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Rheological Properties of Erythrocytes of Healthy Piglets during the Transition from Dairy to Vegetable Nutrition

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AVP and AAP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript.

Authors VIM and TIG managed the analyses of the study. Author NVK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

In the course of ontogenesis mammals can experience some changes of regular elements' micro rheological peculiarities. These changes can influence blood rheological properties in capillaries and, so, the degree of tissues' perfusion. The aim: to determine erythrocytes' micro rheological properties in healthy piglets of dairy-vegetable nutrition. The study used 39 healthy piglets of the breed "Large-White". They were examined on the 21st, 25th, 30th and 40th days of life. All the piglets were kept in the pig complex "Verdazernoprodukt" in Ryazan region, Russia. We evaluated in animals the activity of lipid peroxidation in plasma and erythrocytes in terms of the number of acylhydroperoxides and malon dialdehyde in them. The content of cholesterol and total phospholipids in erythrocytes was determined. In erythrocytes the surface properties of their

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membranes and aggregation activity were evaluated. Statistical analysis of the results was conducted by Student's t-criterion. Against the background of strengthening of erythrocytes' antioxidant protection we noticed lowering of acylhydroperoxides' quantity on 13.2% and the level of malon dialdehyde – on 19.5% in erythrocytes of piglets. During the phase of dairy-vegetable nutrition the piglets were registered to have lowering of erythrocytes-discocytes till $83.3 \pm 0.09\%$ at little rise of the level of reversibly and irreversibly changed erythrocytes. In the course of the experiment the piglets had increase of summary level of erythrocytes' involvement into aggregates on 20.5% and quantity rise of the aggregates themselves on 22.7% at lowering of free erythrocytes' number on 8.6%. Thus, piglets of dairy-vegetable nutrition are characterized by optimum of the processes of lipids' peroxidation in platelets. It is accompanied by some rise of erythrocyte aggregation and quantity increase of reversibly and irreversibly changed erythrocytes in blood.

Keywords: Erythrocytes; aggregation; cytoarchitecture; piglets; dairy-vegetable nutrition.

1. INTRODUCTION

Despite the great successes of modern biology, further studying of morpho-functional properties of productive animals at different stages of their development still remains very urgent. It is necessary to continue improving the technology of their keeping, development and nutrition in different periods of their lives. To do this, the blood system, which provides the basis for the vitality of the body, is actively studied. Continuous circulation of blood along the vessels maintains general resistance [1,2], helping to realize the hereditary potential [3,4]. It is provided by the maintenance of optimal metabolic processes in tissues [5,6] in all the environmental conditions [7,8] and in any functional state of the body [9,10]. There is no doubt that the rheology of blood is largely determined by the characteristics of its regular elements, including erythrocytes [11,12]. They determine hemodynamics in vessels, especially of small diameter, by changing their aggregating activity and surface properties of the membrane [13,14]. The dynamics of these indicators is especially great during the period of active growth, when a large influx of oxygen and nutrients to tissues is needed [15,16]. Its sufficient level ensures the best functioning of all biochemical processes in the body and its active growth [17,18]. When the animal goes over to the consumption of vegetable feeds, the need for oxygen in tissues is especially great [19]. It's necessary to study rheological properties of erythrocytes in calves in this age. Relying on the available information, it becomes clear that micro rheological properties of erythrocytes in piglets should be evaluated during the most significant stage of their life – the period of transition to the consumption of vegetable nutrition. Such information can help modern pig-breeding, which is actively looking for options to increase the productivity of pigs

and biological "points of impact" in the bodies of animals to achieve this result. In this connection, we set ourselves the goal of studying the dynamics of the micro-rheological properties of red blood cells in healthy piglets during their transition from dairy to vegetable nutrition.

2. 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) and the local Ethics Committee of K.I. Scryabin Moscow State Academy of Veterinary Medicine and Biotechnology (record № 14, dated December, 1st, 2015) and the local Ethics Committee of All-Russian Scientific Research Institute of Physiology, Biochemistry and Animals' Nutrition (record № 11, dated December, 4th, 2015).

In our study we examined 39 healthy piglets of the breed "Large-White" on the 21st, 25th, 30th, 35th and 40th days of life. All the animals were kept in the pig complex "Verdazernoproduct" in Ryazan region, Russia. In the course of the study, it was planned to track the dynamics of erythrocytes' micro rheological properties and biochemical parameters of plasma and erythrocytes which can affect them during the phase of dairy-vegetable nutrition. We estimated the activity of lipids' peroxidation (LPO) in piglets' plasma according to the quantity of acylhydroperoxides (AHP) [20] and thiobarbituric acid (TBA)-active products in it by a set of the firm "Agat-Med" (Russia). We also registered antioxidant activity (AOA) of liquid part of blood [20].

Erythrocytes were washed and resuspended by conventional way in order to estimate their biochemical indices. We conducted the estimation of AHP and malon dialdehyde (MDA) levels in them [20]. We determined quantitatively the level of cholesterol (CS) in piglets' erythrocytes with the help of a set produced by the firm "Vital Diagnostikum" (Russia). According to the quantity of phosphorus in their membranes we determined the content of common phospholipids (CPL) [20]. We also estimated the activity of intra-erythrocyte catalase and superoxide dismutase (SOD) [20].

Cytoarchitecture of piglets' erythrocytes was determined with the help of light phase-contrast microscopy in the result of their typing on discocytes, discocytes with one outgrowth, discocytes with a crest, discocytes with numerous outgrowths, erythrocytes like mulberry, dome-shaped erythrocytes (stomatocytes), spherocytes with smooth surface, spherocytes with spinelets on the surface, erythrocytes like "deflated ball", degenerative forms of erythrocytes. The first 5 classes of erythrocytes (with signs of echinocytarous transformation) were considered to be reversibly deformed, the rest classes were considered to be irreversibly deformed forms [21].

The evidence of erythrocytes' aggregation was found in the course of light microscopy at calculation of the number of aggregated and disaggregated erythrocytes and the quantity of erythrocyte aggregates in the meal of washed red corpuscles [22] in Gorjaev's box.

The results were processed by Student's criterion (t). Statistical processing of received information was made with the help of a program package "Statistics for Windows v. 6.0", "MicrosoftExcel". Differences in data were considered to be reliable in case of $p < 0.05$.

3. RESULTS AND DISCUSSION

During the phase of dairy-vegetable nutrition healthy piglets were noticed to have gradual strengthening of plasma AOA (a total of 11.6%). It caused lowering of LPO activity in it. During the observation period, the quantitative content of primary products of AGP in the liquid part of blood decreased on 10.2%, secondary products of lipid peroxidation - TBA-active compounds decreased on 12.1%.

During the phase of dairy-vegetable nutrition erythrocytes' membranes of piglets were found to

have gradual rise of CS quantity from on 7.7% at lowering of CPL on 9.4%.

The level of AHP in erythrocytes of healthy animals gradually decreased between the 21st and the 40th days of life on 13.2%. At the same time, the level of MDA in erythrocytes also decreased on 19.5%. The observed dynamics of lipids' peroxidation in erythrocytes of healthy piglets of dairy-vegetable nutrition was caused by the increase in their catalase activity on 25.1% and SOD on 8.0%. Gradual decrease of discocytes' amount in animals' blood on 3.5% was noted during observation (Table 1). So, piglets of 21 to 40 days of life had the increase of reversibly changed erythrocytes on 22.7% and their irreversibly transformed forms on 18.0% in their blood.

In the course of observation the animals were noted to have gradual lowering of discocytes' quantity in blood till $83.3 \pm 0.09\%$ (Table 1). At the same time, the piglets from the 21st to the 40th days of life were noted to have content rise of reversibly changed erythrocytes in blood till $10.8 \pm 0.07\%$ and their irreversibly transformed forms till $5.9 \pm 0.05\%$.

Besides, the piglets under observation were found to have significant rise of erythrocytes' aggregative ability (Table 1). It was pointed by the level increase of summary erythrocytes' involvement into aggregates (on 20.5%), quantity rise of these aggregates in animals' blood (on 22.7%) at content lowering of freely moving erythrocytes in their blood (on 8.6%).

The course of ontogenesis in mammals is accompanied by changes of micro rheological peculiarities of their regular elements. It inevitably influences rheological blood properties [23,24]. Found enzymes' activity increase of erythrocytes' antioxidation promoted stabilization of LPO processes at the low level in piglets of dairy-vegetable nutrition. In combination with some content rise of CS in their membranes it could provide optimal micro rheological erythrocytes' characteristics for any environmental conditions [25].

Found low LPO activity in piglets' plasma and erythrocytes provided them with preservation of external and internal leaves of lipid bilayer of the erythrocyte membrane [26,27]. It led to preservation of convexoconvex form by most erythrocytes of these piglets what provided optimum of hemodynamics along vessels

Table 1. Indices of plasma and erythrocytes of piglets of dairy-vegetable nutrition

Registered parameters	Phase of dairy-vegetable nutrition, n=39, M±m				
	The 21 st day	The 25 th day	The 30 th day	The 35 th day	The 40 th day
Acylhydroperoxides, D ₂₃₃ /1ml	1.30±0.012	1.28±0.007	1.24±0.005	1.21±0.013	1.18±0.008
TBA-active products, umol/l	2.96±0.010	2.92±0.004	2.85±0.008	2.77±0.011	2.64±0.005
Antioxidant activity of plasma, %	38.0±0.09	38.6±0.07	39.5±0.06	40.7±0.10	42.4±0.09
Cholesterol in erythrocytes, umol/10 ¹² erythrocytes	0.91±0.003	0.92±0.004	0.94±0.002	0.96±0.007	0.98±0.006
Common phospholipids in erythrocytes, umol/10 ¹² erythrocytes	0.70±0.002	0.70±0.008	0.68±0.006	0.66±0.004	0.64±0.003
Acylhydroperoxides of erythrocytes, D ₂₃₃ /10 ¹² erythrocytes	2.83±0.009	2.80±0.007	2.74±0.005	2.63±0.005	2.50±0.007
Malon dialdehyde of erythrocytes, nmol/10 ¹² erythrocytes	0.98±0.004	0.96±0.003	0.93±0.012	0.88±0.002	0.82±0.005
Catalase of erythrocytes, ME/10 ¹² erythrocytes	11170.0±11.6	11280.0±20.4	12220.0±15.7	13250.0±15.2	13980.0±19.3
Superoxide dismutase of erythrocytes, ME/10 ¹² erythrocytes	1781.3±8.36	1790.0±9.92	1826.8±11.82	1880.3±7.34	1923.4±5.22
Erythrocytes-discocytes,%	86.2±0.06	85.8±0.05	85.0±0.13	84.0±0.18	83.3±0.09
Reversibly modified erythrocytes,%	8.8±0.05	9.0±0.02	9.6±0.04	10.3±0.09	10.8±0.07
Irreversibly modified erythrocytes,%	5.0±0.03	5.2±0.05	5.4±0.02	5.7±0.07	5.9±0.05
Sum of all the erythrocytes in an aggregate	33.1±0.16	34.8±0.15	36.5±0.03	38.7±0.08	39.9±0.05
Quantity of aggregates	7.5±0.06	7.8±0.08	8.4±0.03	8.7±0.06	9.2±0.03
Quantity of free erythrocytes	270.2±0.15	265.4±0.11	261.5±0.08	254.3±0.18	248.7±0.15

Conventional signs: p – reliability of differences of accountable indices from the level of 21 days of life

[28,29], especially of small diameter [30,31]. It also maintained optimal conditions for metabolism in a body [32,33]. There's no doubt that it is an important basis for maintenance of optimally low level of reversibly and irreversibly changed erythrocytes' varieties in blood of examined animals at stable predominance of their unchanged forms. It provided optimal conditions for the processes of animals' growth.

Found little strengthening of erythrocytes' aggregation in examined piglets in the course of the phase of dairy-vegetable nutrition was mostly connected with physiological CS increase in their membranes, lowering of CPL and immersion of some proteins with negative charge on their surface into the membrane [34]. It was possible that appearing changes in piglets during the phase of dairy-vegetable nutrition led to stabler connection of erythrocytes with globular plasma proteins which could fulfill the role of "bridges" between erythrocytes in the course of aggregation [35,36]. The increase of the threshold of erythrocytes' disaggregation took place in conditions of growth and changing of piglets' nutrition. It strengthened the linkage of erythrocytes in their aggregates [37,38].

Found changes in micro rheological features of erythrocytes in piglets during the phase of dairy-vegetable nutrition should be considered as physiologically necessary for early ontogeny. Given changes are evidently directed at the maintenance of necessary for the phase of piglets' dairy-vegetable nutrition level of metabolism and can promote their adaptation [39,40].

4. CONCLUSION

The rheological characteristics of erythrocytes, which determine the bloodstream in tissues and the level of metabolism in them, are very important for the successful transition of piglets from dairy to vegetable nutrition. At this age piglets have a slight increase in erythrocyte aggregation and a slight increase of the content of reversible and irreversibly altered erythrocytes in their blood. Apparently, during the dairy-vegetable nutrition phase, the optimal rheological properties of piglets' erythrocytes are largely ensured by the balance in their lipid composition and low lipid peroxidation activity in their membranes. Probably, the revealed rheological properties of erythrocytes of piglets at the age of 21-40 days of life are necessary for their successful transition to the consumption of only

plant feeds while maintaining the necessary level of metabolism in their tissues.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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