Physiological Reaction of Erythrocytes' Micro Rheological Peculiarities in Milk Fed Piglets after the Negative Impact of the Environment

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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
One of physiologically significant and vulnerable stages of productive animals’ (including pigs) ontogenesis is the phase of milk feeding. Microcirculation is very significant for the common functional welfare of a body. It is mostly determined by erythrocytes’ state. The aim of research: to determine the state of erythrocytes’ micro rheological peculiarities in milk fed piglets after the impact of the negative environmental factor – super cooling. We examined 59 milk fed piglets of the breed “Large-White” after the impact of the unfavorable environmental factor (super cooling). In the result, 32 animals had arthritis, 27 – bronchitis. Control group was composed of 32 healthy milk fed piglets. In our study we applied biochemical, hematological and statistical methods of investigation.
We found similar intensity increase of lipids' peroxidation in blood plasma and erythrocytes of piglets after the unfavorable environmental impact which took place in the result of comparable weakening of their antioxidant protectability. Milk fed piglets with arthritis or bronchitis were found to have worsening of erythrocytes' micro rheological features of similar evidence: comparable quantity rise of their reversibly changed (approximately on 60%) and irreversibly changed forms (nearly in 2 times) and strengthening of erythrocytes' ability to aggregation (increase of aggregates' number on more than 29.0%). In case of the impact of super cooling on milk fed piglets, regardless of developing later arthritis or bronchitis, we noticed similar activation of lipids' peroxidation at comparable weakening of antioxidant protectability of plasma and erythrocytes. It was accompanied by comparable quantity rise of erythrocytes' changed forms in their blood and comparable growth of their ability to aggregation.

Keywords: The phase of milk feeding; piglets; arthritis; bronchitis; erythrocytes; aggregation; cytoarchitecture.

1. INTRODUCTION

The process of mammals' vital activity is inseparably connected with interaction of their bodies with the environment which is far not always favorable \[1,2\]. One of physiologically significant and vulnerable stages of productive animals' ontogenesis is the phase of milk feeding \[3\]. There is no doubt that this stage has active adaptation of all the regulatory animal's systems to the conditions of the environment in its basis \[4\]. Microcirculation \[5,6\] is very significant for realization of common functional welfare of a mammal's body and provision of optimum for resistance maintenance. Regular blood elements are its most important components \[7,8\]. Their micro rheological characteristics are physiologically very important \[9,10\] as they mostly determine tissue perfusion and, in such a way, the success of oxygen and nutrients' delivery into all the cells \[11,12\].

At the same time, many parameters of regular blood elements react keenly on external and internal impacts what determines the processes of the whole body's functioning \[13,14\]. It becomes clear that ontogenetic changes in tissues and organs cause dynamics in hemostasis and rheology. It inevitably influences microcirculatory features of blood cells \[15\]. Notwithstanding the great successes of biology, all the aspects of erythrocytes' cytoarchitecture and their aggregation in milk fed piglets, kept in various conditions are not yet fully studied. We decided to determine erythrocytes' micro rheological features in the phase of milk feeding because of its great functional significance for microcirculation and possibility of its light disturbance \[16,17\]. We conducted the estimation of erythrocytes' aggregation and cytoarchitecture in piglets in optimal environmental conditions and after casual impact of unfavorable factors of the environment. As negative environmental impact we chose short-time super cooling of piglets with subsequent development of arthritis or bronchitis.

The aim of our research: to determine peculiarities of erythrocytes' micro rheological features in milk fed piglets after the impact of unfavorable environmental factor – super cooling.

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 approved by the local Ethics Committee of K. I. Skryabin 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' Feeding (record № 11, dated December, 4th, 2015).

We observed 59 milk fed piglets of the breed “Large-White”. They were kept on the farm “Verdazernoprodukt”, Ryazan region (Russia). After the unfavorable impact of the environment – super cooling - 32 piglets had acute arthritis, 27 piglets – acute bronchitis. The presence of arthritis in piglets was determined with the help of the signs of joint inflammation: swelling, redness, skin temperature increase over with difficulty in flexing. The presence of bronchitis in piglets was determined by the following symptoms: coughing, flow of mucous secretions from the
nasal passages, increase of body temperature up to 41.0-41.5°C and wheezing in the lungs. Super cooling of animals was due to unplanned emergency shutdown of the heating system in the pigsty where the piglets were kept. Super cooling lasted 25-30 minutes. The air temperature at this time was 13-14°C in it. Control group was composed of 32 milk fed piglets of the breed “Large-White”. All the piglets, taken into our research, were received in spring from sows of the first or second farrowing with normal course of pregnancy. All the animals were taken into the research on the 6th-7th day of life.

Intensity of the processes of lipids’ peroxidation (LPO) in liquid part of blood was found according to the content of thiobarbituric acid (TBA)-active products in it with the help of a set produced by the firm “Agat-Med” (Russia) and according to quantitative content of acylhydroperoxides (AHP) [18]. We also estimated antioxidant activity (AOA) of all the animals’ blood plasma [18].

Piglets’ erythrocytes were washed and resuspended by traditional method for estimation of their biochemical indices. LPO activity going inside erythrocytes, was registered according to MDA level in the test of thiobarbituric acid reduction and according to the quantity of AHP in them [18]. Functional activity of intraerythrocyte enzymes of antioxidation was determined for catalase and superoxide dismutase (SOD) [18].

Micro rheological erythrocytes’ features were estimated according to their cytoarchitecture with the help of light phase-contrast microscopy and spontaneous aggregation. Erythrocytes were typed into ten classes (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 because of their ability to restore the form spontaneously. The rest classes of erythrocytes were considered to belong to the group of irreversibly deformed forms [19].

Spontaneous aggregative activity of erythrocytes was registered with the help of light microscope by calculating the number of erythrocytes’ aggregates, aggregated and disaggregated erythrocytes in Goryaev’s box [20].

To find out the reliability of differences between experimental groups and control one we used Student’s t-test. Statistical significance of differences was proved at p<0.05.

3. RESULTS AND DISCUSSION

We found no reliable differences in accountable indices when we compared animals from the group with arthritis with the animals from the group with bronchitis. Observed animals had similar LPO activation in plasma and erythrocytes and comparable worsening of micro rheological features after the negative impact of the environment (Table 1).

The quantity of TBA-active products in liquid part of blood in both groups of observation was nearly in 1.6 times higher than in control group. The content of AHP in their plasma also surpassed the normal level more than in 2.1 times. Strengthening of peroxidation became possible in the result of weakening of a body’s antioxidant protection – antioxidative plasma potential of piglets with arthritis lowered till 31.2±0.10%, at bronchitis till 30.8±0.16% (in healthy animals it was equal to 37.5±0.10%).

The concentration of initial LPO-AHP products in erythrocytes at the presence of arthritis in the body of an animal was higher in comparison with one in the control group on 53.5%, at bronchitis - on 54.5%. It showed the activation of LPO initial stages in them. The level of MDA in erythrocytes was also higher on 36.4% and 37.4%, respectively (Table 1).

The intensity of LPO processes in erythrocytes of milk fed piglets after the negative impact of the environment was conditioned by lowering of their antioxidant protection and, first of all, catalase and superoxide dismutase. Their levels in red corpuscles of observed piglets from both groups turned out to be lower on more than 29.0% and 18.0%, respectively; in comparison with control values (Table 1).

Examined animals after the negative impact of the environment were noticed to have quantity lowering of discoid formed erythrocytes in blood (Table 1). It was accompanied by reliable rise of summary quantitative content of erythrocytes’
Table 1. Accountable indices of milk fed piglets under the impact of unfavorable environmental factors

<table>
<thead>
<tr>
<th>Registered parameters</th>
<th>Piglets with arthritis, n=32 M±±</th>
<th>Piglets with bronchitis, n=27 M±±</th>
<th>Control, n=32 M±±</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyl hydroperoxides, D_{233}/1 ml</td>
<td>2.81±0.006**</td>
<td>2.86±0.004**</td>
<td>1.32±0.009</td>
</tr>
<tr>
<td>TBA-active products, umol/l</td>
<td>4.79±0.009**</td>
<td>4.70±0.006**</td>
<td>3.04±0.008</td>
</tr>
<tr>
<td>Antioxidant activity plasma, %</td>
<td>31.2±0.10*</td>
<td>30.8±0.16*</td>
<td>37.5±0.10</td>
</tr>
<tr>
<td>Acylhydroperoxides of erythrocytes, D_{233}/10^{12} erythrocytes</td>
<td>4.39±0.012**</td>
<td>4.42±0.013**</td>
<td>2.86±0.005</td>
</tr>
<tr>
<td>Malon dialdehyde of erythrocytes, nmol/10^{12} erythrocytes</td>
<td>1.35±0.010**</td>
<td>1.36±0.019**</td>
<td>0.99±0.007</td>
</tr>
<tr>
<td>Catalase of erythrocytes, ME/10^{12} erythrocytes</td>
<td>8610.0±32.0**</td>
<td>8450.0±33.4**</td>
<td>11120.0±16.0</td>
</tr>
<tr>
<td>Superoxide dismutase of erythrocytes, ME/10^{12} erythrocytes</td>
<td>1490.0±9.14*</td>
<td>1530.0±16.11*</td>
<td>1767.7±9.31</td>
</tr>
<tr>
<td>Erythrocytes-discocytes,%</td>
<td>75.0±0.36*</td>
<td>74.8±0.26*</td>
<td>86.0±0.17</td>
</tr>
<tr>
<td>Reversibly modified erythrocytes,%</td>
<td>14.7±0.17**</td>
<td>14.6±0.17**</td>
<td>9.1±0.05</td>
</tr>
<tr>
<td>Irreversibly modified erythrocytes,%</td>
<td>10.3±0.07**</td>
<td>10.6±0.08**</td>
<td>5.0±0.04</td>
</tr>
<tr>
<td>Sum of all the erythrocytes in an aggregate</td>
<td>45.2±0.25**</td>
<td>44.7±0.22**</td>
<td>32.8±0.10</td>
</tr>
<tr>
<td>Quantity of aggregates</td>
<td>9.7±0.11**</td>
<td>9.8±0.05**</td>
<td>7.5±0.05</td>
</tr>
<tr>
<td>Quantity of free erythrocytes</td>
<td>236.5±0.28*</td>
<td>239.3±0.27*</td>
<td>246.3±0.21</td>
</tr>
</tbody>
</table>

Note: * p<0.05, **p<0.01 – reliability of differences between the groups of observation and control group. Reliable differences between groups of observation weren’t received.

Taken into the research animals after the impact of negative environmental factors were found to have strengthening of erythrocytes’ aggregation (Table 1). So, these piglets had level rise of summary erythrocytes’ involvement into aggregates (on more than 40.0%) and quantity of aggregates themselves in blood (on not less than 29.0%) at reliable content lowering of freely moving erythrocytes in blood.

The reaction of a body on any external factor is mostly determined genetically [21,22]. It covers the field of behavior [23] and functioning of all the vital processes in tissues [24]. At the same time, the character of changes of blood rheological features of milk fed piglets in response to negative impacts of the environment is studied rather poorly.

We chose acute super cooling of a body with subsequent development either arthritis or bronchitis for our research as such negative factor. The choice was conditioned by the fact that super cooling is still rather often met negative environmental factor which causes this pathology in livestock of pigs’ youngsters in Russia.

At both disease states examined milk fed piglets were found to have comparable lowering of antioxidant protectability of blood liquid part leading to similar quantity increase of initial LPO-AHP products and secondary -TBA-active compounds. It burdened already disturbed processes of metabolism. Found intensity increase of plasma LPO processes inevitably caused damage of regular blood elements’ (including erythrocytes) external membranes [25,26] what additionally worsened their functions and conditioned negative dynamics of their surface geometry and ability to unite into aggregates [27].

Piglets after the impact of super cooling were noticed to have similar depression of antioxidative enzymes in erythrocytes leading to LPO intensity in them. Developing metabolic disturbances in their erythrocytes strengthened depression of these cells’ antioxidant protection [28,29]. It was confirmed by the results of functional activity estimation of catalase and superoxide dismutase in them.
Found LPO activation in animals’ bodies inevitably caused damage of lipid bilayer of their membranes from the inside and outside. In combination with developing disturbances in ion and antioxidant erythrocytes’ homeostasis it promoted development of irreversible disturbances of structural-functional status of some erythrocytes [30,31]. It led to the loss of biconcave form by many erythrocytes what worsened their ability to move along small vessels. So, in conditions of arthritis or bronchitis erythrocytes of milk fed piglets began to get transformed in similar degree into spheres with signs of echinocytosis, when the surface of erythrocytes was covered with conical journals, and stomatocytosis, when erythrocytes were like unilaterally arched disks. Further form changes of piglets’ erythrocytes after the impact of negative environmental factors led to development of spher-o-echinocytosis and spha-ro-stomatocytosis, and finally – to transformation of an erythrocyte into a sphereocyte which was the most rigid structure usually preceding break-up of a cell [10].

Found comparability of evidence’ growth of erythrocytes’ aggregation in milk fed piglets at arthritis or bronchitis was mostly connected with similar charge change of erythrocytes because of comparable degradation of proteins with negative charge on their surface in conditions of strengthened LPO [17]. Similar evidence of active oxygen forms’ formation at these states of piglets caused not only similar oxidative damages of membrane’s proteins with negative charge but also peroxide modification of plasma globular proteins. They played the role of “bridges” between erythrocytes at aggregation. All this promoted comparable growth of evidence of cells’ linkage in aggregates. Erythrocytes with weakened antioxidant enzymes became hyperaggregable having a great amount of ferrihemoglobin [32,33] because of the reaction of hydrogen peroxide with hemoglobin. It promoted further changing of erythrocytes’ forms till hemolysis in the result of oxidation of membrane’s lipids. At the same time, as it’s known, surplus LPO products caused evident increase of the threshold of erythrocytes’ disaggregation. They increased the force of erythrocytes’ linkage in aggregates and rose the speed of aggregation on behalf of oxidative changes from the side of membrane’s lipid bilayer [34,35].

Similar worsening of cytoarchitecture and comparable rise of erythrocytes’ aggregative ability in milk fed piglets, having undergone negative impact of environmental factors, inevitably led to disturbance of microcirculation in all the internals [36,37]. Erythrocytes’ aggregation rose till the state of dense, nonvolatile erythrocyte and erythrocyte-platelet embolus [38,39]. It was shown earlier that even little aggregation strengthening of regular elements (including erythrocytes) blocked bloodstream along vessels [40]. That’s why, a part of small vessels could be fully occluded [41]. Besides, irreversible aggregation promoted break-up of erythrocytes and release of coagulation factors into blood [42]. In this connection, negative changing of erythrocytes’ micro rheological features, leading to partial microcirculatory block, promoted development of tissue hypoxia [43,44] similarly in milk fed piglets with arthritis or bronchitis. It inhibited growth and development of tissues, organs and body on the whole and interfered full realization of productive features according to their genetic program.

4. CONCLUSION

Milk fed piglets have similar activation of lipids’ peroxidation at comparable weakening of antioxidant protectability of plasma and erythrocytes in case of the impact of supercooling on their bodies regardless of development of arthritis or bronchitis in them. It is accompanied by comparable quantity rise of erythrocytes’ changed forms in their blood and comparable growth of their ability to aggregation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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