Functional properties of platelets in piglets when changing methods of nutrition

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Abstract. A functionally significant element in ensuring homeostasis of the internal environment of an animal organism is platelets. The state of their activity greatly influences the rheology of blood in small vessels and, thus, the metabolism in tissues. It becomes clear that the process of growth and development of piglets at any age substantially depends on the level of their activity. In this regard, the assessment of age-related changes in platelet activity in piglets during their early ontogenesis is of great importance. In the work performed, it was found that in piglets during the dairy and vegetable nutrition phase there is an increase in the adhesive, aggregation and secretory properties of platelets. The leading cause of these changes can be the enhancement of receptor processes and activation of the work of post-receptor mechanisms of information transfer in platelets. This is noted in piglets during the milk-plant nutrition phase simultaneously for both strong and weak aggregation inducers. The growth of platelet activity in piglets found during the observation period apparently was due to the intensification of thromboxane generation in platelets as a result of the increased activity of their cyclooxygenase and thromboxane synthetase, as well as an increase in the secretion of ADP molecules from them. The increase in the severity of the hemostatic properties of platelets in piglets during the milk-plant nutrition phase seems to be a serious regulator of their microcirculation and metabolism processes in any environmental conditions.

1 Introduction

Being the main fluid medium of the whole organism, blood continuously maintains its vitality and the necessary level of adaptation to any environmental conditions [1]. Its enormous biological significance is associated with continuous movement and maintaining the vitality of the animal’s body by delivering the right amount of oxygen and necessary substances to all parts of the body and removing unnecessary metabolites [2, 3]. These processes take place in the lumen of the capillary bed, and in this regard, the success of blood flow in it is very biologically significant [4]. The intensity of metabolism, growth and the overall viability of animals strongly depend on the course of microcirculation processes [5].

These processes are important for all productive animals [6]. The degree of development of all their productive indicators, which means the success of their breeding and cultivation, depends on their intensity [7].

In pigs and pigs, aspects of blood physiology are only beginning to be studied in detail. As before, not all moments of microcirculation in growing pigs can be considered clarified [8]. In earlier studies, much attention was paid to the rheological indices of red blood cells, which are the most numerous group of blood cell forms. They managed to find out the presence of their changes with increasing age and the possibility of a violation in response to various environmental factors. At the same time, the functional abilities of the blood platelets, which play a large role in the microcirculation processes, were given little attention to the piglets.

In the work performed on a person, a high functional role of platelets in ensuring the vital processes and work of hemostasis has been repeatedly noted [9]. This is because platelets are recognized as the initiating link in the functioning of hemostasis and participants in all mechanisms of hemostatic reactions [10]. However, in the body of pigs, many aspects of platelet activity require clarification. This is of great relevance in piglets in the third phase of their early ontogenesis. The study of aspects of platelet activity in piglets of dairy and vegetable nutrition will allow stimulating the development of effective options for the intensification of the processes of growth and development of animals.

In the work performed, the goal was set: to establish the physiological dynamics of the physiological properties of platelets in piglets during the milk-plant nutrition phase.

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 18, 1986, and confirmed in Strasbourg in June 15, 2006).
This work was carried out on 37 completely healthy piglets belonging to the large white breed taken under observation at 21 days of age. All animals were obtained from sows of optimal physiological status by 2–3 litters. All animals in the study were examined 5 times: at the age of 21 days, 25 days, 30 days, 35 days and 40 days of their life.

The activity of platelet aggregation (AP) was determined in piglets using a visual micro-method in response to thrombin (0.125 units/ml), on ADP (dose 0.5×10⁻⁴ M), on H₂O₂ (dose 7.3×10⁻³ M), for collagen (dose 1: 2 of the main suspension), for ristomycin (dose 0.8 mg/ml), for adrenaline (dose 5.0×10⁻⁶ M). This was carried out in the plasma of animals, which had previously been standardized on the level of the platelets contained in it to the value of 200×10⁶ platelets per liter. The state of intravascular platelet aggregation ability was performed using phase contrast.

Mediated in the platelets of the examined piglets, the intensity level of thromboxane synthesis was detected and the enzymatic properties of its platelet generation enzymes, cyclooxygenase and thromboxane synthetase, were determined. This was achieved by determining the level of AP in the course of three transfer tests carried out on a photoelectric colorimeter. At the same time, in the platelets of the observed piglets, the available amount of ADP and the level of its secretion were recorded as a result of exposure to thrombin on the platelets.

All digital results obtained in the work were processed using Student's criterion.

### 3 Results

In the piglets at the stage of milk and vegetable nutrition, a gradual increase in the activity of thromboxane formation in their platelets was revealed. This was indicated by an increase in the level of AP during a simple transfer test (from the level of 41.4±0.06 % to a value of 48.8±0.07 %). This was based on the activation of thromboxane synthetase and cyclooxygenase in the blood plates of piglets.

| Considered hemostatic parameters | The term ontogeny of piglets, n=37, M±m |
|----------------------------------|------------------------------------------|
|                                  | 21 day of life  | 25 day of life | 30 day of life | 35 day of life | 40 day of life |
| The severity of the recovery of platelet aggregation in the collagen-aspirin test, % | 75.0±0.08 | 75.7±0.09 | 76.3±0.06 | 77.7±0.09 | 78.7±0.12 |
| The severity of the recovery of platelet aggregation in the collagen-imidazole sample, % | 64.2±0.07 | 64.9±0.05 | 65.5±0.04 | 66.7±0.08 | 69.6±0.09 |
| The level of platelet aggregation in a simple transfer test, % | 41.4±0.06 | 42.6±0.09 | 43.8±0.10 | 45.2±0.09 | 48.8±0.07 |
| The content of ADP in platelet granules, mmol/10⁹ platelets | 3.36±0.07 | 3.39±0.06 | 3.48±0.05 | 3.61±0.10 | 3.78±0.09 |
| The severity of secretion of ADP from platelets during their stimulation, % | 40.4±0.10 | 41.5±0.09 | 42.8±0.09 | 44.2±0.07 | 46.1±0.12 |
| Platelet aggregation time with ADP, s | 39.4±0.10 | 38.5±0.08 | 37.4±0.05 | 36.7±0.09 | 34.2±0.10 |
| Platelet aggregation time with collagen, s | 29.0±0.08 | 28.4±0.05 | 27.7±0.06 | 26.6±0.08 | 24.5±0.05 |
| Platelet aggregation time with thrombin, s | 41.0±0.07 | 40.1±0.06 | 39.2±0.09 | 38.1±0.03 | 36.0±0.07 |
| Platelet aggregation time with H₂O₂, s | 40.7±0.07 | 40.0±0.07 | 39.1±0.09 | 38.2±0.10 | 37.0±0.06 |
| Platelet aggregation time with ristomycin, s | 40.0±0.06 | 39.0±0.08 | 37.5±0.09 | 36.2±0.10 | 35.3±0.12 |
| Platelet aggregation time with adrenaline, s | 93.1±0.08 | 92.0±0.14 | 90.6±0.09 | 88.5±0.11 | 85.0±0.06 |
| The number of platelets in the aggregates, % | 8.0±0.16 | 8.2±0.09 | 8.4±0.12 | 8.6±0.08 | 9.2±0.11 |
| The number of small units per 100 free platelets | 4.0±0.09 | 4.4±0.07 | 4.9±0.03 | 5.3±0.05 | 6.0±0.09 |
| The number of medium and large units per 100 free platelets | 0.23±0.005 | 0.26±0.006 | 0.29±0.008 | 0.32±0.008 | 0.36±0.007 |

Note: p – the reliability of changes in indicators during the dairy nutrition phase.

The intensity of AP recovery during a collagen-aspirin test, which provides an indirect assessment of the level of cyclooxygenase activity in the cytoplasm of platelets, increased from 75.0±0.08 to 78.7±0.12 %. The level of AT recovery during a collagen-imidazole test, which provides an indirect elucidation of platelet thromboxane synthetase activity level in platelets, also increased in the examined piglets during the observation from 64.2±0.07 to 78.7±0.12 %.
to 69.6±0.09%. At the same time, the piglets during the milk-plant nutrition phase showed an increase in the amount of ADP in their platelets (by 12.5%) and an intensification of the activity of its release from the granules (by 14.1%).

In the blood of the piglets taken in the study, normal platelet levels were noted. In animals at the age of 21 days of life, AT in response to collagen occurred in 29.0±0.08 s. This time gradually decreased to 24.5±0.05 s by the end of the observation (Table 1).

A similar acceleration of the AP process in piglets during the milk-vegetable nutrition phase occurred in response to ADP – by 15.2%, H2O2 by 10.0% and ristomycin by 13.3%. A little later, AP appeared in response to thrombin (by the end of the phase, AP appeared with it for 36.0±0.07 s) and AP developed in response to adrenaline (by the end of the phase it was realized in 85.0±0.06 s).

The increase in the number of platelet aggregates freely moving through the blood was found in animals of milk and vegetable nutrition. Their number reached, on the 40th day of life, 6.0±0.09 per 100 free blood plates and 0.36±0.007 per 100 free blood plates. At the same time, an increase in the number of platelets involved in aggregation by 15.0% was observed in the examined piglets during the observation period.

4 Discussion
Increasing the volume of pig production is possible only with continued accumulation of knowledge on all aspects of piglet physiology [11, 12]. This will help in the course of their application in practice to achieve greater vitality of animals and seriously accelerate the processes of their development and growth [13]. Of particular importance in this regard are systems that support homeostasis, including hemostasis [14, 15]. This system includes physiologically significant platelets [16, 17].

It is believed that their activity during ontogenesis essentially determines the state of blood rheology in the capillaries and thus the level of metabolism activity in the tissues [18]. Due to the large physiological role of the level of platelet activity and the main mechanisms for their realization, it was necessary to evaluate their peculiarities during the change in dietary patterns in piglets.

Considering the obtained results, it is possible to assume that the ability of platelets to adhesion increases in piglets during the milk-plant nutrition phase. The basis of this is the increase in the content of von Willebrand factor in their blood, which acts as a cofactor for adhesion of platelets and an increase in the density of receptors to it on platelet membranes [19, 20]. Piglets say this by intensifying platelet aggregation using ristomycin [21].

This is due to the fact that it affects platelets as vascular subendothelial fibers [22]. During adhesion of von Willebrand factor, it binds to collagen, the second end to the platelet via the glycoprotein Ib. This forms a chain that provides an adhesion process consisting of collagen-von Willebrand factor and glycoprotein Ib [23].

Found acceleration of AT in response to other inducers also showed an increase in the number of receptors on blood plates in piglets between 21 and 40 days of ontogenesis [24, 25]. It also spoke of the activation of the platelet mechanisms of antibodies in piglets at this age in response to strong and weak inducers of in vitro aggregation similar to those that implement the process of platelet aggregation in vivo [26].

A significant mechanism of its activation in piglets during the milk-plant nutrition phase is increased expression on the surface of their membranes to the fibrinogen receptor [27].

This is accompanied by an increase in the catalytic activities of their phospholipids, which caused an increase in the formation of active factor X and thrombin on them, which strongly stimulated the functioning of hemostasis in general [28].

5 Conclusion
Strengthening of platelet hemostasis in piglets that are in the dairy-plant nutrition phase is obviously associated with a pronounced increase in the functioning of the mechanisms of perception and post-receptor processes of participation in hemostasis in them. At this age in piglets, this leads to increased platelet adhesion, aggregation and secretion.

The growth of platelet activity in piglets during the milk-plant nutrition phase can be attributed from physiological points of view to the need to maintain microcirculation at the optimal level in their tissues, which, on the one hand, corresponds to piglet species, and, on the other hand, to environmental factors.

An increase in platelet activity in piglets during the milk-plant nutrition phase should be considered as one of the biologically significant adaptive reactions that can affect their growth and development.

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