Restoring the functional state of the hemostasis system in piglets experiencing an episode of transportation

Lyubov P. Solovyova¹, Tamara V. Kalysh¹, Yulia A. Voevodina², and Valery I. Zamuravkin¹

¹Kostroma State Agricultural Academy, Karavaevo village, Kostroma, 156530, Russia
²Vologda State Dairy Farming Academy named after N.V. Vereshchagin, Vologda, 160555, Russia

Abstract. Negative environmental impact can often adversely affect the functional properties of piglets and significantly weaken their viability. It is often possible to judge this by the dynamics of the activity of hemostasis indicators. The level of activity of platelets and coagulation systems very early change activity in the case of negative influences from the outside, causing the appearance of overt hemostasiopathy. This condition leads to the disruption of hemocirculation in the capillaries and a decrease in the severity of metabolism in the tissues. These changes lead to a slowdown in growth and a general weakening of animals, thereby leading to great economic damage. It was found that the appearance of transport stress in piglets leads to increased platelet aggregation and activation of homocoagulation mechanisms during inhibition of fibrinolysis. Due to the fact that this situation is very common, the possibility of eliminating these dysfunctions was studied. The study tested fumaric acid at 0.1 g/kg in the form of a feed additive for 20 days in piglets that underwent long-term transportation. As a result of using this supplement, it was possible to reduce platelet activity and blood coagulation and increase fibrinolysis activity, bringing them to control values. As a result of the study, it was found that the intake of fumaric acid in the body of piglets that have experienced the influence of a high-intensity medium optimizes the functional status of their hemostasis. This minimizes their risk of microthrombosis and weakened animal growth.

1 Introduction

The general activity of processes in the body and their depression at any age can be caused by a large number of external and internal causes [1, 2]. Quite often, strong enough factors can affect the body, which can cause serious changes in its functioning.

The onset of such changes attracts great interest in medicine and biology [3]. In humans, a detailed study of them has a very great social significance and is designed to ensure the growth of the average duration of a large number of people [4, 5]. In animals, especially in productive animals, it is intended to achieve the highest possible level of economically important indicators in productive animals during their rearing and is of great economic importance [6, 7].

The optimal functioning of the body of young animals is ensured by ensuring a balanced diet, creating an optimal microclimate and eliminating stressful situations [8, 9]. At the same time, by no means in all cases during early ontogenesis in productive animals is it possible to observe them fully [10, 11].

Therefore, young animals have a high risk of various dysfunctions [12], which can worsen the general condition and reduce growth [13]. It has been noted that weakening of the optimum microcirculation in tissues is of great importance in weakening the body's vitality, which weakens the metabolic processes in tissues [14, 15].

Due to the fact that microcirculation strongly depends on the rheological properties of blood cells [16], including platelets, and the activity of plasma components of the hemostatic system [17], it is of great practical interest to optimize the functional parameters of platelets, hemocoagulation, and fibrinolysis mechanisms after strong external influences [18, 19]. In addition of great practical importance is the further search for approaches to the correction of hemostasiopathy in piglets that can improve their blood flow and enhance metabolism in cells [20, 21].

The identification of the possibility of normalization in piglets of hemostasis is of great economic importance [22], minimizing economic damage due to the inhibition of their negative changes against any adverse effects [23, 24].

Of particular interest is the reaction of hemostasis of piglets that have experienced a frequent negative environmental factor for them — transportation of an accessible stimulant, fumaric acid, in a closed van [25].

The goal of this work was to assess changes in platelet activity, hemocoagulation indicators and fibrinolysis mechanisms in piglets that experienced an episode of transportation if they added fumaric acid to the feed.
2 Materials and methods

This study was carried out in full compliance with ethical standards defined by the European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes (adopted in Strasbourg on March 18, 1986 and confirmed in Strasbourg on June 15, 2006).

The study was conducted on completely healthy pigs belonging to the breed large white, at the age of 2 months. Piglets are divided into experimental and control groups. The experimental group consisted of 56 animals that underwent long-term transportation in a completely closed van from a pig farm owned by the Kostroma State Agricultural Academy to various farms in the Kostroma region of Russia after selling them to continue growing.

In order to ensure the optimum of their functional status, these piglets were added fumaric acid in the amount of 0.1 g/kg per day for 20 days.

The control group of piglets consisted of 27 healthy pigs at the age of 2 months large white breed, which were kept under normal conditions of a pig farm owned by the Kostroma State Agricultural Academy and never experienced an episode of transportation.

Using a method, platelet aggregation with ADP in a standard dose was studied [26]. The assessment of the state of hemocoagulation was carried out by some parameters. The value of the activated partial thromboplastin time was determined, the level of fibrinogen was estimated, and the concentrations of soluble fibrin-monomer complexes were determined using the orthophenanthroline method [27].

The amount of tissue type 1 plasminogen activator inhibitor was determined in the blood of piglets using a special chromogenic substrate and Coatest PAI-1 test system manufactured by Chromogenix. The fibrinolytic capabilities of the blood were evaluated using the standard method [27].

Statistical processing of the results was carried out using the standard statistical software package. Differences were considered significant at p<0.05.

3 Results and discussion

Recently experienced animals transporting episode found obvious changes in some hematological parameters. In the experimental group of piglets, no differences were found with the control group according to the level of platelets in their blood. Their ability to aggregate in the piglets that made up the experimental group, in the case of getting into the plasma of ADP was accelerated by 33.9 %. At the same time, in experimental piglets, the activated partial thromboplastin time was reduced (by 29.3 %), the concentration of fibrinogen in the blood increased by 77.3 % and the fibrinolytic capabilities of their blood decreased by 38.2 %. Against this background, the number of fibrin-monomer soluble complexes in their blood increased by 41.5 % with an increase in it (by 87.3 %) of the amount of a tissue plasminogen activator inhibitor type 1.

Due to the receipt of fumaric acid with food from piglets that suffered transport stress, the restoration of impaired hemostasis was achieved. In animals of the experimental group, the platelet count in the blood did not change. Moreover, the activity of their aggregation with ADP decreased by 31.5 %, reaching the level of control. As a result of consumption of fumaric acid, these piglets significantly increased the value of activated partial thromboplastin time (by 28.1 %).

By the time observations were completed in experimental animals, the level of fibrinogen in the plasma decreased by 69.4 %, and the activity of the fibrinolytic capabilities of their blood increased by 36.4 %, reaching control values.

The content of soluble fibrin-monomer complexes in the blood of experimental piglets reached the control level by the end of the observation, having decreased by 38.1 % from the outcome level.

At the same time, the consumption of fumaric acid ensured a decrease in the amount of tissue plasminogen activator type 1 inhibitor in the blood of experimental animals by 86.0 %, which ensured its achievement of the control level.

### Table 1. Hemostasis rates in observed piglets

| Registred parameters                           | Control group, M±m, n=27 | Experienced group, M±m, n=56 |
|-----------------------------------------------|--------------------------|-------------------------------|
|                                               | at the beginning of observation | in the end observations      |
| Level platelet, 10⁷/l                         | 196.5±1.33               | 210.1±1.62                   | 200.1±1.27                   |
| The severity of platelet aggregation in response to ADP, s | 49.7±0.34               | 37.1±0.29                    | 48.8±0.26                    |
|                                               |                         | p<0.01                       | p<0.01                       |
| The value of activated partial thromboplastin time, s       | 40.1±0.53               | 31.0±0.43                    | 39.7±0.34                    |
|                                               |                         | p<0.01                       | p<0.01                       |
| The amount of fibrinogen, g/l                  | 2.2±0.34                | 3.9±0.19                     | 2.3±0.25                     |
|                                               |                         | p<0.01                       | p<0.01                       |
| The level of plasma fibrinolytic activity, min   | 7.6±0.47                | 5.5±0.27                     | 7.5±0.20                     |
|                                               |                         | p<0.01                       | p<0.01                       |
| The level of soluble fibrin-monomer complexes, mg %       | 4.1±0.34                | 5.8±0.42                     | 4.2±0.36                     |
|                                               |                         | p<0.01                       | p<0.01                       |
| The concentration of tissue plasminogen activator inhibitor type 1, ng/ml | 29.2±0.48               | 54.7±0.75                    | 29.4±0.69                    |

**Note:** p is the significance of differences in the indicators of the experimental group from the indicators in the control group.

In the presence of various dysfunctions and pathologies, activation of hemostatic mechanisms occurs. This process is associated with the influence of negative environmental factors. The development of
hemostasiopathy threatens the appearance of vasospasm and disturbances in the process of blood rheology in small vessels, which is especially significant during the active growth of the animal [28].

This is always accompanied by platelet activation and increased hemocoagulation, which was also observed in a study on animals that suffered an episode of prolonged transport [29].

Activation of platelet aggregation significantly worsens the microcirculation in the capillaries in animals and is dangerous inhibition of growth due to a decrease in metabolism [30, 31]. The revealed increase in hemocoagulation activity in the examined piglets evidently occurred during the growth of activity of the main number of coagulation factors, which caused a found increase in blood coagulation along the external path [32].

The revealed decrease in the activity of fibrinolytic processes in piglets of the experimental group additionally increased the risk of microthrombosis [33]. In this case, the increase in the amount of fibrinogen should be considered a serious mechanism for stimulating platelet aggregation and blood coagulation. This is ensured by the fact that it is able to bind platelets in the aggregate to each other and at the same time as a substrate for the action of thrombin in the process of the occurrence of a thrombus [34].

The activation of lipid peroxidation in plasma, which stimulates the synthesis of α2-antiplasmin and suppresses fibrinolysis, should be considered a serious mechanism of this dysfunction in the examined piglets. The resulting imbalance in the mechanisms of hemostasis led to its activation and to the deterioration of hemocoagulation in the capillaries [35].

The addition of fumaric acid to the piglets of the experimental group ensured the elimination of hemostatic dysfunctions and mitigation of the risk of pathology. Their hemostasiopathy was completely leveled, which minimized the threat of vascular spasm and hemorheological disorders. In piglets treated with fumaric acid, platelet activity and blood coagulation decreased to normal levels.

Reducing the severity of platelet aggregation activated microcirculation, eliminating the risk of weakening growth. The onset of a decrease in the level of blood coagulation activity occurred due to a decrease in the number of activated coagulation factors, which led to the normalization of plasma coagulation properties [36].

Strengthening of fibrinolytic mechanisms in piglets who consumed fumaric acid with food reduced the risk of thrombosis in their vessels. A decrease in the level of fibrinogen in the blood should be considered as an important mechanism for weakening platelet activity and hemocoagulation mechanisms.

This fact weakened the connection of platelets in the aggregates, and also reduced the amount of substrate for the action of thrombin, which inhibited the growth of the thrombus. The use of fumaric acid in experimental piglets no doubt inhibited the lipid peroxidation in plasma [37]. This, in turn, weakened the synthesis of α2-antiplasmin and stimulated plasma fibrinolysis.

Thus, the disappearance of piglets due to the use of fumaric acid imbalance in hemostasis with food created a normalization of the conditions for microcirculation and optimal growth in them.

4 Conclusion

The optimal functioning of organ systems in a piglet is strongly determined by their response to the influence of environmental factors on them and the state of the components of the hemostasis system. Against the background of the transport episode, piglets often experience hemostasiopathy, which is manifested by activation of platelets and hemocoagulation mechanisms with a significant decrease in fibrinolysis activity. Developing hemostasiopathy is eliminated due to the use of fumaric acid in piglets for 20 days, which is mixed with food. The results obtained suggest that with the help of fumaric acid, hemostasis dysfunctions in piglets under adverse environmental conditions can be eliminated.

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