition concentration acylhydroperoxides - primary products of lipid peroxidation of plasma and secondary – thiobarbituric acid-active compounds for 45 days dramatically increases of 22.7% and 19.8%, respectively, decreasing it to a level close to the initial 75 days of the subsequent further reduction by the end of this phase of early ontogeny. Found dynamics of the intensity of peroxidation was possible as a result of authentic depression in calves 45 days of life antioxidant protection of organism of animals by 20.2%, with further gradual increases to 75 days to the original level with additional enhancement they have antioxidant activity of plasma to the 90 th day of life by 4.0% (33.7±0.13%).

In healthy calves, a significant change in the erythrocyte lipid composition was noted during the milk-plant nutrition phase. Thus, by the 45th day of life, the content of cholesterol in the membranes of red blood cells increased to the level of 0.99±0.004 μmol/10^{12} erythrocytes against the background of a decrease in total phospholipids to 0.70±0.001 μmol/10^{12} erythrocytes with increasing cholesterol / total phospholipids their membranes up to 1.41±0.006. Further, in calves, until the end of the phase, cholesterol content in erythrocyte membranes was reduced to 0.96±0.008 μmol/10^{12} erythrocyte and cholesterol/total phospholipids gradient to 1.28±0.005 against the background of an increase in the total phospholipids in them to 0.75±0.008 μmol/10^{12} erythrocyte.

In calves, a low level of intra-erythrocyte lipid peroxidation was observed at the beginning of the milk-plant nutrition phase when the antioxidant protection of the red blood cells was activated. However, by the 45th day of life, calves showed a weakening of the functional ability of erythrocyte catalase to 10150.0±9.2 IU/10^{12} erythrocytes and superoxide dismutase to 1680.0±6.01 IU/10^{12} erythrocytes, subsequently experiencing a reliable dynamics to activation, reaching a level higher than the initial (catalase 10780.0±14.6 IU/10^{12} erythrocytes, superoxide dismutase 1850.0±3.71 IU/10^{12} erythrocytes). As a result of the detected dynamics of red blood cells antioxidant protection in calves, the content of lipid peroxidation products in them experienced regular fluctuations - the number of acylhydroperoxides in them on the 45th day was 3.41±0.03 D_{233}/10^{12} erythrocytes, at the level of basal malonic dialdehyde –1.19±0.04 nmol/10^{12} erythrocytes), followed by their decrease to the end of the phase by 20.1% and 27.9%, respectively.

In healthy calves of milk-plant nutrition, a 15.7% decrease in the red blood cell level of the discoid form was noted in the bloodstream, followed by an increase in their content by the end of the phase to a level comparable to that at its beginning. The found dynamics of the level of blood discocytes was accompanied by a sharp increase in the 45 days of life of the total blood content of reversibly and irreversibly modified forms of red blood cells by 90.6% and 52.6%, respectively. In further periods of observation, their levels gradually decreased, reaching values close to those at the beginning of the phase (11.2±0.13% and 4.1±0.06%, respectively) by the end of the phase. At the same time, in the examined calves, a similar dynamics of the value of the transformation index was recorded with a peak on the 45th day and a subsequent decrease in its value to 0.18±0.003 by the end of the phase.

A sharp increase in calves by the 45th day of life of the content in the bloodstream of reversibly altered erythrocytes determined the peak increase in these periods of the reversible transformation index to 0.27±0.003 with its subsequent decrease to the outcome values by the end of the phase. At the same time, similar dynamics in the calf's blood content of irreversibly altered forms of erythrocytes provided similar changes in the value of the index of irreversible transformation during the milk-plant nutrition phase.

The observed increase in the index of reversibility in calves in the middle of the milk-vegetable nutrition phase was followed by its decrease to its end, due to the revealed dynamics of the level in the bloodstream of reversibly and irreversibly deformed erythrocytes. These changes in calves were accompanied by a peak increase in the aggregation of red blood cells in the region of 45 days of life, followed by the elimination of these changes.

ISSN: 0975-8585

DISCUSSION

The dairy plant nutritional phase is strictly hereditary [27,28] and is characterized by complex exchange changes associated with changes in the nutritional composition, which inevitably affects the rheological properties of red blood cells [29]. Maintaining optimal hemodynamics and fluctuations in plasma antioxidant activity determine the elucidated dynamics of lipid peroxidation in the liquid part of the blood, ensuring stabilization of the erythrocyte outer membrane [30,31]. The decrease in the level of activity of the antioxidation enzymes of red blood cells at the beginning of the phase causes a peak enhancement of lipid peroxidation activity in them, followed by its weakening by the end of the phase, which, combined with a peak increase in the level of cholesterol in their membranes, enhances their functional activity, which decreases by the end of the phase. In this regard, in calves of milk-plant nutrition in the bloodstream, by 45 days of life, a short increase in the content of reversibly and irreversibly modified forms of erythrocytes is observed with a certain decrease in the level of discoocytes. Short-term deterioration of the surface geometry of erythrocytes led to a short-term increase in their aggregation, providing not very favorable rheological properties of blood, temporarily worsening the perfusion of internal organs and probably the inevitable element of the optimal course of early ontogenesis [32,33,34].

Achieving the optimality of the rheological properties of red blood cells and blood in general in healthy calves by the end of the milk-plant nutrition phase is undoubtedly an element of the calf's body's adaptation process. It is implemented by ensuring an adequate supply of nutrients and oxygen to the developing tissues of the body [35-39]. This is an important element of protection against possible adverse environmental factors affecting the organism of a living being [40,41].

Table. Indicators of the micro-rheological properties of erythrocytes in calves dairy and vegetable nutrition

Registrated parameters	Phase of dairy and vegetable food, n=34, M±m					The average value for the milky phase vegetable nutrition n=34, M±m
	31 day	45 day	60 day	75day	90 day	
Discocytes, %	85.6±0.15	74.0±0.29 p<0.01	78.9±0.36 p<0.05	85.0±0.30 p<0.05	84.7±0.23	81.6±0.27
Reversibly modified erythrocytes, %	10.6±0.12	20.2±0.07 p<0.01	17.2±0.15 p<0.05	11.0±0.09 p<0.05	11.2±0.13	14.0±0.11
Irreversibly changed erythrocytes, %	3.8±0.01	5.8±0.05 p<0.05	3.9±0.04 p<0.05	4.0±0.09	4.1±0.06	4.3±0.05
The sum of all erythrocytes in the unit	37.1±0.10	46.6±0.18 p<0.01	40.2±0.05 p<0.05	37.0±0.04 p<0.05	37.6±0.08	39.7±0.09
Number of units	8.3±0.07	9.9±0.09 p<0.01	9.0±0.06 p<0.05	8.2±0.02 p<0.05	8.4±0.05	8.8±0.06
The number of free erythrocytes	251.6±0.08	234.2±0.07 p<0.01	248.2±0.12 p<0.05	252.4±0.19 p<0.05	250.1±0.17	202.5±0.13
Percent of non-aggregated erythrocytes	87.1±0.05	95.9±0.07 p<0.01	85.9±0.18 p<0.01	87.2±0.17 p<0.05	86.9±0.19	88.6±0.12
The average size of the aggregate, cells	4.5±0.02	4.7±0.07	4.5±0.03 p<0.05	4.5±0.06 p<0.05	4.5±0.02	4.5±0.04

Legend: p - the reliability of the ontogenetic dynamics of the estimated indicators.

It becomes clear that the peak activation of erythrocytes in calves in the dairy-plant nutrition phase provides the level of fluid properties of the blood typical for this stage of ontogenesis and a certain degree of perfusion of the internal organs in response to the transition to a new diet [42,43]. This largely supports the

level of reactivity necessary for the organism, contributing to the further growth and development of the animal [44]. There is no doubt that the revealed dynamics of the activity of the rheological properties of erythrocytes is an important element of the overall adaptation process of the organism in early ontogenesis, which is necessary for the growth and development of the organism.

CONCLUSION

For calves during the dairy-plant nutrition phase, the peak red blood cell activation is characteristic, which ensures the level of blood fluid properties typical for this stage of ontogenesis and the necessary degree of perfusion of the internal organs during the transition to a new type of nutrition. The current situation largely supports the level of reactivity necessary for the organism, contributing to the further growth and development of the animal. The revealed dynamics of the activity of the rheological properties of red blood cells should be considered as an important element of the general adaptation process of the organism in early ontogenesis, necessary for the growth and development of the organism.

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