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DYNAMICS OF OXIDATIVE STATE INDICATORS IN RABBITS (*Oryctolagus cuniculus* L.) UNDER SIMULATED TECHNOLOGICAL STRESS AND ITS PHARMACOLOGICAL CORRECTION

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Abstract

Stress is the most important livestock problem, causing great damage to the industry. The emergence of technological stress contributes to a large number of factors, from transportation to conditions of keeping and feeding. The development of pathological processes under stress intensifies free radical processes in the body, with the excessive formation of free radicals. Therefore, there is a need in drugs based on substances with high antioxidant activity to pharmacologically correct technological stress in farm animals. In our experiment, we simulated conditions of technological stress in Soviet chinchilla rabbits aged 6-7 months by immobilization. Antioxidant and anti-stress drugs developed at Stavropol State Agrarian University were used as agents. Group 1 of animals was control. Rabbits of group 2 received Drug to correct stress in farm animals (Patent RU 2428992 of 09.20.11), group 3 received Mebisel (Patent RU 2418579 of 05.20.11), these drugs have a pronounced anti-stress effect; group 4 received Antioxidant preparation for animals (Patent RU 2435572 of 12.10.11) and group 5 received Polyoxidol (Patent RU 2538666 of 01.10.15), the antioxidants. Blood levels of cortisol, thyroxine, lipid peroxidation and antioxidant protection were assessed. It was shown that immobilization of experimental animals provokes a significant production of cortisol (5,8 times higher) and a decrease in the thyroxine level up to 60,9 % ($p \le 0,01$), the blood concentration of diene conjugates increases 2.6 times ($p \le 0,01$), malondialdehyde by 55,8 % ($p \le 0,01$) and fluorescent Schiff bases 2,2 times ($p \le 0,01$). The restricted mobility adversely affectes the activity of antioxidative defence enzymes, with a significant decrease in glutathione peroxidase activity (by 35.2 %), superoxide dismutase (by 36.4 %), catalase (by 40.7 %) ($p \le 0.01$) and the content of reduced glutathione (by 33.3 %, $p \le 0.01$). Administration of antioxidant and antistress preparations contributes to the normalization of the studied parameters in experimental animals, the values of which during the experiment were statistically significantly different from the data recorded in the control group. In the dynamics of activity of antioxidant enzymes and products of lipid peroxidation, there were significant differences between the indices of animals from the control group and rabbits which received preventive agents. The animals of the control group showed a progressive increase in the concentration of lipoperoxides and a decrease in the activity of glutathione peroxidase, superoxide dismutase, catalase, and reduced glutathione. The use of antioxidant and antistress drugs three days before immobilization contributed to the optimization of these indicators. The applied prevention regimens allowed reduction of negative impact of stress, which resulted in statistically significant differences in the numerical values of the results of the laboratory blood test of animals from the groups 2, 3, 4 and 5 conoared to the control. At the end of the experiment glutathione peroxidase was 48.2-107.4 % higher ($p \le 0.01$), superoxide dismutase 31.1-85.9 % higher ($p \le 0.01$), catalase 12.9-40.1 % higher ($p \le 0.05$ in groups III, IV and V), while glutathione was 34.8-60.8 % lower ($p \le 0.01$), thyroxine 27.2-82.7 % lower ($p \le 0.05$). The cortisol level declined by 83.5-207.0 % ($p \le 0.01$), diene conjugates by 37.2-84.3 % ($p \le 0.01$), malondialdehyde by 26.1-46.9 % ($p \le 0.05$), and fluorescent Schiff bases by 22.03-118.1 % ($p \le 0.05$). The use of drugs accelerates post-stress adaptation, which was expressed in an increase in the average daily weight gain of rabbits from experimental groups, i.e. 28 g for group 2, 34 g for group 3, 36 g for group 4, and 38 g for group 5 compared to 24 g for the control group. Our results on the stress-born hormone dynamics are indicative of significant changes in the antioxidant defense system functioning and lipid peroxidation. These data allow us to recom-

mend the developed tranquilizers and antioxidants for physiological correction of technological stresses in animals.

Keywords: *Oryctolagus cuniculus* L., rabbits, technological stress, immobilization, antistress agent, antioxidant preparation, antioxidant system, lipid peroxidation, hormones, enzymes

Stress is one of the most important factors disturbing homeostasis in animals and humans. Biologically active substances balance changes under the influence of stress reaction leading to the development of pathologies. Many researchers agree that the imbalance of antioxidant and pro-oxidant processes is one of the first manifestations of metabolic disorders under stress [1-4]. The stress problem in animal husbandry is extremely acute. Stress leads to a decrease in productivity and in the quality of products, an increase in animal morbidity, a decrease in the rate of reproduction and, as a consequence, the profitability of animal husbandry [5-7]. Animal welfare is important not only in the context of humanizing human activities but also in terms of economic benefits and therefore attracts the attention of researchers and practitioners.

Unfortunately, it has to be stated that in animal husbandry technological stress accompanies almost all production process. It occurs during animals' transportation, during feeding and care operations, can be observed at sharp changes in diets and maintenance conditions, its development can be affected by changes in the microclimate and many other factors [8-10]. In addition to technological stress, animals experience physiological stress due to the most intense periods of exploitation, such as pregnancy and parturition [11, 12].

Normally, free radical oxidation ensures normal cell functioning and metabolic processes in the cell [13, 14]. Pathological changes in these processes trigger the mechanism of chain lesions of cells and tissues due to the ability of free radicals to disrupt the structure and integrity of biological membranes [15-17]. The intensification of free-radical reactions out of control of the antioxidant protection system is the most probable mechanism of organism damage under stressful load [18, 19].

Currently, animal husbandry is focused on improving the welfare of livestock. However, due to the need to increase production at minimal cost, it is not always possible to modify technologies to reduce the number of stress factors and their impact [20]. Consequently, the pharmacological prevention of stress-related disorders remains appropriate. The mode of action of modern means and methods of correction of the changes caused by the negative influence of stress factors on the body must be effective and safe [21, 22].

In the present study, based on the assessment of the state of the antioxidant protection system, cortisol and thyroxine status in the simulation of technological stress, we have shown for the first time the anti-stress effect of the developed antioxidant and anti-stress drugs.

The aim was to investigate the influence of experimental stress on the dynamics of free-radical oxidation and antioxidant protection in rabbits under correction by complex antioxidant and anti-stress drugs.

Techniques. According to the principle of analogs, 6 groups of Soviet Chinchilla rabbits (*Oryctolagus cuniculus* L.) aged 6-7 months (20 animals per group) were formed. Technological stress was simulated by placing the test animals in specially manufactured modules of 0.12 m^2 for 5 days (immobilization with space limitation). Group II rabbits were intramuscularly injected with a drug for correction of stress states in farm animals (anti-stress drug) [23] at a dose of 3.9 mg/kg of live weight (by active substance) 3 days and 1 hour before immobilization, Group III used Mebisel [24] at a dose of 6.0 mg/kg, Group IV

used antioxidant agent for animals [25] at a dose of 5.4 mg/kg, and Group V used Polyoxidol at a dose of 5.0 mg/kg [26]. No drugs were used in Group I (control group). All used drugs are developed at the Department of Therapy and Pharmacology of Stavropol State University. The test drugs in Group II and Group III contain active substances with an anti-stress effect, in Group IV and Group V with an antioxidant effect.

Blood was taken from the auricular vein before drug administration, immediately before immobilization, in 1 day and 5 days after the beginning of stress simulation and in 5 days after the end of restriction of animal mobility, at the same time the animals were weighed. Blood cortisol and thyroxine concentrations, lipid peroxidation and antioxidant protection parameters were determined. Cortisol was measured on a Chem Well Combi automatic enzyme-linked analyzer (Awareness Technology, USA) with reagent kits (Hema LLC, Russia). The antioxidant protection parameters were determined as per the description [27]. A UNICO 2800 UV/VIS spectrophotometer (United Products & Instruments, Inc., USA) was used to measure the activity of catalase, superoxide dismutase, glutathione peroxidase, reduced glutathione content, and lipid peroxidation product concentrations.

The mean (*M*) and standard error of mean (±SEM) were calculated during data processing. The reliability of the differences was assessed by Student's *t*-test. Differences were considered statistically significant at $p \leq 0.05$.

Results. It is known that space-constrained immobilization is one of the strongest stressors [28-31]. The analysis of the data obtained in the laboratory blood test indicates that the immobilization stress simulated in rabbits leads to a multiple increase in the amount of cortisol and a decrease in the thyroxine production (Table 1). It was found that in animals not subjected to prophylactic treatment, the concentration of blood cortisol for 1 day in a limited space increased 5.8 times and remained high during the whole experiment. It should be noted that in the blood of animals injected with anti-stress and antioxidant drugs before the provocation of the stress response, cortisol also increased significantly, and the peak of such an increase was on day 1 of stressing. Even at its peak, it was less than in the control group, by 65.2% in Group II, by 44.7% in Group III, by 22.9% in Group IV, and by 29.5% in Group V; the difference between the groups in this and subsequent stages of the experiment was statistically significant ($p \leq 0.05$). After the cessation of the stress factor, the tendency to normalize the amount of cortisol in the blood was more pronounced in animals undergoing pharmacological preparation. In 5 days after the end of immobilization, rabbits in Group I had the highest values for this parameter (they were 1.8 times or higher than in other groups).

The use of anti-stress and antioxidant drugs contributed to an increase in the amount of blood thyroxine in rabbits (see Table 1). Three days after the drug introduction, the concentration of this hormone in animals of Group II increased by 42.3%, in Group III by 48.6%, in Groups IV and V by 9.1 and 31.1%, respectively. After the rabbits were moved to limited space, the amount of thyroxine in all groups decreased, in most of them by more than 50%. In blood samples taken 5 days after the end of immobilization, the hormone concentration increased in all groups. This indicator was statistically significantly lower in the control group, by 64.6% ($p \leq 0.01$) compared to Group II, by 82.7% ($p \leq 0.01$) compared to Group III, by 37.7 ($p \leq 0.01$) and 27.2% ($p \leq 0.02$), respectively, compared to Group IV and Group V. The dynamics of thyroxine indicates that stress response has a pronounced inhibitory effect on its synthesis, and the drugs used have a significant preventive effect.

1. Blood concentration of stress hormones and lipid peroxidation products in Soviet Chinchilla rabbits (*Oryctolagus cuniculus* L.) under simulation of immobilization stress (M±SEM, n = 20)

Group	Cortisol, nmol/l	Thyroxin, nmol/l	Diene conjugates, OD/mg lipids	Malone dialdehyde, μmol/l	Schiff bases, rel. units/ml
Before drug administration					
I	38.26±2.69	27.18±1.94	0.33±0.03	1.29±0.09	0.30±0.02
II	34.67±2.12	29.43±2.15	0.29±0.02	1.17±0.08	0.27±0.02
III	37.19±2.74	26.83±1.71	0.34±0.03	1.31±0.09	0.30±0.03
IV	35.72±2.44	33.62±1.98	0.31±0.02	1.24±0.08	0.31±0.02
V	39.12±2.81	28.52±2.03	0.34±0.03	1.32±0.09	0.28±0.02
Before immobilization					
I	40.73±2.56	25.44±1.58	0.34±0.03	1.32±0.09	0.31±0.02
II	22.21±1.70[a]	42.27±2.99[a]	0.27±0.02	1.19±0.08	0.27±0.02
III	24.96±1.94[a]	39.87±3.11[a]	0.30±0.02	1.28±0.09	0.31±0.02
IV	36.09±2.48[e]	36.70±2.78[a]	0.24±0.02[c]	1.20±0.09	0.29±0.02
V	38.47±2.61[e]	37.39±2.63[a]	0.21±0.02[d]	1.23±0.08	0.26±0.02
1 day after the beginning of immobilization					
I	238.23±16.89	13.47±1.00	0.62±0.05	1.57±0.11	0.35±0.03
II	144.19±11.17[a]	20.53±1.46[a]	0.46±0.04[a]	1.48±0.10	0.31±0.02
III	131.74±9.75[a]	23.66±1.59[a]	0.42±0.03[a]	1.43±0.09	0.33±0.03
IV	183.51±13.90[d]	17.32±1.34[c]	0.37±0.03[a]	1.25±0.08[a]	0.27±0.02[a]
V	167.91±12.07[c]	19.48±1.48[a]	0.33±0.02[d]	1.22±0.08[b]	0.26±0.02[a]
5 days after the beginning of immobilization					
I	181.14±12.93	9.93±0.72	0.87±0.07	2.01±0.15	0.67±0.05
II	74.60±5.21[a]	16.58±1.26[a]	0.62±0.05[a]	1.73±0.13	0.56±0.04
III	86.89±6.34[a]	18.21±1.33[a]	0.58±0.04[a]	1.59±0.11[a]	0.52±0.04[a]
IV	111.24±7.72[d]	12.97±0.93[d]	0.41±0.03[d]	1.41±0.10[a]	0.48±0.04[a]
V	100.76±7.14[b]	13.22±1.18[c]	0.45±0.03[d]	1.36±0.10[b]	0.41±0.03[b]
5 days after the immobilization completed					
I	149.12±11.65	11.84±0.89	0.59±0.04	1.88±0.14	0.72±0.05
II	48.57±3.65[a]	19.49±1.39[a]	0.43±0.03[a]	1.49±0.10[a]	0.59±0.04[a]
III	60.08±4.62[a]	21.63±1.70[a]	0.39±0.03[a]	1.42±0.10[a]	0.46±0.03[b]
IV	81.24±5.76[d]	15.06±1.12[d]	0.36±0.03[a]	1.31±0.09[a]	0.39±0.03[b]
V	76.43±5.53[d]	16.31±1.21[c]	0.32±0.02[b]	1.28±0.09[a]	0.33±0.03[d]

Note. [a] – between this group and Group I the difference is statistically significant; [b] – between this group, Group I and Group II the difference is statistically significant; [c] – between this group, Group I and Group III the difference is statistically significant; [d] – between this group, Group I, Group II and Group III the difference is statistically significant; [e] – between this group, Group II and Group III, the difference is statistically significant ($p \leq 0.05$).

Our findings indicate that the effect of the stress factor is accompanied by the intensification of lipid peroxidation, as evidenced by the change in the concentration of peroxidation products. The concentration of diene conjugates in the control group for the 5 days of immobilization increased 2.5 times. Under the conditions of 5-day immobilization, the index increased by 82.3% in animals from Group II that received the anti-stress drug, by 93.3% in rabbits that received Mebisel, by 70.8% in Group IV where another antioxidant drug was used, and more than 2-fold in Group V where Polyoxidol was administered. After immobilization, the concentration of diene conjugates decreased in all five groups, but in the control group, it was significantly higher ($p \leq 0.05$) than in the test groups.

Comparing the values for malondialdehyde (MDA) between the groups, it is worth noting that the highest content of this peroxidation product was in the control group throughout the experiment. In blood samples obtained 5 days after immobilization, the MDA concentration in Group I was 20.7% ($p = 0.16$) higher than in Group II, 24.5% ($p \leq 0.05$) higher than in Group III, 30.3% ($p \leq 0.05$) and 31.9% ($p \leq 0.05$) higher than in Group IV and Group V, respectively (see Table 1).

The dynamics of accumulation of fluorescent Schiff bases in the blood of animals from all groups practically did not change after 3 days from the drug administration. Differences began to appear in 1 day after the beginning of simulation of stress exposure. During the 5 days of immobilization, the concentration of

Schiff bases in Group I was 16.4% higher than in Group II ((p = 0.09), 22.4% higher than in Group III (p = 0.02), 28.4% higher than in Group IV (p ≤ 0.01), and 38.8% higher than in Group V (p ≤ 0.01) (see Table 1).

Assessing the dynamics of the enzymatic link of the antioxidant protection system, it should be noted that the use of antioxidant and anti-stress drugs has led to an increase in the activity of enzymes (Table 2). At the end of the experiment, the activity of glutathione peroxidase in Group I was 48.2% lower than in Group II (p ≤ 0.01), 76.8% lower (p ≤ 0.01) than in Group III, 2.0 times and 2.1 times lower than in Group IV and Group V, respectively. This difference can be explained by the fact that the active substances of all used drugs include a selenium-containing compound.

2. Antioxidant blood protection parameters and dynamics of bodyweight in Soviet Chinchilla rabbits (*Oryctolagus cuniculus* L.) under simulation of immobilization stress (*M*±SEM, *n* = 20)

Group	GPx, μmol G-SH/(l · min · 10^3)	SOD, units/mg hemoglobin	Catalase, μmol H_2O_2/(l · min · 10^3)	Reduced glutathione, mmol/l	Bodyweight, kg
Before drug administration					
I	7.39±0.53	4.72±0.36	24.13±1.78	0.31±0.02	3.58±0.26
II	8.43±0.64	5.11±0.42	23.27±1.51	0.35±0.03	3.44±0.23
III	7.87±0.57	4.93±0.39	23.79±1.82	0.33±0.03	3.61±0.29
IV	8.18±0.69	5.02±0.46	24.42±1.96	0.34±0.03	3.49±0.24
V	7.34±0.56	4.81±0.34	22.94±1.35	0.29±0.02	3.70±0.31
Before immobilization					
I	7.22±0.36	4.64±0.39	24.05±1.66	0.30±0.02	3.64±0.30
II	10.31±0.76[a]	5.63±0.58	23.89±1.47	0.35±0.03	3.42±0.28
III	11.13±0.69[a]	5.49±0.43	24.01±1.59	0.35±0.03	3.65±0.32
IV	12.41±0.88[a]	6.18±0.53[a]	26.95±1.91	0.37±0.03	3.57±0.29
V	12.93±0.82[b]	5.92±0.55	27.11±2.13	0.32±0.03	3.72±0.34
1 day after the beginning of immobilization					
I	5.07±0.42	3.22±0.25	19.47±1.28	0.26±0.02	3.37±0.21
II	10.56±0.71[a]	5.41±0.44[a]	21.60±1.43	0.30±0.02	3.31±0.29
III	13.22±0.94[b]	5.78±0.50[a]	22.11±1.52	0.32±0.02[a]	3.48±0.27
IV	14.49±1.09[b]	5.89±0.52[a]	24.76±1.73[a]	0.35±0.03[a]	3.34±0.31
V	15.01±1.03[b]	6.14±0.57[a]	25.31±1.80[a]	0.30±0.02	3.51±0.33
5 days after the beginning of immobilization					
I	4.68±0.34	2.95±0.23	14.53±1.12	0.20±0.02	3.09±0.24
II	9.69±0.67[a]	4.26±0.29[a]	16.82±1.20	0.27±0.02[a]	3.24±0.27
III	10.92±0.83[a]	4.71±0.33[a]	18.63±1.36[a]	0.30±0.02[a]	3.51±0.32
IV	12.45±0.88[b]	5.04±0.40[a]	21.26±1.49[b]	0.32±0.02[a]	3.22±0.25
V	12.95±0.96[b]	5.63±0.44[b]	23.15±1.42[c]	0.34±0.03[a]	3.45±0.29
5 days after the immobilization completed					
I	6.33±0.51	3.41±0.27	18.49±1.54	0.23±0.02	3.21±0.28
II	9.38±0.59[a]	4.47±0.32[a]	20.88±1.41	0.31±0.02[a]	3.38±0.31
III	11.19±0.82[a]	5.23±0.41[a]	24.31±1.89[a]	0.34±0.03[a]	3.68±0.32
IV	12.86±0.89[b]	5.79±0.38[b]	26.52±2.33[b]	0.36±0.03[a]	3.40±0.26
V	13.13±0.97[b]	6.34±0.52[b]	25.91±2.07[a]	0.37±0.03[a]	3.64±0.32

Note. GPx — glutathione peroxidase, SOD —superoxide dismutase.
[a] — the difference between this group and Group I is statistically significant; [b] — the difference between this group, Group I and Group II is statistically significant; [c] — the difference between this group, Group II and Group III is statistically significant (p ≤ 0.05).

The activity of blood superoxide dismutase significantly increased in animals that received antioxidant and anti-stress drugs, with a decrease in the control. The simulated technological stress led to a marked decrease in the activity of blood superoxide dismutase in rabbits from Group I (control). During 5 days of immobilization, the statistically significant differences were 44.4% (p ≤ 0.01) between Group I and Group II, 59.6% between Group I and Group III, 70.8% (p ≤ 0.01) between Group I and Group IV, and 90.8% (p ≤ 0.01) between Group I and Group V.

After the rabbits were returned to their usual conditions, their catalase activity normalized, but in the control group this indicator was much lower than in other groups, the difference with Group II was 12.9% (p = 0.25), with Group

III was 31.5% ($p \leq 0.02$), with Group IV was 43.4% ($p \leq 0.01$), and with Group V was 40.1% ($p \leq 0.01$) (see Table 2).

Glutathione is one of the most important factors of antioxidant protection [32, 33] and critical markers of its functioning [34, 35]. After 5-day immobilization of rabbits, the concentration of reduced glutathione in Group I was 35% lower than in Group II ($p \leq 0.02$), 50% lower ($p \leq 0.01$) compared to Group III, 60% ($p \leq 0.01$) and 70% ($p \leq 0.01$) lower compared to Group IV and Group V, respectively (see Table 2).

The simulated technological stress had a negative impact on the dynamics of the rabbits' bodyweight. The use of antioxidant and anti-stress drugs accelerated post-stress adaptation, resulting in an increase in the average daily bodyweight gain of animals. Over 5 days since the cessation of restriction of mobility, the average daily gain of rabbits was 24.2 g in Group I, 28.4 g in Group II, 34.1 g in Group III, 35.6 g in Group IV, and 38.3 g in Group V.

So the developing stress response appears as a sharp increase in the blood cortisol level, up to 238.23±16.89 nmol/l, with the maximum values on day 1 of stressing. Based on this, it can be assumed that day 1 is the most critical for the course of the pathological process during immobilization stress in animals. Also, at technological stress during the whole period of its influence, there is a decrease in the concentration of thyroxine from 27.18±1.94 to 9.93±0.72 nmol/l. The simulated stress affected the antioxidant protection function, as it was shown by a decrease in the activity of glutathione peroxidase enzymes by 36.7%, superoxide dismutase by 37.9% and catalase by 39.8%, as well as a decrease in the concentration of reduced glutathione by 35.9%. The depressive state of the antioxidant system is accompanied by a significant accumulation of lipid peroxidation products in the blood (diene conjugates, malondialdehyde and fluorescent Schiff bases). A decrease in bodyweight by 490 g in 5 days of immobilization was also a result of the negative impact of stress on rabbits.

The use of four developed anti-stress drugs to prevent the negative effects of technological stress ensures stabilization of the level of cortisol, thyroxine, the activity of antioxidant enzymes and the concentration of lipid peroxidation products. At the same time, tranquilizers to a greater extent reduce the concentration of cortisol and normalize the amount of thyroxine, which contributes to improving free radical oxidation and antioxidant protection. Antioxidants activate the function of the enzymatic link of antioxidant protection and reduce the accumulation of lipid peroxidation products in rabbits, resulting in a decrease in the amount of thyroxine and cortisol. This allows the developed means to be suggested for pharmacological prevention of technological stress.

Thus, it has been established that under the experimental stress (immobilization), rabbits develop oxidative stress, which is expressed in an increase in the blood concentration of lipid peroxidation products, i.e. diene conjugates, malondialdehyde and fluorescent Schiff bases, as well as a reduced activity of glutathione peroxidase, superoxide dismutase and catalase in the presence of increased concentration of cortisol and decreased thyroxine. The use of new medicines for animals (i.e. a drug for stress correction in farm animals, an antioxidant drug for animals, Mebisel and Polyoxidol) effectively prevents technological stress and can be used in practical veterinary medicine. Normalization of the antioxidant status in rabbits leads to a decrease in the blood level of cortisol and an increase in the concentration of thyroxine. These results allow us to recommend the drugs with antioxidant activity in the scheme of technological stress prevention in animals.

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