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Effects of lipoperoxidation and mitochondrial state on milk yield of dairy cows under technological stress

A.V. Deryugina[1*], M.N. Ivashchenko[1], V.B. Metelin[2,3], D.A. Danilova[1], A.V. Polozova[1], M.N. Talamanova[1]

[1]Department of Physiology and Anatomy, Lobachevsky State University, Nizhny Novgorod, Russia
[2]Vladimirsky Moscow Regional Clinical Research Institute, Moscow, Russia
[3]Russian State University named after AN Kosygin, Moscow, Russia

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KEYWORDS

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Hematological parameters

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ABSTRACT

Evaluation of the physiological state of cattle is crucial in creating healthy, high-performing dairy cattle herds. Technological stress is one of the most critical factors determining the biological potential of higher-yielding cows. This work aimed to assess the effect of technological stress on various oxidative parameters and mitochondrial states in dairy cows' blood, milk yield and milk composition. The study was conducted on the black-and-white breed of healthy herds. Regrouping, changing service personnel, and carrying out veterinary and sanitary manipulations were considered technological stress factors. The concentration of cortisol in the blood serum was studied by the immunological method. The concentrations of malonic dialdehyde (MDA), diene conjugates (D.C.), Schiff bases (S.B.), reduced glutathione and catalase activity were measured spectrophotometrically. The mitochondrial state was estimated by laser interference microscopy. While the milk yield, protein and lipid composition of cow milk were studied using an ultrasound analyzer. The researched indicators were analyzed before and for 30 days after the effect of technological stress. Results of the study suggested that technological stress caused an increase in oxidative processes, along with a reduction of antioxidant activity of blood and milk at the initial stages of registration (1-7 days). The concentration of glutathione remained reduced for 30 days after technological stress. A decrease in mitochondrial refractoriness and disintegration accompanied these processes. The milk yield indicator decreased was not restored to the values of intact animals by 30 days after technological stress. Further, the protein and lipid composition also reduced.

* Corresponding author
E-mail: derugina69@yandex.ru (A.V. Deryugina)

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Thus, a decrease in the quantity and quality of milk under technological stress may be mediated by the development of oxidative stress, which the refractoriness and disintegration of mitochondria might trigger.

1 Introduction

Industrial technologies are widely used in modern agricultural enterprises; sometimes, these technologies show adverse impacts and might create stressful conditions for animals. New equipment, noise exposure, table size, maintenance method, and change of personnel care are the main factors of technological stress for cattle (Breuer et al. 2003; Gupta et al. 2007; Hernandez et al. 2014). The severity of the stress reaction depends on the duration and the type of stress factors, and these two factors causing the things to disturb the regulatory mechanisms of the animal body and a violation of physiological, behavioural and metabolic parameters (Mandal et al. 2021; Chikkagoudara et al. 2022). The sympathoadrenal and hypothalamus-pituitary-adrenal (HPA) axes are crucial in the implementation of the action of stress factors (Bagath et al. 2019). Recent studies have suggested that catecholamines alter the number and function of lymphocytes exerting an inflammatory effect, while cortisol causes a decrease in the immune system of animals (Ibrahim et al. 2023). It has been shown that stress worsens the immune response and causes immunosuppressive effects (Chen et al. 2018). In this regard, the consequences of stress are decreases in susceptibility to infections (Akinmoladun 2021). The effect of any stress factors is associated with the activation of oxidative stress and depletion of the antioxidant system.

Further, stress factors are also associated with the disturbance of the delicate balance between the lipid peroxidation processes and the antioxidant defence system, including the disruption of Mitochondria (Chauhan et al. 2014). Additionally, the acid-base status is also changed. Due to their wide-ranging impact, the development of acidosis and increased oxidative stress can lead to a deterioration of the physiological state of animals and a substantial diminution of milk yield (Raghunandan et al. 2022; Semsirmboon et al. 2023).

Consequently, comprehending the stress reaction mechanisms and analyzing more accurate stress indicators give advanced opportunities to eliminate damaging factors, avoid animal diseases and increase milk yield. Therefore, this research aim was to evaluate the relationship between oxidative parameters and the mitochondria state in the blood serum, milk yield and milk quality under technological stress.

2 Materials and Methods

2.1 Experimental animals and design

This study was carried out on a clinically healthy dairy population of 2nd lactation (n=20) Black-and-White breed of Holstein cows under the conditions of the Nizhny Novgorod region industrial complex. All the experimental conditions, like feeding and keeping animals were the same. The research was conducted as per the suggestions of the European Convention for the Protection of Vertebrate Animals used for Experimental or Scientific Purposes (ETS No. 123, Strasbourg, 1986) and the Ministry of Health of the Russian Federation No. 708 N dated August 28, 2010. Further, the Russian Academy of Agricultural Sciences norms were followed, and the experimental animals were kept tethered in standard barns throughout the year, taking food and water according to the standards.

As stress factors, regrouping, changing service personnel, and conducting veterinary and sanitary manipulations were employed in this study. The research was carried out in the winter season. After the morning feeding, the blood samples were collected from the jugular vein of animals before and after 1, 3, 14, and 30 days of selected stress exposure. This dynamic made it possible to analyze the role of stress in the short-term (up to 3 days) and long-term (up to 30 days) periods. Cortisol concentration, indicators of oxidative stress (concentration of MDA), diene conjugates (D.C.), Schiff bases, catalase activity, and reduced glutathione content in blood serum were recorded in the blood as per the standard methodology, as suggested in subsequent paragraphs.

2.2 Analysis of Blood samples

Serum cortisol level was determined using an automatic ELISA analyzer (Evolis Twin Plus, Russia) (Asuzu et al. 2023). The MDA concentration was determined by reaction with thiobarbituric acid to form a coloured trimethine complex, and the absorption of this complex was recorded with the help of spectrophotometrically at 530 nm (Deryugina et al. 2019a).

Further, the level of serum peroxide reduction was used to determine the catalase activity (Deryugina et al. 2018a).

A spectrophotometric assay (using a wavelength of 240nm) was implemented immediately after adding H_2O_2 into the serum and after 20 seconds. Catalase activity was calculated by the formula: A = (log E1/E2 × 120000)/Hb, where A is catalase activity, the E is molar extinction coefficient (E1 - immediately, E2 - after 20 sec); Hb is the amount of hemoglobin in the sample. Catalase activity was expressed in μM H_2O_2 / l min 10^3.

The reduced glutathione level was determined using Ellman's method (1959) with 5,5'-di-thio-bis(-2-nitrobenzoic) acid. A sulfosalicylic acid solution was used for protein precipitation in the

studied samples to avoid the spontaneous transition of glutathione from the reduced to the oxidized form. The reduced glutathione concentration was expressed in mmol/l.

The intensity of oxidative damage was studied by the content of molecular products of lipid peroxidation (LPO) such as diene conjugates and Schiff's bases by the spectrophotometric method (Volchegorsky et al. 1989) using an SF-2000 spectrophotometer (St. Petersburg, Russian Federation).

The content of diene conjugates and Schiff bases were estimated by relative values of E232/E220 and E400/E220 and expressed in relative units. Diene conjugates (232 nm is absorption wavelengths) and Schiff bases (400 nm is absorption wavelengths) phase was measured in comparison with the corresponding control (220 nm is absorption wavelengths of isolated double bonds) by relative values of E232/E220, E400/E220 and expressed in relative units.

2.3 Isolation and estimation of mitochondria

60-100 ml of venous blood sample was mixed with 25 ml of medium containing 5% dextran, 0.12 M NaCl, 10 mM EDTA, pH 7.4, and centrifuged for 45 min at 4 °C for erythrocytes precipitation. Further, the precipitate was gathered and centrifuged at 5000 × g for 10 minutes. Then the sediment was suspended in the hypotonic medium (pH 7.6) containing sucrose (0.25 m) as an osmotic shock inhibitor. This suspension was centrifuged at 600 × g for 10 minutes; the received precipitate was exposed to a second osmotic shock and re-centrifuged. The supernatants were stored, combined and centrifuged at 12,000 × g for 20 min to precipitate the mitochondria. The mitochondrial precipitate was suspended in a medium containing 0.25 M sucrose, 2 mM EDTA, and pH 7.4 (Egorova and Afanasiev 2011).

Structural changes in mitochondria were studied using a laser interference microscope MIM-340 (Yekaterinburg, Russia) with a 30x objective (NA=0.65), λ laser=650 nm, and images were captured using high-resolution VS-415U CCD video camera, and a mirror substrate was used for the signal enhancement.

Consequently, a double-sideband phase shift of a coherent light source beam at each point of the object was recorded, and an extra wave from the same source was used to form an interference image of the organelle. Images of 10 sites with the one-layer placement of organelles in the interference channel were obtained for research. The mitochondrial state was evaluated by recording the optical path difference (OPD) mean relevance and diameter of the mitochondria's phase image. For reliability, the indices were measured using a minimum of 20 mitochondria from each sample (Deryugina et al. 2018b).

2.4 Methods of studying milk and milk productivity of cows

The control milking estimated the milk productivity of animals for a month from the start of the study. When investigating milk productivity, fat and protein content were also determined using an ultrasonic analyzer, "Lactan 1-4" (Russia). The lipoperoxidation intensity in animal milk samples was assessed by determining the primary and secondary lipoperoxidation products by Spectrophotometry of the lipid extract was performed at three wavelengths, i.e., 220, 232, and 278 nm, which allowed determining the content of primary oxidation products (diene conjugates "D.C."), the content of secondary oxidation products (ketodienes and conjugated trienes "CD/CT"). The final products of lipoperoxidation-Schiff bases were determined by the method of Lvovskaya et al. (1991). The content of free radical lipid oxidation products was expressed in units of the oxidation index.

2.5 Statistical analysis

The obtained data were processed using the Statistica program; subsequent analysis to determine statistically significant differences was carried out using the Student's T-test.

3 Results and Discussion

3.1 Cortisol concentration analysis

The development of a stress reaction is accompanied by an increase in the content of corticolibyrin in the blood, which increases the production of adrenocorticotropic hormone (Mormede et al. 2007; Deryugina et al. 2019b). Cortisol concentrations indicate the stress level in the cows. It was shown that before the technological stress, the cortisol levels were within the physiological parameters characteristic of cattle and amounted to 17.68 ± 0.79 nmol/l. A 2.5 times increase in the hormone cortisol concentration was recorded by 44.77 ± 5.61 mol/l on the first day. By days 7 and 14, the cortisol level was recorded as 29.43±1.69 and 25.89 ±2.19, respectively. The amount of cortisol in the blood decreased after the 30th day of stress exposure, but this value also exceeded the values obtained before the technological stress (19.32 ± 0.60 nmol/l). The percentage changes in the cortisol level compared to the before treatment are represented in Figure 1.

3.2 Lipoperoxidation and blood antioxidant system

An integral part of the imbalance of internal homeostasis in animals under stress is a change in the concentration of free radicals and the development of oxidative stress against these technological stresses (Slimen et al. 2016). Considering the dynamics of the LPO products obtained a day after the onset of exposure, a 2-fold rising of the level of D.C. was recorded with the

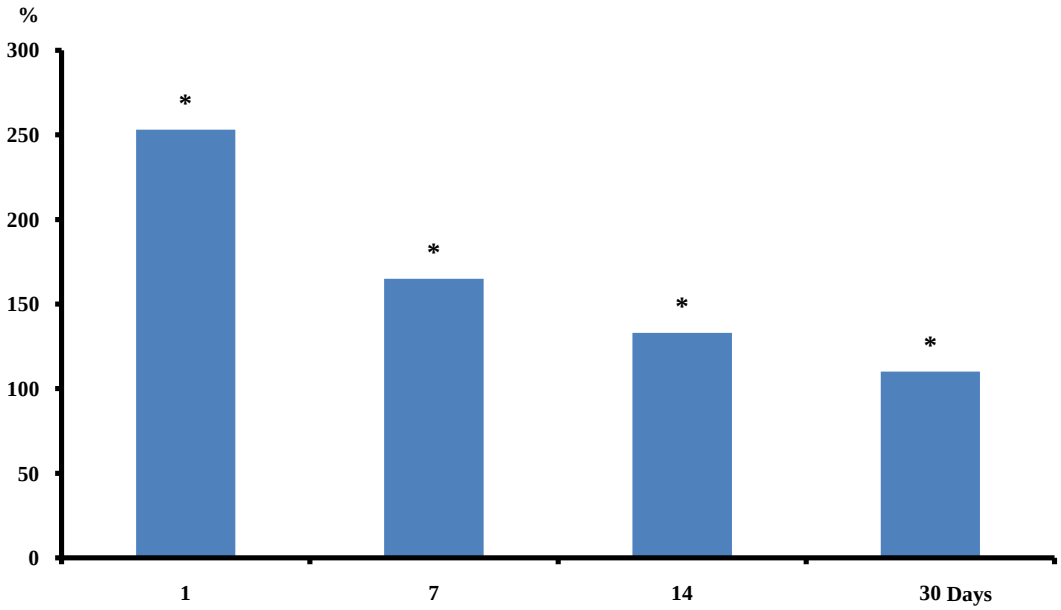

Figure 1 Dynamics of blood cortisol concentration after technological stress (Here 100 % represented the level of the indicator before technological stress, * mark on each bar represented the statistically significant differences concerning the indicators before technological stress at $p < 0.05$)

Table 1 The level of peroxidation products and indicators of the antioxidant capacity system in the blood of cows

Indicator	Initial value Before stress	Days After Technological Stress			
		1	3	14	30
D.C. (rel. units/ml serum)	0.34 ± 0.02	0.70 ± 0.01*	0.73 ± 0.03*	0.62 ± 0.02*	0.39 ± 0.02
MDA (µmol/l)	1.45 ± 0.03	1.71 ± 0.01*	1.74 ± 0.02*	1.96 ± 0.04*	1.39 ± 0.02*
S. B. (rel. units/ml serum)	0.33 ± 0.02	0.34 ± 0.02	0.40 ± 0.01*	0.58 ± 0.02*	0.34 ± 0.04
Catalase (µM H_2O_2 / l min 10^3)	18.87 ± 1.29	15.43 ± 1.55*	14.45 ± 1.53*	15.13 ± 1.27*	17.88 ± 0.73
Glutathione reduced (mmol/l)	0.25 ± 0.02	0.14 ± 0.01*	0.12 ± 0.01*	0.18 ± 0.01*	0.19 ± 0.03*

Diene conjugates (D.C.); Schiff bases (S.B.), value followed by * showing statistically significant differences with indicators before technological stress ($p < 0.05$)

maintenance of elevated values during 14 days of observation relative to the indicator before stress. The concentration of malondialdehyde (MDH) increased from the first day; the peak of the increase in this product was found in blood samples obtained 14 days after the technological stress by 24% relative to the initial values. The same dynamic was observed for the concentration of fluorescent Schiff bases. Studies have shown that the level of Schiff bases was ultimate by day 14 relative to the data before stress (Table 1). The effect of stress also affected the antioxidant activity in the blood of cows (Table 1). In particular, the level of catalase was below the initial level for 14 days after technological stress. The amount of reduced glutathione during the experiment was reduced by 30-50% over 30 days, depending on the timing of exposure.

3.3 Mitochondrial analysis

The study of mitochondria by laser interference microscopy showed that the phase characteristics of the organelles changed under technological stress (figure 2). It was shown that the ratio of mitochondrial height to diameter allows for calculating mitochondrial refractoriness (Yaguzhinsky et al. 2008). Under technological stress, the refractoriness of individual mitochondria decreased, which may be related to the inhibition of the electron transport chain. The number of disintegrated mitochondria under technological stress increased 2-fold by day 1 compared to the indicators of the control group. Mitochondria are the primary source of reactive oxygen species (Long et al. 2009; Guevera et al. 2011), and the growth of disintegrated mitochondria with an

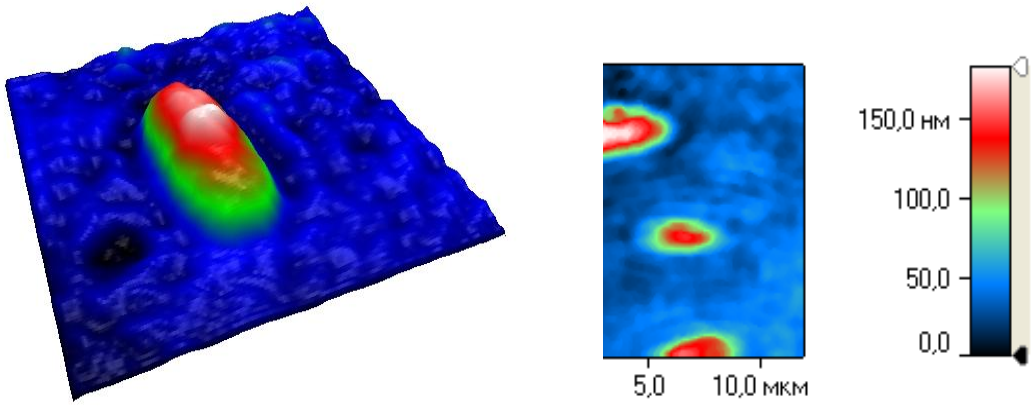

Figure 2 Phase portraits of mitochondria obtained by interference microscopy

Table 2 The effect of low-intensity laser radiation on dairy productivity of cows and the content of lipoperoxidation products of cow's milk

Indicator	Initial level before stress	after stress, hours	
		1	30
Milk productivity, kg	44.9±2.20	30.1±2.17*	32.3±2.13*
Mass fraction of fat, %	5.13±0.56	4.47±0.7	4.52±0.70
Mass fraction of protein, %	3.20±0.08	2.82±0.18*	3.06±0.24
Diene conjugates (c.u.)	0.94±0.16	1.32±0.17*	1.29±0.21
Kettani and related trienes (c.u.)	0.085±0.01	0.103±0.012*	0.104±0.014
Schiff base (c.u.)	0.015±0.001	0.023±0.01	0.022±0.013

Value followed by * showing statistically significant differences with indicators before technological stress ($p < 0.05$)

altered refractory level can enhance the development of oxidative stress in cows, which will have a negative effect on their productivity.

3.4 Milk analysis

The analysis of milk productivity in cows on day 1 after technological stress showed a decrease in milk productivity by 32% relative to the values of cows without technological stress and preservation of the reduced milk productivity indicator at day 30 of the study. Under technological stress, the amount of protein significantly decreased by 13% on day 1 after technological stress and recovered by day 30. The mass fraction of fat tended to decline. An increase in lipoperoxidation products in milk was recorded under technological stress. The number of diene conjugates, Kettani and related trienes increased significantly by day 1 of technological stress, while on day 30, the indices tended to decrease (Table 2).

Results of the study suggested that the cortisol level after 30 days of technological stress reached the initial values but, after this, also remained above the standard limit. Glucocorticoids function as checkpoints for energy homeostasis and mediate many effects of stress on metabolism. A high cortisol level suppresses the animal immune system and increases the incidence of diseases (Fomichev et al. 2012).

Additionally, in most deviations of the diverse etiologies, lipid peroxidation activity enlarges, leading to pronounced changes in the physicochemical properties of lipids. The structure and, consequently, the main functions of membrane proteins are more regulated by the protein–lipid interaction (Hammerschmid et al. 2023). Violating lipid and protein components with increased lipid peroxidation leads to cellular dysfunction. A detrimental impact of lipid peroxidation is manifested by a violation of lipid and protein components of the membranes and leads to cellular dysfunction (Villalón-García et al. 2023).

Furthermore, it is assumed that cytotoxic free radical processes' activity reduces the SS-groups and raises the level of S.H. groups. The detrimental impact of various factors on thiol compounds arises explicitly due to their ability to instantly and, at the same time, convertible oxidize. Among the multiple antioxidant mechanisms that prevent cell damage, the critical

place is controlled by thiol–disulfide exchange, and glutathione is the essential component that maintains cell REDOX balance (Asanuma and Miyazaki 2021). Glutathione (γ-glutamyl-cysteinylglycine) is a thiol-tripeptide that exists in two interconvertible forms, reduced glutathione (GSH) and oxidized glutathione (GSSG). The reduced glutathione is the principal intracellular antioxidant buffer which is crucial for maintaining the level of cysteine in proteins. Additionally, glutathione controls the maintenance of the normal oxidation-antioxidant equilibrium states and scavenges the hydrogen and lipid peroxide (Kuhn et al. 2017; Ighodaro and Akinloye 2018; Bayır et al. 2020).

The study's results also demonstrated that the content of the GSH was reduced after technological stress. The reduced level of glutathione remained on the 30th day of registration, which indicates a decrease in the adaptation and resistance to oxidative stress. Oxidation of fatty acid esterified in membrane phospholipids leads to the primary mechanisms of cellular oxidative damage.

The primary substrates for free radical-inducted damages are the double bonds of unsaturated fatty acids in phospholipids (Gaschler and Stockwell 2017). Mitochondrial membranes are particularly susceptible to reactive oxygen species (ROS) because cardiolipin is localized in the inner mitochondrial membrane (Schenkel and Bakovic 2014). Under technological stress, the functional activity of mitochondria decreases, which is caused by the disruption of the antioxidant system and the formation of non-selective mitochondrial pores. The opening of these pores leads to irreversible disruption of mitochondrial functions (Bernardi et al. 2015). At the same time, the enzyme glutathione peroxidase (GPx) is required to maintain the levels of ROS in mitochondria (Shimura et al. 2022).

Consequently, the decrease in glutathione during the study may negatively affect the efficiency of mitochondria. Meanwhile, mitochondrial dysfunction can be considered an oxidative stress trigger in cows. The separation of respiration and phosphorylation process in mitochondria leads to a superoxide anion radical production by the respiratory chain (Skulachev et al. 2012). Thus, under technological stress, it is necessary to consider its intensity so that a vicious circle does not develop, increasing free-radical oxidation, damage to mitochondria, and increasing oxidative stress.

Conclusion

The study shows that technological stress significantly affects the processes of lipid oxidation in blood serum and milk, which is accompanied by a decrease in milk productivity. The most pronounced changes were registered on the 1st day after the action of technological stress. The revealed disorders of oxidative processes may be mediated by mitochondrial disintegration. It is shown that the index of the antioxidant system, i.e., reduced glutathione, was not restored to the initial values, which was combined with a decrease in milk productivity on day 30 of registration. Effects on mitochondrial energetics can significantly increase the efficacy of therapeutic drugs. However, the mechanisms of regulation of these processes are not fully understood. The answer to the extent to which cell energy modulation will contribute to the adaptation of the organism to stress is crucial for developing an effective direction of prevention and therapy.

Declaration of interest

The authors declare that there is no conflict of interest.

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