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## Functional Features Of Erythrocytes In Calves Of Vegetable Nutrition.

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### ABSTRACT

The final phase of early ontogenesis in calves is characterized by the final maturation of organs and systems, including the blood system, and in particular the completion of the formation of indicators determining its hemorheology. Of great importance in this process is the age dynamics of the surface geometry of erythrocytes. The maximum of these features determine the hemodynamics in the microvasculature, causing the inflow of the required amount of oxygen and nutrients, inevitably changing during ontogenesis under the influence of various environmental influences. The functional activity of erythrocytes in calves that have switched to vegetable nutrition is very important for the maximum possible deployment of their potential functional capabilities of the organism during the period of active growth. The purpose of the work is to assess the state of the surface geometry of erythrocytes in early ontogenesis in healthy calves of plant nutrition. The object of observation was 36 healthy calves of vegetable nutrition, which do not have deviations in the objective status and the results of instrumental and laboratory research methods, the state of which was traced from 91 days to the end of 12 months of life. In calves of plant nutrition, a high level of blood fluid properties necessary for this stage of ontogenesis is noted, providing an optimal degree of perfusion of internal organs, which largely supports the level of metabolism in tissues necessary for the organism, contributing to the further growth and development of the animal.

**Keywords:** healthy calves, the phase of plant nutrition, the rheological properties of red blood cells.

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## INTRODUCTION

The process of growth and development of a living organism is associated with the sequential switching on and off of a large number of mechanisms [1,2,3]. Integrating systems of a living organism [4,5,6] play a huge role in this, among which blood is prominent [7,8]. The degree of phenotypic expression of all hereditarily determined traits depends on the degree of success of the functioning of all regulatory mechanisms [9-12]. The implementation of these mechanisms in productive animals [13], including cattle during its active growth [14], is of great economic importance.

The final phase of early ontogenesis in calves is characterized by the final maturation of organs and systems, including the blood system [15], and in particular the completion of the formation of indicators determining its hemorheology [16,17]. The age dynamics of the surface geometry of erythrocytes is of great importance in this process [18]. Maximally these features determine the hemodynamics in the microcirculatory bed, causing the inflow of the required amount of oxygen and nutrients, inevitably changing during ontogenesis under the influence of various environmental influences [19]. The functional activity of erythrocytes in calves that have switched to plant nutrition is very important for the maximum possible deployment of their potential functional capabilities of the organism during the period of active growth [20]. However, many aspects of age-related changes in the surface geometry of erythrocytes in healthy calves of plant nutrition in early ontogenesis remain insufficiently clarified. In this regard, the goal of the study is formulated: to assess the state of the red blood cell surface geometry in healthy calves of plant nutrition.

## MATERIALS AND METHODS

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) and approved by the local ethic committee of Russian State Social University (Record №12 dated December 3, 2015).

The object of observation was 36 healthy calves of vegetable nutrition, which do not have deviations in the objective status and the results of instrumental and laboratory research methods, the state of which was traced from 91 days to the end of 12 months of life.

In washed and resuspended erythrocytes, cholesterol levels were quantified by the enzymatic colorimetric method using the Vital Diagnosticum set and total phospholipids for their phosphorus content [21], followed by calculating the ratio of cholesterol / total phospholipids in erythrocytes.

Intraperitoneal peroxidation of lipids was determined by the concentration of malondialdehyde in the reduction of thiobarbituric acid in washed and resuspended red blood cells and the acylhydroperoxide content by traditional methods [22]. The activity of intraerythrocyte antioxidant enzymes was established for catalase and superoxide dismutase [23].

Evaluation of the structural and functional properties of the erythrocyte membrane was carried out taking into account their surface geometry using light phase-contrast microscopy of cells.

A quantitative assessment of the ratio of pathological and normal forms of erythrocytes was carried out by calculating the transformation index = (percentage of reversibly deformed erythrocytes + percentage of irreversibly deformed erythrocytes) / percentage of discocytes. A reversible transformation index was also determined = percentage of reversibly deformed red blood cells / percentage of discocytes, an irreversible transformation index = percentage of irreversibly deformed red blood cells / percentage of discocytes, and reversibility index = percentage of reversibly deformed red blood cells / percentage of irreversibly deformed red blood cells.

Statistical processing of the results was carried out by t-student criterion.

## RESULTS

In the calves during the plant nutrition phase, in early ontogenesis, the composition of erythrocyte membranes showed a tendency to increase the level of cholesterol and total phospholipids to  $1.03 \pm 0.004 \mu\text{mol}/10^{12}$  erythrocytes and  $0.78 \pm 0.004 \mu\text{mol}/10^{12}$  erythrocytes, respectively, at cholesterol level/total phospholipids  $1.28 \pm 0.003$ . This was accompanied by a slight weakening of the activity of lipid peroxidation in red blood cells, ultimately contributing to their low functional activity, providing the best conditions for microcirculation in this phase of ontogenesis.

The content of the primary products of lipid peroxidation - acylhydroperoxides in erythrocytes of healthy calves at the age of 91 days was at the level of  $2.83 \pm 0.12 \text{ D}_{233}/10^{12}$  erythrocytes, gradually decreasing to 12 months of erythrocytes by 12 months. At the same time, the level of malondialdehyde in erythrocytes, the end product of lipid peroxidation, had a similar dynamics, reaching  $0.80 \pm 0.05 \text{ nmol}/10^{12}$  erythrocytes by a year of life.

The tendency to the weakening of lipid peroxidation in erythrocytes of healthy calves of vegetable nutrition was possible due to the increase in their activity of the antioxidant system and, above all, catalase and superoxide dismutase. Thus, the levels of catalase and superoxide dismutase in the red blood cells of the animals under observation reached  $11,350.0 \pm 11.5 \text{ IU}/10^{12}$  red blood cells and  $21200.0 \pm 4.27 \text{ IU}/10^{12}$  red blood cells, respectively.

In calves of vegetable nutrition, in early ontogenesis a normal level of discocytes in the blood flow was noted, averaging  $82.7 \pm 0.20\%$ , with a trend towards an increase in the transformation index from  $0.18 \pm 0.010$  on the 91st day to  $0.24 \pm 0.006$  by the end 12 months of life (table). At the same time, the observed calves showed an increase by the end of 12 months of life of the level of reversibly altered erythrocytes to  $14.8 \pm 0.18\%$ . The low content in the bloodstream of the observed calves of reversibly altered erythrocytes determined a low level of their reversible transformation index during early ontogenesis, reaching  $0.18 \pm 0.004$  by the end of the observation. At the same time, in these animals, the number of irreversibly altered erythrocytes experienced a statistically insignificant increase from  $4.1 \pm 0.02\%$  on the 91st day of life to  $4.5 \pm 0.04\%$  after 12 months, accompanied by invariance of the index of irreversible transformation (average  $0.05 \pm 0.004$ ). It was revealed that in the examined animals, as the chronological age increases during the phase, the reversibility index experiences a slight tendency to increase (on average  $3.02 \pm 0.005$ ), emphasizing the similar dynamics of the total red blood cells and the specific gravity in the bloodstream of their irreversible forms.

## DISCUSSION

In the last phase of early ontogenesis in calves, the final formation of metabolic and hemostatic processes, inevitably affecting the rheological properties of blood, is noted [24]. Optimal hemodynamics stabilizes the outer erythrocyte membranes in animals. The high level of activity of the anti-oxidation enzymes of the red blood cells causes them to maintain a low activity of lipid peroxidation, which, combined with a slight increase in the level of cholesterol in their membranes, provides the necessary rheological and functional properties of red blood cells [25]. This is the physiological basis for maintaining low levels in plant calves in the bloodstream with a tendency to increase reversibly and irreversibly altered forms of erythrocytes with a high level of discocytes in the blood [26]. The optimality of cytoarchitecture of erythrocytes in many respects contributes to the low aggregation of red blood cells, ensuring proper rheological properties of blood, sufficient perfusion of internal organs, promoting optimal ontogenesis.

Minor changes in the cytoarchitecture of erythrocytes in the blood of healthy calves of plant nutrition are undoubtedly an element of the process of their body's adaptation to the external environment, thus providing an adequate supply of nutrients and oxygen to the developing tissues of the animal. Undoubtedly, the low severity of deformational changes in the surface of erythrocytes in the vascular bed of plant-fed calves is an important element in protecting their bodies against possible adverse environmental factors.

**Table. Surface geometry of erythrocytes in healthy calves**

Registered parameters	Phase of plant nutrition, n=36, M±m				The average values for the phase of plant nutrition, n=36, M±m
	91 day of life	6 months of life	9 months of life	12 months of life	
Discocytes, %	84.7±0.22	83.1±0.17	82.2±0.16	80.7±0.24	82.7±0.20
Reversibly modified erythrocytes, %	11.2±0.08	12.7±0.09	13.4±0.13	14.8±0.18	13.0±0.12
Irreversibly changed erythrocytes, %	4.1±0.02	4.2±0.04	4.4±0.03	4.5±0.04	4.3±0.03
Transformation index	0.18±0.010	0.20±0.007	0.22±0.002	0.24±0.006	0.21±0.006
Reversible transformation index	0.13±0.002	0.15±0.004	0.16±0.002	0.18±0.004	0.16±0.003
Irreversible transformation index	0.05±0.003	0.05±0.002	0.05±0.004	0.05±0.006	0.05±0.004
Reversibility index	2.73±0.003	3.02±0.004	3.04±0.008	3.29±0.004	3.02±0.005

### CONCLUSION

In calves of vegetable nutrition, the lipid composition of erythrocytes is normal and the level of lipid peroxidation in them is normal. In the blood of plant-fed calves, there is a slight tendency to an increase in the blood level of reversibly and irreversibly altered red blood cells with a slight decrease in discoid forms of red blood cells.

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