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# Physiological Dynamics Of Microrheological Characteristics Of Erythrocytes In Piglets During The Phase Of Milk Nutrition.

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**ABSTRACT**

Hemocirculation by microvessels determines to a large extent the functional activity of the whole organism of young animals of productive animals, including piglets. It provides the necessary level of all processes of their life activity. In this connection, the age dynamics of microrheological properties of erythrocytes in productive animals can be considered as an important element in maintaining the required level of anabolic processes in tissues and one of the mechanisms for realizing their productive potential. At the same time, despite the great practical importance of these indices, the features of the surface properties of the membrane and the aggregation of red blood cells in piglets in individual phases of early ontogenesis remain insufficiently investigated. For healthy piglets of milk nutrition, the increase of cytoarchitectonics and erythrocyte aggregation is characteristic, which, apparently, is a necessary element of the process of their adaptation to environmental conditions in the second phase of early ontogeny. The tendency towards increasing the aggregation of erythrocytes in piglets during the phase of milk nutrition noted in the study is largely due to the age-related change in the charge of red blood cells, due to the masking of negatively charged proteins against the background of the increase in the amount of cholesterol detected in them. This inevitably increases the adhesion strength of these erythrocyte membrane proteins to globular plasma proteins that act as bridges between red blood cells in aggregates. This allows them to maintain the level of metabolism necessary for the body in the cells, contributing to the further growth and development of the animal. Undoubtedly, the revealed features of cytoarchitectonics and the aggregation of erythrocytes in piglets of dairy nutrition largely ensure the transition of the organism to nutrition with plant foods.

**Keywords**: erythrocytes, aggregation, cytoarchitectonics, blood coagulation, piglets, milk feeding phase.

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## INTRODUCTION

Blood is a fluid organism that ensures a clear integration of the organism into one whole and the metabolism in all its cells [1,2,3]. Of great importance in this are all its components - both the blood elements [4,5] and the plasma [6,7]. Continuously circulating through blood vessels, blood provides in the capillaries metabolism [8,9] and gas exchange [10,11]. For this reason, hemocirculation processes in capillaries have a very high physiological significance [12,13] and largely depend on the rheological properties of blood [14,15]. These properties in capillaries are largely ensured by the state of its constituent elements and, in the first place, by its most abundant group, erythrocytes [16,17]. Particularly of great importance in this respect is their aggregation and surface properties of the membrane. One can think that their condition largely determines the degree of development of economically significant signs of productive animals [18-20], including pigs. It becomes clear that hemocirculation by microvessels largely determines the functional activity of the whole organism of young animals of productive animals, including pigs, providing the necessary level of all processes of their vital activity [21]. In this connection, the age-related dynamics of microrheological properties of erythrocytes in productive animals can be considered as an important element in maintaining a certain level of anabolic processes in tissues and the mechanism for realizing their productive potential in them [21]. At the same time, despite the great practical importance of these indices, the features of the surface properties of the membrane and the aggregation of red blood cells in piglets in individual phases of early ontogenesis remain insufficiently investigated.

In this connection, the goal of the present study was to establish the characteristics of the microrheological properties of erythrocytes in healthy piglets during the phase of milk nutrition.

## MATERIALS AND METHODS

The 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 in March, 18th, 1986, and confirmed in Strasbourg in June, 15th, 2006), approved by the Local Ethics Committee of K. I. Skryabin Moscow State Academy of Veterenary Medicine and Biotechnology (record №14, dated December, 1st, 2015), the Local Ethics Committee of Russian State Social University (record №11, dated December, 4th, 2015) and the Local Ethics Committee of All-Russian Scientific Research Institute of Physiology, Biochemistry and Animals' Nutrition (record №11, dated December, 4th, 2015).

We examined 32 healthy piglets of large white breed, under physiological conditions. The examination was conducted on the 6th, 10th, 15th and 20th days of their life. In all animals, the activity of lipid peroxidation (LPO) of plasma was estimated by the number of acyl hydroperoxides, thiobarbituric acid-active products in the kit "Agat-Med" (Russia), and the antioxidant activity of the liquid part of the blood was recorded [22].

To assess the intensity of biochemical processes in erythrocytes, they were washed and resuspended. The expression of lipid peroxidation in the erythrocytes was recorded by the number of acyl hydroperoxides in them and by the level of malonicdialdehyde in the reduction reaction of thiobarbituric acid. Using the "Vital Diagnosticum" kit (Russia), the content of cholesterol was quantitatively measured in erythrocytes, and the level of total phospholipids was estimated from the level in their phosphorus membranes, based on the obtained data on the cholesterol / total phospholipids ratio. The functional activity of intra-erythrocyte antioxidation enzymes was determined for catalase and superoxide dismutase [22].

The pigs were evaluated cytoarchitectonics of erythrocytes with the use of light phase-contrast microscopy. The registered erythrocytes were typified by the following varieties: discocytes, discocytes with one outgrowth, discocytes with a crest, discocytes with multiple outgrowths, erythrocytes in the form of mulberry, dome-shaped erythrocytes (dentites), spherocytes with a smooth surface, spherocytes with spines on the surface, erythrocytes in the form of " degenerated forms of erythrocytes). The first five classes of erythrocytes (with signs of echinocyte transformation) were considered reversibly deformed in view of their ability to spontaneously restore the form. The remaining classes of erythrocytes were referred to the group of irreversibly deformed or pre-hemolytic forms.

Taking into account the ratio of reversibly and irreversibly altered forms of erythrocytes, a number of indices were calculated [22]:

Transformation index = (percentage of reversibly deformed erythrocytes + percent of irreversibly deformed red blood cells) / percentage of discocytes.

Index of reversible transformation = percentage of reversibly deformed erythrocytes / percentage of discocytes.

Index of irreversible transformation = percentage of irreversibly deformed erythrocytes / percentage of discocytes,

Index of reversibility = percentage of reversibly deformed erythrocytes / percentage of irreversibly deformed red blood cells.

The aggregation activity of erythrocytes was recorded with the aid of a light microscope by counting aggregated and non-aggregated erythrocytes in the Goryaev chamber and the number of aggregates themselves in the suspended plasma erythrocyte slurry with the calculation of the average aggregate size = the sum of all the erythrocytes in the aggregate / the number of aggregates. The aggregation index was also calculated = (average aggregate size x number of aggregates + number of free red blood cells) / (number of aggregates + number of free erythrocytes). The percentage of unaggregated erythrocytes = (the number of free erythrocytes x 100) / (the average size of the aggregate x number of aggregates + the number of free erythrocytes) was calculated [23].

For statistical processing of the results obtained, Student's t-test was used.

**RESULTS**

During the phase of dairy nutrition, a small gradual increase in the antioxidant activity of the plasma (from 36.9±0.16% at the beginning to 38.2±0.12% at the end) is registered in healthy piglets, which contributes to the weakening of the activity of LPO in it. Thus, the quantitative content of the primary products of LPO-acyl hydroperoxides in the liquid part of the blood was 1.34±0.10 $D_{233}$/1ml at the beginning of the phase, 1.29±0.16 $D_{233}$/1ml at the end of the phase, and secondary lipid peroxidation products - thiobarbituricacid -active compounds were 3.10±0.13 µmol/l and 2.95±0.008 µmol/l, respectively.

The observed piglets during the milk feeding phase in erythrocyte membranes showed a tendency to increase in the amount of cholesterol from 0.90±0.005 µmol/$10^{12}$ erythrocytes to 0.92±0.005 µmol/$10^{12}$ erythrocytes with a slight decrease in total phospholipids from 0.70±0.006 µmol/$10^{12}$ erythrocytes up to 0.69±0.008 umol/$10^{12}$ erythrocytes.

The number of acyl hydroperoxides in erythrocytes of healthy six-day-old piglets was 2.90±0.07 $D_{233}$/$10^{12}$ erythrocytes, gradually decreasing at 20 days old (2.82±0.04 $D_{233}$/$10^{12}$ erythrocytes). At the same time, the level of malonicdialdehyde in erythrocytes - the final product of LPO - also tended to decrease from 1.01±0.02 nmol/$10^{12}$ erythrocytes to 0.98±0.07 nmol/$10^{12}$ erythrocytes. The revealed dynamics of LPO level in erythrocytes of healthy piglets of milk nutrition was provided with a tendency to enhance their antioxidant system, estimated by the functional activity of catalase, which was 11040.0±10.8 IU/$10^{12}$ erythrocytes and 1750.0±9.24 IU/$10^{12}$ at the beginning of the phase erythrocytes at the end, and superoxide dismutase, the functional capacity of which reached 11190.0±17.6 IU/$10^{12}$ erythrocytes at the beginning of the phase and 1788.4±6.82 IU/$10^{12}$ erythrocytes, respectively, at the end.

In piglets of milk nutrition, the optimal number of discocytes in the blood was recorded with a tendency to increase to 86.5±0.26%, ensuring a low level of the transformation index at the end of the phase - 0.16±0.009 (Table). In this case, in animals from 6 to 20 days of life, a slight decrease in the content of reversibly altered erythrocytes in the blood to 8.8±0.06% and their irreversibly transformed forms to 4.7±0.05% was noted. The revealed dynamics of erythrocyte cytoarchitectonics has provided a tendency for piglets of dairy nutrition to reduce their index of reversible transformation to 0.10±0.003 when the index of irreversible transformation is 0.05±0.005 and the index of reversibility is 1.87±0.010.

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**Table: Microrheological properties of erythrocytes in piglets of milk nutrition in physiological conditions**

| Registered parameters | Milk phase, n=32, M±m | | | | |
|---|---|---|---|---|---|
| | 6 dayoflife | 10 dayoflife | 15 dayoflife | 20 dayoflife | Medium meanings |
| Erythrocytes-discocytes, % | 85.5±0.14 | 85.8±0.17 | 86.1±0.12 | 86.5±0.26 | 86.0±0.17 |
| Reversibly modified erythrocytes, % | 9.3±0.07 | 9.1±0.03 | 9.0±0.05 | 8.8±0.06 | 9.1±0.05 |
| Irreversibly modified erythrocytes, % | 5.2±0.02 | 5.1±0.04 | 4.9±0.03 p<0.05 | 4.7±0.05 p<0.05 | 5.0±0.04 |
| Index of transformation | 0.17±0.007 | 0.16±0.005 p<0.05 | 0.16±0.007 | 0.16±0.009 | 0.16±0.007 |
| Index of reversible transformation | 0.11±0.002 | 0.11±0.006 | 0.10±0.007 | 0.10±0.003 | 0.11±0.005 |
| Index of irreversible transformation | 0.06±0.005 | 0.06±0.006 | 0.06±0.009 | 0.05±0.005 p<0.05 | 0.06±0.006 |
| Index of reversibility | 1.78±0.008 | 1.78±0.005 | 1.83±0.007 p<0.05 | 1.87±0.010 p<0.05 | 1.82±0.008 |
| The sum of all red blood cells in the aggregate | 32.4±0.14 | 32.6±0.12 | 32.9±0.08 | 33.2±0.07 | 32.8±0.10 |
| Number of aggregates | 7.3±0.05 | 7.4±0.02 | 7.5±0.06 | 7.6±0.05 | 7.5±0.05 |
| The number of free red blood cells | 280.1±0.19 | 278.1±0.25 | 276.3±0.17 | 270.6±0.23 | 276.3±0.21 |
| Aggregation rate | 1.09±0.07 | 1.09±0.03 | 1.09±0.06 | 1.09±0.04 | 1.09±0.05 |
| The percentage of non-aggregated red blood cells | 89.7±0.09 | 89.5±0.14 | 89.3±0.16 | 89.0±0.13 | 89.4±0.13 |
| The average size of the aggregate, cells | 4.4±0.06 | 4.4±0.05 | 4.4±0.03 | 4.4±0.02 | 4.4±0.04 |

Legend: p-trustworthiness of the age dynamics of the indicators considered.

Piglets during the phase of milk nutrition showed a gradual increase in the aggregation capacity of erythrocytes (Table) with an increase in the level of total involvement of erythrocytes in aggregates (by 2.5%), an increase in the number of these aggregates in the bloodstream (by 4.1%), a decrease of 3.5% of the blood in the blood of freely moving red blood cells with a constant average aggregate size (on average 4.4±0.04 cells) and the aggregation index (average 1.09±0.05) and a slight decrease in the percentage of non-aggregated red blood cells (mean 89.0±0.13).

Thus, healthy piglets of dairy nutrition are characterized by an increase in cytoarchitectonics and erythrocyte aggregation with an undetectable increase in the activity of clotting factors and acceleration of the basic coagulation tests, which apparently is an indispensable element of the process of their adaptation to environmental conditions during the second phase of early ontogeny.

## DISCUSSION

In all productive animals, ontogeny is obligatory marked by a number of hematological changes that inevitably affect the rheological properties of the blood [24]. The high activity of antioxidation enzymes of red blood cells existing in piglets of dairy nutrition contributes to the stabilization of LPO processes at a low level, which, in combination with a low content of cholesterol in their membranes, ensures optimal microreological

properties of red blood cells [25]. Undoubtedly, this is the physiological basis for maintaining in the bloodstream in piglets of a low level of reversibly and irreversibly altered varieties of erythrocytes with persistent prevalence in the blood of their unchanged forms. This provides the best rheological properties of blood, sufficient perfusion of the internal organs and, in this connection, the optimality of the growth process of the animal [26].

The tendency in the study to increase erythrocyte aggregation in piglets during the milk feeding phase is largely due to the age-related change in the erythrocyte charge due to the masking of negatively charged proteins [27] against the background of the increase in the amount of cholesterol detected in them [28]. This inevitably increases the adhesion strength of these erythrocyte membrane proteins to globular plasma proteins, which act as "bridges" between red blood cells in aggregates [29].

Optimum microrheological properties of erythrocytes in piglets of dairy nutrition in many ways provide the liquid properties of blood necessary for this stage of ontogeny and, thus, sufficient perfusion of internal organs [30]. This allows them to maintain the level of metabolism necessary for the body in the cells, contributing to the further growth and development of the animal. Undoubtedly, the revealed features of cytoarchitectonics and the aggregation of erythrocytes in piglets of dairy nutrition largely ensure the transition of the organism to nutrition with plant foods.

## CONCLUSION

For healthy piglets of milk nutrition, the optimal lipid composition of erythrocytes and the low activity of lipid peroxidation in them with a slight increase in erythrocyte aggregation and an unbiased increase in the content of their discocytes as a result of a decrease in the reversibly and irreversibly altered forms are observed.

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