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Physiological Aspects Of Platelet Aggregation In Piglets Of Milk Nutrition.

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ABSTRACT

A particularly physiologically significant mechanism for maintaining homeostasis in the animal's body is platelet hemostasis. Its activity largely determines the rheology of blood in the capillaries and thereby trophic tissues and the rate of development of the animal. This statement is also valid for the phase of dairy nutrition. There is a point of view that the success of the formation and development of the body structures of piglets, as well as the development of their functional parameters, largely depends on the level of functional activity of platelets. In this regard, great importance is the study of the age-related dynamics of platelet activity in piglets during the phase of dairy nutrition. The study found that the piglets of milk nutrition has an increase in the adhesive and aggregation capacity of platelets. At its base, apparently, lies the intensification of the functioning of receptor and postreceptor mechanisms of platelets. The found regularity was characteristic of piglets to the same extent with respect to strong and weak inducers of platelet aggregation. A major role in increasing the activity of platelets in piglets during the phase of milk nutrition belongs to the intensification of the metabolism of arachidonic acid in platelets by cyclooxygenase and thromboxane synthetase and the activation of secretion from them of ADP. The increase in the functional activity of platelets in piglets during the phase of milk nutrition, apparently, is an important component of adaptation of their microcirculation and regulation of trophic tissue to the current conditions of life.

Keywords: piglets, milk nutrition phase, platelets, aggregation, adhesion, secretion.

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INTRODUCTION

It is now recognized that the hemostasis system is very complex [1,2]. The processes of its functioning ensure the preservation of the liquid state of blood in the vessels [3,4] and rapid local formation of the thrombus when the vessel wall is damaged [5,6,7]. The uninterrupted functioning of the entire system of hemostasis ensures the effective preservation of the viability of the organism [8,9]. In view of the fact that hemostasis strongly influences the fluidity of blood along the vessels [10, 11], there is no doubt that it is of great importance for the realization of trophic tissue and the course of metabolic processes in mammalian tissues [12,13], including productive animals [14].

It was previously shown that the optimal activity of hemostasis is accompanied by high viability of animals at all stages of their ontogenesis [15, 16]. In this connection, the need for further detailed study of many aspects of the physiology of hemostasis becomes clear. The receipt of new information on its functioning will allow further intensification of breeding of farm animals, including pigs. This is due to the fact that hemostasis is an important "point" of potential impact on the functional state of the whole organism, especially those that have fallen into unfavorable environmental conditions [17,18,19].

High functional significance of platelets for the work of all hemostasis is due to the fact that they are the initial link and the participant of almost all haemostatic processes in the body [20,21]. Of great interest is the clarification of the features of platelet activity in pigs in the second phase of early ontogeny. High interest in the functional activity of platelet hemostasis in piglets during the phase of dairy nutrition is associated with the effect of their activity on the formation of body structures and the development of their functional activity [22,23]. In this regard, obtaining information on various aspects of platelet activity in piglets of dairy nutrition can be a serious basis for developing options for intensifying their growth and development [24].

In this connection, the goal was formulated: to study the physiological features of platelet activity 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 18, 1986, and confirmed in Strasbourg in June 15, 2006) and approved by the local ethic committee of Federal State Budgetary Educational Institution of Higher Education "Vologda State Dairy Farming Academy by N.V. Vereshchagin" (Record №12 dated December 3, 2015), the local ethic committee of All-Russian SII of Physiology, Biochemistry and Animals' feeding (Record №11, dated December 4, 2015) and the local ethic committee of Russian State Social University (Record №16, dated December 7, 2015).

The study was performed on 35 healthy pigs of large white breed, taken in the study on the 6th day of life. Piglets were examined and examined 4 times: 6 days, 10 days, 15 days, 20 days of life. All the examined piglets were obtained from healthy sows 2-3 farrowing.

In animals, the levels of platelet aggregation (AP) activity in vitro were determined using a visual micrometer using inductors: thrombin (0.125 U/ml), ADP (0.5×10^{-4} M), H_2O_2 (7.3×10^{-3} M), collagen (1: 2 dilution of the main suspension), ristomycin (0.8 mg/ml), adrenaline (5.0×10^{-6} M) in plasma, which was standardized for platelets to 200×10^9 platelets [25]. Intravascular platelet aggregation activity was assessed using a phase contrast microscope [26].

In the thrombocytes of all the pigs examined, an indirect method was used to determine the intensity of the exchange of arachidonic acid contained in the membranes, taking into account the level of enzymatic activity of platelet cyclooxygenase and thromboxane synthetase. This occurred during the registration of AP in three transport samples on a photoelectric colorimeter [27]. In platelets of all animals, the content of ADP and the intensity of its secretion during the process of thrombocyte stimulation by thrombin were evaluated [26].

The results of the study were processed using the Student's test.

RESULTS

In the blood of the pigs taken under observation a normal amount of platelets was detected. At them on the 6th day of life, the AP with collagen attacked 32.3 ± 0.07 s. Its time gradually decreased to 28.8 ± 0.05 s by the end of the phase of dairy nutrition (Table 1). Similar acceleration of AP in piglets during milk feeding was noted under the action of ADP - by 7.9%, H_2O_2 by 9.3% and ristomycin by 12.4%. Somewhat later, trombin AP appeared (by the end of the phase in 50.3 ± 0.10 s) and adrenaline AP (by the end of the phase in 92.0 ± 0.12 s).

In the blood of piglets of dairy nutrition, the levels of free-flowing small and large platelet aggregates gradually increased, amounting to 4.1 ± 0.07 per 100 free-standing platelets and 0.24 ± 0.005 per 100 free-standing platelets on day 20 of life. Under these conditions, the number of platelets in the piglets at the end of the observation, which entered the aggregation process, increased by 10.9%, which indicated an increase in their platelet aggregation *in vivo*.

A physiologically important mechanism for increasing the hemostatic activity of platelets in piglets of milk nutrition can be considered to enhance the intensity of the metabolism of arachidonic acid with activation of thromboxane formation. This was indirectly indicated by an increase in AP in a simple transfer sample (from $38.6 \pm 0.03\%$ to $41.9 \pm 0.05\%$). This intensification of the metabolism of arachidonic acid in the blood plates of pigs was due to the revealed activation of both enzymes of its transformation in platelets - cyclooxygenase and thromboxane synthetase. The degree of AP recovery in the collagen-aspirin test, which allows to indirectly assess the activity of cyclooxygenase in platelets, increased during the observation from $69.1 \pm 0.07\%$ to $75.3 \pm 0.08\%$. The severity of the recovery of AP in the collagen-imidazole sample, which allows to indirectly evaluate the functional properties of thromboxane synthetase in blood plates, also increased in the course of observation in piglets from $59.3 \pm 0.05\%$ to $64.7 \pm 0.09\%$.

A very important mechanism for increasing the functional activity of platelets in piglets during the phase of milk feeding can be considered as the increase in the content of ADP (by 8.7%) found in the study and the activity of its secretion (by 16.0%).

DISCUSSION

At present, a very large body of knowledge on the physiology of piglets is collected, however, some of their aspects need to be supplemented and refined [28,29]. This is especially important for practical biology, which applies the results of studying vital systems in the animal organism for the intensity of their development [30]. These systems, supporting homeostasis, include a system of hemostasis, in which platelets play an important role [31]. The state of their activity throughout the ontogenesis largely determines the rheology of blood in the microcirculatory bed and, due to this, the activity of metabolism in tissues [32,33]. Given the physiological importance of platelet hemostasis activity and the mechanisms of its realizing, it was necessary to clarify their condition in piglets during the phase of milk nutrition [34].

Estimating the results obtained in the study, it becomes clear that in healthy piglets during the phase of the dairy diet, the adhesive capacity of the blood platelets gradually increases. This was due to a simultaneous increase in the level of their von Willebrand factor (WF) factor in their blood, which is a cofactor of platelet adhesion and an increase in the number of receptors for it (GPIb) on their membranes [35]. The increase in the level of activity of these mechanisms in the examined pigs was judged by the acceleration of the process of aggregation of their platelets in response to ristomycin. It is known that it has the ability to influence platelets, like subendothelial vascular structures [36]. During adhesion, WF is connected by one end of the molecule to the collagen, and the other to the platelet through the platelet receptor-glycoprotein Ib [37]. As a result, there is a chain of adhesion: collagen - WF - GPIb. For this reason, the acceleration of AP with ristomycin indicates the development in piglets during milk feeding of an increase in the number of these receptors on the platelet membranes. The detected acceleration of AP under the influence of other inducers also showed an increase from 6 to 20 days of life in piglets of the number of receptors to them on the surface of the blood platelets.

The registration of AP in response to strong and weak inducers of *in vitro* aggregation in piglets aged between 6 and 20 days of life made it possible to reveal the specific features of their influence on platelets along the ways of their activation, which works with platelet aggregation *in vivo* [38].

An increase in the number of platelet aggregates in the blood of piglets of milk nutrition is undoubtedly caused by increased work and platelet receptors and postreceptor mechanisms [39]. This was manifested by an increase in the hemostatic activity of platelets, as judged by the dynamics of their adhesion, aggregation and secretion. Great importance in this was the growth of expression on their membrane of different receptors and especially fibrinogen receptors (GP IIb – IIIa). The inevitable enhancement of the catalytic properties of the phospholipids of their membranes in these conditions increased the generation of factor Xa and thrombin on it, which stimulated the work of all hemostasis [40,41,42].

Significant intra-platelet mechanisms of increasing the functional activity of platelets in piglets during the phase of milk nutrition can be considered the developing intensification in them of the metabolism of arachidonic acid due to the increase in the activity of platelet enzymes - cyclooxygenase and thromboxane synthetase. This was also enhanced by the accumulation of ADP in the platelet granules and an increase in the intensity of its secretion from them.

CONCLUSION

The increase in the number of platelet aggregates in the blood of piglets of milk nutrition was associated with an increase in the functioning of receptor and postreceptor mechanisms in platelets. This provided the piglets at this age with an increase in their adhesion, aggregation and secretion. The growth of platelet activity in piglets during the phase of milk nutrition determines the optimal level of microcirculation in tissues, which is adequate, on the one hand, to their genetic program, and on the other hand, to the influences of the external environment. In this connection, the increase in platelet activity in piglets during the phase of milk nutrition can be considered a species adaptive reaction that affects their growth and development.

Table 1. Parameters of functional activity of thrombocytes in piglets of milk nutrition

Indicators	Milk phase, n=35, M±m			
	6 day of life	10 day of life	15 day of life	20 day of life
Aggregation of platelets with ADP, s	42.2±0.12	41.9±0.10	40.3±0.07	39.1±0.09 p<0.05
Aggregation of platelets with collagen, s	32.3±0.07	30.6±0.06	29.7±0.09	28.8±0.05 p<0.05
Aggregation of thrombocytes with thrombin, s	54.3±0.06	53.2±0.08	52.4±0.11	50.3±0.0 p<0.05
Aggregation of platelets with H ₂ O ₂ , s	44.5±0.08	43.6±0.09	42.4±0.11	40.7±0.07 p<0.05
Aggregation of platelets with ristomycin, s	44.3±0.10	43.2±0.06	40.8±0.08	39.4±0.06 p<0.05
Aggregation of platelets with adrenaline, s	97.5±0.15	96.0±0.14	94.4±0.10	92.0±0.12
Recovery of platelet aggregation in a collagen-aspirin test, %	69.1±0.07	70.2±0.08	72.4±0.10	75.3±0.08 p<0.05
Restoration of platelet aggregation in a collagen-imidazole sample, %	59.3±0.05	60.5±0.06	62.8±0.09	64.7±0.09 p<0.05
Aggregation of platelets in a simple transfer sample, %	38.6±0.03	39.8±0.05	40.7±0.04	41.9±0.05 p<0.05
The content of ADP in platelets, mmol/10 ⁹ platelets	3.11±0.11	3.18±0.08	3.25±0.10	3.38±0.09
The degree of secretion of ADP from platelets on the background of stimulation, %	35.0±0.10	36.8±0.09	38.5±0.07	40.6±0.11
The number of platelets in the aggregates, %	7.3±0.12	7.5±0.10	7.7±0.09	8.1±0.08 p<0.05

The number of small aggregates of 2-3 platelets per 100 freely lying platelets	3.4±0.06	3.5±0.08	3.7±0.09	4.1±0.07 p<0.05
The number of medium and large aggregates, 4 or more platelets, per 100 free-lying platelets	0.16±0.005	0.18±0.004	0.21±0.006	0.24±0.005 p<0.05

Legend: p - reliability of the dynamics of the phase of the dairy nutrition taken into account with respect to the beginning.

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