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The Effectiveness Of The Joint Use Of Antioxidant And Antistress Agents In The Experimental Modeling Of Technological Stress For Rabbits.

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ABSTRACT

One of the factors in reducing the efficiency of livestock production is technological stress, the impact of which leads to loss of productivity, a decrease in reproductive potential and the development of various pathologies in the organism of farm animals. It is assumed that one of the links in the pathogenesis of the stress reaction is the intensification of free radical oxidation processes against the background of a malfunctioning of the body's antioxidant defense system. This article presents the results of studying the effectiveness of the combined use of antioxidant and antistress agents in the prevention of technological stress in the conditions of ego modeling in laboratory animals. It has been established that as a result of immobilization of rabbits, the level of cortisol, products of lipid peroxidation increases manyfold, and the level of thyroxine decreases and the activity of the enzymatic link of the antioxidant system decreases. The use of the "Preparation for the correction of stress conditions for farm animals" in combination with the "Antioxidant preparation for animals" and the preparation "Polyoxidol" together with the preparation "Mebisel" with the preventive purpose of rabbits for three days and one hour before immobilization contributes to a reliable normalization of the hormonal background, an increase in the level of activity of glutathione peroxidase, catalase and superoxide dismutase, an increase in the amount of reduced glutathione and a decrease in the concentration of peroxidic products in the blood, as well as an increase in body weight gain in the post-stress period.

Keywords: stress, rabbits, antioxidant drugs, antistress agents, antioxidant system, hormones, lipid peroxidation.

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INTRODUCTION

Technological stress is today a significant problem in productive livestock production, and its impact on animals is inevitably more or less inevitable and is accompanied by damage to the industry through reduced productivity, increased morbidity, shortened animal life, and reduced reproductive potential. With the development of intensive technologies for the maintenance and operation of farm animals, the prerequisites for increasing the stress on them are created.

Stress in the modern sense from the point of view of physiology is a series of biochemical reactions in the body that arise and proceed under the control of biochemical active substances. It is well established that its development provokes a violation of the internal homeostasis of the body and one of its inevitable manifestations is a change in the antioxidant prooxidant balance that provokes the pathological course of free radical processes [2, 3, 7].

Free radical oxidation is a normal physiological process and with its standard flow provides cellular metabolism in warm-blooded [5, 8]. But under extreme conditions, under the influence of exogenous and endogenous factors, a change in the rate of flow of free radical reactions develops, there is an excessive accumulation of their products in the body [4, 6]. This process is under the control of the body's antioxidant defense system, which at various levels ensures the maintenance of a normal concentration of free radicals. With its depressive state, free radical oxidation becomes uncontrollable and leads to the development of pathological conditions in the body [1].

Given the relationship between the stress response and the violation of free radical processes in the animal body, it is advisable to develop means and methods for complex pharmacological prophylaxis aimed at achieving an antistress and antioxidant effect and to test their effectiveness when applied in laboratory and production conditions.

MATERIAL AND METHODS

The aim of the work was to study the effect of antioxidant and antistress preparations in their combined application to rabbits under the conditions of experimentally modeled technological stress.

Experimental studies were carried out on rabbits «Sovetskaya shinshilla» breed aged 6-7 months, which, taking into account the principle of analogues, were divided into three groups. The first group of animals was used as a control and no treatment-prophylactic drugs were used in it. Rabbits from the second group were injected intramuscularly in the form of water-soluble complexes, "Preparation for the correction of stress conditions for farm animals" (Anti-stress drug) at a dose of 3.9 mg / kg and "Antioxidant preparation for animals" (Antioxidant preparation) at a rate of 5.4 mg / kg. Animals from the third group received in the form of oil solutions the preparation "Mebisel" at the rate of 6.0 mg / kg and "Polyoxidol" in a dose of 5.0 mg / kg by the active substance. After three days, all the animals were placed in individual modules with an area of 0.12 m^2, which were constructed taking into account the fact that rabbits were not able to move freely. It is believed that the restriction of mobility is one of the most powerful stress factors. One hour prior to immobilization and one day after its inception, rabbits from the second and third group were re-injected with the corresponding preparations in a manner similar to the first administration. Animals under simulated technological stress were kept for five days. Blood for the study was obtained from the ear vein prior to application of the preparations, one hour before the immobilization of the rabbits, one and five days after the restriction of mobility and five days after the return of the rabbits to their usual conditions of detention. Blood was used to determine the level of cortisol and thyroxine, the concentration of lipid peroxidation products, and certain indicators characterizing the functional state of the body's antioxidant defense system, and also weigh animals.

RESULTS AND DISCUSSION

When analyzing the results of a laboratory blood test, it was noted that during the first three days since the first administration of prophylactic agents, a slight increase in the level of cortisol occurred in rabbits from the first group, while in the second and third groups this index decreased and was significantly lower than in the control by 31.3% and 34.9%, respectively (Table 1).

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The placement of animals in conditions of limited space provoked a marked increase in numerical values reflecting the concentration of cortisol in the blood of laboratory animals. So, for the first day of the experiment, in the first group there was an increase in this indicator by 4.6 times, in the second group - the level of this hormone in the blood increased 3.9 times, and in the third group there was an increase of 3.4 times. At this stage of the experiment, the level of cortisol was the highest, which indicates that in the first day, it is likely that the stress response of the organism in response to the stress-factor effect develops to the maximum. Comparing the level of cortisol after the day of immobilization of rabbits in the context of experimental groups, it was noted that in the first group it was statistically significantly more than in the second group by 72.5% and more than in the third group by 108.7%, respectively.

Table 1: Level of stress hormones and products of lipid peroxidation in blood of rabbits, (n = 20)

Group №	Cortisol, nmol / l	Thyroxine, nmol / l	DK, units of optical density / mg lipids	MDA, µmol / l	Foundations of Schiff, rel. u / ml serum
Before drugs introduction					
H	15-45	30	0,2-0,4	1-1,45	0,25-0,4
1	42,19±3,11	21,74±1,98	0,31±0,02	1,27±0,11	0,28±0,02
2	37,63±2,87	25,06±1,70	0,28±0,02	1,19±0,09	0,26±0,02
3	43,47±3,26	22,59±1,73	0,32±0,02	1,30±0,10	0,28±0,02
Before immobilization					
1	44,83±3,23	20,24±1,56	0,33±0,03	1,33±0,09	0,30±0,02
2	30,77±2,56*	28,13±1,82*	0,25±0,02*	1,17±0,10	0,25±0,02
3	29,18±2,61*	26,54±1,74*	0,27±0,02	1,22±0,09	0,26±0,02
A day after the immobilization					
1	206,18±15,38	15,15±1,09	0,57±0,04	1,64±0,14	0,41±0,03
2	119,52±8,50*	21,85±1,48*	0,41±0,03*	1,25±0,10*	0,27±0,02*
3	98,80±7,65*	22,16±1,63*	0,37±0,03*	1,28±0,09*	0,25±0,02*
Five days after the start of immobilization					
1	173,29±12,33	8,79±0,65	0,96±0,07	2,23±0,16	0,71±0,05
2	69,41±5,06*	19,05±1,37*	0,47±0,04*	1,29±0,12*	0,39±0,03*
3	63,96±4,51*	17,23±1,24*	0,42±0,03*	1,17±0,09*	0,34±0,03*
Five days after the overestimation of immobilization					
1	135,78±10,36	10,48±0,79	0,66±0,05	1,95±0,13	0,81±0,06*
2	45,82±3,32*	21,72±1,42*	0,29±0,02*	1,19±0,09*	0,31±0,02*
3	43,12±3,57*	23,69±1,61*	0,26±0,02*	1,26±0,09*	0,27±0,02

Note: * p≤0,05 - the difference is statistically significant between this and control groups

In the next four days of the maintenance of animals in the conditions of immobilization, a decrease in the level of cortisol in the blood of animals from all groups was noted. Thus, in the first group, the average value for this indicator decreased by 15.9%, in the second group - by 41.9% and in the third group - by 35.2%, respectively. Thus, at the end of the period of space limitation in rabbits from the first group, the concentration in the blood of this hormone was 2.5 times higher than in the second group, and 2.7 times in comparison with the third group.

For five days, since the moment immobilization was completed, a noticeable decrease in the amount of cortisol in blood samples of rabbits was recorded. At the same time in the first group, this indicator repeatedly exceeded the values fixed in the other groups. So, in control, his level was 2.9 times higher in the second group, and 3.1 times in the third group, respectively.

The use of drugs promoted an increase in the level of thyroxin in the blood of rabbits, as a result, the day before the beginning of modeling of technological stress in the second group, the concentration of this

hormone was higher than in the first group by 39%, and in the third group - 31.1% higher than in the first group.

Immobilization negatively affected the level of thyroxine in experimental animals, whose level decreased by 25.15% in the first day of space restriction in the first group, in the second group - by 22.3% and in the third group by 16.5%. A day after the beginning of modeling of technological stress, the level of this hormone in the blood of rabbits from the control group was less than in the second group by 44.2% and less than in the third group by 46.3%, respectively.

The level of thyroxine decreased throughout the period of finding rabbits in conditions of limited space and for five days of immobilization decreased in the blood of animals from the first group by 42%, in the second group by 12.8% and in the third group by 22.2%. At this stage of the experiment, the difference in this indicator between the control group and the rest was at the level of multiple values. For example, in the second group, the level of thyroxine in the blood was 2.1 times higher, and in the third group, 1.9 times than in the first group.

Five days after completion of immobilization, the amount of thyroxin increased in all groups, but the number in the control group was 2.1 times lower than in the second group and 2.3 times lower than in the third group, respectively.

At the beginning of the experiment in blood samples of all experimental animals, the values of parameters characterizing the state of lipid peroxidation processes not exceeding the limits of physiological parameters were fixed. The use of preventive regimens for rabbits from the second and third groups contributed to the optimization of the level of peroxidation products, expressed in a decrease in their level in the body.

During the day the rabbits were kept under restricted conditions, the concentration of dienic conjugates in the blood of the first group increased by 72.7%, in the second group by 64% and in the third group by 37%, respectively. As a result, the day after the beginning of immobilization in the control group, this indicator was 28.1% more than in the second group and 35.1% more than in the third group, with the differences being statistically significant.

Five-day immobilization of rabbits led to an increase in the level of diene conjugates by 68% in the first group, 14.6% in the second group and 13.5% in the third group, respectively. When the blood of rabbits was examined at the moment of completion of the restriction of mobility in animals from the second group, the level of this product was 2 times lower and in the third group 2.3 times than in the control group. Five days after the immobilization was completed, a decrease in this indicator in all groups of experimental animals, but in spite of this, the difference between the values recorded in the rabbit samples used by the preventive agents in the control group was significant. Thus, in the first group, the level of diene conjugates was 2.3 times higher than in the second group and 2.5 times greater than in the third group.

Considering the dynamics of malonic dialdehyde (MDA), it can be noted that its level changed insignificantly in the decrease in the second and third groups, and in the first group, on the contrary, there was a slight increase. As a result, there was no significant difference between the groups before immobilization. At the same time, in the first group this indicator had the highest values and was more than in the second group by 12.3% and more than in the third group - by 8.3%.

During the first day of modeling of technological stress, the level of malonic dialdehyde increased in the first group by 23.3%, in the second group - by 6.8% and in the third group - by 4.9%, respectively. At this stage of the experiment, the differences between the control group and animals injected with antistress and antioxidant drugs reached statistically significant values for this parameter. In the first group, this parameter after 24 hours of immobilization was higher than in the second by 23.8% and higher than in the second by 22%, respectively.

In the next four days, the concentration of malonic dialdehyde in the blood of rabbits from the first group increased by 36%, in the second group by 32% and in the third group by 8.6% in the next four days of exposure to the artificially created stress factor in the experimental animals. At that time, there was a

statistically significant difference in this parameter in the first group relative to the second and third groups, which was 42.1% and 47.5%, respectively.

After the rabbits were placed in habitual conditions for them, the level of malonic dialdehyde decreased in the first and second groups and slightly increased in the third group. Five days after the completion of immobilization in the second and third group, the level of malonic dialdehyde was statistically significantly lower than in the first group by 39% and 35.4%, respectively.

A similar dynamics was observed for the fluorescent Schiff bases (Schiff bases). The placement of rabbits in conditions of limited space also provoked a surge in the level of this final product of lipid peroxidation, which during the first day of immobilization increased in the first group by 36.6%, in the second group by 8% and in the third group by 3.8% . Such changes led to the fact that at this stage there was a significant difference in the values for this indicator between the control group at 34.1% relative to the second group and 39% relative to the third group.

The most critical period of the study, in which the most significant increase in the Schiff base concentration occurred, was recorded from the second to the fourth day of immobilization. During this time, their level in the first group increased by 73.1%, in the second group - by 44.4% and in the third group - by 36%, respectively. In blood samples obtained five days after the beginning of the restriction of the mobility of animals, the values for this indicator were significantly higher in the first group than in the second group by 45.1% and higher than in the third group by 52.1%.

Five days after the completion of immobilization, the first group experienced an increase in the level of Schiff bases in the blood, and in the second and third there was a significant decrease in this parameter. As a result, the difference between the control group and the second group was expressed in 2.6 times, and relative to the third - in 3 times, respectively.

The simulated technological stress significantly affected the functioning of the system of antioxidant protection of rabbits (Table 2). The administration of drugs to animals from the second and third groups promoted a marked increase in the activity of glutathione peroxidase, which was higher in the rabbits before the immobilization, when the antioxidant preparation for animals was used in combination with the preparation for the correction of stress conditions for farm animals, by 61.5% than in the control group , and in rabbits who received Mebisel together with Polyoxidol, this indicator was higher by 69.5% compared to the control group.

Table 2: Indicators of antioxidant protection in blood and dynamics of live weight of rabbits, (n = 20)

Group №	GnR activity, µM G-SH / l • min • 10^3	SOD activity, unitary act. / mg hemoglobin	Catalase activity, µM H_2O_2 / L • min • 10^3	Glutathione reduced, mmol / l	Live weight, kg
Before drugs introduction					
H	8-10	4-6	23-27	0,3-0,4	
1	8,23±0,69	4,58±0,33	24,13±1,76	0,32±0,02	3,24±0,25
2	8,44±0,63	4,71±0,36	23,87±1,62	0,30±0,02	3,16±0,21
3	8,29±0,51	4,37±0,29	24,21±1,69	0,33±0,02	3,41±0,23
Before immobilization					
1	8,31±0,73	5,11±0,42	23,79±1,58	0,33±0,02	3,30±0,27
2	13,42±0,93*	6,23±0,44	27,76±1,94	0,38±0,03	3,28±0,24
3	14,09±1,04*	6,98±0,51*	29,13±2,07*	0,40±0,03	3,52±0,29
A day after the immobilization					
1	6,16±0,46	3,69±0,27	17,08±1,23	0,23±0,02	3,03±0,20
2	15,31±1,19*	5,93±0,42*	24,42±1,75*	0,35±0,03*	3,19±0,23
3	15,87±1,33*	7,21±0,50*	26,76±1,98*	0,37±0,03*	3,45±0,25
Five days after the start of immobilization					

1	5,01±0,35	3,12±0,21	13,34±1,03	0,18±0,02	2,74±0,18
2	13,07±0,97*	6,04±0,49*	24,66±2,15*	0,35±0,03*	3,14±0,21
3	13,22±1,13*	6,71±0,53*	25,58±2,22*	0,36±0,03*	3,39±0,26*
Five days after the overestimation of immobilization					
1	7,12±0,52	3,74±0,28	19,29±1,42	0,26±0,02	2,98±0,21
2	14,48±1,06*	7,42±0,54*	27,34±2,31*	0,37±0,03*	3,41±0,26
3	15,23±1,21*	8,01±0,62*	28,22±2,04*	0,41±0,03*	3,69+0,28*

Note: * p≤0,05 - the difference is statistically significant between this and control groups

During the first day of immobilization in the first group, the level of activity of glutathione peroxidase (GPO) decreased by 25.9%, and in the second group it increased by 14.1% and in the third group - an increase of 18.3%, respectively. As a result, the difference between the groups increased sharply and was 2.5 times at this stage between the first and second group, and 2.6 times, respectively, between the first and the third group.

Five-day finding of rabbits in conditions of limited space for the next four days contributed to a decrease in the activity of glutathione peroxidase in the first group by 18.7%, in the second group by 14.6% and in the third group by 16.7%, respectively. At this stage of the experiment, the difference between the control group and the rest of the groups was 2.6 times. For five days, since the moment immobilization was completed, this indicator has increased. At this time in the first group it was lower than in the second 2 times and lower than in the third group 2.1 times.

The level of activity of superoxide dismutase (SOD) after application of drugs has increased in all groups, but in the first group in much less than in the second and third groups. Such changes led to a significant difference in the values of this indicator in different groups. Thus, in the first group, the activity of this enzyme was lower by 21.9% than in the second group and 36.6% lower than in the third group.

During the first day of immobilization in the blood of rabbits from the third group, the activity of superoxide dismutase increased by 3.3%, while in the first and second groups it decreased by 4.8% and 27.8%, respectively. At this stage in the blood samples from animals in the control group, the activity level of this enzyme was lower than in the material taken from rabbits from the second and third groups by 60.7% and 95.4%, respectively.

Over the next four days of immobilization, a decrease in activity of superoxide dismutase in the first group was registered by 15.4%, in the third group - by 6.9%, and in the second group an increase by 1.8% was recorded. Such changes led to an increase in the difference between the animals of their control group and colic, which used preventive drugs. As a result, five days after the beginning of immobilization, in the first group this indicator was lower than in the second group by 93.6% and lower than in the third group - by 115.1%.

After the animals were returned to their habitual conditions, there was an increase in the activity of superoxide dismutase, but there were significant differences between the groups. In the first group, values by this criterion were lower than in the second group by 98.4% and lower than in the third group - by 114.2%, respectively.

The dynamics of catalase activity indicates that the use of antistress and antioxidant drugs to experimental animals leads to an increase in this indicator. So, after three days from the beginning of the blood test, the activity of this enzyme was less in the first group than in the second one by 21.9% and less than in the third group by 36.6%.

As a result of the stress-factor effect for the first day, catalase activity decreased by 28.2% in the first group, 12% in the second group and 8.1% in the third group. For the next four days in the first group, this indicator decreased by another 21.9%, and in the third group - by 4.4%, while in the second group it remained practically unchanged. The greatest difference between control rabbits and animals treated with preventive treatment was observed after the immobilization was completed. At the same time, in the blood of animals from the first group this indicator was lower than in the second group by 84.7% and lower than in the third

group - by 91.7%, respectively. Five days after the immobilization, the catalase activity increased in all three groups, and the difference between them under this criterion was significantly reduced. At the final stage in the control group, this critical indicator was less than in the second group by 42.3% and less than in the third group - by 57.7%, respectively.

At the beginning of the experiment, the level of reduced glutathione in the blood of rabbits from all groups was within the lower limits of the physiological norm. The use of drugs led to an increase in this indicator in the second and third groups, while in the first group it remained almost unnamed. As a result, at this stage, the difference in the level of reduced glutathione between the first group and the second group was 15.1%, and between the first group and the third group - 21.2%, respectively.

The simulated stress reaction caused a decrease in the level of reduced glutathione during the first day of immobilization in the first group by 30.3%, in the second group by 7.9% and in the third group by 7.5%, respectively. This was due to a lower value of this criterion in the control relative to the second group by 52.2% and relative to the third group - by 60.9%.

During the next four days, during which the rabbits were kept in space-constrained conditions, the level of this product did not change in the second group, while in the first group it decreased by 21.7% and in the second group by 0.5%, respectively. At this time, the significant difference between the control animals and rabbits from the second group was 94.4%, and relative to the third group - 100%.

The five days that have elapsed from the moment immobilization was completed were characterized by a noticeable increase in the level of reduced glutathione in the blood of rabbits from all groups. But at the same time, the difference in the amount of this product between control animals and those using preventive drugs was significant and statistically reliable. In the first group, this marker was lower by 42.3% than in the second and lower by 57.7% than in the third group, respectively.

The dynamics of the live weight of rabbits was associated with a decrease in weight under the influence of a stress reaction. So for the first day of immobilization, the average body weight of rabbits from the control group decreased by 8.2%, in the second group by 2.7% and in the third group by 2%, respectively. For the next four days of the limitation of mobility, the decrease in this parameter in the first group was 9.6%, in the second group - 1.6% and in the third group - 1.8%. The average daily gain for the past five days after immobilization in the first group was 48 grams, in the second group 54 g and in the third group 56 g, respectively.

CONCLUSION

As a result of the studies, data have been obtained that make it possible to judge that immobilization of animals as a laboratory model of technological stress leads to significant changes in internal homeostasis in rabbits. As a result of this stress factor in the body of animals, the level of cortisol is repeatedly increased and the production of thyroxine is reduced. The dynamics of the level of cortisol, as a specific marker of stress, allows us to say that the peak of the stress reaction occurs one day after the stressor, and then, apparently, a certain adaptation comes. It is established that one of the pathogenetic links changes in the rabbits exposed to the process of stress is the increase in the concentration of lipid peroxidation products due to lower activity of the main antioxidant enzymes and reduce the concentration of reduced glutathione. One of the clinical manifestations of stress in animals is a significant decrease in body weight.

It is found that the integrated process scheme prevention of stress, comprising the combined use of dosage forms of drugs with antioxidant and anti-stress effect, is an effective method to reduce the negative effects of pathogenic stress reaction. In this application of the complex "preparation for correction of stress conditions for farm animals" and "animal Antioxidant drug" in appropriate doses comparable performance using "Mebisel" complex drugs and "Polioksidol". Their administration to rabbits accompanied by normalization dynamics of thyroxine and cortisol, and also reduces the concentration of malondialdehyde, conjugated dienes and fluorescent Schiff bases. Use of prophylactic schemes data contributes to the normalization operation of the system of antioxidant protection of animals, as evidenced by an increase under their influence in rabbits glutathione peroxidase activity of superoxide dismutase and catalase in the blood, as well as the level of reduced glutathione. The obtained results indicate that the applied preventive measures

can reduce the loss of body weight of animals as a result of exposure to technological stress and increase the average daily weight gain during the post-stress recovery period.

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