







Erythron and protein system in piglets blood under stress influence

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Abstract

The article presents the results of research on the development of adaptive syndrome in the system of erythron and protein of piglets at the effects of weaning stress. After one (anesthetic stage) and seven days (beginning of G.Selle's resistance stage), after the echoing, the adaptation of the erythron system was shown by a decrease in the number of red blood cells on the contrary of increasing the mean corpuscular volume and hemoglobin indicating the inhibition of erythrocytosis with simultaneous macrocytosis, and a decrease in mean Corpuscular hemoglobin concentration for 14.0–13.0 % that indicated hypochromia. In these stressful periods, the catabolism of protein substrates was discovered, the redistribution of albumins between blood and tissues in the form of a decrease in the total protein content by 6.1–7.1 % due to albumin by 14.8–10.0 %, and the deficit of the humoral link of the immune response due to reduction of γ -globulins by 29.9–18.7 %. In the later stages of the resistance stage (after 20 and 60 days after weaning), there was a stabilization of erythrocytic indices and the development of anisocytosis in the form of an increase in red cell distribution width by 19.0 %, with a simultaneous decrease of 6.1 % of the total protein content, redistribution of its fractions in the side of the growth of γ -globulins by 18.4 %. A practical, prolonged way of preventing disorders in the erythron and protein of extramarital piglets was feeding the additive “Biovir”, which stimulated the intensity of oxidative-reducing processes and the exchange of proteins.

Keywords: stress, weaning, piglets, adaptation, red blood cells.

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1. Introduction

The realization of the genetic potential under the conditions of agricultural pig farms depends upon the adaptation possibilities of an animal's organism, which is characterized by the imperfection of the compensated mechanisms at an early age (Khalak et al., 2020; 2021; Masiuk et al., 2021). This is connected with the activity of the suprarenal cortex, which is aimed at the support of the optimal concentration of the adaptive hormones – glucocorticoids and is formed by the age of 6 months (Kovalchuk et al., 2020). According to Boiko O. et al. (2021), the early weaning of piglets is quite a stressful factor due to reduced productiveness, and the animals' deaths are observed, which is quite undesirable for the cost-efficient activity in the sphere. The primary chain of the mechanism of the negative influence of stress upon an animal's organism is changed in the homeostatic, immunological balance, and antioxidant defense system as a consequence of hormonal rebuild with an increase in the intensity of catabolic processes (Garmatyk et al., 2020). Under such

conditions in the liver, the pharmacodynamics of the biotransformation of medicines changes, and the expressed pathogenic modifications in the microsomes of hepatocytes, their lysosomal system, and organospecific macrophages, which testifies to the difficulties in the prophylaxis of the consequences of stressful reactions development, therefore, as adaptogens it is recommended to apply feed additives of natural origin (Slivinska et al., 2019; Gutyj & Khalak, 2020). The thesis aims to clarify the condition of the erythronus and protein metabolism in the organism of the piglets of Poltava meat breeds before and after the stress ablation with the introduction of “Biovir” additive as an adaptogen into the diet.

2. Materials and methods

All manipulations with animals were carried out according to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the law of Ukraine “On

protection of animals from cruelty” (Kyiv, 2001). The tests are conducted at the Davydivsky scientific training center of the Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies of Lviv, Ukraine, on clinically healthy piglets 5–90 days old of Poltava White Breed. For the research, two groups of piglets were formed – reference group (R) and study group (S), with ten piglets in each group. Starting from the 5-day age, the piglets of all groups were fed with the pre-starter feed, and piglets of the S group from 5- to 45-day old were additionally fed with “Biovir” additive in the form of dry powder with the calculation of 10 mg/kg of the body weight per day. Its application and dose were agreed upon according to the instruction and were suggested by the manufacturer – Ariadna Biotechnological Company of Odesa, Ukraine. The additive contained the complex of activated bacteria cell-walls low-molecular-weight peptide *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium mlongum*, and the products of their metabolism (lactic acid, acetic acid, lysozyme, reuterin, plantaricine, lactocidin, lactoline, acydoifiline) generally amounting to 700 g/kg. The succinic acid generally amounting to 300 g/kg.

Technological stress associated with animals weaning on the 28th day of piglets' life was complex. It included the following stress factors: piglets weaning, formation of groups from different clusters, and additional antigenic loading on the immune system of the digestive system due to change of the diet structure during the completion of growing.

The research material was constituted by the blood selection before the morning feeding of animals through the puncture of vena cava cranialis on the 15-th day of life (preparation period), one day after the ab lactation (which corresponded to the alert phase, according to H. Selye), 7, 20 and 60 days after the ab lactation (which corresponded to different resistance development periods according to H. Selye). An absolute number of red blood cells (RBC), mean corpuscular volume (MCV), red cell distribution width (RDW), the concentration of hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were conducted using the Hematology Analyzer MITKH-18, (Switzerland). The total protein concentration of the blood serum was determined with the help of a refractometer RDU. Protein fractions determination (albumen, α 1-, α 2-, β -, γ -globulins) was conducted using the vertical microelectrophoresis in the polyacrylamide gel tubes used at the Institute of Animal Biology of the National Academy of Agrarian Sciences of Ukraine. The level of difference (P) probability was determined between the studied indexes of the piglets of R and S groups and statistically processed using Statistics software for Windows XP with the application of T-Student's criterion.

The results of average indexes were considered statistically probable at – * P < 0.05.

3. Results and discussion

Table 1 contains the results of piglets' blood system of erythronus during different stress periods of ontogeny with the feeding “Biovir” additive in animals of the R group; before weaning, the amount of RBC was 6.02 ± 0.60 b/l, MCV and RDW – 51.48 ± 2.65 mcm³ and 21.20 ± 1.34 %, respectively, indicating the functioning of erythropoiesis within the limits of the physiological norm. The value of

HGB in the amount of 71.33 ± 2.30 g/L, its MCH, and MCHC indicated an adequate level of hemoglobin saturation and an intense oxygen-transport function of the blood. In the piglets of the S group, which in the form of supplements received “Biovir”, the studied indicators corresponded to the ones of the R group.

After one and seven days after weaning, in animals of the R group, the amount of RBC reduced by 16.3–8.9 %, the MCV and RDW increased by 7.9–19.8 %, the HGB – by 4.0–7.1 % with the decrease of MCHC by 14.0–13.0 % without changes in the MCH, compared with the numerical values of the period before the weaning. The number of RBC in piglets of the S group was higher by 7.9–5.6 %, MCV, RDW, and MCH remained at the control level, HGB increased by 10.8 % (P < 0.05), MCHC by 13.1–14.3 % (P < 0.05) concerning control. At later stages of the resistance stage, namely, after 20 and 60 days after weaning in the blood of animals in Group R, there was a decrease in the amount of RBC by 3.8–15.1 %, RDW by 19.0 %, a tendency to stabilize MCV, MCHC and HGB increased by 21.8 %, compared to the period before weaning. In these stressful periods, the piglets of the S group, which received the additive “Biovir”, the amount of RBC increased by 36.8 % (P < 0.05), MCV and RDW – by 6.8 and 23.9 % (P < 0.05), HGB – by 8.9 % (P < 0.05) compared with control, and the values of MCH and MCHC were close to the ones of the group R.

Several homeostatic mechanisms include protein balance, the violation of which can be considered as a criterion for assessing the intensity of metabolic processes for the development of the adaptive syndrome. Table 2 contains the results of the piglets' blood proteinogram during different stress periods of ontogeny with the feeding “Biovir” additive.

Before weaning, it should be noted that the numerical value of the protein in the serum of the blood of piglets from the R and S groups was within the physiological norm with a predominance of a numerical value for 7.9 % due to redistribution of protein fractions in the direction of increase of albumins by 6.3 % in animals receiving the additive “Biovir”. After one and seven days after weaning in animals in the R group, the content of total protein decreased by 6.1–7.1 % due to albumin content – by 14.8–10% and γ -globulins – by 29.9–18.7% with the simultaneous growth of α 1-, α 2- and β -globulins compared with the numerical values of the period before weaning. In the piglets of the S group, the protein content was higher by 8.3–16.9 % (P < 0.05), while on the first day of the development of the stress reaction, due to albumin and γ -globulins, 14.7 % and 21.6 % (P < 0.05), respectively, on the seventh day – due to α 2- and γ -globulins by 26.3 and 16.4 % (P < 0.05), respectively, compared with control. At later periods of the resistance stage (after 20 and 60 days after weaning), the content of total protein in the blood serum of piglets in the group R remained lower by 6.1 % at the expense of albumin by 14.3–18.8 %, however, the amount of α 2-, β - and γ -globulins exceeded their indices in the period before weaning at 14.4–28.8 %, 29.1–14.2 % and 18.4 % respectively. Feeding piglets of the S group with supplements “Biovir” provided them with high protein metabolism in the form of an increase of the content of total protein by 9.4 %, β -globulins – by 18.3 % (P < 0.05), γ -globulins – by 27.2 – 26.8 % (P < 0.05) compared with control.

Table 1

State of the erythronine of the blood of piglets during different stress periods with the feeding “Biovir” additive (M ± m, n = 10)

Groups	Periods before weaning	Periods after weaning (24 hours)			
		One	seven	20	60
RBC, T/L					
R	6.02 ± 0.60	5.04 ± 0.33	5.48 ± 0.75	5.79 ± 0.51	5.11 ± 0.47
S	5.94 ± 0.49	5.44 ± 0.70	5.79 ± 0.58	6.08 ± 0.49	6.99 ± 0.65*
MCV, mkm ³					
R	51.48 ± 2.65	52.66 ± 2.74	55.53 ± 2.56	54.26 ± 2.73	51.42 ± 2.98
S	54.89 ± 2.89	53.10 ± 2.17	55.26 ± 2.67	53.56 ± 2.37	54.93 ± 2.53
RDW, %					
R	21.20 ± 1.34	25.40 ± 1.34	23.93 ± 1.34	21.76 ± 1.76	17.16 ± 1.35
S	22.27 ± 1.38	22.33 ± 1.55	23.56 ± 1.66	23.63 ± 1.27	21.26 ± 1.33
HGB, g/L					
R	71.33 ± 2.30	74.20 ± 2.34	76.42 ± 1.52	84.30 ± 2.01	86.91 ± 1.90
S	64.66 ± 1.18	75.42 ± 2.87	84.71 ± 2.77*	85.71 ± 3.84	94.66 ± 2.05*
MCH, pg					
R	14.06 ± 1.01	13.56 ± 1.19	13.63 ± 1.37	13.13 ± 1.19	13.84 ± 1.15
S	14.41 ± 1.06	14.59 ± 1.32	13.33 ± 0.83	12.46 ± 0.97	13.23 ± 1.20
MCHC, g/L					
R	249.20 ± 6.02	214.33 ± 6.33	216.90 ± 6.56	230.20 ± 6.02	250.14 ± 7.02
S	247.66 ± 8.66	242.57 ± 8.30*	247.84 ± 9.66*	240.21 ± 7.09	259.33 ± 6.09

Note. In this table and table 2nd, the differences are statistically probable in the studied group concerning the reference group and are marked as follows: *P < 0,05

Table 2

Piglets' blood proteinogram during different stress periods with the feeding “Biovir” additive (M ± m, n = 10)

Groups	Periods before weaning	Periods after weaning (24 hours)			
		One	seven	20	60
Total protein, g/L					
R	48.02 ± 1.97	45.09 ± 1.61	44.59 ± 2.42	47.20 ± 1.73	45.50 ± 2.44
S	51.82 ± 1.68	48.82 ± 2.01	52.12 ± 1.75*	49.12 ± 1.66	49.80 ± 1.87
Albumins, %					
R	50.52 ± 2.60	43.06 ± 1.80	45.45 ± 2.21	43.30 ± 1.88	41.02 ± 2.52
S	53.57 ± 2.58	49.37 ± 1.93*	39.35 ± 2.57	40.35 ± 1.96	39.82 ± 1.70
α1-globulins, %					
R	2.45 ± 0.31	3.40 ± 0.51	3.60 ± 0.62	2.89 ± 0.22	3.00 ± 0.66
S	2.27 ± 0.45	2.97 ± 0.49	2.82 ± 0.88	2.98 ± 0.78	2.30 ± 0.73
α2-globulins, %					
R	13.92 ± 1.88	19.35 ± 1.33	17.37 ± 1.20	15.90 ± 0.80	17.92 ± 1.34
S	13.14 ± 1.60	15.70 ± 1.47	21.93 ± 1.44*	15.02 ± 1.38	14.27 ± 1.67
β-globulins, %					
R	13.40 ± 1.20	19.74 ± 1.52	17.70 ± 1.24	17.35 ± 1.87	15.30 ± 0.85
S	13.35 ± 1.46	15.51 ± 1.73	17.28 ± 1.81	16.72 ± 1.48	18.10 ± 0.99*
γ-globulins, %					
R	18.87 ± 1.11	13.40 ± 0.90	15.84 ± 0.85	19.82 ± 1.20	22.35 ± 1.55
S	17.27 ± 1.27	16.30 ± 0.80*	18.43 ± 0.71*	25.22 ± 1.66*	28.35 ± 1.22*

The obtained results of the study of the amount of RBC and protein, as well as the indicators characterizing their functional state (MCV, RDW, HGB, MCH, MCHC) and (albumin, α1-, α2-, β -γ-globulins), indicate active erythropoiesis, no violations in gas exchange, stable functioning of the system of erythron and protein metabolism of piglets of R and S groups. Due to the lack of potential intergroup differences, the effect of the additive “Biovir” on the erythrocytic indices of the blood of animals of the S group was not detected. However, an increase in albumin by 6.3 % indicated an increase in the transport function of the blood within the limits of the physiological norm. According to [Martyshuk T. et al. \(2021\)](#), the influence of the weaning stress causes discrepancies between the biological nature and physiological capabilities of the piglets' organism. However, its development is due not only to the nature of the influ-

ence of stress factors but also to the functional activity of adaptive systems that provide the homeostasis of the organism. The blood system, as a reflection of the state of the body as a whole, is a criterion for assessing the stress response ([Lukashchuk et al., 2018; 2020](#)). It was determined that the development of the adaptive syndrome in the piglet organism was accompanied by a violation of the erythron system and the intensity of the protein metabolism. In the R group, the number of RBCs decreased by 16.3–8.9 % in one and seven days, against a 19.8 % increase in MCV and 4.0–7.1 % in HGB. The obtained results indicate the inhibition of erythropoiesis with a simultaneous increase in the size of erythrocytes (macrocytosis) and their hemoglobin content due to digestive tract digestion and vitamin B₁₂ absorption from the intestine. The established RDW norm of heterogeneity of red blood cells allowed minor deviations (7.9 %),

which could be due to the redistribution of white blood cells, since according to [Vyslotska et al. \(2021\)](#), in the period of stress, neutrophilia and eosinopenia are observed. However, the obtained RDW erythrocyte's anisotropy coefficient affected the ability to transport oxygen and carbon dioxide. While there was an increase in HGB, no abnormalities were detected in the MCH, indicating a sufficient content of iron-containing protein in one erythrocyte. However, the reduction of MCHC by 14.0–13.0 %, which reflects the filling density of the erythrocyte hemoglobin, was a sign of hypochromia, that is, a reduced transport function of the blood due to possible abnormal iron metabolism. In general, changes in the erythronine status of piglets in the group R can be due to the functional activity of the hypothalamic-pituitary-adrenocorticotrophic system and the complex reaction of the hypothalamic-pituitary-thyroid gland under stress ([Karami Kheirabad et al., 2016](#); [Kerry et al., 2018](#)). Under these conditions, a decrease in the total protein content by 6.1–7.1 % was found due to albumin by 14.8–10 % and γ -globulins by 29.9–18.7 % with the simultaneous growth of α 1-, α 2- and β -globulins, compared with the numerical values of the period prior to communication. The results showed a decrease in the functional activity of the synthetic liver function ([Danchuk & Karpovskiy, 2019](#); [Smyslov et al., 2021](#)), a deficiency of the humoral link of the immune response. They could occur due to the partial catabolism of protein substrates, albumins redistribution between the blood and tissues under the influence of the glucocorticoids (stress hormones).

At the stage of anxiety and the beginning of the stage of resistance to feeding, the additive “Biovir” did not affect the homogeneity and size of erythrocytes since MCV and RDW remained at the control level. However, the amount of RBC was higher by 7.9–5.6 %, HGB by 10.8 % ($P < 0.05$), and MCHC by 13.1–14.3 % ($P < 0.05$) relative to control, indicating the positive effect of supplements on the erythropoiesis and respiratory function of the blood of piglets after the stress of weaning. Probable increase in protein content by 8.3 %–16.9 % ($P < 0.05$) due to albumin (14.7 %, $P < 0.05$), α 2-globulins (26.3 % $P < 0.05$) and γ -globulins (21.6–16.4 %, $P < 0.05$) compared to control, which characterized the development of the stress reaction in the S group piglets' organism by some intensification of the catabolic process against the background of the improvement of the synthetic liver function and immune system activity.

From 20 to 60 days after the stress-weaning, a functional adaptation of the system of erythron and protein metabolism of piglets to the group was observed as a sign of stabilizing the protective mechanisms of their organism. The MCV indicators did not differ from the numerical values before ex-communication, indicating the compensatory reaction of the organism to intense gas exchange. The increase in HGB by 21.8 %, in the absence of changes in MCH and MCHC stabilization, reflected both the sufficient amount and the density of red blood cells in hemoglobin, indicating an increased ability of erythrocytes to transport oxygen. Although the decrease in the amount of RBC (3.8–15.1 %) did not go beyond the lower limits of the physiological norm for this age of animals, however, the degree of deviation of RDW in the amount of 19.0 % indicated anisocytosis development, which could be a sign of the insufficient exchange of iron in the detached piglets. Under these conditions, the metabolism of protein substrates varied since the content of total protein in the blood serum of piglets in the group R

remained lower by 6.1 % at the expense of albumin by 14.3–18.8 %, as a consequence of hormonal reorganization of the organism in the context of the implementation of the adaptive syndrome. The increase in the number of α 2-, β - and γ -globulins by 14.4–28.8 %, 29.1–14.2 %, and 18.4 %, respectively, indicated a recovery of transport and protective function of the blood.

Adaptation of the erythron system to the weaning of piglets of the S group after 20 and 60 days after the stress was characterized by an increase in the number of RBC and HGB by 36.8 and 8.9 % ($P < 0.05$), with an increase in MCV and RDW by 6.8 and 23.9 % ($P < 0.05$) concerning the control that was determined by the norm of heterogeneity of red blood cells in the period before weaning, and the stable value of MCH and MCHC was a sign of normochemistry. The use of the additive was positive for leveling changes in the protein metabolism system due to the development of an adaptive syndrome, which was manifested by an increase in the content of total protein by 9.4%, β -globulins – by 18.3 % ($P < 0.05$), γ -globulins – by 27.2–26.8 % ($P < 0.05$) compared with control. Our previous studies ([Stoyanovskyy et al., 2020](#)) showed that peptides of cell walls of lactobacillus and bifidobacteria supplements of “Biovir” had immunostimulatory properties for lymphoid tissue of the intestinal mucosa of piglets and served as substrate for the growth and development of its microbiocenosis. An increase in the number of bifidobacteria, which is the basis of the perineal and cavity microflora, led to an increase in the vitality of vitamin products B₆ and B₁₂ in process of vitality, which had a positive effect on the intensity of oxidative-reducing processes in the system of erythron and hemopoiesis of piglets in the S group. Adaptation of the system of protein metabolism in animals of this group was shown by an increase in the synthetic function of hepatocytes, transport and immune function of the blood, due to the basic properties of amber acid, as the main component of the “Biovir” ([Stoyanovskyy et al., 2020](#)). It is a universal biogenic metabolism stimulator in cells, that is, the natural endogenous substrate of oxidation-reduction processes (Krebs cycle) without additional energy expenditure, which leads to the activation of biosynthesis of proteins at different levels ([Roman et al., 2020](#)).

4. Conclusions

It can be concluded that the adaptation of the system of erythronine piglets at the stage of anxiety and the initial stage of resistance was manifested by a reduction in erythrocytosis, macrocytosis, and hypochromy with subsequent stabilization of erythrocytic indices and signs of anisocytosis. In contrast, in the protein metabolism system a reduction in the total protein content was found due to albumins and γ globulins from the further increase of hepatocytic and immune proteins at the later stages of implementing the adaptive syndrome. A practical, prolonged way of preventing disorders in the erythron and protein of extramarital piglets was feeding the additive “Biovir”, which stimulated the intensity of oxidative-reducing processes and the exchange of proteins.

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Conflict of interest

The authors declare that there is no conflict of interest.

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