Rheological Properties of Erythrocytes and Blood Coagulation Characteristics of Healthy Milk-Fed Piglets

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Abstract—Investigations are carried out on 32 healthy Large White piglets in the milk-fed stage at ages of 6, 10, 15, and 20 days. By the end of this stage, a tendency toward an increase in the number of discocytes in the piglets’ blood to 86.5% and a decrease in the level of reversibly modified erythrocytes to 8.8% and irreversibly modified to 4.7% is noted. An insignificant increase in the level of total involvement of erythrocytes into aggregates (by 2.5%) and number of aggregates themselves (by 4.1%) with a 3.5% reduction of freely moving erythrocytes is revealed. Piglets aged 6–20 days are characterized by a tendency toward intensification of the activity of individual clotting factors (I, II, VII, VIII, IX, and XII) with unchanged activity of factors V, X, and XI. Gradual acceleration of activated partial thromboplastin time from 38.9 s on day 6 to 37.7 s on day 20 is noted. In this case, prothrombin time decreases slightly by day 20, to 16.7 s. Thrombin time, reflecting the intensity of transition of fibrinogen to fibrin, also accelerates, reaching 16.8 s.

Keywords: piglets, milk-fed stage; erythrocytes, aggregation, cytoarchitecture, blood clotting

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The state of blood circulation has a considerable effect on general functional activity of productive animals, including piglets, supporting all tissue metabolic processes. The most important components of blood rheology needed for preserving an optimal internal environment of piglets at initial stages of ontogeny are erythrocytes determining in many respects the fluid properties of blood due to their adequate aggregation activity and state of their surface geometry with optimal blood clotting capacity. Consequently, age-related dynamics of the functional characteristics of the microrheological properties of erythrocytes and blood coagulation in various biological objects, including in productive animals, is a serious physiological element in maintaining in them tissue anabolic processes, providing the course of development under established conditions of their existence [1, 2].

Despite great progress in biology, veterinary science, and zootechny, the characteristics of the cytoarchitecture and aggregation of erythrocytes as well as the dynamics of activity of clotting hemostasis in healthy piglets in individual stages of ontogeny remain unexplained. In connection with this, the purpose of the present work was to establish the characteristics of the rheological properties of erythrocytes and coagulation properties of blood plasma in healthy milk-fed piglets.

METHOD

For the experiment we selected 32 healthy Large White piglets in which at ages 6, 10, 15, and 20 days we determined lipid peroxidation (LPO) activity of blood plasma on the basis of the content in it of acyl hydroperoxide (AHP) [3], thiobarbituric acid (TBA) reactive substances by an Agat-Med kit, and antioxidant activity (AOA) of the fluid part of blood [4]. Erythrocytes were washed and resuspended for evaluating the intensity of biochemical processes. The expression of LPO occurring inside erythrocytes was determined from the content of AHP in them [3] and from the level of malondialdehyde (MDA) in the thiobarbituric acid reduction reaction [5]. By means of the Vital Diagnosticum kit, we determined in erythrocytes the total cholesterol (TCS) content, and from the level of phosphorus in their membranes the total phospholipid (TPL) content [6] with calculation of the TCS/TPL ratio on the basis of the data obtained. The functional activity of intr erythrocytic antioxidant enzymes was determined for catalase and superoxide dismutase (SOD) [7].

The cytoarchitecture of erythrocytes was evaluated with the use of light phase-contrast microscopy. They were typed according to the following varieties: discocytes; discocytes with one process, with a crest, with multiple processes; mulberry-like erythrocytes, dome-like (stomatocytes), in the form of a flat ball, degenerative forms; spherocytes with a smooth surface and with spines on the surface. The first five classes of erythro-
cytes (with signs of echinocytic transformation) were considered reversibly deformed in view of their ability to spontaneously recover their shape; the others were considered irreversibly deformed or prehemolytic forms [8]. With consideration of the ratio of reversibly and irreversibly altered forms of erythrocytes, the following indices were calculated [1]:

transformation index (TI):
\[ TI = \frac{(RD + ID)}{D}, \]

where D is the percentage of discocytes; RD and ID are the percentage of respectively reversibly and irreversibly deformed erythrocytes;

reversible transformation index (RTI):
\[ RTI = \frac{RD}{D}; \]

irreversible transformation index (ITI):
\[ ITI = \frac{ID}{D}; \]

Reversibility index (RI):
\[ RI = \frac{RD}{ID}. \]

The aggregation activity of erythrocytes was determined with the aid of a light microscope by counting in a Goryaev chamber aggregated and unaggregated erythrocytes and number of aggregates themselves in a suspension of washed erythrocytes with calculation of the average aggregate size (AAS): AAS = SEA/NA; where SEA is the sum of all erythrocytes in the aggregate; NA is the number of aggregates. We also calculated the aggregation index (AI): AI = (AAS × NA + NFE)/(NA + NFE) (where NFE is the number of free erythrocytes) and the percentage of unaggregated erythrocytes (PUA): PUA = (NFE × 100)/(AAS × NA × NFE) [1].

The activity of clotting factors (I, II, VII, VIII, IX, X, XI, and XII) and activated partial thromboplastin time (APTT), prothrombin time, and thrombin time of the animals were evaluated in each investigation [9].

Student’s t test was used for statistical processing of the results obtained.

RESULTS AND DISCUSSION

During the milk-fed stage, we noted an insignificant increase in plasma AOA, from 36.9 ± 0.16 to 38.2 ± 0.12%, promoting a decrease of LPO activity in it. Thus, the content of primary LPO–AHP products in the fluid portion of blood at the start of the stage was 1.34 ± 0.10 D233/ml and at the end 1.29 ± 0.16 D233/ml, and of secondary lipid peroxidation products (TBA reactive compounds), respectively 3.10 ± 0.13 and 2.95 ± 0.08 μmol/l.

In the membranes of erythrocytes (Erc) we noted a tendency toward an increase in cholesterol content from 0.90 ± 0.005 to 0.92 ± 0.005 μmol/1012 Erc with an insignificant decrease in total phospholipid content from 0.70 ± 0.006 to 0.69 ± 0.008 μmol/1012 Erc.

The content of AHP in erythrocytes 6-day-old piglets was 2.90 ± 0.07 D233/1012 Erc and at day 20, 2.82 ± 0.09.4 D233/1012 Erc. In this case, the level of MDA in erythrocytes (end product of LPO) also decreased insignificantly, from 1.01 ± 0.02 to 0.98 ± 0.07 nmol/1012 Erc. The noted dynamics in the level of LPO in erythrocytes was related to a tendency toward intensification of their antioxidant activity, evaluated from the functional activity of catalase, having amounted at the start of the milk-fed stage to 11040 ± 10.8 and at the end to 1750.0 ± 9.24 IU/1012 Erc, with a SOD level of respectively 11 190.0 ± 17.6 and 1788.4 ± 6.82 IU/1012 Erc.

An optimal number of discocytes in blood with a tendency toward an increase by age 20 days and a low level TI were noted (Table 1). In this case, an insignificant reduction in the content of reversibly and irreversibly altered erythrocytes was detected. The noted dynamics of the cytoarchitecture of erythrocytes affected the tendency toward a decrease in RTI and ITI and increase of RI. We found a gradual intensification of the aggregation ability of erythrocytes (Table 1) with increase in the level of total involvement of erythrocytes into aggregates in the bloodstream (by 4.1%) and decrease of the blood content of freely moving erythrocytes (by 3.5%) with constancy of the average aggregate size, aggregate index, and percentage of unaggregated erythrocytes.

A tendency toward intensification of the activity of most clotting factors (I, II, VII, VIII, IX, and XII) with unaltered change in activity of factors V, X, and XI was noted in piglets during the milk-fed stage (Table 2). The length of time of clotting tests is related to the activity of individual clotting factors. Thus, the length of APTT gradually accelerated from 38.9 to 37.7 s. In this case, prothrombin and thrombin times (reflecting the intensity of transition of fibrinogen to fibrin) accelerated insignificantly.

Thus, healthy milk-fed piglets are characterized by an increase in cytoarchitecture and aggregation of erythrocytes with unexpressed intensification of the activity of clotting factors and acceleration of the main clotting tests, which is probably a necessary element of the process of their adaptation to environmental factors in the second stage of early ontogeny.

Hematological changes, inevitably affecting the rheological properties of blood, occur in all productive animals during ontogeny [2]. The high activity of antioxidant enzymes of erythrocytes existing in milk-fed piglets promotes stabilization at a rather low level of LPO processes, which in combination with a decreased content of cholesterol in their membranes provides optimal microrheological properties of erythrocytes. Undoubtedly, this is the physiological basis of the maintenance in the piglets’ bloodstream of a low level of reversibly and irreversibly altered varieties of erythrocytes with a stable prevalence of unaltered forms in their blood. This provides the best rheological properties of blood, sufficient perfusion of internal