Functional features of vascular hemostasis in piglets of milk and vegetable nutrition

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Abstract. The beginning of the intake of plant feed by the piglet is a serious stage in the maturation of its regulatory systems. Under these conditions, vascular hemostasis, which significantly controls the level of blood flow to tissues, is an essential element in maintaining homeostasis of a growing animal organism. Examination of 25 healthy piglets during the phase of milk and vegetable nutrition revealed a gradual decrease in the amount of peroxidation products in their blood due to increased activity of the antioxidant potential of their plasma. Healthy piglets of dairy and plant nutrition are characterized by a low level of endotheliocythemia, combined with an age-related increase in the synthesis of substances with anti-aggregation activity, antithrombin III and tissue plasminogen activators in the vascular wall. Piglets of dairy and plant nutrition are characterized by a weakening of lipid peroxidation in the liquid part of the blood, which in many respects increases the antiaggregation, anticoagulation and fibrinolytic capabilities of the vessel walls. This supports the adequacy of the vascular control of piglets at this age over hemostasis as a whole.

1. Introduction
The beginning of the intake of plant feed by the piglet is a serious stage in the maturation of its regulatory systems [1]. Under these conditions, vascular hemostasis, which significantly controls the level of blood flow to tissues, is an essential element in maintaining homeostasis of a growing animal organism [2]. The age-related dynamics of hemostatic activity of the vascular wall in piglets during a change in nutrition, consisting of their antiplatelet, anticoagulant, and fibrinolytic abilities, largely determines the state of microcirculation in tissues and, therefore, the level of metabolic processes in them [3].

The state of control of the vascular wall over hemostasis in the phase of milk-vegetable nutrition significantly determines the growth and development of the animal throughout the entire subsequent ontogenesis, laying the foundations of the productive properties of the pig [4]. Despite the high scientific and practical importance of the hemostatic activity of the vascular wall in piglets during the phase of dairy and plant nutrition, its features remained poorly understood, dictating the need to plan and conduct a real study.
The purpose of the work is to find out the age-related dynamics of hemostatic activity of the vascular wall in healthy piglets during the phase of milk-vegetable nutrition.

2. Materials and research 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 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).

Large white piglets under the supervision of 25 healthy dairy and plant nutrition pigs were examined and examined 5 times: 21 day, 25 day, 30 day, 35 day and 40 day.

The intensity of plasma lipid peroxidation was recorded in all animals, evaluating the level of acyl hydroperoxides [5] and thiobarbituric acid, the active products in it, using the Agat-Med company kit (Russia) with the manifestation of the antioxidant activity of the liquid part of the blood [6].

The value of endotheliocythemia in piglets was evaluated [7]. The anti-aggregation ability of the vascular wall [8] was recorded based on the visual micromethod of platelet aggregation registration [9] using ADP (0.5 × 10−4 M), collagen (1: 2 dilution of the main suspension), thrombin (0.125 units/ml), ristomycin (0.8 mg/ml) and adrenaline (5.0 × 10−6 M.) in plasma with a standardized platelet count of up to 200 × 10^9 platelets per liter of plasma before and after temporary venous occlusion. The index of anti-aggregation activity of the vascular wall was calculated by dividing the time of platelet aggregation against the background of venous occlusion by the time of platelet aggregation without it.

The examined piglets also calculated the index of anticoagulation activity of the vessel wall by dividing the activity of antithrombin III [10] in plasma taken after venous occlusion by its activity in plasma taken without it [8].

The fibrinolytic ability of the vascular wall was determined by recording the lysis time of the fibrin clot before and after temporary venous occlusion, which causes the release of tissue plasminogen activator from the vessel wall into the blood [8]. The index of fibrinolytic activity of the vascular wall was calculated by dividing the time of lysis of a fibrin clot in plasma taken without venous occlusion by the time of lysis without it.

The results obtained in the course of research were processed using Student's criterion (td).

3. Research results
Piglets during the phase of milk and plant nutrition showed a significant decrease in the content of acyl hydroperoxides and thiobarbituric acid-active compounds in the blood plasma from 1.37±0.08 D_233/1ml to 1.25±0.05 D_233/1ml and from 3.18±0.13 μmol/l to 3.01±0.10 μmol/l, respectively. This was due to a gradual increase in their antioxidant plasma protection from 34.7±0.12% at the beginning of the third phase of early ontogenesis to 38.2±0.11% at its end.

Piglets of dairy and plant nutrition turned out to be characterized by a high integrity of the endothelial lining, which could be judged by a stably low endotheliocythemia (on average 1.3±0.08 cells/μl). This very much ensured in these piglets a high synthesis of hemostatically active substances in the vessel wall.

In the observed animals, during the third phase of their early ontogenesis, a gradual increase in the values of the index of antiplatelet activity of the vessel walls with all the inducers used was noted (table 1).

The minimum values of the index of anti-aggregation activity of the vessel walls are noted for thrombin in view of the highest activity of this inducer and the least severity of inhibition of platelet aggregation with it in a sample with venous occlusion. With other inducers, a higher level of the index of anti-aggregation activity of the vessel walls was recorded, the values of which were observed in piglets to increase during the entire phase of milk-vegetable nutrition.
The observed piglets showed a gradual increase in the anticoagulant activity of the vascular wall from the 21st to the 40th day of life, as indicated by the found increase in the activity of antithrombin III in their blood before the test with temporary venous occlusion and after it (table 1). An increase in the activity of antithrombin III to 99.6 ± 0.12% was detected in the blood of piglets of milk-vegetable nutrition against the background of an increase in its production by 7.1% of endotheliocytes. This ensured their growth with age of the index of anticoagulation activity of the vessel wall in animals to 1.36±0.11.

**Table 1.** Hemostatic properties of blood vessels in piglets of milk and vegetable nutrition.

| Considered indicators | Milk plant phase, n=25, M±m | 21 day of life | 25 day of life | 30 day of life | 35 day of life | 40 day of life |
|-----------------------|-----------------------------|---------------|---------------|---------------|---------------|---------------|
| Vascular wall antiaggregation index with ADF | 1.72±0.05 | 1.76±0.07 | 1.81±0.06 | 1.87±0.09 | 1.92±0.05 |
| Collagen vascular wall antiaggregation activity index | 1.70±0.08 | 1.74±0.06 | 1.82±0.03 | 1.86±0.05 | 1.93±0.08 |
| Vascular wall antiaggregation index with thrombin | 1.50±0.06 | 1.53±0.03 | 1.56±0.02 | 1.60±0.04 | 1.65±0.07 |
| Vascular wall antiaggregation index with ristomycin | 1.68±0.05 | 1.71±0.04 | 1.75±0.05 | 1.80±0.06 | 1.85±0.09 |
| Vascular wall antiaggregation index with adrenaline | 1.73±0.03 | 1.77±0.08 | 1.80±0.10 | 1.84±0.08 | 1.89±0.08 |
| Plasma antithrombin III activity before compression test, % | 97.9±0.05 | 98.2±0.08 | 98.5±0.12 | 98.8±0.06 | 99.6±0.12 |
| Plasma antithrombin III activity after compression test, % | 126.2±0.16 | 128.9±0.05 | 130.8±0.10 | 131.9±0.08 | 135.2±0.13 |
| Vascular wall anticoagulant activity index | 1.29±0.08 | 1.31±0.07 | 1.33±0.06 | 1.33±0.09 | 1.36±0.11 |
| The fibrin clot lysis time before compression, min. | 8.2±0.04 | 8.1±0.05 | 7.8±0.03 | 7.7±0.06 | 7.6±0.16 |
| The fibrin clot lysis time after compression, min. | 5.8±0.07 | 5.4±0.09 | 5.2±0.12 | 5.0±0.10 | 4.8±0.08 |
| Vascular wall fibrinolytic activity index | 1.41±0.05 | 1.50±0.08 | 1.50±0.05 | 1.54±0.08 | 1.58±0.05 |

Legend: p - the reliability of the age dynamics of the considered indicators.

When assessing the fibrinolytic activity of the vascular wall in piglets of milk and vegetable nutrition, a decrease in the time of spontaneous lysis of the fibrin clot by 7.9% was noted. Moreover, during the phase of dairy and plant nutrition, the secretion of tissue plasminogen activators, provoked by the creation of temporary venous wall ischemia, increased (the index of fibrinolytic activity of the vascular wall increased by 12.0%).
4. Discussion
The beginning of consumption of plant-based feed is considered one of the key stages in the early ontogenesis of the piglet. During this period, the foundations of its future productive properties and adequate adaptation to the external environment of all organs and systems of the animal are laid [11]. In this case, one of the most important integrative elements in the body of the animal are vessels. Their work plays a leading role in maintaining the fluid properties of the blood [12]. This ability of the vessel walls in young pigs is provided by a certain age-related dynamics of the secretion of hemostatically active factors from them [13].

The low activity of peroxidation of plasma lipids in piglets during the phase of milk and plant nutrition largely ensures low alteration of endotheliocytes, which is the morphological basis of the age-related increase in the anti-aggregation ability of blood vessels as a result of increased generation of prostacyclin and NO in it, which control the state of microcirculation in tissues [14].

In healthy piglets of dairy plant nutrition, an increase in the control of the vascular wall over the adhesive ability of blood plates was found, which is realized through at least two mechanisms. The first is increased control of the vascular wall over the number of collagen receptors (glycoproteins Ia - IIa and VI) on the surface of platelets. This was indirectly determined by the increase in inhibition of platelet aggregation with collagen in a sample with temporary venous occlusion. The second mechanism was to reduce the production of von Willebrand factor by vascular structures while reducing the expression of receptors for it - (GPI c) on the surface of blood plates in response to the entry of physiological vascular antiplatelet agents into the blood [15].

An increase in the production of substances with antiplatelet activity in the vessel walls also ensured a weakening of the fixation of strong aggregation inducers — collagen and thrombin to their receptors on the platelet membrane. This is inevitably accompanied by a decrease in the activity of phospholipase C and inhibition of the phosphoinositol activation pathway of platelets [16]. It is also accompanied by a decrease in the effect of weak aggregation inducers - ADP and adrenaline on platelet receptors. This is apparently supplemented by a decrease in the expression of fibrinogen receptors (GPIIc-IIIa), a weakening of the activity of phospholipase A2, a decrease in the yield of arachidonic acid from platelet phospholipids and some weakening of thromboxane generation [17].

An important component in ensuring the height of the atrombogenic activity of the vascular wall in piglets during the phase of dairy and plant nutrition is a marked gradual increase in its anticoagulant and fibrinolytic capabilities [18]. The former are associated with an increase in the production of one of the most powerful physiological anticoagulants, antithrombin III, in the structures of the walls of their vessels. The latter were determined by a gradual increase in the synthesis of plasminogen activators in them.

Thus, piglets of dairy and plant nutrition are characterized by a weakening of lipid peroxidation in the liquid part of the blood, which in many respects increases the antiaggregation, anticoagulation and fibrinolytic capabilities of the vessel wall. This supports the adequacy of the vascular control of piglets at this age over hemostasis as a whole.

5. Conclusion
In healthy piglets of dairy and plant nutrition, an increase in the antioxidant protection of plasma is noted, which weakens the processes of lipid peroxidation in it. For healthy piglets during the phase of milk-vegetable nutrition, a gradual increase in the anti-aggregation ability of the vascular wall is characteristic. During the phase of milk and plant nutrition in piglets, a gradual increase in coagulation-significant effects from the vascular wall (anticoagulant and fibrinolytic vascular activity) on hemostasis is recorded.

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