Morpho-biochemical parameters of blood and antioxidant protection of the body of repair pigs using natural metabolites

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Abstract. The authors conducted experiments to study the effect of natural metabolites fed to repair pigs in the last three months before insemination on their growth, changes in their live weight, the chemical composition of milk and the content of immunoglobulins in it, as well as morphological and biochemical blood compositions, protein, carbohydrate and lipid metabolism in pregnant and suckling sows. Four groups of repair pigs were formed for research white breed (control and three experimental) at the age of 6 months, 20 heads each, which were kept in separate machines. The control group received the basic diet, without additives. The pigs of the experimental groups were fed natural metabolites from the sixth to the ninth month inclusive. The animals of the I experimental group were fed organic selenium (Selenium) as part of the compound feed – 0.3 kg / t of feed, II experimental group – succinic acid at the rate of 20 mg / kg of live weight (frequency of 10 days), III experimental group - Carolin preparation (beta-carotene oil solution) – 15 ml / head per day. At the ages of 6.5 and 9 months, 5 pigs from each group had blood taken from the ear vein in the morning before feeding for research. The object of research was repair pigs of large white breed and Krasnodar meat type. Feeding of animals both in this experiment and subsequent ones was carried out taking into account detailed feeding standards. The composition and nutritional value of the diets were calculated using the «Kormoptimaexpert» program.

1 Introduction

The duration of production use and productivity of sows are largely determined by the quality of breeding repair pigs [1,2]. One of the conditions for preparing repair pigs for long-term productive use is to increase their antioxidant and immune systems of body

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protection, hormonal status, nonspecific resistance and the formation of a stable reproductive ability [3,4].

The antioxidant defense system plays an important role in protecting the body from adverse factors [5,6]. Many natural biological processes in the body, such as respiration, digestion of food, conversion of fats into energy, produce harmful compounds called free radicals [7,8]. Free radicals are usually destroyed by the body's natural antioxidant system [9,10]. If this system does not cope properly, free radicals can cause a negative chain reaction in the body, a reaction that can destroy the cell membrane, block the action of basic enzymes, interfere with cellular processes necessary for the proper functioning of the body, interfere with the normal functioning of cells. fission, destroy deoxyribonucleic acid (DNA) and block energy production. Since free radicals are essential for life, there are several enzymatic mechanisms in the body that minimize damage caused by radicals and protect against excessive formation of free radicals [11,12]. Antioxidants play a vital role in these defense mechanisms [13]. In healthy organisms, protection from the harmful effects of reactive oxygen species is achieved by maintaining a delicate balance between oxidants and antioxidants [14,15].

2 Materials and methods

The experiment was carried out in the conditions of CJSC "SQUAW" of the Zernogradsky district, LLC "Southern Milk" of the Peschanokopsky district and on pigs of the Krasnodar meat type in LLC "Energia" of the Proletarian district of the Rostov region on repair pigs of large white breed and Krasnodar meat type.

For this purpose, studies have been conducted on the use of biologically active drugs: Selenium (organic selenium), succinic acid, and Carolin (beta-carotene), in the cultivation of repair pigs, according to the scheme (Table 1).

Table 1. Scheme of experience on repair pigs

Group	Number of pigs	Additives used	The scheme of application of additives
Control	20	BD	-
I experienced	20	BD + Selenium	0,3 kg/t of feed (throughout the entire period of experience)
II experienced	20	BD + succinic acid	20 mg / kg of body weight (frequency 10 days after 10 throughout the entire period of the experiment)
III experienced	20	BD + Caroline	15 ml/100 kg of live weight (throughout the entire period of the experiment)

3 Results

To determine to what extent the biological additives used affect the morphological composition of the blood, which characterizes the intensity of redox processes in the body and to a certain extent the level of its cellular defense system, the content of erythrocytes, leukocytes, hemoglobin and hematocrit in the blood of repair pigs was determined (Table 2).

The use of the studied biologically active additives in diets repair of guinea pigs for two weeks (from 6 to 6.5 months of age) led to some changes in blood composition.

Table 2. Morphological parameters of blood in repair pigs (n=5)

Group	Red blood cells x 10^{12}/l	Leukocytes x 10^9/l	Hemoglobin, g/l	Hematocrit, %
Age 6,5 months				
Control	6,15±0,17	13,20±0,20	120,54±1,55	39,05±0,19
I experienced	6,29±0,26	14,12±0,22*	131,62±1,68**	39,86±0,23*
II experienced	6,36±0,33	14,15±0,24*	127,86±1,71*	40,53±0,34**
III experienced	6,32±0,21	14,48±0,17**	130,84±1,84**	39,61±0,17
Age 9 months				
Control	5,60±0,17	13,72±0,29	116,44±1,82	34,53±0,98
I experienced	6,17±0,13*	14,84±0,31*	130,78±2,64**	39,28±0,93**
II experienced	6,28±0,18*	15,22±0,33**	139,40±3,57***	38,39±0,82*
III experienced	6,04±0,12	14,57±0,21*	123,55±1,48*	37,94±0,77*

Thus, the level of erythrocytes had a slight tendency to increase, with an unreliable difference, and the hematocrit content significantly increased in the I and II experimental groups by 2.07 ($P < 0.05$) and 3.79% ($P<0.01$). During this time, the hemoglobin content in the blood in all experimental groups increased significantly by 9.19 ($P \leq 0.01$), 6.07 ($P \leq 0.05$) and 8.55% ($P \leq 0.01$), respectively. Activation of leukocytes was recorded within the physiological norm: in the I experimental group by 6.97 ($P \leq 0.05$), in the II experimental group by 7.20 ($P \leq 0.05$) and in the III experimental group by 9.70% ($P \leq 0.01$).

At 9 months of age, after feeding the studied supplements for 3 months, in terms of the number of erythrocytes, leukocytes and hemoglobin, the dominant position among the experimental groups was occupied by animals of group II, exceeding the control group in terms of these indicators by 12.14 ($P < 0.05$), 10.93 ($P<0.01$) and 19.72% ($P \leq 0.001$). These indicators were slightly lower, but also significantly exceeded the control in repair pigs of the I experimental group by 10.18 ($P \leq 0.05$), 8.16 ($P \leq 0.05$) and 12.32% ($P \leq 0.01$%), but inferior to peers of the II group by 1.78%, 2.56 and 6.59. The advantage of these indicators over the control in pigs of the III experimental group was less impressive and amounted to 7.86%, 6.19 ($P<0.05$) and 6.11% ($P<0.05$).

The hematocrit level was highest in pigs of the I experimental group, which reached 39.28%, which is 4.75% ($P < 0.01$) higher than the control. The excess of this indicator in pigs of the II and III experimental groups over the control groups was 3.86 ($P \leq 0.05$) and 3.41% ($P \leq 0.05$).

The nature of the processes occurring in the body is significantly influenced by the protein composition of the blood (Table 3).

At 6.5 months of age, two weeks after the start of dietary supplements, a significant increase in total protein by 6.29 (P<0.05), 3.74 (P<0.05) and 4.91% (P<0.05), respectively, was detected in the blood serum of repair pigs of the experimental groups. The albumin level in the I experimental group increased by 8.98 (P <0.05), in the II experimental group – by 7.81 (P< 0.05) and in the III experimental group – by 7.58% (P<0.05), relative to the control group. The total number of globulin fractions significantly exceeded the control only in the I and III experimental groups by 4.42 (P<0.05) and 3.06% (P<0.05), mainly due to gamma globulins - by 6.47 (P<0.05) and 4.36% (P<0.05).

In the II experimental group, these indicators tended to increase relative to the control. The activation of globulins was within the physiological norm, and an increase in the gamma globulin fraction indicates an increase in the immunity of repair pigs of the experimental groups.

It follows that even with such short-term feeding

of natural metabolites such as organic selenium, succinic acid and beta-carotene oil solution, protein metabolism processes begin to activate in the body of pigs.

Table 3. Indicators of protein metabolism in repair pigs (n = 5)

Indicator	Group			
	Control	I experienced	II experienced	III experienced
Age 6,5 months				
Total protein, g/l	62,52±0,75	66,45±1,08*	64,86±0,54*	65,59±0,96*
Albumins, g/l	25,60±0,58	27,90±0,62*	27,60±0,56*	27,54±0,51*
Globulins, g/l including:	36,92±0,32	38,55±0,41*	37,26±0,29	38,05±0,27*
Alpha- globulins	13,24±0,52	13,57±0,37	13,23±0,47	13,67±0,42
Beta- globulins	8,08±0,20	8,37±0,15	8,02±0,18	8,10±0,16
Gamma- globulins	15,60±0,27	16,61±0,33*	15,98±0,45	16,28±0,47*
AST, mmol/(h-l)	0,88±0,03	0,90±0,03	0,88±0,03	0,89±0,04
ALT, mmol/(h-l)	0,53±0,02	0,51±0,04	0,49±0,03	0,52±0,03
Age 9 months				
Total protein, g/l	70,54±1,12	77,04±1,26**	76,28±1,08**	74,14±1,13*
Albumins, g/l	28,15±0,57	31,06±0,0,62**	31,46±0,69**	29,92±0,49*
Globulins, g/l including:	42,39±0,68	45,98±0,75**	44,82±0,56**	44,22±0,37*
Alpha- globulins	16,00±0,48	15,10±0,32	14,91±0,41	14,58±0,44
Beta- globulins	9,90±0,19	11,12±0,23**	10,60±0,17*	11,07±0,18**

Gamma- globulins	16,49±0,67	19,76±0,62**	19,21±0,43**	18,57±0,37*
AST, mmol/(h-l)	2,07±0,07	2,38±0,09*	2,34±0,08*	2,29±0,06*
ALT, mmol/(h-l)	1,81±0,06	1,59±0,05*	1,52±0,09*	1,58±0,08*

Studies of the protein composition of the blood of pigs at the age of 9 months have shown that protein metabolism largely depends on the biological activity of the additives used.

The most significant effect on the protein metabolism of repair pigs was provided by the Selenium supplement (experimental group I). The advantage over the control group in terms of total protein content was 9.21% ($P<0.01$), the level of albumin fraction – by 10.34% ($P<0.01$), the total number of globulin fractions – by 8.47% ($P<0.01$), including due to beta-globulins by 12.32% ($P<0.01$) and gamma globulins - by 19.83% ($P\leq0.01$). Significantly high rates were also found in the II experimental group, where animals received succinic acid. An increase in total protein and albumin fraction was recorded by 8.14 ($P\leq0.01$) and 11.76% ($P\leq0.01$), activation of globulin fractions – by 5.73% ($P\leq0.01$), especially gamma globulins - by 16.49% ($P\leq0.01$) relative to the control group.

In terms of the total protein content in the blood, pigs of the experimental group III who received an oil solution of veta-carotene as part of the Karolin feed additive were inferior to their peers of the I and II experimental groups, but had an advantage over intact animals: in terms of total protein, albumins and globulins by 5.10 ($P\leq0.05$), 6.29 (P0.05) and 4.32% ($P<0.05$), in terms of beta and gamma globulin levels by 11.82 ($P<0.05$) and 12.61% ($P<0.05$), respectively.

Evidence of high protein metabolism is also provided by the indicators of transamination enzymes, which in our experience emphasize the effectiveness and safety of the studied drugs. The AST content in the blood serum of animals of the I experimental group increased by 14.98 ($P\leq0.05$), and ALT decreased by 13.84% ($P\leq0.05$), in the II experimental group – by 13.04 ($P\leq0.05$) and 19.08% ($P\leq0.01$), and in the III experimental group – by 10.63 ($P \leq0.05$) and 14.56% ($P<0.05$) compared to the control group.

In general, it should be noted that the introduction of the studied feed additives into the body of repair pigs contributes to the activation of protein metabolism in their body and has a positive effect on the physiological status of the liver, as evidenced by a decrease in ALT activity.

There were some changes in the indicators of carbohydrate-lipid metabolism, under the influence of biologically active feed additives (Table 4).

Table 4. Indicators of carbohydrate-lipid metabolism (n = 5)

Values	Control	I experienced	II experienced	III experienced
Age 6,5 months				
Total lipids, g/l	0,82±0,03	0,90±0,04	0,89±0,04	0,88±0,03
Cholesterol, mmol/l	1,82±0,05	1,85±0,04	1,83±0,05	1,80±0,06
Triglycerides, mmol/l	0,54±0,03	0,59±0,03	0,57±0,03	0,56±0,03
Glucose, mmol/l	5,73±0,20	5,40±0,25	5,28±0,32	5,36±0,35
Lactic acid, mmol/l	12,57±0,25	13,31±0,26	13,14±0,18	13,28±0,31
Age 9 months				

Total lipids, g/l	1,54±0,04	1,73±0,07*	1,69±0,05*	1,88±0,08**
Cholesterol, mmol/l	3,17±0,06	2,95±0,07*	2,76±0,09**	2,88±0,08*
Triglycerides, mmol/l	0,63±0,023	0,75±0,031*	0,71±0,015*	0,69±0,11*
Glucose, mmol/l	6,12±0,13	6,65±0,12*	6,87±0,19*	7,03±0,21**
Lactic acid, mmol/l	16,08±0,41	18,02±0,42*	17,34±0,27*	18,32±0,38**

At the age of 6.5 months, repair pigs of the experimental groups showed a tendency to increase the blood content of total lipids (9.76; 8.54 and 7.32%), triglycerides (9.26; 5.56 and 3.70%), lactic acid (5.89; 4.53 and 5.65%), while reducing glucose (6.11; 8.52 and 6.90%), which is probably due to an increase in the energy expenditure of the body for the intensive growth of muscle mass of the body and organs of the reproductive system. No pattern has been revealed in the change in cholesterol levels in the blood.

The results of studies of animal blood serum at 9 months of age showed that the total lipid content of the experimental groups exceeded the control by 12.34 (P<0.05), 9.74 (P<0.05) and 22.08% (P<0.01), respectively, while the highest number was recorded in the III experimental group, where pigs daily an oil solution of beta-carotene was obtained. Among the experimental groups, pigs of the II experimental group, whose animals received succinic acid, differed in the lowest cholesterol content, as well as total lipids. The decrease in cholesterol levels in this group, relative to the control, reached 14.86 (P < 0.01), in the III experimental group – 10.07 (P<0.05), and in the I experimental group – 7.46% (P<0.05).

The content of triglycerides, whose function is to provide energy to the processes occurring in the body, was more significantly influenced by the additives "Selenium" (I experimental) and succinic acid (II experimental), which increased the level of the component by 19.05 (P ≤0.05) and 12.70% (P≤0.05), respectively. The feed additive "Karolin" (III experimental) contributed to an increase in the triglyceride content by 9.52% (P<0.05).

By the age of 9 months, the highest intensity of carbohydrate metabolism was observed in animals of the III experimental group who received the Karolin supplement, where the lactic acid content increased by 13.93% (P≤0.01), glucose by 14.87% (P≤0.01), in the I experimental group – by 12.06 (P≤0.05) and 8.66% (P≤0.05), in the II experimental group – by 7.83 (P≤0.05) and 12.25% (P≤0.05).

4 Discussion of the results

Natural immunity monitoring parameters can provide useful information about animal health. The natural resistance of repair pigs was determined at the ages of 6.5 and 9 months (Table 5).

Table 5. Indicators of natural resistance of repair pigs (n=5)

Values	Group			
	Control	I experienced	II experienced	III experienced
Age 6,5 months				
Phagocytic activity of neutrophils, %	38,73±2,11	40,48±2,64	39,19±3,04	39,25±2,88
Phagocytic index, mk./leukocytes	1,60±0,04	1,70±0,03	1,66±0,02	1,68±0,03

Phagocytic capacity, 10^9L	12,24±0,09	12,89±0,15	12,65±0,25	12,70±0,17
Bactericidal activity, %	55,75±2,24	57,90±2,19	57,32±1,73	56,23±1,97
Lysozyme activity, %	40,06±1,23	42,68±1,32	43,09±0,95	41,92±1,15
Age 9 months				
Phagocytic activity of neutrophils, %	34,80±0,89	39,87±0,76**	38,85±0,63**	37,18±0,49*
Phagocytic index, mk./leukocytes	3,06±0,08	3,52±0,07**	3,43±0,06**	3,28±0,04*
Phagocytic capacity, 10^9L	18,34±0,35	20,63±0,42**	20,57±0,36**	19,86±0,41*
Bactericidal activity, %	52,42±1,12	59,18±1,55**	60,05±1,67**	58,83±1,50**
Lysozyme activity, %	36,35±0,71	41,42±0,84**	43,58±0,93***	38,25±0,42*

It was found that after two weeks of using drugs in the blood of pigs, there was a tendency to increase the indicators characterizing the level of natural resistance. To the greatest extent, this was reflected in an increase in the lysozyme activity of blood serum, which in animals of the experimental groups increased by 2.62; 3.03 and 1.86% relative to the control. The indicators of phagocytic activity of neutrophils in the blood of repair pigs of the experimental groups also exceeded the control by 1.75; 0.46 and 0.52%, which led to an increase in phagocytic capacity by 5.31; 3.35 and 3.76%, respectively. A change in the bactericidal activity of the blood of animals of the experimental groups was recorded, the difference in indicators with the control group was 2.15; 1.57 and 0.48%.

At the age of 9 months, both in terms of cellular and humoral body protection factors, the pigs of the experimental groups significantly exceeded the analogues of the control group. The level of phagocytic activity of neutrophils exceeded the same indicator from the control in the experimental group I by 5.07 ($P \leq 0.01$), experimental group II by 4.05 ($P \leq 0.01$) and experimental group III by 2.38% ($P \leq 0.05$), phagocytic index and phagocytic capacity by 15.03 ($P \leq 0.01$) and 12.49% ($P \leq 0.01$), 12.09 ($P \leq 0.01$) and 12.06% ($P \leq 0.01$), 7.19 ($P \leq 0.05$) and 8.29% ($P \leq 0.05$), respectively. Humoral resistance factors have also changed under the influence of biostimulants. The level of bactericidal activity increased relative to the control in the I experimental group by 6.76 ($P \leq 0.01$), in the II experimental group by 7.63 ($P \leq 0.01$) and in the III experimental group by 6.41% ($P \leq 0.05$), and the level of lysozyme by 5.07 ($P \leq 0.01$), 7.23 ($P \leq 0.001$) and 1.90% ($P<0.05$), respectively. The data obtained indicate that the most formed resistance factors, at the time of insemination, were in repair pigs treated with Selenium and succinic acid.

The antioxidant defense system plays an important role in protecting the body from adverse factors. Many natural biological processes in the body, such as respiration, digestion of food, conversion of fats into energy, produce harmful compounds called free radicals. Free radicals are usually destroyed by the body's natural antioxidant system. If this system does not cope properly, free radicals can cause a negative chain reaction in the body, a reaction that can destroy the cell membrane, block the action of basic enzymes, interfere with cellular processes necessary for the proper functioning of the body, interfere with the normal functioning of cells. fission, destroy deoxyribonucleic acid (DNA) and block energy production. Since free radicals are essential for life, there are several enzymatic mechanisms in the body that minimize damage caused by radicals and protect against excessive formation of free radicals. Antioxidants play a vital role in these defense mechanisms. In healthy organisms, protection from the harmful effects of reactive oxygen species is achieved by maintaining a delicate balance between oxidants and antioxidants.

In the blood of 6.5- month-old repair pigs of the experimental groups, under the influence of natural metabolites involved in the experiment, a decrease in lipid peroxidation and an increase in the antioxidant defense system of the body were recorded (Table 6), this is confirmed by a reduction in malondialdehyde by 7.74; 7.21 and 6.69%, diene conjugates – by 6.70; 5.25 and 5.32%, lipid hydroperoxides – by 7.51; 6.47 and 7.28% compared with the control. At the same time, catalase activity increased by 7.34; 6.51 and 5.45%, superoxide dismutase by 4.99; 5.62 and 4.57%, glutathione peroxidase – by 6.94; 3.47 and 5.56% compared to the control group.

In the 9-month-old pig population of the control group, a high level of lipid peroxidation products was found compared with the control groups MDA by 24.01 ($P \leq 0.05$), 19.29 ($P \leq 0.05$) and 20.43% ($P \leq 0.05$), DC – by 17.77 ($P \leq 0.05$), 14.55 ($P \leq 0.05$) and 16.70% ($P<0.05$), GPL – by 22.56 ($P<0.05$), 16.90 ($P<0.05$) and 19.52% ($p<0.05$) correspond.

Table 6. Indicators of lipid peroxidation and antioxidant body protection system of repair pigs (n =5)

Values	Control	I experienced	II experienced	III experienced
Age 6,5 months				
Malondialdehyde (MDA), nmol/ml	6,54±0,33	6,07±0,37	6,10±0,45	6,13±0,41
Diene conjugates (DC), nmol/ml	52,85±2,30	49,53±2,06	50,21±1,87	50,18±2,42
Lipid hydroperoxides (HPL), nmol MDA/ml	35,20±1,72	32,74±1,60	33,06±1,41	32,81±1,67
Catalase, EA/ml	82,38±2,65	88,43±2,77	87,74±2,49	86,87±3,14
Superoxide Dismutase (SOD), A/ml	25,41±1,07	26,68±0,85	26,84±1,16	26,57±1,03
Glutathione peroxidase (GPO), mmol NADP/min-ml	1,44±0,08	1,54±0,12	1,49±0,09	1,52±0,07
Age 9 months				
Malondialdehyde (MDA), nmol/ml	5,01±0,24	4,04±0,21*	4,20±0,18*	4,16±0,16*
Diene conjugates (DC), nmol/ml	54,80±2,12	46,53±2,25*	47,84±2,07*	46,96±1,97*
Lipid hydroperoxides (HPL), nmol MDA/ml	34,11±1,43	27,83±1,65*	29,18±1,54*	28,54±1,37*
Catalase, EA/ml	73,18±2,49**	89,64±2,81**	88,05±2,46**	87,82±1,83**
Superoxide Dismutase (SOD), A/ml	28,43±0,86	32,11±1,14*	33,82±0,93**	31,76±0,82*
Glutathione peroxidase a (GPO), mmol NADP/ min-ml	1,49±0,07	1,76±0,09*	1,65±0,05	1,71±0,06*

It is known that the harmful effects of reactive oxygen species are controlled by various cellular defense systems consisting of enzymatic components (catalase, glutathione peroxidase and superoxide dismutase, and others).

The body is provided with energy mainly due to the breakdown of fats, as a result of which, in our opinion, a certain amount of peroxidation products is formed, the content of which in pigs of the II experimental group, which showed a high growth rate during cultivation, turned out to be the highest. In pigs of the I and III experimental groups, the blood content of malonic aldehyde was lower by 3.96 and 0.96%, diene conjugates by 2.82 and 1.88%, lipid hydroperoxides by 4.85 and 2.24% compared with the II experimental group.

Of all the metabolites used in the experiment, selenium is considered the most active antioxidant, the addition of which increased the activity of catalase and glutathione peroxidase. In the first experimental group, catalase activity increased by 22.49% ($P < 0.01$) relative to the control, and glutathione peroxidase activity increased by 18.12% ($P<0.05$). The activity of superoxide dismutase in the blood was more influenced by the addition of succinic acid, increasing the enzyme content by 18.95% ($P<0.01$).

5 Conclusions

Thus, it was found that the natural metabolites included in the diet of repair pigs, for three months, had a significant effect on all types of metabolism (protein, lipid, carbohydrate). It follows that even with such short-term feeding of natural metabolites such as organic selenium, succinic acid and beta-carotene oil solution, protein metabolism processes begin to activate in the body of pigs.

The analysis of the body's defense system as a whole allows us to conclude that repair pigs that received succinic acid and Selenium preparations as part of the diet turned out to be the most resistant to various kinds of stress factors. In general, it should be noted that the introduction of the studied feed additives into the body of repair pigs contributes to the activation of protein metabolism in their body and has a positive effect on the physiological status of the liver, as evidenced by a decrease in ALT activity.

There were some changes in the indicators of carbohydrate-lipid metabolism, under the influence of biologically active feed additives.

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