Research Article

Biochemical Screening of Lipid Peroxidation and Antioxidant Protection in Imported Cows During Adaptation

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Abstract | The work aimed to evaluate the changes in LPO parameters and the AOP system during adaptation to new living conditions in highly productive dairy cows in the dynamics of dry and postpartum periods. The research was carried out in 2019 in LLC "Ermolovskoe", Voronezh region, the Russian Federation. A total of 32 heifers and first-calf red-motley Holstein cows of the German selection were included in the experiment. When being transferred to dry cows, catalase activity decreased from 35.8 ± 0.99 μM N2O2 / l-min-10^3 to 33.2 ± 2.45 μM N2O2 / l-min-10^3 or by 7.8% after a month and a half, and to 32.4 ± 0.58 μM N2O2 / l-min-10^3, or by 10.5% ($p<0.05$), after another week. There was also an increase in triglycerides and cholesterol by 6.7 and 19.1% ($p<0.001$), respectively. Biochemical studies of various blood parameters have the greatest relevance in controlling the state of animal homeostasis. It has been established that the processes of lipids peroxidation intensify with the increasing pregnancy period in imported heifers and the first days after calving. At that, the AOP indices are quite low. After delivery, the activity of enzymatic and non-enzymatic parts of AOP in the blood of first-calf cows significantly increases, leading to the stabilization and reduction of LPO products. As this work shows, the stress load on the antioxidant system is fairly high in highly productive cows after giving birth and is restored only after 3-4 weeks.

Keywords | Adaptation; Antioxidant protection; Dairy cows; Lipid peroxidation; Screening.

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INTRODUCTION

In modern cattle farming, considerable economic damage is caused by the effects of oxidative stress suffered by high-production dairy cows at the beginning of the post-partum period, as also by hyperketoanemia (Ma et al., 2020). In economic conditions in the Russian Federation, the problem of dairy cattle breeding has acquired high importance. Thus, the development of dairy cattle based on the rational use of local breeds with the involvement of the imported gene pool of cultivated dairy breeds is being constantly improved. However, continuous monitoring of biochemical and physiological systems of imported animals is required to achieve and consolidate the positive results of this issue.

One of the priority research areas in studying the biochemical mechanisms in animals during different physiological periods and manifestations of nosologically differentiated pathology is the evaluation of lipid peroxidation (LPO) and antioxidant defense system activity (antioxidant protection, AOP) (Kunwar and Priyadarsini, 2011). Hence, the oxidative phosphorylation, regulation of cell biomembrane permeability, synthesis of prostaglandins and steroids, and functioning of many enzyme systems are directly connected with lipids peroxidation (Dobson and Smith, 2000; E. Dushkin and A. Dushkin, 2012; Fridovich, 2001). From the physiology point of view, normal animal health requires, first of all, the fulfillment of two conditions related to each other, namely, the balance between lipid peroxidation (LPO) and the antioxidant protection of the body (AOP). Usually, the normal functioning of the animal organism is characterized by the low intensity of LPO processes. The main role of LPO, in this case, is the renewal or regeneration of phospholipids building the cell membrane (Esposito et al., 2014).

In non-enzymatic oxidation, the reaction results in the production of peroxides, which, in turn, are non-specific regulators of such processes as phagocytosis, lipocytosis, and destruction of foreign compounds by oxidation. Also, they can act as regulators of membrane permeability in lysosomes. Another type of LPO, enzymatic oxidation, is a catalyst for progesterone and prostaglandins synthesis, as well as other biologically active compounds (Yu et al., 2020). Certain extreme (stress) factors can increase the intensity of LPO processes resulting in the accumulation of excessive side products, developing, thus endogenous intoxication accompanied by a decrease in AOP activity. Outwardly, intoxication is manifested as diseases of non-contagious etiology. During intensive milking, for example, the majority of highly productive cows (80%) experience the AOP disturbance, inciting the deviation from the normal level of some biochemical parameters (Sies, 2015). LPO processes can also increase under the influence of $Fe2+$ and $Cu+$ ions, contributing to the formation of the OH radical, which is the most reactive among oxygen forms. The consequence of this can be cytolysis of the cell or disruption of its structures. In this regard, high concentrations of copper or iron that can develop during stressful situations can pose a health risk to the animal (Mozduri et al., 2018). There is a compound in the body that provides copper- binding and transport, ceruloplasmin. In addition, ceruloplasmin is part of AOP, being the main component of this system in blood plasma. Ceruloplasmin is also characterized by both specific and nonspecific antioxidant activity (Konvičná et al., 2015).

Two other compounds included in AOP are tocopherol and vitamin C (ascorbic acid). The first compound has a protective effect on unsaturated fatty acids of phospholipids that are part of cell membranes from lipoperoxidation. Vitamin C contributes to the preservation of vitamin E in the body. In turn, cobalt is responsible for the preservation of necessary vitamin C reserves in the body (Gross et al., 2011).

Thus, the intensity of the LPO determines the normal functioning of the membrane permeability, the synthesis of prostaglandins and steroids, the stable functioning of other enzymatic systems and the phosphorylation system. Hence, it can be seen that deviations in free-radical metabolism, including in cattle, can cause not only serious productivity reduction but even the development of diseases with a fatal outcome.

Studies show that significant changes in the level of LPO products content and activity of individual AOP system links in the blood of cows are expressed during the drying period and after delivery (Kireev et al., 2017; Naziroglu and Gur, 2000) when the adaptation and compensatory mechanisms of mother and fetus organisms are in the state of maximum tension (Sundrum, 2015). Besides, the period of body adaptation, in response to any changes in the environment, maintenance factors, transportation, has a negative impact on all systems of the animal as a whole, forcing all metabolic links to work in the most intense mode. The need to monitor the homeostasis in imported cattle arises due to the need to prevent the development of pathological conditions during acclimatization and adaptation periods, as the most stressogenic (Karpenko et al., 2018). In this regard, biochemical screening of lipid peroxidation processes and antioxidant protection system (AOP) of highly productive imported cows remain highly relevant.

According to new information, oxidative stress reactions and antioxidant protection are related to nitric oxide (NO). Possible reasons for this protective effect include the ability of nitric oxide to increase the activity of enzymes associated with the antioxidant properties. In addition to enzyme activation, nitric oxide is capable of neutralizing superoxid anion radicals, thereby contributing to the detoxification process of reactive oxygen radicals (Safonov et al., 2018; Sayiner et al., 2020).

At the same time, under the influence of unfavorable external factors or due to the disorders of internal physiological LPO processes, the concentration of products of free-radical reactions can increase, resulting in the development of oxidative stress and pathologies caused by an excessive concentration of free radicals. Furthermore, structural and metabolic changes occur, primarily related to the reproductive system. The result of it is the development of such diseases as fetoplacental insufficiency, or late toxemia in pregnancy, antenatal embryonic hypoxia, placentitis, delayed placenta, or postpartum uterine subinvolution, as well as endometritis (He et al., 2017). This can also include various kinds of chronic pathologies, namely, uterine and ovarian, resulting in infertility. Due to such a wide range of pathologies, the works devoted to studying the LPO significance in the molecular mechanisms associated with adaptation and the disease genesis in productive animals, remain necessary and relevant (Baimishev et al., 2019).

In this study, the authors examined the changes occurring with LPO and AOP during the adaptation of cows. The authors suggest that an active increase of peroxidation products in the blood of heifers in the final stage of pregnancy is associated with the increased stress of the animals. It may be a consequence of the maximum intense metabolic processes occurring in the body of pregnant animals.

This study aimed to examine changes in LPO-AOP system parameters during adaptation to new living conditions in highly productive dairy cows in the dynamics of dry and postpartum periods.

MATERIALS AND METHODS

The studies were carried out on imported heifers and red-motley Holstein cows of the German selection belonging to LLC Ermolovskoe, Liskinsky district, Voronezh region of the Russian Federation. The experiments were conducted in the winter and stall autumn period (October-April). The study group consisted of 32 animals of the same age kept under the same conditions and fed the same fodder.

Study design

Blood for the study was taken from the jugular vein two, one month, two weeks, one week before delivery, and one to four or more weeks after delivery. Animals under experiment were monitored continuously. The study was conducted in compliance with international standards for the ethical treatment of animals and was approved at a meeting of the Voronezh Agricultural University Ethics Committee (protocol #234-01). All the animals were kept in the same conditions of temperature, humidity, lighting, in the same rooms and received the same food and the same potable water. Before delivery, silage, root vegetables and concentrated foodstuffs were excluded from the average daily intake. Only quality forage remained. During the winter, cows were fed at least two times a day with constant access to drinkers. Cows preparing to give birth received less feed but had unrestricted access to water. In the early hours after the calves were born, the cow was fed 1 kg of wheat bran diluted in hot water. Next, high-quality dry and mixed fodder was gradually added to the cow's diet, with the addition of green fodder.

Blood metabolic parameters determined in the work

The intensity of lipid peroxidation processes was evaluated based on the determination of malondialdehyde (MDA) content in the blood of cows, and the power of the AOP system – by determining the catalase activity in blood, glutathione reductase (GR) activity, ferroxidase activity of ceruloplasmin, vitamin E and carotene content using conventional methods.

The catalase activity was determined by permanganometry. Glutathione reductase activity was measured using a Screen Master biochemical analyzer (manufacturer: Hospitex, Italy-Switzerland). RANDOX reagents (United Kingdom) were used for this purpose. Ceruloplasmin activity parameters were determined based on the oxidation reaction of paraphenylenediamine. The concentration of malonic dialdehyde was detected by reaction with thiobarbituric acid. At high temperature and an acidic pH reaction, a pink-colored trimethylene complex was formed. Further, this complex was subjected to extraction with butanol, with maximum absorption values at 532 nm. The concentration values of vitamin E were established by reaction with α,α'-dipyridyl, and the staining intensity of the red complex of Fe2+ cation as well as α,α'-dipyridyl was measured at wavelength values of 520 nm.

Afterward, the state of lipid metabolism was analyzed based on the content of total lipids, triglycerides, and cholesterol in the blood of cows (Retskiy et al., 2005). These parameters were determined using the kits of «Vital Diagnostica» (Russia) and «Lachema» (Czech Republic).

Mathematical data processing

The data obtained were entered into a Microsoft Excel 2010 database (Microsoft Corp., USA). The experimental data were processed using mathematical statistics accepted in biology and medicine, using computer programs «Statistica 6.0». Quantitative indices were described using mean (M) and standard deviation (m), presenting the data in tables as M±m. Changes in the LPO and AOP values are presented as percentages because such standardization allows for a better understanding of which parameter has undergone changes and to which extent. A two-sample t-test was used to compare various parameters or the same parameter but at different study periods. The minimum level of difference significance between the samples was taken as $p \le 0.05$.

RESULTS AND DISCUSSION

As follows from Table 1, the content of LPO-MDA was 1.03±06 μM/l at the beginning of the dry period with a further increase to 1.12±0.04 μM/l, or by 8.7%, a month later. With approaching parturition, its concentration increased to 16.5% ($P<0.01$). It indicates the activation of LPO processes associated with the neuro-endocrine restructuring of the body in pregnant animals and the development of tension in the metabolic processes, ensuring the formation of the parturition dominant.

Excessive accumulation of LPO products and maintaining

Table 1: Changes in LPO and AOP parameters in heifers in the dynamics of the final pregnancy period

Indicators	Reference value	Periods of observation before parturition			
		2 months	1 month	2 weeks	1 week
Malone dialdehyde, μM/l	0.5-1.5	1.03±0.06	1.12±0.04	1.16±0.06	1.20±0.03*
Vitamin E, μM/l	10-30	32.4±2.40	30.9±1.92	25.7±2.88	21.4±1.92*
Carotene, μM/l	3.5-18	4.83±0.98	5.25±0.12	4.68±0.77	3.98±0.91
Ceruloplasmin, μM benzoquinone/L- min.	180-450	329.2±11.04	315.4±19.68	299.1 1±0.03*	278.3±8.72*
Catalase activity, μM H2O2 /l-min-103	30-40	35.8±0.99	37.4±0.37	33.2±.45	32.4±0.58*
Glutathione reductase activity, μM G-SS-G/L-min.	150-400	429.5±13.99	424.3±10.15	412.6±7.51	414.2±11.22

Note: * P<0.05-0.001

Table 2: LPO and AOP parameters in cows after parturition

Indicators	Reference value	Follow-up periods after parturition			
		1 week	2 weeks	3 weeks	4 weeks or more
Malone dialdehyde, μM/l	0.5-1.5	1.27±0.04	1.25±0.05	1.21±0.03	1.17±0.06
Vitamin E, μM/l	10-30	17.5±2.16	16.3±1.20	17.8±2.40	20.9±1.92
Carotene, μM/l	3.5-18	3.4±1.01	3.52±0.39	4.21±1.13	4.14±1.06
Ceruloplasmin, μM benzoquinone/L- min.	180-450	325.6±12.01	319.9±7.21	302.8±15.22	300.5±11.75
Catalase activity, μM H2O2 /l-min-103	30-40	26.1±1.76	26.7±2.15	29.4±0.88	32.7±1.13*
Glutathione reductase activity, μM G-SS- G/L-min.	150-400	394.6±10.98	368.4±10.15*	371.2±11.05	400.3±14.69

Note: *P<0.05-0.001

them at a stationary physiological level is ensured by primarily non-enzymatic link of antiradical protection included in these processes. Thus, the blood concentration of vitamin E, an effective «quencher» of singlet oxygen, its anion-radical acceptor and «interceptor» of free radicals (Fukuzawa et al., 1998), decreases in the month before labor by 4.2%, in two weeks by 20.7%, and in one week by 33.9% (P<0.01). Similar dynamics can be traced in the activity of ceruloplasmin, which exhibits both specific and nonspecific antioxidant properties. The decrease in its activity during the indicated periods was 4.2%, 9.1%, and 15.5%, respectively (P<0.01).

Carotene also shows antioxidant activit through the regeneration of the antiradical activity of vitamin E. Its amount in the blood decreased markedly in the week before parturition by 21.3%.

A similar pattern was revealed in the indices of the enzymatic component of AOP-catalase. However, it is actively involved in the process of lipid peroxidation only in the last two weeks of pregnancy. Thus, catalase activity decreased by 7.8% in a month and another week later by 10.5% (P<0.05). It should be assumed that the decrease in the activity of this enzyme at the final stage of pregnancy is associated with its active involvement in the process of neutralizing the mass of formed peroxide agents.

In the indicators of glutathione reductase, responsible for maintaining a sufficient level of the glutathione defense system, only a tendency toward a decrease in the activity of this enzyme was revealed.

The activity of lipid peroxidation processes continues to grow after parturition as evidenced by the MDA increase in blood in prenatal period by 5.8%. And only in the second half of the postpartum period, its content gradually decreases.

At the same time, there was a 31.3% decrease in vitamin E concentration, a 17.1% decrease in carotene concentration, a 24.1% decrease in catalase activity (P<0.05), and a 12.4% decrease in glutathione reductase activity (Table 2), while the ferroxidase activity of ceruloplasmin, in contrast, increased by 14.9%. Only one month after parturition, the AOP system indices increased and reached the prenatal level.

Consequently, postnatal involution as in genital organs, so in the whole body of animals requires large energy expenditure accompanied by the inclusion of lipid metabolism products in this process as evidenced by the content of total lipids,

Table 3: Lipid metabolism indices in cows in the prenatal period

Observation period	Indicators		
	total lipids, g/l	triglycerides, mM/l	cholesterol, mM/l
A – prenatal period			
2 months	5.26±0.78	0.49±0.01	2.59±0.24
1 month	4.93±1.11	0.52±0.09	3.16±0.25*
2 weeks	4.02±0.58	0.42±0.02	2.81±0.07
1 week	3.54±0.22*	0.39±0.04	2.94±0.15
Reference value	2.5-6.0	0.2-0.6	4.7-6.2
B – postpartum period			
1 week	3.67±0.45	0.30±0.06	3.25±0.15
2 weeks	3.43±0.16	0.32±0.03	3.87±0.07*
3 weeks	3.98±0.23	0.45±0.03*	4.52±0.13*
4 weeks or more	4.37±1.09*	0.48±0.05*	4.28±0.07*

Note: *P<0.01-0.001

triglycerides, and cholesterol in the blood of cows (Table 3).

Thus, the content of total lipids in animals two months before calving was 5.26± 0.78 g/l. One month before calving, their concentration decreased by 6.3%, and by the week of parturition – to 32.7% lower than in the initial observation period ($P<0.05$).

Almost the same situation occurred in the dynamics of triglycerides and cholesterol. Although their concentrations slightly increased in the eight months of pregnancy. Afterwards, as labor approached, both triglyceride and cholesterol concentrations decreased – two weeks before delivery by 19.2% and 11.1%.

Such a decrease in the content of some components in the blood serum lipid spectrum indicates, probably, an increased accumulation in the body for their subsequent use as energy material during childbirth. Besides, decrease in total lipids and cholesterol concentration with the approach to parturition might be associated with an increased synthesis of estrogenic and corticosteroid hormones, the precursors of which they are. At the same time, these hormones are inducers of labor (Shabunin et al., 2018).

Furthermore, the excessive accumulation of lipid metabolism products in the body can adversely affect the health and the course of the birth and postpartum period due to the activation of lipid peroxidation and formation of its toxic products.

The following processes were observed in lipid metabolism after parturition. The concentration of total lipids slightly increased by 3.7%, in the first week due to cholesterol, whose content also increased in the first week after delivery by 10.5%. In contrast, triglyceride concentrations during this period decreased by 23.1% compared to those at a week before.

Such an increase in the levels of total lipids and cholesterol is due to a decrease in the synthesis of estrogenic and corticosteroid hormones with the release of cholesterol from the ether-bound state. The increase in triglycerides in the blood of animals may indicate the beginning of the milk period since triglycerides are the main precursors of milk fat.

Two weeks after parturition, some decline in the intensity of lipid metabolism was observed. The total lipid content decreased from 3.67±0.45 to 3.43±0.61 which might be attributed to the higher intensity of involutional processes in reproductive organs of animals. At the same time, there was an increase in triglycerides and cholesterol by 6.7 and 19.1% ($P<0.001$), respectively. The same trend in their content in the blood of animals was observed three weeks after parturition. Thus, triglyceride concentration increased by 40.6% and cholesterol concentration – by 16.8% ($P<0.001$). That, in turn, promoted an increase in the concentration of total lipids by 16.0% ($P<0.001$). Such changes indicate a decrease in hormone-producing function, particularly, in the concentration of estrogens, whose precursor is cholesterol as one of the components in total lipid fractions. Triglyceride levels increased probably due to their reduced elimination into the colostrum.

Four and more weeks after parturition, the concentration of total lipids and triglycerides continued to increase but rather insignificantly, reaching 9.8 and 6.7%, respectively. During the same observation period, there was a slight decrease in cholesterol levels by 5.3%, which may indicate a possible onset of the sexual cycle and an increase in ovarian estrogen-synthesizing function. The increase in the level of total lipids in the blood is most probably related to the completion of involutional processes in the uterus.

At present, dairy cattle breeding in Russia remains one of the important agriculture branches. The reduction in the number of cattle in the early 2000s led to the destabilization of the domestic gene pool of various cattle breeds and the need to improve it by importing highly productive animals. Import substitution of cattle is still going on in many farms of the Russian Federation. The imported animals combine high productivity, have good health, acclimatize, and adapt quite successfully to new living conditions (Kibkalo et al., 2009). Therefore, the study and control of economic and biological features, as well as adaptation abilities of imported cattle, currently continues to be relevant. Violation of biochemical mechanisms of adaptation leads to a decrease in genetically determined productivity, reproductive qualities, and the development of pathological processes in the body of animals.

Stress reactions in the body are known to be accompanied by a set of general responsive stereotypic and functional shifts, which also include significant biochemical changes in the blood of animals (Dobson and Smith, 2000). One of the manifestations of stress reactions of the body is the physiological stress of pregnancy and parturition. These periods and the early postnatal period, are characterized by the most striking changes in the metabolic processes of the animal organism.

The results of this study showed that cows with higher milk productivity respond more strongly to physiological stress caused by delivery. This results in a higher concentration of lipid peroxide products. Due to this negative factor, recovering the antioxidant potential in cows takes more time in the post-partum period. Involutional processes in the organs of the reproductive system have slowed down. Under some circumstances, this may lead to the development of an obstetric pathology.

One of the most impressive examples of adaptive capabilities at the biochemical level is the LPO-AOP processes. The maintenance of LPO reactions at a stable physiological level is ensured by the coordinated functioning of the enzymatic and non-enzymatic mechanisms of the AOP system. The antioxidant defense system controls the level of reactive oxygen species, free radicals, and molecular LPO products and ensures the utilization of their excessive amounts and the normal course of oxidative processes (Fridovich, 2001; Fukuzawa et al., 1998). The analysis of the data obtained also testifies to the containment of lipids peroxidation products due to the active functioning of the AOP system biomechanisms in the periods under study. In turn, it leads to the general normalization of homeostasis in the cow organism, and the formation of morpho-physiological adaptation of animals.

Metabolic processes in animals are based on redox reactions. Among these, reactions involving free radicals are of particular importance. Free radicals have an unpaired electron in one of the oxygen atoms, which determines their increased activity. Free radicals reactions result in the formation of peroxide compounds. As believed, oxidation associated with free radicals can regulate at least one-quarter of all physicochemical reactions in the body (Gong and Xiao, 2016). The toxicity of free radicals is also reported as they expose phospholipids and proteins that build up the cell membranes to oxidation (Sharma et al., 2011). As a result, membrane integrity is compromised, while the membrane and cellular enzymes are deactivated. The level of LPO changes when a stressful situation develops and membrane degradation occurs, resulting in increased membrane permeability. Therefore, when free radicals accumulate in the animal organism, irreversible changes occur in physiological processes, leading to various pathologies.

Usually, animal adaptation to changing conditions, such as climate, is associated with a stress process (Sciorsci et al., 2020). Adaptation, as well as pathology, is dynamically associated with the background process of the reactive oxygen species formation, which leads to increased oxidation of biosubstrates by free radicals. Ultimately, adaptation leads to the adjustment of the organism to new conditions, or the development of pathological reactions if the adaptation process fails. The mechanisms of LPO are studied well enough, in particular, in the comparison of normal and pathological modes of cell functioning (Paiano et al., 2019). Mainly, molecules from cell membranes as well as nuclear chromatin are subjected to reoxidation. However, there is also a change in the structure of proteins, which is associated with their oxidative modification. During oxidative stress, reactive oxygen species attack proteins rather than the lipid component of membranes in the first place (Ingvartsen and Moyes, 2015; Vicente et al., 2014). Therefore, when protein molecules change their structure under the influence of free radicals, they become «alien» to the organism and are, therefore, subjected to immune attack by antibodies (Pilarczyk et al., 2012). At the same time, antibodies can be formed not only in relation to macromolecules of proteins, drugs of low molecular origin (Nakov et al., 2016). The level of antibody production can be related to the number of antigens, such as proteins of the intracellular origin or intramembrane complexes. These are available for uptake and processing by presenting cells and are further recognized by T- and B-lymphocytes (von Soosten et al., 2011). If large amounts of compounds are produced in the body to be recycled (e.g., free radicals, as was shown in this study), more autoantibodies are produced in response, which initiates macrophage-mediated recycling after binding to these substances.

CONCLUSION

Hence, an active increase of lipid peroxidation products in the blood of imported heifers in the final stage of pregnancy may indicate the increased stress condition of the animals and intense stress of metabolic processes occurring in the body during pregnancy. Changes in the intensity of lipid peroxidation processes depend on the functioning of the antioxidant defense system in these periods. The state of certain constituents of the AOP system also changes with the growing stress situation that occurs with an increase in gestation. At the same time, the indicators of the antioxidant defense system are of the opposite nature. Thus, the most significant changes occur in the non-enzymatic link and ceruloplasmin at the end of pregnancy. Ultimately, it leads to the normalization of lipid peroxidation processes in the postpartum period, preventing their deleterious effect on involution in the uterus and the development of obstetric pathology, which is also facilitated by the corresponding processes in the lipid metabolism. Besides, the data obtained in this study show that all the blood parameters during all observation periods were within the physiological norm. In turn, it reflects the stability of the metabolic processes and emphasizes the high positive adaptive capacity of the body of the studied animals.

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CONFLICT OF INTERESTS

Authors declare that they have no conflict of interests.

AUTHORS CONTRIBUTION

Both authors contributed equally to the experimentation, read and approved the final manuscript.

Data Availability

Data will be available on request.

ETHICAL STATEMENT

The authors declare that the work is written with due consideration of ethical standards. The study was conducted in accordance with the ethical principles approved by the Ethics Committee of Voronezh State Agricultural University Named After Emperor Peter the Great and Vernadsky Institute of Geochemistry and Analytical Chemistry of Russian Academy of Sciences.

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