Hematological and hemostasiological characteristics of piglets during the plant feeding phase

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Abstract. Preservation of all parameters of homeostasis in pigs of any age is largely ensured by the state of their hematological parameters. The parameters of primary hemostasis are of great importance in this. For piglets in the phase of plant nutrition, a decrease in the intensity of peroxidation in the blood due to an increase in its antioxidant protection is characteristic. At this stage of development, the levels of total protein, urea, albumin, triglycerides in the blood increase in piglets, and a stable sufficient level of glucose is maintained. At the end of early ontogenesis in piglets, there is a clear balance between the development of platelet aggregation properties and the severity of the disaggregation characteristics of blood vessels. The balance of changes in platelet aggregation and the severity of control over it from the walls of blood vessels found during the phase of plant nutrition is an important basis for maintaining the balance in the activity of individual components of hemostasis in piglets.

1 Introduction

Modern pig breeding is a highly profitable and knowledge-intensive sector of the economy, capable of providing meat products to the general population of many countries of the world. The profitability of pig breeding is largely based on the high growth rate of pigs and the large number of offspring. Now pig breeding is considered in many countries as a highly promising industry with serious development potential [1]. The great need for the growth of pig production makes it necessary to intensify various aspects of pig breeding, primarily through the use of modern technologies for keeping, feeding and treating animals based on the latest achievements in physiological science [2].

An important biological significance for the implementation of early ontogenesis in productive animals is recognized in the phase of plant nutrition. This stage of development of the animal organism is considered as a stage at which it is possible to provide a clear acceleration of their development without loss in terms of productivity and without risk to the health of young animals [3]. This opinion is supported by various scientific papers devoted to various biological phenomena in the body at this stage of development. At this

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age, young animals develop a gradual increase in the consumption of plant foods, with the release of all parameters of their body of the level of adult animals [4].

In piglets, at the end of early ontogenesis, anabolism is highly active, which is more pronounced than in many other types of productive animals. These features of pigs, apparently, are related to the state of their hematological parameters at different ages. In view of this, blood is recognized as a marker of their current condition. Tracking its composition, you can quickly and accurately determine the functional state of the animal and make a clear forecast for the near future [5]. Therefore, the assessment of age-related changes in blood parameters and hemostasis in piglets is of high relevance.

An important role in the work of the whole organism of animals is played by hemostasis associated with the work of the walls of blood vessels, platelets and blood coagulation [6]. The severity of their hemostatic properties determines the manifestations of the fluid parameters of the blood and the state of microcirculation in all organs, providing the desired level of anabolism and development of animals according to the parameters [7]. There are reasons to attach great importance to the state of platelet aggregation and vascular control over it at the end of early ontogenesis due to their influence on the intensity of anabolism in animals [8]. Hematological and, especially, hemostasiological parameters of piglets of optimal functional status during the fourth phase of their early ontogenesis have not been fully elucidated. For this reason, the aim of the work is to find out the state of hematological parameters and indicators of their primary hemostasis in piglets of the plant nutrition phase.

2 Materials and methods

This study was carried out in full compliance with the ethical aspects of the European Convention for the Protection of Vertebrate used for all scientific purposes, which was adopted in Strasbourg on March 18, 1986 and was confirmed in Strasbourg on June 15, 2006.

The work was carried out on 32 completely healthy piglets of the Large White breed, taken under observation at the beginning of the fourth phase of early ontogenesis. All piglets were in standard conditions of a pig farm in central Russia. The first examination of piglets was carried out on the 41st day of their life. Subsequently, their condition was monitored daily during the entire phase of early ontogenesis. Subsequently, the examination was carried out at the stage of 90 days, at the stage of 150 days, at the stage of 200 days and at the stage of 230 days of their ontogenesis.

In the examined piglets, the plasma levels of acyl hydroperoxides and compounds that can interact with thiobarbituric acid were evaluated using a set of reagents manufactured by Agat-Med (Russia). In young animals, the antioxidant capabilities of plasma were determined by the traditional method. In piglets, plasma levels of total protein, urea, albumin, triglycerides, glucose, and cholesterol were determined using standard methods.

Using the micro method, a visual assessment of the severity of platelet aggregation (AP) was carried out, using as inducers of this process collagen obtained during the dilution of its main suspension 1:2, ADP, having a final concentration of 5 μg/ml, thrombin, having a final concentration of 0.125 units / ml, epinephrine, having a final concentration of 5 μg/ml, ristomycin, having a final concentration of 0.8 mg/ml. AP was assessed only in platelet-rich plasma after its standardization by the content of platelets in it.

The ability of vessels to disaggregate was assessed by the value of the indices of antiaggregation activity of the vascular wall for each indicator. This was done by dividing the duration of plasma AP obtained from temporary venous occlusion by the duration of plasma AP obtained from blood without any compression of the venous wall.

The level of platelet aggregation developing under blood flow conditions was determined using phase-contrast microscopy.

The digital data found in the work were processed with the calculation of the value of the criterion (td) Student.

3 Research results and Discussion

During the study, there were changes in acyl hydroperoxides in the blood of piglets associated with their decrease from 1.38±0.016 D_{233}/1 ml to a concentration of 1.23±0.019 D_{233}/1 ml and a decrease in the level of metabolites that can interact with thiobarbituric acid, from 3.25±0.031 µmol/l to 2.99±0.022 µmol/l, respectively. This dynamics was associated with an increase in the level of plasma antioxidant capacity in piglets from 36.2±0.19% to 39.8±0.11%.

During the study, a significant dynamics of the main metabolites was found in the blood of animals taken into work (Table 1).

Table 1. Blood metabolites in piglets during the plant feeding phase.

Hematological parameters	Animal age in days, n=32, M±m				
	41	90	150	200	230
Cholesterol, mmol/l	3.20±0.27	3.29±0.32	3.38±0.41	3.57±0.45*	3.82±0.32**
Triglycerides, mmol/l	0.40±0.08	0.43±0.11	0.48±0.05**	0.52±0.04**	0.58±0.09**
Urea, mmol/l	3.48±0.39	3.51±0.27	3.57±0.39	3.63±0.24	3.86±0.39*
Glucose, mmol/l	4.3±0.29	4.4±0.16	4.2±0.22	4.3±0.29	4.5±0.34
Total protein, g/l	82.1±0.33	83.6±0.36	84.2±0.41	86.9±0.32	94.9±0.49*
Albumins, g/l	40.0±0.25	42.1±0.30	43.8±0.25	45.8±0.32*	47.7±0.29**

Note: the reliability of changes in the levels of recorded parameters compared to their level in the first study: *$p<0.05$; **$p<0.01$. The rest of the tables have similar designations.

In the examined piglets at the beginning of the observation, the content of total protein in the blood reached 82.1±0.33 g/l. During the entire phase of plant nutrition, its amount in the blood of piglets increased by 15.6% ($p<0.05$), amounting to 94.9±0.49 g/l in the last study. An increase in the level of total protein in the plasma of animals during the observation is considered as a consequence of increased absorption in the digestive tract of all amino acids against the background of the assimilation of the proteins of the consumed feed. In piglets, at the beginning of the phase of plant nutrition, the level of albumin in plasma was 40.0±0.25 g/l, subsequently gradually increasing over the entire phase by 13.2% ($p<0.05$).

The content of urea in the plasma of piglets at the first observation was 3.48±0.39 mmol/l. During the observation period, its level increased in the blood and, by the end of the phase of plant nutrition, was 3.86±0.39 mmol/l. An increase in the content of urea in the blood by 10.9% was the result of intensification in piglets during the observation of protein metabolism.

During the study period, the young animals showed a constant implementation of carbohydrate metabolism. This was indicated by the stability of the concentration of glucose in their plasma, which turned out to be 4.5 ± 0.34 mmol/l in the last study.

In animals during the entire observation, physiologically desirable changes in lipid parameters favorable for their growth were found (Table 1). The amount of cholesterol in their plasma at the beginning of the study was the smallest - 3.20±0.27 mmol/l. During the follow-up observation, its level in the blood of piglets increased significantly by 19.4%, reaching the level of 3.82±0.32 mmol/l by the end of the phase of plant nutrition. The obtained changes in blood lipid parameters indicated an increase in fat metabolism in piglets at the end of early ontogenesis, which is very significant for their development [4].

During the last phase of early ontogeny, the observed animals had a shortening of the onset of AP. The most rapidly AP was formed in them in response to the contact of platelets with collagen (Table 2). A little later, AT developed under the influence of ADP, ristomycin, and hydrogen peroxide. The introduction of solutions with thrombin or adrenaline into the plasma with platelets led to the appearance of AP a little later.

During the entire study in the blood of the observed piglets, there was an increase of 45.2% in the number of aggregates having the smallest size and an increase of 42.3% in platelet aggregates with medium and large volume. In their blood, during the observation period, the inclusion of platelets in the composition of aggregates moving through the blood increased to 11.5±0.03% (Table 2).

Table 2. The state of platelet aggregation activity in piglets during the phase of plant nutrition.

Hematological parameters	Animal age in days, n=32, M±m				
	41	90	150	200	230
The number of medium and large aggregates of 4 or more platelets, per 100 free-lying platelets	0.26±0.05	0.28±0.03	0.31±0.05**	0.34±0.07**	0.37±0.03**
The number of small aggregates of 2-3 platelets, per 100 free-lying platelets	4.2±0.09	4.5±0.05	4.9±0.08*	5.5±0.04**	6.1±0.05**
Inclusion of platelets in aggregates, %	8.7±0.09	9.0±0.06	9.4±0.10*	9.8±0.05*	11.5±0.03**
Platelet aggregation with thrombin, s	49.0±0.10	47.2±0.14	44.3±0.05*	41.3±0.11**	38.4±0.04**
Platelet aggregation with collagen, s	23.6±0.08	21.5±0.12	20.0±0.15*	18.7±0.06**	15.9±0.07**
Platelet aggregation with ADP, s	34.1 ±0.12	32.2±0.10	30.1±0.14 $p<0.05$	28.2±0.10 $p<0.01$	25.7±0.08 $p<0.01$
Platelet aggregation with ristomycin, s	34.9±0.14	32.1±0.06	29.8±0.19*	26.9±0.14**	24.3±0.09**
Platelet aggregation with adrenaline, s	85.6±0.15	83.2±0.18	78.5±0.17*	75.2±0.13*	71.4±0.10**
Platelet aggregation with H_2O_2, s	36.5±0.13	34.0±0.18	31.6±0.10*	28.2±0.08**	25.5±0.12**

In the course of the entire work, the piglets showed an increase in the values of the indices of antiaggregatory activity of the vascular wall in relation to all agonists of platelet aggregation (Table 3). The value of the index of antiaggregatory activity of the vascular wall in response to the influx of adrenaline into the plasma turned out to be the largest due to a very significant weakening of adrenaline AP in the plasma after temporary venous occlusion. This index was slightly lower in piglets in response to hydrogen peroxide and in response to ristomycin. Its value was even lower for collagen (at the end of early ontogenesis it was 1.65±0.03), for the ADP inducer (at the end of early ontogenesis it was 1.66±0.04) and for the thrombin inducer (at the end of early ontogenesis it was 1.67±0.06).

The end of early ontogenesis in animals is associated with the continued growth and maturation of their organs [9]. At this stage, animals gradually increase the work of their organs to the level characteristic of an adult organism [10]. Of particular importance in achieving the coordinated work of all body systems is the blood and the mechanisms of hemostasis. Its optimal functioning is ensured by a number of processes, which largely depends on the preservation of the liquid state during the movement of blood through the vessels and the formation of a thrombus at the site of vessel alteration [11].

In the animals taken under observation, there was a decrease in the severity of plasma lipid peroxidation, which favored the preservation of the optimum structure of platelets and their low hemostatic activity. Quite significantly, this was ensured by some gradual inhibition of the synthesis of thromboxane in the platelets of piglets. The emerging conditions favored the implementation of hemocirculation through the small vessels of the internal organs and the sufficient supply of oxygen and nutrients to them [12].

Table 3. Disaggregation characteristics of the vessel walls in piglets during the plant feeding phase**.**

Hematological parameters	**Animal age in days, n=32, M±m**				
	41	**90**	**150**	**200**	**230**
Index of antiaggregation activity of the vascular wall with thrombin	1.53±0.08	1.55±0.08	1.58±0.17	1.62±0.05	1.67±0.06*
Index of antiaggregation activity of the vascular wall with collagen	1.51±0.04	1.53±0.05	1.56±0.08	1.60±0.07	1.65±0.03* $p<0.05$
Index of antiaggregatory activity of the vascular wall with ADP	1.49±0.07	1.53±0.07	1.57±0.06	1.61±0.07	1.66±0.04*
Index of antiaggregatory activity of the vascular wall with ristomycin	1.54±0.02	1.57±0.05	1.61±0.08	1.64±0.06	1.69±0.05*
Index of antiaggregatory activity of the vascular wall with adrenaline	1.56±0.08	1.58±0.07	1.61±0.05	1.64±0.06	1.70±0.09*
Index of antiaggregation activity of the vascular wall with H_2O_2	1.53±0.07	1.55±0.06	1.58±0.06	1.63±0.10	1.68±0.03*

The functional general status of piglets is directly determined by the state of metabolism in animals, manifested by the levels of various metabolites in the blood. Their amounts significantly affect the activity of all growth processes and the functional capabilities of the formation of all internal organs [8].

Strengthening of hemostatic processes in platelets during the phase of plant nutrition in piglets was associated with an increase in the synthesis of aggregants that reduce the activity of various receptors on the surface of platelets. One of the manifestations of increased platelet adhesion was the acceleration of AT in response to the influx of collagen into the plasma with platelets. It was also associated with an increase in the blood level of von Willebrand factor, which also implements the adhesive process of platelets [7].

Strengthening of the adhesive manifestations of platelets in piglets was accompanied by an increase in the severity of their aggregation capabilities. Activation of the platelet response to the entry of a strong aggregation stimulator into the plasma enhanced the work of pro-aggregation platelet mechanisms, including the phosphoinositol pathway of platelet activation, activation of phospholipases in them, and an increase in the activity of phosphorylation of elements of the platelet contractile apparatus [2]. Reducing the time of platelet aggregation should be associated with an increase in the number of glycoproteins on the outer surface of platelets that can act as their receptors, as well as with an increase in the biochemical capabilities of platelet enzymes that generate thromboxane during hemostasis [10].

In healthy piglets, during the last phase of early ontogenesis, an increase in the disaggregation capabilities of vascular walls was found, which was associated with the activation of the synthesis of various antiaggregants in them. It becomes clear that under conditions of tissue maturation, piglets developed a strict balance between the severity of platelet aggregation and disaggregation manifestations from the vascular walls.

An increase in the content of antiplatelet agents in the plasma at the end of early ontogenesis in animals effectively controlled the phenomena of platelet reception, signal transmission from the membrane into platelets, and the reaction of platelets to realize their hemostatic capabilities. This was associated with the development of a functional balance between the activity of substances released into the plasma with the activity of proaggregants and the activity of antiplatelet agents that can affect the implementation of the entire hemostasis.

During the observation in animals, an increase in platelet aggregation occurring directly in the bloodstream was found. Its growth in vivo in piglets was inhibited by the increasing activity of the hemostatic capabilities of the vessels, as evidenced by the increase in the values of the indices of the antiaggregation activity of the vessels with all the studied stimulants. Their high values proved sufficient disaggregation manifestations on the part of the vessels under conditions of increasing hemostatic capabilities of platelets. This moment can be considered as a significant mechanism for restraining the hemostatic capabilities of platelets in piglets during the last phase of early ontogenesis and maintaining the optimum hemocirculation in them at this age. The onset of a physiologically extremely important balance between the activity of substances with anti-aggregation and pro-aggregation properties in the observed animals indicates the development of large hemostatic capabilities of the vessel walls, which implement the formation of molecules that affect the level of platelet activity. The activity of the disaggregation properties of the vascular endothelium, which increases during the fourth phase of early ontogenesis, is a significant mechanism for maintaining homeostasis in all tissues of piglets at the end of growth and development.

4 Conclusion

Achieving the optimum functional status of piglets during the last phase of their early ontogenesis is very important for the realization of their productive potential. A serious role in this process is played by changes in hematological parameters associated with the age-related dynamics of all types of metabolism in the body of pigs at this stage of ontogenesis. It is extremely important to maintain the optimum hematological parameters, as well as the balance of hemostatic manifestations of blood vessels and platelets. In the examined animals, during the entire observation, a strict balance was found between the functionality of platelets and vascular antiaggregation manifestations. The optimum of their hemostatic manifestations in piglets at the end of early ontogenesis creates the conditions for ensuring a high intensity of hemocirculation in all internal organs. The balance of platelet activity and disaggregation properties at the last stage of early ontogenesis provides piglets with physiological conditions for their normal growth and development of their productive qualities.

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