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# Oxidant and Antioxidant Status in the Poor Environmental Conditions to Biological Active Substances in Cows

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## ABSTRACT

The constant influence of inadequate chemical, biological and other factors on the animal organism causes a stress reduction in resistance and an immunosuppressive state in animals. In the body, it is characterized by a deficit in the energy supply of the function of the genetic apparatus and enzymes, a toxic blockade of the specific activity of enzymes, the prevalence of catabolic processes and the stay of the body in a state between norm and pathology.

The aim of the research was to study the effectiveness of the use of α- and γ-interferons alone and in combination with dimethyldipyrazolyl selenide for the correction of the oxidative-antioxidant status in cows under environmental conditions.

Materials and methods. The studies were carried out on three groups of cows taken into the experiment 3 weeks before calving, of which one group (n = 12) served as a control without the use of drugs, the animals of the other two groups were prescribed pharmacologic agents: one of them (n = 12) - only α - and γ-bovine recombinant interferons and other (n = 14) - α- and γ-interferons in combination with dimethyldipyrazolyl selenide. Blood samples were taken from 5 cows from each group before the administration of drugs and 4 days after the last injection to determine the content of malondialdehyde, indicators of endogenous intoxication and antioxidant protection.

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Results and Discussions. Cows with the administration of α- and γ-interferons alone and in combination with dimethyldipyrazolyl selenide, compared with animals in the control group, contained less malondialdehyde, respectively, by 12.7% and 21.8%, MSM at a wavelength of 238 nm - by 4 , 1% and 6.3%, MSM at a wavelength of 254 nm - by 12.5% ($p < 0.05$) and 15.6% ($p < 0.05$), SMP - by 13.5% and 8 ,one%. 21.6% ($p < 0.05$). Of the indicators of the antioxidant defense system, they had higher values of glutathione peroxidase (GPO) activity by 4.8% and 14.0%, catalase - by 5.2% and 9.9%, vitamin A - by 8.8% and 10.4%, vitamin E - by 12.5% and 9.5%, vitamin C - by 14.6% and 17.0%. Generic pathology was recorded less often 3.0 and 3.5 times, postpartum - 2.0 and 4.7 times. The period from calving to insemination was shorter by 11.9 and 23.0 days, fertility was higher by 8.3% and 9.6%, the insemination index was lower by 15.4% and 26.9%.

Conclusion. Thus, the use of recombinant bovine α- and γ-interferons in cows in the last month of pregnancy alone and in combination with dimethyldipyrazolyl selenide under conditions of environmental disadvantage contributed to a decrease in the accumulation of a toxic product of lipid peroxidation in the body of animals - malondialdehyde, a decrease in the manifestation of endogenous intoxication, stabilization of the antioxidant system. protection, which had a positive effect on the state of the reproductive system.

**Keywords:**malondialdehyde, endogenous intoxication, antioxidant protection, α- and γ-interferons, dimethyldipyrazolyl selenide, reproductive organs.

## INTRODUCTION

With the increase of industrial production and increasing chemicalization in agricultural production in the environment, the number of toxicants that have a negative impact on the livelihoods of the established biocenosis increases [1-6]. Technogenic biogeochemical zones, as a rule, are formed in the neighborhood of large industrial enterprises and ore mining. Separate sources of man-made pollution may also occur far from industrial enterprises as a result of the transfer of pollutants by air or water flows [6-10]. Industrial emissions imprint on all biological objects in the zone of the enterprise, and on the state of health of productive animals [11-15]. At the same time, toxic substances, when released into the soil, water, atmosphere, feed, cause a metabolic disorder in the animals, a change in the immunological and endocrine status, a disorder of the reproductive function [15-25].

As a result of the weakening of the functioning of the systems that control the body's defense reactions, the course of inflammatory and reparative processes is disturbed in animals,

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which leads to metabolic disorders and the development of pathological changes [26-30].

One of the key links in the development of the inflammatory process in reproductive organs is endogenous intoxication of the body with bacterial toxin, which, being a stimulator of macrophage function, in the area of inflammation activates the generation of reactive oxygen species and contributes to the development of oxidative stress leading to hyperproduction of free radicals and destruction of membranes with impaired function antioxidant protection [31-35].

Endogenous intoxication syndrome accompanies many diseases and pathological conditions, determining their severity. In various pathological conditions and diseases of inflammatory nature, accompanied by a syndrome of endogenous intoxication, an increase in the level of medium molecular peptides has been established, the role and importance of which is an indicator of endogenous intoxication of the body in a number of inflammatory diseases and pathological conditions are given much attention [36-42].

Highly productive cows with intensive metabolism and a sensitive neurohumoral regulatory system are most susceptible to disturbances in environmental conditions and react to this by a pronounced disturbance of metabolic processes, decreased reproductive function, natural resistance and immunobiological reactivity, which ultimately leads to their premature culling [43-50].

In this regard, in environmentally unfriendly zones, it is shown that animals use means that reduce the anthropogenic load on the body and increase its adaptive capacity to the conditions created by the external medium [51-58].**The aim of the research** was to study the effectiveness of the use of α- and γ-interferons alone and in combination with dimethyldipyrazolyl selenide for the correction of the oxidative-antioxidant status in cows that are in conditions of ecological distress.

## MATERIALS AND METHODS

The studies were performed under the conditions of a dairy complex located in the zone of operation of a large chemical enterprise with flare emissions into the atmosphere.

Experimental animals and treatment management. The experience included 38 dry cows taken in the experiment 3 weeks before calving and divided into 3 groups. Cows of the first group (n = 12) without prescription served as controls, cows of the second group (n = 12) were subcutaneously injected with α-and γ-interferons bovine recombinant at a dose of 10 ml each per animal three times with an interval of 24 hours, the third (n = 14) - α- and γ-interferons bovine recombinant were used according to the above scheme and intramuscularly with the first injection

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of interferons injected selector (organic selenium preparation) once at a dose of 1 ml / 100 kg body weight.

Preparations α- and γ-interferons bovine recombinant, which are low molecular weight proteins and related to cytokines, have the ability to regulate the sensitivity of cells to foreign factors. Under their action increases the likelihood of recognition and the production of antibodies against infection of the body. They cause changes in the cell that interfere with exposure to a foreign antigen. Interferons lead to a change in the homeostasis of the body, enhance its immunity [59-63]. Dimethyldipyrazolyl selenide is a preparation of organic selenium, has an oxidative effect. It reduces and prevents the accumulation of toxic lipid peroxidation products, helps to normalize the metabolism and increase the resistance of animals, shows immunostimulating and immunomodulatory effects [64-70].

Before administration of drugs and 4 days after their last injection, blood samples were taken from 5 cows from each group for laboratory tests. In the blood and serum, certain parameters of the lipid peroxidation system and antioxidant defense (LPS-AOD) were determined. The concentration of malondialdehyde (MDA), the activity of glutathione peroxidase (GPO) and catalase was determined spectrophotometrically using a Shimadzu-1700 spectrophotometer according to the Methodological Guidelines for the Study of Free Radical Oxidation and the Antioxidant Defense System of the body (Voronezh, 2010) [21]. The principle of determining MDA is based on its ability to react with 2-thiobarbituric acid at high temperature in an acidic environment to form a colored trimethyl complex (TMK) having an absorption maximum at a wavelength of 532 nm. The MDA content is calculated by the formula: $C \times 10^6 \times 3 / 1.5 \times 10^3$, where C is the concentration of MDA, μM / L; E is the optical density of the sample; $10^6$ — conversion factor in μM / L; $1.56 \times 10^3$ is the molar extinction coefficient of TMK MDA with 2-thiobarbituric squint; 3 - dilution factor.

The method for determining the activity of the enzyme glutathione peroxidase (GPO) in the blood is based on determining the decrease in reduced glutathione in the incubation medium during the reduction of hydroperoxides with glutathione peroxidase. The blood GPO activity is calculated by the formula: $A = (Eo – Ek) \times 10.55 \times 10^6 \times 166,4 / 13100$, where A is the enzyme activity in μM reduced glutathione / (l × min); Eo is the optical density of the control sample in comparison with the experimental one during the enzymatic oxidation of glutathione; Ek - the optical density of the control sample compared with the experimental non-enzymatic oxidation of glutathione; 10,55 - final sample volume, ml; 166.4 - dilution factor; $10^6$ - conversion of mMol to μM; 13100 - the molar extinction coefficient of the thionitrophenyl anion (THFA).

The determination of catalase activity in the blood is based on the ability of hydrogen

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peroxide to form a stable colored complex with ammonium molybdate with a maximum absorption at a wavelength of 410 nm. Catalase activity is calculated by the formula: A = (Ek-Eo) × 4.1 × 16 × $10^5$ × $10^6$ / 22.2·× $10^6$ × 3, where A is the enzyme activity, M.E. (μCH2O2 / l min); Ek is the optical density of the control sample; Eo is the optical density of the experimental sample; 4.1 - final sample volume; 16 × $10^5$ - breeding factor; $10^6$ - conversion factor μMot in μmol; 22.2 × $10^6$ - the molar extinction coefficient of $H_2O_2$; 3 - incubation time.

The concentration of average molecular peptides (AMP) was established by a modified method for determining the average molecular peptides in biological fluids [71-74]. The principle of the modified method for determining the content of AMP is to use 96% ethanol to precipitate proteins in biological fluid, which is able to completely precipitate high molecular weight proteins and, when performing spectrophotometry, determine the optical density of the tested solutions at a wavelength of 205-210 nm, at which the maximum absorption of protein substances is achieved .The content of medium-mass molecules (MMM) according to Grebneva et al. (2006) [8]. This indicator is determined based on the registration of the absorption spectrum of biological samples at four wavelengths in the range of 238-298 nm. The endogenous intoxication index was determined by M.Ya. Malakhova (1995).

The content of vitamins A, E and C in the blood serum was determined spectrophotometrically using an SF-2000 spectrophotometer [75-80]. The method for determining vitamin A in blood serum is based on alkaline hydrolysis and its extraction from blood serum and subsequent spectrophotometric measurement of light absorption by a solution at a wavelength of 328 nm before and after the destruction of vitamin A by ultraviolet rays. The calculation of the amount of vitamin A is carried out according to the formula: A - (E1 - E2) × 1274, where A is the concentration of vitamin A, μg / 100 ml; E1 - extinction of the solution before exposure to a wave of 315 nm; E2 - extinction of the solution after exposure to a wave of 315 nm; 1274 is a coefficient for determining the amount of vitamin A.

Determination of vitamin E in blood serum is based on its oxidation with ferric chloride and detection of the formed Fe2 + in the form of a colored complex with α, α′-dipyridyl at a wavelength of 520 nm. Correction for carotenoids is made by absorption at a wavelength of 460 nm. To determine the content of vitamin E, its optical density is calculated by the formula: De = D520 - (Dк + 0.217) · D460, where De is the optical density of vitamin E; D520 - the optical density of the experimental sample at a wavelength of 520 nm; Dк is the optical density of the control sample at a wavelength of 520 nm; 0.217 is an experimentally determined correction for optical density due to the presence of carotenoids; D460 is the optical density of the experimental sample at a wavelength of 460 nm. The principle of determining vitamin C is based on its ability

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to restore ferric iron to ferrous iron, which forms a pink complex with α, α′-dipyridyl. Calculation of vitamin C is carried out using a calibration graph.

During the experiment, cows took into account the nature of the course of labor and the postpartum period, the length of time from calving to fertilization, and the insemination index was determined.

The results of the studies were processed statistically and presented in tables.

## RESULTS AND DISCUSSION

It was established that when re-examining blood obtained 4 days after the completion of the gastrointestinal tract, changes occurred in the LPS-AOP system of varying severity (Table 1). These are the indicators characterizing the intensity of the flow of the LPS and endogenous intoxication, in cows of the first group underwent a slight change. The concentration of MDA decreased by 2.9% compared with the initial period, the MWM values at a wavelength of 238 nm decreased by 4.5%, at a wavelength of 254 nm - by 5.9%.

**Table 1. The content of malondialdehyde and indicators of endogenous intoxication in cows**

| Indicators | Terms of research | | |
|---|---|---|---|
| | Before use of drugs | | |
| | 1 group | 2 group | 3 group |
| **MDA, umol/l** | 3.73±0.38 | 3.57±0.35 | 3.75±0.86 |
| **$MWM_{238}$, y.e.** | 1.00±0.06 | 0.94±0.01 | 0.99±0.04 |
| **$MWM_{254}$, y.e.** | 0.34±0.02 | 0.30±0.03 | 0.30±0.02 |
| **MMP, y.e.** | 0.76±0.03 | 0.70±0.05 | 0.68±0.04 |
| **IEI, y.e** | 21.58±0.92 | 21.62±0.52 | 22.49±0.71 |
| | After the use of drugs | | |
| **MDA, umol/l** | 3.62±0.23 | 3.16±0.35 | 2.83±0.26 |
| **$MWM_{238}$, y.e.** | 0.95±0.03 | 0.91±0.02** | 0.89±0.01* |
| **$MWM_{254}$, y.e.** | 0.32±0.01 | 0.28±0.01 | 0.27±0.01 |
| **MMP, y.e.** | 0.74±0.06 | 0.64±0.05 | 0.58±0.05* |
| **IEI, y.e** | 20.80±0.92 | 20.10±0.54 | 19.96±0.67* |

Note: * – p<0,05; ** – p<0,01 – to the original

The content of the MMP, which is an indicator of endogenous intoxication in the body, was lower by 2.7%, while the IEI had a lower rate of 3.6%. In animals of the second group, there was a decrease in the number of MDA by 11.5%, values of MWM at a wavelength of 238 nm - by 7.1% (p <0.01), at a wavelength of 254 nm - by 6.7%, the concentration of MMP - by 8.1%, indicator of IEI - by 7.0%. In cows of the third group, these changes were more pronounced. The concentration of MDA decreased by 24.5%, the values of MWM decreased at a wavelength of 238 nm by 10.1% (p <0.05), at a wavelength of 254 nm - by 10.0%, the content of MMP - by

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14.7% and the indicator of IEI - by 11.2% ($p < 0.05$).

Compared with cows of the first group, animals of the second and third groups contained less malondialdehyde by 12.7% and 21.8%, MWM at a wavelength of 238 nm - by 4.1% and 6.3%, MWM at a wavelength 254 nm - by 12.5% ($p < 0.05$) and 15.6% ($p < 0.05$), MMP - by 13.5% and 21.6% ($p < 0.05$). The endogenous intoxication index had lower values, respectively, by 3.4% and 4.5%.

**Indicators of an antioxidant defense system.** Against the background of a decrease in the intensity of lipid peroxidation in cows with the prescription of α- and γ-interferon alone and in combination with dimethyldipyrazolyl selenide, activation of the AOP system was observed (Table 2).

**Table 2 Indicators of an antioxidant defense system in cows**

| Indicators | Terms of research | | |
|---|---|---|---|
| | Before use of drugs | | |
| | 1 group | 2 group | 3 group |
| **GPO, umol G-SH/l· min ·$10^3$** | 17.69±0.58 | 17.61±0.50 | 16.74±0.68 |
| **Catalase, umol $H_2O_2$/l ·min·$10^3$** | 41.98±2.48 | 42.69±3.35 | 41.28±3.65 |
| **Vitamin A, umol /l** | 1.22±0.11 | 1.24±0.13 | 1.21±0.10 |
| **Vitamin E, umol /l** | 15.36±1.03 | 16.24±1.42 | 15.28±1.52 |
| **Vitamin C, umol /l** | 22.74±2.08 | 22.68±1.44 | 22.30±2.10 |
| | After the use of drugs | | |
| **GPO, umol G-SH/l· min ·$10^3$** | 17.23±0.21 | 18.06±0.24 | 19.64±0.54** |
| **Catalase, umol $H_2O_2$/l ·min·$10^3$** | 43.17±2.40 | 46.62±3.15 | 47.43±3.92 |
| **Vitamin A, umol /l** | 1.25±0.06 | 1.36±0.08 | 1.38±0.07 |
| **Vitamin E, umol /l** | 16.26±1.43 | 18.30±1.50 | 17.81±1.41 |
| **Vitamin C, umol /l** | 23.20±1.06 | 26.58±1.28** | 27.14±3.32* |

Note: * – $p<0,05$; ** – $p<0,01$ – to the original

If in the control group animals with repeated blood tests, the rate of GPO activity was lower by 2.6%, then after the administration of α and γ-interferon to the animals, the GPO activity increased by 4.8%, after the injection of α and γ-interferon with selecor - by 17.3% ($p < 0.01$). At the same time, with an insignificant (by 2.8%) increase in the activity of catalase in cows of the first group in animals of the second and third groups, the increase in its activity was 9.2% and 14.9%, respectively.

From the indicators of the nonenzymatic level of the AOP system, the concentration of vitamins A, E and C in the first group of cows tended to increase. Increasing the concentration of vitamin A 2.5%, vitamin E - 2.3%, vitamin C - 1.5%. In animals of the second and third groups,

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the content of vitamin A increased respectively by 9.7% and 14.0%, vitamin E - by 12.7% and 16.6%, vitamin C - by 17.2% ($p < 0.01$ ) and 21.7% ($p < 0.05$).

In comparison with cows of the first group, animals of the second and third groups had higher rates of GPO activity by 4.8% and 14.0%, respectively, catalase - by 5.2% and 9.9%, vitamin A - by 8.8% and 10.4%, vitamin E - by 12.5% and 9.5%, vitamin C - by 14.6% and 17.0%.

Observing the nature of the course of labor and the postpartum period of the first, second and third cows, the pathology of calving was recorded respectively in 25.0%, 8.3%, 7.1% of animals, including difficult births in 8.3%, 8, 3%, 7.1% and post-mortem retention in 16.7%, 0%, 0% of animals. Compared with intact animals in groups of cows, which were used only α- and γ-interferons and α- and γ-interferons in combination with selector, the pathology of calving was recorded less frequently in 3.0 times and 3.5 times, respectively. Postpartum complications were detected in 33.3% of cows of the first group, in 16.7% of the second group, in 7.1% of the third group, occurring in cows of the first group in 8.3% of cases in the form of uterus subinvolution and in 25, 0% of cases in the form of endometritis, the second group - in 8.3% of cases in the form of subinvolution of the uterus and in 8.3% of cases in the form of endometritis, the third group - in 7.1% of cases in the form of endometritis. In comparison with animals of the first group, postnatal pathology in cows of the second group was recorded less frequently by 2 times, in animals of the third group - less often by 4.7 times.

The positive effect of drugs on the course of labor and the postpartum period in cows had a positive effect on the functional state of the reproductive system. The period from calving to productive insemination in cows using only α- and γ-interferons, α- and γ-interferons in combination with selector was 84.4 ± 5.04 and 73.3 ± 5.41 days, respectively, which was less in comparison with intact animals (96.3 ± 12.9 days), respectively, by 11.9 and 23.0 ($p < 0.05$) days. The fertility of animals of the second and third groups was respectively 91.6% and 92.9% and was respectively 8.3% and 9.6% higher than in the control (83.3%). The insemination index in animals using only α- and γ-interferons, α- and γ-interferons in combination with selector was 2.2 ± 0.37 units, respectively. and 1.9 ± 0.20 units. and was 15.4% and 26.9% ($p < 0.05$) less than that of intact cows (2.6 ± 0.34 units).

Thus, the use of α- and γ-interferons and their combination with dimethyldipyrazolyl selenide and aminoseleton in cows last month helped reduce the manifestation of generic and postnatal pathology, reduce the time from calving to insemination and increase their fertility.

The results of studies that indicate the stabilizing effect of bovine recombinant α- and γ-interferons on the LPS-AOD system are consistent with N.T. Klimov et al. (2018), which, when

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assigned to cows with subclinical mastitis, showed a decrease in malondialdehyde by 42.3%, average molecular peptides by 45.4% [81-85].

When prescribing bovine recombinant α- and γ-interferons in combination with estrofan for prophylaxis in postpartum endometritis cows according to V.N. Skorikova (2018), the concentration of catalase compared to its content in intact animals was higher by 13.0%, and malondialdehyde was lower by 25% and the difference in these indicators was more pronounced compared with the use of estrofan alone. This is also consistent with the results of our studies, when the combined administration of bovine recombinant α- and γ-interferons with dimethyldipyrazolyl selenide showed a more pronounced stabilizing effect on the POL-AOP system than the use of α- and γ-interferons alone [86-90].

The use of bovine recombinant ones alone and in combination with dimethyldipyrazolyl selenide in dry cows of α- and γ-interferons made it possible to reduce the pathology of childbirth and the postpartum period, shorten the period from calving to insemination, increase fertility and decrease the insemination index, which is consistent with V.N. Skorikova (2018), according to which when bovine recombinant α- and γ-interferons with estrofan were given to cows, the incidence of animals with postpartum endometritis decreased 3.4 times, the period from calving to fertilization and the insemination index decreased 1.4 times [91-96].

## CONCLUSION

The use of α-and γ-interferon bovine recombinant for cows in the last month of pregnancy, the combination with selector under conditions of environmental distress has helped stabilize the LPS-AOP system, which consists in reducing the accumulation of toxic lipid peroxidation in animals, reducing the concentration of molecules the average mass and medium molecular peptides with an increase in enzymatic activity (an increase in the activity of GPO and catalase) and non-fermentative the active (increased concentration of vitamins A, E and C) of the antioxidant defense links, which had a positive effect on the state of the reproductive organs.

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**Competing Interests**

The authors declare that they have no competing interests.

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