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Physiological Features of Platelets in Milk and Vegetable Nutrition Piglets

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A very important mechanism for maintaining homeostasis in the animal body is platelet hemostasis. The level of his activity very strongly determines the rheology of blood in the capillaries and due to this the state of metabolism in the tissues. There is reason to believe that the growth and development of piglets depends on the level of functional activity of platelets. For this reason, studies of the age-related dynamics of platelet activity in piglets during early ontogeny are of great importance. In the study, it was established that piglets of milk and vegetal nutrition showed an increase in the adhesive, aggregation and secretory capacity of platelets. The main reason for this may be an intensification of the work of receptor and postreceptor mechanisms of platelets. This was characteristic of piglets during dairy and plant nutrition in the same degree with respect to strong and weak inducers of platelet aggregation. At the base of the revealed increase in the activity of platelets in piglets throughout the course of the observation, the increase in the synthesis of thromboxane in platelets was largely due to the increase in the activity of cyclooxygenase and thromboxane synthetase in them and activation of the secretion of ADP from them. The increase in hemostatic activity of platelets in piglets during the phase of milk and plant nutrition should be considered an important regulator of their microcirculation and metabolism in tissues under changing conditions of existence.

Key words: piglets, milk and vegetable nutrition phase, platelets, aggregation, adhesion, secretion.

Being a liquid medium of the body, the blood ensures its integration and regulation in accordance with constantly changing environmental conditions[1,2]. The main role of blood is to maintain the viability of the organism every minute due to the constant transfer of oxygen[3] and nutrients to all cells of the body[4] and the removal of metabolic products from them[5,6]. These processes occur in the capillary channel[7,8] and therefore the success of the blood flow in it has a particularly great physiological significance[9,10]. From the success of microcirculation all metabolic processes[11], growth[12] and the level of resistance of the living organism depend[13].

These processes play a particularly important economic role in productive animals[14]. This is due to the fact that the level of realization of the productive qualities of animals depends on their success and, ultimately, the economic efficiency of their breeding and rearing[15,16].

In pigs, the physiology of the blood is only beginning to be studied. Not all microcirculation issues for piglets of different ages have been clarified. In previous studies, attention was

mainly paid to the rheological properties of erythrocytes, which are the largest population of blood cells[17]. The possibility of their age dynamics and disturbance due to environmental factors was elucidated[18]. At the same time, the functional aspects of platelets, which strongly influenced microcirculation processes in pigs, were given unnecessarily little attention.

In human studies, the high functional significance of platelets for the work of all hemostasis is recognized. It is associated with the fact that platelets are the initial link in the work of hemostasis and participants in almost all haemostatic processes in the body[19,20]. It is necessary to clarify the features of platelet activity in pigs in the third phase of early ontogeny. A special interest in the functional activity of platelet hemostasis in piglets at this age is associated with the effect of their activity on the formation of the body's structures and the development of their functional activity during the change in the mode of feeding[21]. The new information obtained on various aspects of platelet activity in piglets of milk and plant nutrition can serve as the basis the development in the future of effective options for intensifying their growth and development[22].

In this connection, the goal was formulated: to evaluate the physiological dynamics of platelet activity in piglets during the phase of milk and vegetable 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 work was carried out on 37 healthy pigs of large white breed, taken in the study for 21 days of life. All piglets were obtained from healthy sows with 2-3 farrowing. Then the piglets were inspected and examined 5 times: for 21 days, for 25 days, for 30 days, for 35 days and for 40 days of life.

In a study in piglets, platelet aggregation (AP) was assessed in vitro by a visual micromethod with a series of inducers: with thrombin (0.125 U/ml), with ADP (0.5×10^{-4} M), with H_2O_2 (7.3×10^{-3} M), with collagen (dilution 1: 2 main suspension), with ristomycin (0.8 mg/ml), with adrenaline (5.0×10^{-6} M). This was done using their plasma, which was standardized for platelet count to a level of $200x10^9$ platelets[23]. The expression of intravascular platelet aggregation was determined using a phase contrast microscope[23].

Indirectly in the platelets taken in the study of piglets, the intensity of formation of thromboxane was determined and the enzymatic activity of the platelet enzymes of its synthesis (cyclooxygenase and thromboxane synthetase) was determined. This was done by recording the AP in three transfer samples on a photoelectric colorimeter. In addition, in the platelets of all animals, the quantitative content of ADP and the degree of its secretion after platelet thrombin stimulation were evaluated.

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

RESULTS

The blood of the pigs examined contained a normal number of platelets. In pigs on the 21st day of life, AT with collagen attacked 29.0±0.08 s. This value gradually decreased to 24.5±0.05 s by the end of the phase of milk and vegetable nutrition (Table 1). A similar acceleration of AT in piglets during the phase of milk and vegetable nutrition was observed under the action of ADP - by 15.2%, H_2O_2 by 10.0% and ristomycin by 13.3%. Somewhat later, AT developed with thrombin (by the end of the phase for 36.0±0.07 s) and AP with adrenaline (by the end of the phase in 85.0±0.06 s).

In the blood of piglets of milk and vegetable nutrition, a gradual increase in the number of free-moving small and large platelet aggregates is revealed. Their number at the 40th day of life was 6.0±0.09 per 100 free-standing platelets and 0.36±0.007 per 100 free-standing platelets. This was accompanied by an increase

in piglets during the observation of the number of platelets that entered the aggregation process by 15.0%.

Piglets of milk and vegetable nutrition found a gradual increase in intensity in platelets of thromboxane formation. This was judged by the increase in the AP level in a simple transfer sample (from 41.4±0.06% to 48.8±0.07%). This turned out to be possible as a result of activation in the blood plates of piglets of both enzymes of its synthesis - 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 period from 75.0±0.08% to 78.7±0.12%. The severity of AP recovery in the collagen-imidazole sample, which allows to indirectly ascertain thromboxane synthetase activity in blood plates, also increased in piglets during observation from 64.2±0.07% to 69.6±0.09%. This was accompanied in piglets during the phase of milk and vegetable nutrition by increasing the content of their platelets ADP (by 12.5%) and increasing the activity of its secretion (by 14.1%).

Table 1. Thrombocyte activity in piglets of milk and vegetable nutrition

Parameters	Milk phase, n=37, M±m				
	21 days of life	25 days of life	30 days of life	35 days of life	40 days of life
Aggregation of platelets with ADP, s	39.4±0.10	38.5±0.08	37.4±0.05	36.7±0.09	34.2±0.10 p<0.05
Aggregation of platelets with collagen, s	29.0±0.08	28.4±0.05	27.7±0.06	26.6±0.08	24.5±0.05 p<0.05
Aggregation of platelets with thrombin, s	41.0±0.07	40.1±0.06	39.2±0.09	38.1±0,03	36.0±0,07 p<0.05
Aggregation of platelets with H_2O_2, s	40.7±0.07	40.0±0.07	39.1±0.09	38.2±0.10	37.0±0.06 p<0.05
Aggregation of platelets with ristomycin, s	40.0±0.06	39.0±0.08	37.5±0.09	36.2±0.10	35.3±0.12 p<0.05
Aggregation of platelets with adrenaline, s	93.1±0.08	92.0±0.14	90.6±0.09	88.5±0.11	85.0±0.06 p<0.05
Restoration 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 p<0.05
Restoration of platelet Aggregation in the Collagen-imidazole test, %	64.2±0.07	64.9±0.05	65.5±0.04	66.7±0.08	69.6±0.09 p<0.05
Aggregation of platelets 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 p<0.05
The content of ADP in Platelets, mmol /10^9 Platelets	3.36±0.07	3.39±0.06	3.48±0.05	3.61±0.10	3.78±0.09
The degree of secretion Of ADP from platelets on The background of Stimulation,%	40.4±0.10	41.5±0.09	42.8±0.09	44.2±0.07	46.1±0.12
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 p<0.05
The number of small Aggregates of 2-3 Platelets per 100 freely Lying platelets	4.0±0.09	4.4±0.07	4.9±0.03 p<0.05	5.3±0.05 p<0.05	6.0±0.09 p<0.01
The number of medium and large aggregates, 4 or more platelets, per 100 free-lying platelets	0.23±0.005	0.26±0.006	0.29±0.008	0.32±0.008 □<0.05	0.36±0.007 □<0.01

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

DISCUSSION

Further intensification of pig production is possible with an additional accumulation of knowledge on the physiology of pigs[16,18]. Their application in practice to strengthen the work of vital systems can seriously accelerate their development and growth[24]. These systems, supporting homeostasis, include a system of hemostasis. In its functioning, a very large role is played by platelets[25,26]. It is their activity during the whole ontogenesis that largely determines the rheology of blood in the microcirculatory bed and, thereby, the activity of metabolism in all tissues[27,28]. Taking into account the physiological importance of platelet hemostasis activity and the mechanisms of its realization, it was necessary to clarify their condition during the change in their feeding patterns in piglets, that is, during the phase of dairy-plant nutrition[22].

Based on the results obtained, we can say that in healthy piglets during the phase of dairy-plant nutrition the adhesive capacity of blood plates gradually increases. This is based on a simultaneous increase in the level of their factor of von Willebrand (FW) 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[29]. Both processes were judged by the acceleration of the process of aggregation of their platelets in response to ristomycin. This is due to the fact that it is able to influence platelets, as well as subendothelial vascular structures[30,31]. With the development of adhesion, FW is joined by one end of the molecule with collagen, and the other with a platelet through the platelet receptor - glycoprotein Ib[32,33]. This leads to the emergence of a chain of adhesion: collagen - FW - GPIb. The detected acceleration of AP under the influence of other inducers also proved an increase between 21 and 40 days of life in piglets of the number of receptors to them on the surface of their blood plates. In addition, this indicated activation of platelet AP pathways in response to strong and weak inducers of in vitro aggregation similar to those that realize platelet aggregation in vivo[34,35]. An important mechanism for its amplification in the observed pigs with all inducers should be considered an increase in the expression on their membrane of fibrinogen receptors (GP IIb-IIIa). In this case, the catalytic properties of the phospholipids of their membranes inevitably increase with the growth of active factor X and thrombin on them, which further stimulates the work of all hemostasis[36,37].

The increase in the content of platelet aggregates in the blood of piglets during the phase of milk and vegetarian nutrition proves the strengthening of their in vivo functioning of platelet receptors and postreceptor mechanisms[38]. This led to an increase in their activity of adhesion, aggregation and secretion of platelets.

Also, the highly functional intra-platelet mechanisms that promote the growth of platelet activity in piglets during the phase of dairy-plant nutrition should be considered to increase their synthesis of thromboxane by increasing the activity of enzymes of its synthesis - cyclooxygenase and thromboxane synthetase. This was supplemented in piglets at this age by an increase in accumulation in dense platelet granules of ADP and an increase in its secretion from them.

CONCLUSION

The increase in platelet activity in piglets of milk and vegetable nutrition should be associated with the intensification of the functioning of receptor and postreceptor mechanisms in them. In piglets at this age, this is manifested by an increase in their adhesion, aggregation and secretion. The revealed growth of platelet activity in piglets of milk and vegetable nutrition should be associated with the need to maintain microcirculation in tissues at a necessary level, which corresponds, on the one hand, to the specific features of the animal, and, on the other hand, to the action of environmental factors. The increase in platelet activity in piglets during the phase of milk and plant nutrition should be considered one of the species adaptive reactions that can affect their growth and development.

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