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# Effect of Lithium Ascorbate on the Biochemical Parameters of Sows

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## ABSTRACT

The aim of this work is to develop an effective way of dealing with the manifestations of stress in sows are able to increase their productivity. The study was conducted on 5 groups of pregnant sows of the Irish Landrace breed (4 experimental and 1 control) with 5 heads each. 30 days after successful insemination, the sows of the 1st, 2nd, 3rd, and 4th experimental groups began receiving daily lithium ascorbate in powder form in a dose of 10 mg/kg, 5 mg/kg, 2 mg/kg, and 0.5 mg/kg of live weight, respectively. Studies have revealed that as a result of the introduction of lithium ascorbate with food to sows at a dose of 10, 5 and 2 mg/kg of body weight, a pronounced adaptogenic and stress-protective effect develops, which contributes to maintaining cortisol and progesterone at the physiological level throughout pregnancy. At the same time, lithium ascorbate can increase the nonspecific resistance of sows, the intensity of their growth during pregnancy, and provide protection against technological and spontaneous stressors. The use of lithium ascorbate in animals has a positive effect on lipid-cholesterol metabolism, the glutathione reduction system, which increases the safety, increases the build-up of live weight and stimulates the reproductive function of sows. The conducted test, based on the use of lithium ascorbate in pregnant sows, opens up a new scientific direction for economically beneficial regulation of biochemical and physiological parameters in animals in conditions of their industrial maintenance with the increase of non-specific resistance and productivity of animals.

**Keywords:** Pregnant sows, Lithium ascorbate, Antioxidant status, Glutathione reduction system, Lipid-cholesterol metabolism.

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## INTRODUCTION

The effectiveness of the development of modern pig breeding is closely related to the biotechnology of pig reproduction[1]. The practical application of effective methods to increase the productivity and reproductive indicators of animals significantly increases the effectiveness of using breeding stock, while ensuring high stability of production indicators and profitability of pork production technology [2,3].

At the current stage of development of physiological science, a special place is occupied by the elucidation of the fundamental mechanisms of ensuring the vital functions of the mammalian organism [4,5] and their use in practical pig breeding [6]. This will make it possible to develop new and improve existing adaptive technologies for reproduction of livestock, to properly organize the technology of reproduction of the herd in accordance with the laws of growth and development of pigs[7]. Currently, the practice of animal husbandry often resorts to methods of regulating functional homeostasis (the use of new generation adaptogens), which include lithium ascorbate [8,9].

By combining the mineral lithium salt with ascorbic acid, it was assumed to obtain an organic lithium salt, which will provide new biologically beneficial properties that are not present in the starting substances. This is facilitated by the fact that lithium ascorbate is an adaptogenic drug based on minimizing stress in animals. It claims to be one of the new effective components of cheaper pork production technology [10].

The observed increase in stress tolerance and productivity causes a positive effect on lipid-cholesterol metabolism, the system of glutathione reduction with associated processes of reducing the intensity of free radical processes and lipoperoxidation in animals, leading to optimization of their neuro-metabolic processes [11,12]. In this regard, these parameters can be considered as markers of the effectiveness of the use of lithium ascorbate in sows, which must be taken into account.

The purpose of the study: to develop an effective way to combat stress in sows, which can increase their productivity.

## METHOD

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 All-Russian research Institute of Physiology, Biochemistry and

Nutrition of animals – branch of the Federal Science Center for Animal Husbandry named after Academy Member L. K. Ernst(Record №12, dated December 7, 2017).

The study was conducted in the conditions of the "Rodina" pig farm in the Maloyaroslavetsky district of the Kaluga region of Russia.

The experimental part of the work was carried out on 25 heads of pregnant sows of the Irish Landrace breed (on the second farrowing). For this study, the animals were randomly divided into 5 groups (4 experimental and 1 control) with 5 heads each. Pregnant sows of the experimental and control groups were kept in the same room in individual machines from the moment the groups were organized; accurate feed dosing and the content of lithium ascorbate in it were ensured. The diet and technological process did not differ from the one adopted in the farm (Table 1).

**Table 1.** Food composition of the examined sows during gestation

| The nutritional value of feed | The first 84 days of pregnancy | Mid-pregnancy | Last 30 days of pregnancy |
|---|---|---|---|
| Energy feed unit | 2.87 | 3.10 | 3.54 |
| Metabolizable energy, MJ | 28.7 | 32.2 | 35.4 |
| Dry matter, kg | 2.47 | 2.87 | 3.05 |
| Crude protein, g | 346.0 | 385.0 | 427.0 |
| Digestible protein, g | 260.0 | 290.0 | 320.0 |
| Lysine, g | 14.8 | 17.2 | 18.3 |
| Threonine, g | 10.1 | 11.3 | 12.5 |
| Methionine + cysteine, g | 8.9 | 9.7 | 11.0 |
| Crude fiber, g | 287.0 | 217.0 | 354.0 |
| Salt, g | 14.0 | 16.0 | 18.0 |
| Calcium, g | 21.0 | 24.0 | 27.0 |
| Phosphorus, g | 18.0 | 20.0 | 22.0 |
| Iron, mg | 200.0 | 220.0 | 247.0 |
| Copper, mg | 42.0 | 46.0 | 52.0 |
| Zinc, mg | 215.0 | 230.0 | 265.0 |
| Manganese, mg | 116.0 | 128.0 | 143.0 |
| Cobalt, mg | 4.0 | 4.5 | 5.0 |
| Iodine, mg | 0.8 | 0.9 | 1.1 |
| Carotene, mg | 28.0 | 31.0 | 35.0 |
| Vitamins: A, thousand IU | 14.0 | 16.0 | 18.0 |
| Ergocalciferol (D), IU | 1.4 | 15.0 | 1.8 |
| Tocopherol (E), mg | 101.0 | 110.0 | 125.0 |
| Thiamin ($B_1$), mg | 6.0 | 7.0 | 8.0 |
| Riboflavin ($B_2$), mg | 17.0 | 19.0 | 21.0 |
| Pantothenic acid ($B_3$), mg | 57.0 | 62.0 | 70.0 |
| Choline ($B_4$), g | 2.8 | 3.1 | 3.5 |
| Pantothenic acid ($B_5$), mg | 200.0 | 225.0 | 247.0 |
| Cyanocobalamin ($B_{12}$), μg | 72.0 | 77.0 | 88.0 |

30 days after successful insemination, the sows of the experimental groups were given long-term daily administration of various doses of lithium ascorbate in the form of powder together with the feed. In the first experimental group, the dose of lithium ascorbate was 10 mg/kg; in the 2nd–5 mg/kg; in the 3rd – 2 mg/kg; in the 4th experimental group – 0.5 mg/kg. The control group of sows was on the main diet without adding the drug. Animals were weighed before administration of lithium ascorbate, 2, 3 and 4 months after fertilization. At the same time, blood was taken from sows and placed in vacuum tubes with the addition of a 10 % solution of Trilon B for biochemical analysis.In their blood plasma, at each examination, the concentration of malondialdehyde (nm/ml), reduced glutathione (μM/L), oxidized glutathione (μM/L), triacylglycerols (mM/L), low-density lipoprotein cholesterol (mM/L) was determined, very low density lipoprotein cholesterol (mM/L), high density lipoprotein cholesterol (mm/L), thiol-disulfide ratio, superoxide dismutase activity (Eg), glutathione peroxidase activity (Eg).

**Chromatographic determination of glutathione**

Reduced glutathione (GSH) reacts with orthophthalaldehyde to form a stable, highly fluorescent tricyclic derivative at pH = 8, while GSSG reacts with orthophthalaldehyde at pH = 12.When measuring oxidized (GSSG) glutathione in blood plasma, GSH was mixed with N-ethylmaleimide. For separation, the discovery C18, 150 mm × 4 mm, 5 microns reversed phase columns were used. A mixture of methanol and 25 mm sodium hydrophosphate (15:85), pH = 6.0, was used as the mobile phase. The analytical efficiency of this method is satisfactory for GSH and GSSG. Within the analysis, the coefficients of variation were 4.3 and 5.2 % for plasma. The coefficients between the analyses of variation for plasma were 6.9 and 7.8 %. The plasma yield is distributed as follows: 94.1 % (7.5 %) and 103.5 (8.5 %). The calibration curve was linear over the entire range studied. The liquid chromatography method is effective and sensitive for determining the amount of glutathione, glutathione disulfide, cysteine mixed with glutathione disulfide and 20 related sulfur-containing amino acids or their derivatives. The procedure is based on the initial

stage of formation of S-carboxymethyl free thiol-diacetic acid derivatives with subsequent conversion of free amino groups to 2,4-dinitrophenyl derivatives by reaction with 1-fluoro-2,4-dinitrobenzene.After chromatography of the reaction mixture without isolation, the sample is placed on a 3-aminopropylsilane derivatized column with silicon dioxide, eluting with a gradient of sodium or ammonium acetate in a water–methanol–acetic acid solvent at pH = 4.5.

The method allows us to quantify simultaneously reduced and oxidized glutathione, cysteine, cystine and other SH/SS in various tissues, including blood and plasma, liver, and hippocampus. Using metaphosphoric acid and high–performance liquid chromatography-DEC, optimal conditions for typical processing and analysis were determined. True standards of 10 General SH/SS compositions were established and eluted for 15 min, with all standard curves being linear from 5 to 1600 nmol. The test was based on the following: first, the tissue size was proportional to the peak area in the eightfold range; second, the recovery of SH/SS added to the samples before treatment was 96–101 %; third, the results are equivalent and strongly correlated with high values for total SH using the 5.5'-dithiobis (2-nitrobenzoic acid) (DNBA) assay ($r^2$ = 0.996), as well as for the analysis of total glutathione reductase DNBA-GSSG ($r^2$ = 0.998). The lifetime of the Au/Hg electrode was limited to 200–500 samples based on a linear range of standard curves.

**Method for evaluating superoxide dismutase activity**

**Superoxide dismutase (SOD)** catalyzes the formation of hydrogen peroxide from a superoxide radical. In contrast to spontaneous $O_2$dismutation, hydrogen peroxide and oxygen are formed:

$$O_2 + O_2 + 2H^+ \rightarrow O_2 + H_2O_2.$$

In a SOD-catalyzed dismutation reaction, the product is triplet oxygen, rather than excited singlet oxygen, as in spontaneous dismutation. SOD is an extremely active enzyme: its number of revolutions ($k_{cat}$) is one of the highest for enzymes – about $10^6$ $s^{-1}$, and the speed constant ($k_{cat}/K_m$) > $10^8$ $M^{-1}s^{-1}V^1$. Therefore, it should almost immediately eliminate the resulting $O_2$ to a negligibly low stationary level. Therefore, it should almost immediately eliminate the resulting $O_2$ to a negligibly low stationary level. According to a number of available estimates, the stationary level of $O_2$ in cells and tissues, despite the presence of many and quite intensive sources of its generation, is extremely low – about $10^{-10}$–$10^{-11}$ M. This just helps to ensure that cells and tissues do not develop oxidative processes that damage their structures, but at the same time, there is a sufficient "reserve" of ROS necessary for the generation of electronically excited States.

Superoxide dismutase of higher animal and human cells consists of two subunits containing one copper atom and one zinc atom. This form of SOD is called Cu, Zn-SOD. It is a dimer with a molecular weight of 32,000 daltons, found in all eukaryotic cells. It is believed that the copper atom provides the catalytic activity of the enzyme, and the zinc atom gives it stability. Cu, Zn-SOD in humans and higher animals is an intracellular enzyme. In Cu and Zn cells, SOD is localized mainly in the cytosol and in the intermembrane space of mitochondria.

A high-molecular extracellular (extracellular) SOD, referred to as ECTA-SOD (ECSOD), consisting of 4 subunits was found in the intercellular space. This Cu, Zn-containing glycoprotein is the main isoform of intercellular fluids – plasma, lymph, synovial fluid (and is found in small amounts in almost all tissues).

The method is based on the ability of SOD to inhibit the reduction of tetrazoliumnitrosine (TNS) under conditions of generation of a superoxide anion radical.

*Sampling and storage.* Blood was collected in a test tube with heparin (200 units/ml) and centrifuged at 3000 rpm for 10 minutes. Plasma was collected and stored in the cold, and red blood cells were washed three times with saline. The washed red blood cells were hemolysed for this purpose, 2.7 ml of chilled bidistilled water was added to 0.3 ml of Packed red blood cells using a pipette. The suspension was shaken periodically for 5 minutes, and then centrifuged at 14,000 rpm for 20 minutes to precipitate Strom cells. The resulting 10 % hemolysate was diluted to 1 % (0.1 ml of hemolysate + 0.9 ml of water). An aliquot was selected from the diluted hemolysate for analysis of the hemoglobin content.

*Equipment.* Cooling centrifuge, magnetic stirrer, thermostat.

*Reagents and their preparation.* Bidistilled water was used in the preparation of reagents.

0.067 M $KH_2PO_4$ solution – in a measuring flask per 1000 ml, 0.907 g of $KH_2PO_4$ is dissolved in distilled water.

0.067 M $Na_2HPO_4{\cdot}2H_2O$ solution-in a measuring flask per 1000 ml in distilled water, 11.866 g $Na_2HPO_4{\cdot}2H_2O$ is dissolved.

0.067 M phosphate buffer (pH 7.8) – 36 ml of 0.067 M $KH_2PO_4$ solution mixed with 964 ml of 0.067 M $Na_2HPO_4{\cdot}2H_2O$ solution under pH control on the pH meter.

26.9 microns EDTA solution – 10 mg of EDTA disodium salt is dissolved in 100 ml of water (before working, the initial solution is diluted 10 times);

4.04 mm solution of tetrazoliumnitrosine – 33 mg TNS is dissolved in 10 ml of phosphate buffer (prepared before the experiment, stored in the dark in the cold);

65 microns solution of phenazinemetasulfate (PMS) – 2 mg of the reagent is dissolved in 100 ml of phosphate buffer; stored in the dark for no more than a week (frozen in the dark is stored for several months);

1 mm solution of NAD·H – 7.63 mg of the reagent is dissolved in 10 ml of phosphate buffer (prepared before the experiment and stored in the cold).

0.9 % sodium chloride solution. 96 % ethyl alcohol. Gelatin.Reagents for the determination of hemoglobin by the ammonia method. Reagents for protein determination using the Lowry method. The NADH content in dry commercial reagent preparations is preliminarily determined using the Butler method. To do this, first measure the optical density of a Tris-EDTA solution without NADH, and then add a NADH solution containing 2 mg of NADH in 1 ml of Tris-EDTA buffer, and again record the optical density of the solution at a wavelength of 340 nm.

The concentration of NADH in mM is calculated by the formula:

$$T = ((E_x - E_{oн}) / E_к) \times 100\ \%,$$

where$E_o$ is the optical density with NADH; $E_k$ is the optical density of the Tris-EDTA buffer; 0.311– coefficient of molar extinction of NADH.

*Determination progress.* For the precipitation of hemoglobin and partial purification of SOD, 0.3 ml of 96 % ethanol and 0.15 ml of chloroform are added to 1 ml of 1 % hemolysate. The mixture is stirred on a magnetic stirrer in the cold for 15 minutes, then the same time is left in the cold, shaking from time to time. The chloroform-ethanol mixture is centrifuged at 10,000 rpm for 15 minutes. The top layer is taken and used as the source of the enzyme. The supernatant should be clear or slightly opalescent with no yellow tinge.

Immediately before determining the activity of the enzyme, an incubation mixture of the following composition was prepared: 1 ml of 26.9 μM EDTA; 1 ml 4.04 mMTNS; 1 ml 65 μM PMS; 1 mg gelatin; 26 ml 0.067 M phosphate buffer (pH 7.8).

2.85 ml (in the case of plasma, 2.7 ml) of the incubation mixture were poured into test tubes and heated for 5 min at 25 °C. Then, 0.05 ml of hemolysate supernatant (0.2–0.3 ml of plasma supernatant) and 0.1 ml of 1 mM NADH were added to the incubation mixture. In the control sample, 0.1 ml of phosphate buffer was added instead of the supernatant. Incubation was performed for 10 min in a thermostat at 25 °C under aerobic conditions in the dark. The reaction was stopped by illuminating the samples. The optical density of the samples was measured at a wavelength of 540 nm in a cuvette with an optical path width of 5 mM against a mixture containing all components of the incubation mixture, except for NADH.

Fluctuations in the degree of inhibition of the enzymatic reaction should be in the range from 30 to 70 %. If the percentage of inhibition due to SOD went beyond the indicated limits, the amount of enzyme introduced into the incubation mixture was changed.

One conventional unit of SOD activity was taken to be 50 % inhibition of the TNS recovery process during incubation. The enzyme activity was expressed in arbitrary units per mg of hemoglobin (mg of protein, ml of plasma).

**Method for the determination of malonicdialdehyde**

**Malonicdialdehyde (MDA)** is one of the products of free radical lipid oxidation, the accumulation of which reflects the degree of oxidative stress in the body. An assessment of this indicator is necessary to determine the causes and mechanisms of development of a particular pathological process and to suggest ways to treat diseases. A well-known method for assessing the level of MDA is its reaction with thiobarbituric acid, as a result of which a pink chromogen is formed, which is called the "trimethine complex". The concentration of MDA was determined from the value of the optical density of this chromogen, obtained on a spectrophotometer. The object of the study was plasma or serum. A 1 % detergent solution (Triton X-100 or deoxycholate), 0.6 M HCl solution and 0.06 M TBA working solution were sequentially added to the serum sample; the sample was incubated at a constant temperature (95 °C) for 30–60 min. The resulting mixture had a characteristic pink color. Optical density was measured on a spectrophotometer at 532 nm. A mixture with the same reagents, where the serum was replaced by distilled water, served as a control. The amount of the formed trimethine complex corresponds to the amount of reacted MDA; therefore, its concentration can be calculated knowing the optical density of the sample under study. This method for determining the level of oxidative stress according to MDA is very sensitive.Even a small sample volume (less than 0.1 ml) allows you to accurately identify the concentration of a given substance.

**Method for the determination of glutathione peroxidase (GPO)**

For the deactivation of hydrogen peroxide, there is an important family of enzymes – glutathione peroxidases. This family of enzymes includes selenium, which contains 4 selenium atoms in each molecule. In addition to this cellular isoform, which received serial number 1 (GPO1) in the classification, glutathione peroxidase is represented by selenium isoenzymes: “gastrointestinal” (GPO2 is isolated from the cytosol of liver and intestinal cells); extracellular (GPO3 is detected in plasma and milk); GPO of phospholipid hydroperoxides (GPO4) and Se-free isozymes – “secretory” GPO5 contained in the epididymis and GPO7. In addition to all, exotic GPO6 is also isolated, which contains selenium (man, pig), or not (mouse, rat).

All GPOs reduce $H_2O_2$ (as opposed to GST) and organic ROOHs, respectively, to water or alcohol ROH. With an insufficient amount of selenium in the diet, the GPO level decreases, which reduces the body's resistance to oxidative stress and thus can lead to the development of free radical pathology. GPO neutralizes lipid peroxides such as linoleic and linolenic acid hydroperoxides, 7P cholesterol hydroperoxide and some synthetic substances. GPO also neutralizes peroxynitrite: 2GSH + ONOO → G-S-S-G + $H_2O$ + $NO_2$. The highest GPO activity is observed in the liver, erythrocytes, adrenal glands; medium activity – in the lungs and heart, low – in the muscles. Deficiency or inhibition of GPO leads to lipid peroxidation. The enzyme activity depends on the amount of peroxides formed. The enzyme is resistant to the action of cyanide and azide; it is most pronounced in the presence of reduced glutathione GSH.

Method principle: glutathione peroxidase (GPO) catalyzes the reaction of interaction of glutathione (GSH) with tert-butyl hydroperoxide (TBHP, model substrate):

*GSH + TBHP = (GPx) = GSSO + reduced TBHP.*

The enzyme activity is assessed by the change in the GSH content in samples before and after incubation with a model substrate during a color reaction with DTNBA.

*Reagents:* 0.1 M Tris-HCl buffer with 0.01 % EDTA content, pH = 8.5; complex buffer (78 mg of sodium azide, 100 mg of reduced glutathione is dissolved in 100 ml of 0.1 M Tris-HCl buffer with 0.01 % EDTA, pH = 8.5); 0.14 % solution of TBHP (commercial preparation, 10 μl of TBHP is diluted in 5 ml of distilled water); 20 % TCA solution; methanol; 0.4 % solution of DTNBA diluted in methanol. A new reagent was prepared before each determination.

*Determination:* the reagents were added to the experimental and control samples. Serum 0.2 ml; Complex buffer 0.73 ml; incubated for 10 min at 37 °C; TBHP 0.07 ml; thermostated for 5 min at 37 °C; TCA 20 % – 0.2 ml; centrifuged for 10 min at 1700 g; supernatant 0.1 ml; 0.1 M Tris-HCl– 2.65 ml; DTNBA– 0.025 ml.

*Measurement:* The supernatant was used to determine the amount of reduced glutathione. For this purpose, 2.65 ml of 0.1 M Tris-HCl buffer and 0.025 ml of DTNBA solution were added to 0.1 ml of the supernatant. After stirring, the samples were photometric on a spectrophotometer at a wavelength of 412 nm, in a cuvette with an optical path length of 1.0 cm against distilled $H_2O$.

*Calculation:*

$$A = \frac{AD \times V \times 1000}{V_{np} \times s \times \text{protein} \times d},$$

where *AD* is the difference in optical density of the experimental and control samples; *V* is the volume of the supernatant used to determine the concentration of GPO (0.1 ml); *d* is the length of the optical path of the cuvette (1 cm); $V_{np.}$– the volume of the reaction mixture (2.775 ml); *1000/*protein– conversion factor per g/protein.

Total cholesterol and fractions: high density lipoproteins, low density lipoproteins, very low density lipoproteins, triglycerides, B-lipoproteins, immunoglobulins, were determined on an acoustic analyzer "AKBa-01-BIOM", firm "BIOM" (Russia) by an acoustic method and an electrophoretic system "Paragon" by "Beckman" (USA).

Statistical processing of the data obtained was carried out using Statistics for Windows software, version

6.0(Microsoft Excel). Differences in data were considered statistically significant at a value of $p<0.05$.

## RESULTS

The live weight of the animals varied depending on the content of lithium ascorbate in the feed. An increase over the entire period of gestation was noted in individuals of the control group and the group with a dosage of 0.5 mg/kg of lithium asorbate. The greatest increase for the entire period of gestation was recorded in sows of the 1st and 2nd experimental groups and exceeds the control by 5.6 and 4.3 %. It should be noted that in groups 1 and 2 there was the highest live weight of piglets at birth (Table 2).

**Table 2.** Dynamics of changes in body weight of pregnant pigs after administration of lithium ascorbate

| Group no. | 1 month of gestation (kg) | 2 months pregnant (kg) | 3 months pregnant (kg) | 4 months of gestation (kg) |
|---|---|---|---|---|
| 1st group | 213.40±4.45* | 227.35±4.53* | 247.40±5.50* | 269.20±5.07* |
| 2nd group | 215.20±5.17* | 228.86±4.95* | 243.80±8.23* | 266.02±9.30 |
| 3th group | 202.60±6.69 | 215.20±5.97 | 229.50±8.23 | 252.60±5.73 |
| 4th group | 207.20±4.32* | 218.95±4.96 | 235.45±5.67* | 260.04±5.37* |
| control | 206.80±7.76 | 217.85±7.43 | 232.89±7.83 | 255.00±8.69 |

*Note: * p<0.05 Student's t test compared to control*

The introduction of lithium ascorbate with feed helps to increase the fertility of sows during the gestation period, in the 1st group – by 37 %, in the 2nd – by 30 %, in the 3rd – by 13 %, relative to the control animals. In the 4th group at a dosage of 0.5 mg/kg of lithium ascorbate, no changes in fertility were observed. At the same time, the number of stillborn pigs decreased in the noted experimental groups. So, in groups 1 and 2, stillborn pigs were not detected. All the piglets born were viable, and the weight indices corresponded to the norm, which is confirmed by the increased weight of the nest. The reproductive qualities of purebred sows during their operation in an industrial environment are presented in Table 3.

**Table 3.** Reproductive qualities of sows after administration of lithium ascorbate

| Group no. | Received piglet heads | | | Birth weight (kg) | |
|---|---|---|---|---|---|
| | total | alive | stillborn | nests | 1 head |
| 1st group | 13.40±1.14* | 13.40±1.14* | 0 | 26.53±1.41* | 1.98±0.09* |
| 2nd group | 12.60±1.52* | 12.60±1.52 | 0 | 24.70±2.14* | 1.96±0.10* |
| 3th group | 11.80±1.58 | 11.80±1.52 | 0 | 22.65±2.35 | 1.92±0.09 |
| 4th group | 8.00±1.14 | 6.80±1.82 | 1.20±1.82 | 12.17±2.94 | 1.79±0.03 |
| control | 7.90±1.67 | 6.20±1.58 | 1.70±1.11 | 10.78±2.72 | 1.74±0.09 |

*Note: * p<0.05 Student's t test compared to control*

**Indicators of lipid-fat metabolism in sows during gestation**

The direct and indirect participation of proteins in the formation of fat in the animal organism was noted in his works in 1908 by E.A. Bogdanov. Modern publications indicate that the negative impact of stress factors of various etiologies is accompanied by an increase in blood lipids[13].

In experiments on sows with the introduction of lithium ascorbate with feed, an increase in the level of total protein in the serum of their blood during pregnancy was found within the physiological norms of lipogenesis indicators. In the blood of the uterus of the experimental groups on the 110th day of gestation, an increase in the concentration of triglycerides was noted by 0.5 times, total cholesterol – by 20 %, β-lipoproteins – by 34 % (Table 4).

**Table 4.** Indicators of lipid-cholesterol metabolism in the blood

| Group no. | 2 months pregnant | | | | | |
|---|---|---|---|---|---|---|
| | TAG | TC | HDL-C | LDL-C | VLDL | β-LP |
| 1st group | 0.86±0.05 | 3.52±0.03 | 1.60±0.05 | 1.62±0.02 | 0.31±0.02 | 0.78±0.14 |
| 2nd group | 0.79±0.04 | 3.48±0.05 | 1.55±0.06 | 1.63±0.03 | 0.30±0.03 | 0.75±0.16 |
| 3th group | 0.60±0.23 | 3.41±0.06 | 1.43±0.10 | 1.68±0.13 | 0.31±0.03 | 0.60±0.14 |
| 4th group | 0.68±0.14 | 3.37±0.07 | 1.38±0.14 | 1.65±0.14 | 0.33±0.02 | 0.61±0.13 |
| control | 0.69±0.13 | 3.38±1.40 | 1.40±0.12 | 1.65±0.08 | 0.33±0.03 | 0.64±0.12 |
| | 3.5 months of gestation | | | | | |
| 1st group | 0.93±0.07* | 4.32±0.42 | 2.12±0.19* | 1.93±0.26 | 0.27±0.02* | 0.76±0.15 |
| 2nd group | 0.81±0.04* | 4.02±0.38 | 1.99±0.12 | 1.74±0.30 | 0.29±0.02 | 0.78±0.16 |
| 3th group | 0.53±0.20 | 3.85±0.32 | 1.86±0.17 | 1.67±0.16 | 0.32±0.03 | 0.60±0.13 |
| 4th group | 0.47±0.23 | 3.53±0.20 | 1.47±0.29 | 1.72±0.15 | 0.35±0.03 | 0.60±0.11 |
| control | 0.52±0.17 | 3.58±0.16 | 1.48±0.21 | 1.75±0.30 | 0.34±0.03 | 0.58±0.08 |

*Note:* TAG – triacylglycerols, mmol/l; TC – total cholesterol, mmol/l; HDL-C – high density lipoprotein cholesterol, mmol/l; LDL-C – low density lipoprotein cholesterol, mmol/l; VLDL – very low density lipoprotein cholesterol, mmol/l; β-LP – beta-lipoproteins, mmol/l. * $p<0.05$ by *t*-criterion when compared with control.

In sows of the first, second and third experimental groups on the 110th day of gestation there was a significant increase in the concentration of high-density lipoprotein cholesterol fractions by 20 and 12 % $p<0.05$: 7 % respectively. In the fourth group, an increase in these fractions relative to the control group was not observed. This circumstance was considered as very positive. Currently, the term and concept "hyperlipemia" almost completely lose both their meaning and the relevance of a clinical test. The concentration of total lipids and phospholipids is not an informative criterion. Even the total cholesterol concentration is of limited value. It is not the total amount of lipids of various fractions that is important, but their ratio. In 1970, experts from the World Health Organization proposed to abolish the term hyperlipemia and replace it with the concept of "dyslipaemia". This term emphasizes the importance for the characterization of lipid metabolism not of the total concentration of lipids, but of the ratio of their various fractions.

The increased content of chylomicrons in the balanced lipoprotein system of VLDL and LDL-C determines the risk of excess cholesterol deposition in the vascular endothelium. At the same time, the removal of cholesterol from the endothelium and the body accelerates the increase in the concentration of HDL-C [10,14]. The main pathway of chemical transformation of lipoproteins is excessive peroxidation of lipids that make up their composition. On the one hand, peroxide-modified LDL-C undergoes capture by macrophages and smooth muscle cells of the arterial wall, which leads to a massive accumulation of cholesterol esters in them belonging to the "atherogenic" fraction, which initiates the formation of atherosclerotic plaques. Peroxide modification of LDL-C is accompanied, on the other hand, by a significant increase in their immunogenicity. The formation of autoantibodies to altered LDL-C, captured by the cells of the arterial wall, is an additional factor in arterial damage (destruction under the influence of immune complexes). That is why it was announced that HDL-C is “good” or “useful”, and LDL-C is “bad” or “harmful”. Further, knowledge about their negative role deepened even more [15].

Hypertrophied antigenic stimuli emanate from peroxidized low density lipoproteins. They are also considered as the main factors of the structural and functional destruction of cell membranes and individual molecules, which is the main reason for the emergence of a variety of pathological conditions, the most common of which are cholesterol plaques. These studies are driven by the desire to fight atherosclerosis of the vessels of the brain and heart. In connection with the quality of food supplied by these animals, the state of lipid-cholesterol metabolism of animals is of interest to humans.

The concentration of lipoprotein cholesterol of low and very low density in sows of the third and fourth groups did not undergo statistically significant changes. However, there is a very clear tendency for lower values of these two indicators relative to the animals of the control group. The revealed changes in the increase in the concentration of the high density lipoprotein fraction with a simultaneous decrease in the content of low and very low density lipoprotein fractions indicate a favorable course of lipid and cholesterol metabolism in animals of the first and second groups. Taking into account the importance of cholesterol fractions in lipoproteins of various densities, lithium ascorbate is considered as a drug that has an antiatherogenic effect due to the positive effect of the drug on the systems responsible for the stress resistance of the body of pregnant sows. There is a manifestation of neuroleptic, normotimic, tranquilizing, sedative function.

**The functional state of the glutathione reduction system in the blood of sows**

*Thiol-disulfide ratio.Glutathione reduction system.* Moving on to the consideration of the next group of studied indicators, we proceed from the fact that the redox balance in the body is determined by donor-acceptor relationships: from whom, where and how many electrons and protons will be taken away, as well as to whom, where and how many will be donated. Thus, the regulation of redox metabolism is determined by some kind of dynamic balancing between these fundamental processes. Changing this balance within acceptable limits is used to regulate multiple private functions and going beyond these limits leads to pathology and cell death [16]. From this point of view, the assessment of the functional state of the systems responsible for the nonspecific resistance of the organism should be supplemented with the characteristics of the thiol-disulfide system, since the thiol-disulfide ratio (TDR), i.e. the ratio of the number of sulfhydryl and disulfide groups plays the role of an important regulatory parameter in the processes of redox metabolism.

The TDR values in the sows of the experimental groups were higher than the corresponding values in the experimental group during the 60th and 110th days of gestation, the effect size decreased as the dose of the drug decreased (Table 5).

**Table 5.** Activity of the glutathione reduction system in the blood against the background of lithium ascorbate

| Group no. | 2 months pregnant | | | | | |
|---|---|---|---|---|---|---|
| | SH | SS | SH/SS | MDA | SOD | GPO |
| 1st group | 1.005±0.062 | 0.385±0.041 | 2.63±0.34 | 5.84±0.42 | 1082±197 | 2569±240 |
| 2nd group | 1.004±0.126 | 0.420±0.039 | 2.39±0.25 | 6.04±0.14 | 1023±85 | 2525±177 |
| 3rd group | 0.938±0.079 | 0.522±0.172 | 1.91±0.47 | 6.16±0.75 | 1018±94 | 2393±128 |
| 4th group | 0.934±0.032 | 0.513±0.170 | 1.98±0.47 | 6.36±0.68 | 1007±94 | 2353±266 |
| control | 0.933±0.130 | 0.520±0.129 | 1.87±0.40 | 6.35±1.04 | 1024±157 | 2376±116 |
| | 3.5 months of gestation | | | | | |
| 1st group | 1.112±0.058 | 0.432±0.019* | 2.62±0.11* | 4.34±0.48* | 1284±55* | 2803±396 |
| 2nd group | 1.089±0.127 | 0.444±0.050 | 2.45±0.12 | 5.04±0.91 | 1249±239 | 2778±236 |
| 3rd group | 1.013±0.048 | 0.464±0.056 | 2.21±0.25 | 6.68±0.52 | 1157±180 | 2560±161 |
| 4th group | 0.912±0.189 | 0.513±0.170 | 1.96±0.85 | 7.09±1.26 | 1013±141 | 2323±254 |
| control | 0.914±0.185 | 0.524±0.083 | 1.83±0.66 | 7.20±1.18 | 1140±70 | 2346±177 |

*Note:* SH – reduced glutathione + cysteine, μmol/ml; SS – oxidized glutathione + cystine, μmol/ml; SH/SS –thiol-disulfide ratio; MDA –malonicdialdehyde, nmol/ml; GPO – activity of glutathione peroxidase, Units; SOD – activity of superoxide dismutase, Units.

## DISCUSSION

In most pathologies of an infectious and non-infectious nature in a state of oxidative stress of any etiology, there is a decrease in the content of SH-groups and an increase in the concentration of SS-groups [17].

It is known that the severity of the disease, the periods of its exacerbation, the impact of adverse environmental factors in healthy people and animals correlate with the degree of decrease in the thiol-disulfide ratio. The dynamics and magnitude of changes in the thiol-disulfide ratio (thiol-disulfide system) are a reflection of the development of an adaptive response. An increase in the content of SH-groups and a decrease in SS-groups are associated with the active extraction of the reserve of low molecular weight thiols from the liver in response to the depletion of the redox system of the blood and with the mobilization of the body's reserves for the reduction of oxidized thiols. Thiol compounds (low molecular weight and high molecular weight), due to their ability to quickly but reversibly oxidize, are most sensitive to adverse effects of various nature and intensity[18].

The nodal component of the thiol-disulfide system is glutathione. Glutathione is a gamma-glutamyl-cysteinyl-glycine tripeptide with a free sulfhydryl group [19].

It is never found in the products of protein hydrolysis; therefore, glutathione is synthesized by the body to perform specific functions. The reduced form of glutathione serves in the intracellular space as the main sulfhydryl buffer to maintain the reduced state of cysteine residues in all proteins – from hemoglobin, preserving it in ferroform, to numerous enzymes containing SH-groups in the active center, as well as various vitamins, hormones and cysteamine.

According to its chemical properties, glutathione is able to independently participate in detoxification processes, reacting with both hydrogen peroxide and organic peroxides. It belongs to the group of the most important thiol antioxidants with antitumor and radioprotective properties. Many enzymes in the active site contain sulfhydryl groups, the oxidation of which leads to the loss of catalytic activity.

An interesting specificity of glutathione is that no amount of feeding it, no intramuscular or intravenous injections at all helps. It is very poorly transported across cell membranes, and only the reduced glutathione that is formed directly in the intracellular space works. Therefore, by initiating and maintaining reactions leading to the preservation of reduced thiol equivalents, we increase the adaptive capacity of the organism and its resistance to adverse factors.

The special place that thiols occupy among tissue antioxidants is due to their following characteristic properties: 1) extremely high reactivity of sulfhydryl groups, due to which thiols are oxidized at a phenomenally high rate;2) the reversibility of the oxidation reaction of sulfhydryl groups into disulfide groups, which suggests the possibility of the most energetically beneficial maintenance of the homeostasis of thiol antioxidants in the cell without activating their biosynthesis; 3) the ability of thiols to exhibit both anti-radical and anti-peroxide action;4) the hydrophilicity of thiols determines their high content in the aqueous phase of the cell and the ability to protect biologically important hydrophilic molecules, including hemoglobin, from oxidative damage. At the same time, the presence of non-polar groups in thiols provides them with the possibility of manifesting antioxidant activity in the lipid phase of the cell [14].

Thus, a group of indicators characterizing the functional state of the system for the reduction of glutathione and lipid-cholesterol metabolism was assessed, which can be combined into a single set of criteria for obtaining an informative and objective assessment of the state of antioxidant-prooxidant processes in the body of animals. They determine the health of animals, productivity and quality of the products. As for the complex of indicators characterizing the state of the glutathione reduction system, it is possible to note with certainty the changes that have occurred in the blood of sows in the experimental groups (Table 5).

In the 1st, 2nd, 3rd experimental groups, the concentration of malondialdehyde decreased from 38 to 7 %, depending on the dose of lithium ascorbate used. The results obtained indicate a decrease in the intensity of lipid peroxidation processes in animals.

A very accessible and widely recognized criterion for the ratio of antioxidant-prooxidant processes in any cell of the body and the functional activity of systems responsible for nonspecific resistance has long been considered the content of malondialdehyde in the blood. An increase in its concentration characterizes the inability of the body's defense systems to cope with the processes of lipid peroxidation and oxygen oxidation along the one-electron path, during which the bulk of super-reactive free radicals are formed – under-oxidized oxygen products that lower the nonspecific resistance of animals and birds [20].

The information obtained in the study on a decrease in the content of malondialdehyde in the blood of animals of all experimental groups can serve as reasoned confirmation of the above. This indicates a decrease in the intensity of lipid peroxidation processes in pigs [1].

One of the main components of the antioxidant-antiradical defense system is considered to be superoxide dismutase, which converts a super-reactive superoxide anion into molecular oxygen and hydrogen peroxide, which have high oxidative activity. The task of their neutralization is solved by the following enzyme: glutathione peroxidase, which catalyzes the hydrolysis reaction of organic hydroperoxides.

The main biological purpose of both of these enzymes is to protect cellular structures, primarily biomembranes, from oxidative attack, which work in conjunction. Their activity can sharply increase under conditions of activation of oxidative stress reactions. In relation to the activity of these two enzymes of the first echelon, the antioxidant-antiradical defense of the body was higher in the experimental groups. Thus, in the first group, SOD and GPO were 26 and 19 % higher than in the control group. In the 2nd and 3rd groups, such increases in SOD were 23 and 14 %; GPO 18 and 9 % respectively. The values obtained in the studies, which fit within the limits of their natural biological fluctuations, indicate the normal state of redox processes in the body of the observed sows. In the control group, these data were at the lower limit of the norm, which indicates the depletion of their antioxidant-antiradical system and the potential possibility, under these conditions, of weakening protection against stress factors of various etiologies.

## CONCLUSION

Thus, it is possible to realize the biological necessity of creating new highly effective methods of physiologically adequate pharmacological correction of technological and spontaneous stresses in farm animals. Lithium ascorbate at a dosage of 10, 5 and 2 mg/kg when administered with

feed exhibits pronounced adaptogenic and stress-protective properties with the greatest effect when administered to sows. Lithium ascorbate increases nonspecific resistance, accelerates the growth of pregnant sows, and provides protection against technological and spontaneous stressors. It can be considered that lithium ascorbate effectively affects lipid-cholesterol metabolism and the glutathione reduction system in animals, increasing the safety of the livestock and increasing the live weight of pregnant sows. The performed verification of the tested dose, scheme and method of administration of lithium ascorbate in terms of the level of biochemical parameters confirmed the validity of the assumption made about the possibility of improving the state of nonspecific resistance and productivity of animals with its help.

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## CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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## ETHICAL APPROVAL

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 All-Russian research Institute of Physiology, Biochemistry and Nutrition of animals – branch of the Federal Science Center for Animal Husbandry named after Academy Member L. K. Ernst(Record №12, dated December 7, 2017).

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