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Research paper

Antioxidant System and its Functioning in Animal Organisms

R.R. Fatkullin, A.A. Ovchinnikov, E.M. Ermolova, Y.V. Matrosova, S.A. Chulichkova

South Ural State Agrarian University, Russia, Chelyabinsk Region, Troitsk, Gagarin Street, 13

Abstract

According to the opinion of F. Z. Meyerson, one of the main reasons for disruption of organs' plastic functions under stress is insufficient intake of substrates and cofactors required for synthesis of nucleic acids and protein, as well as partial or complete fasting. In the first stage, according to the author, activity of amino acids' gluconeogenesis and transamination enzymes increases through the neurohumoral effect, which is the reason of reduced synthesis of protein and transformation of amino acids into glucose, i.e. plastic resources of the organism, its structural proteins, and energy resources in particular, are transformed.

Keywords*: antioxidant enzymes, technogeneously-polluted areas, stress-realizing system, morphofunctional growth indicators, antioxidant, free radical oxidation, Vitartil, lipids' peroxidation, productivity, adaptation mechanisms, metabolism, homeostasis*.

1. Introduction

1.1 Relevance

Limitation of stress systems at the level of organs and tissues occurs due to activation of stress-limiting systems, antioxidant systems that include antioxidant enzymes (catalase, superoxidedismutase, glutathione peroxidase, and antioxidants' tocopherol, vitamin A, ascorbic acid). [1]

Given the above, study of the physiological status of animals is required, including analysis of the functional state of the antioxidant system of their organisms, as it best reflects the ability of youngsters to adapt to the technogeneous factors of the environment. This will make it possible to find ways of correcting the physiological status of the animals contained in technogeneously contaminated territories. Insufficient attention to this topic in the literature has determined the topic of this research which was studying peculiarities of the physiological state of stress-realizing and stress-limiting systems. [2, 3]

In addition, it is necessary to assess adaptive ability of the organism through the use of corrective additives by activating stress-limiting systems.

The goal is to set the ratio of the stress-realizing and stress-limiting systems, the dynamics of morphological and functional growth and development of white-faced calves.

The influence of the Vitartil biological active additive on intensification of lipid peroxidation and on the changes in the nature of concentration of major plasma antioxidant - hepatocuprein - has been identified. The intensity of stress-limiting systems of the organism and the nature of their interaction that contribute to stimulation of antioxidant defensive mechanisms that limit free radical oxidation under stress have been studied. During the study, it has been shown for the first time that Vitartil inhibits lipid peroxidation and activates antioxidant protection system of the organism.

1.2 Scientific Novelty

Age peculiarities of growth, development, meat productivity, interior indicators and ethological characteristics of white-faced bulls during intensive fattening on the background of using the Vitartil biologically active additive have been comprehensively studied. The use of Vitartil in the diet of calves with the aim of increasing their productivity has been scientifically substantiated.

According to the opinion of F. Z. Meyerson, one of the main reasons for disruption of organs' plastic functions under stress is insufficient intake of substrates and cofactors required for synthesis of nucleic acids and protein, as well as partial or complete fasting. In the first stage, according to the author, activity of amino acids' gluconeogenesis and transamination enzymes increases through the neurohumoral effect, which is the reason of reduced synthesis of protein and transformation of amino acids into glucose, i.e. plastic resources of the organism, its structural proteins, and energy resources in particular, are transformed [4, 5, 6].

Based on the literature data about the role of environmental factors in disrupting metabolism in the organisms of agricultural animals, one can draw the conclusion that metabolism and chemical composition of the internal environment change in new environmental conditions as a result of adaptive mechanisms. The organism passes on to the new level of homeostasis. The idea of the internal environment constancy regains its importance, but in a new capacity. In the context of acute or chronic stress, individual homeostatic systems rebuild not only their activity, but also the area of allowable changes to relevant systemically important factors (factors of homeostasis) [1, 7, 8].

Transition to a new level of homeostasis often involves changes of not only certain intermediate values that relate to the work of the homeostatic system, but also the area of permissible fluctuations of vital characteristics. This implies a fundamentally new practical conclusion about the necessity of developing "regional" standards for animal health in specific climatic and geographical, industrial conditions with the purpose of prepathological diagnostics and prevention of diseases, where differentiation of environmental health is closely related to updating animals' feeding norms for individual geochemical provinces [2, 3, 5].

Maintaining productive health of animals depends on the organism's ability to adapt to, and maintain its homeostasis in inadequate environmental conditions.

2 Methods

3 groups of white-faced calves, 10 calves in each, were formed according to the principle of analogous pairs. Calves of all groups were loosely kept indoors in group cages on slatted floors. The calves feeding and keeping conditions in all groups were the same. The total of 4 series of experiments were made.

The scheme of feeding the Vitartil biologically active additive: the second experimental group received 120 g of Vitartil added to the basic diet of 1 head for 15 days in month 6; the 3rd experimental group received Vitartil for 30 days.

Blood serum, liver tissue and heart muscle were taken for the study. Hematological parameters: the number of erythrocytes and leukocytes by counting the Goryaev's chamber were studied (A. M. Smirnov et al, 1978). Biochemical parameters: total protein was studied using the refractometric method on refractometer IRF–11 (I. P. Kondrakhin and others, 1985), and total lipids were studied using the photocolorimetric method.

Lipid peroxidation in the tissues was studied by the method of J. Stocksetal (1974) in the modification of I. A. Volchegorsky and coauthors (1989). Malondialdehyde (hereinafter referred to as MDA) was determined according to V. I. Orekhovich (1977). Activity of superoxide dismutase (hereinafter referred to as SOD) (EC number 1.15.1.1.) was determined by the method of S. Chevari, et al. (1985). Activity of catalase (EC number 1.11.1.6.) was determined by the method of N. S. Mamontov et al. (1994). Hepatocuprein (HC) in blood serum was determined by the modified method of Revin (S. V. Bestuzheva, V. G. Kolb, 1976).

3 Results

The Vitartil biologically active additive was used as a feed additive to the basic diet of white-faced calves during intensive growing.

The obtained results in studying the increase in weight have shown that calves in groups 2 and 3 that received Vitartil for 15 and 30 days at the age of 6 months grew most intensively. In the age of 6 months, live weight of calves on the background of using Vitartil exceeded the live weight of animals in the reference group by 4.1 kg, or 2.4% ($p<0.05$).

By the end of the experiment, when the calves were 15 months of age, calves in the 3rd experimental group with high degree of veracity surpassed their peers in the reference group by 35.4 kg (8.6%, $p<0.05$). It should be noted that the statistical significance in terms of live weight between the animals in groups 2 and 3 with the detected high values has not been determined.

Analysis of the dynamics of average daily weight gain has shown that the highest average daily growth was observed in the period between months 9 and 12, and was equal to 890.6±18.4; 942.6±17.3 and 1,017.5±16.2 g, in respective groups.

During the entire period of experiment, the average daily growth of calves in group 3 was by 101.1 g ($p<0.01$) higher than that of their counterparts in the reference group. The difference between calves in the control group and the calves that received Vitartil was more significant towards the end of the experiment, and reached 111.8 g ($p<0.001$).

The absolute growth of body weight was also the highest in the calves in groups 2 and 3. Same as in analyzing the relative growth rate in calves in the age of 6-9 months, no significant difference has been noted between the groups of calves. In the subsequent period, superiority of calves in groups 2 and 3 over their counterparts in the reference group was again observed, which was 3.10 and 2.00 kg, respectively. A similar pattern was observed in the period of 12 to 15 months.

It should also be noted that the biologically active additive has a positive effect on meat qualities' formation in calves, since the body type of cattle grown for meat is closely related to meat productivity.

Physiological and productive characteristics of animals are formed in the period of ontogenesis under the influence of genetic factors and the environment. Genetic factors (breed, origin of animals, etc.) determine the upper limit of growth, and the conditions of keeping and feeding determine the lower limit. During growth and development, significant quantitative and qualitative changes occur in the animals.

Based on the foregoing, the morphological and biochemical blood composition of calves was studied. Blood samples were taken from experimental calves in the age of 6, 9, 12 and 15 months.

In the age of 6 months, the leukocyte count in blood of the calves in group 3 decreased by 9.4% ($P<0.05$), compared to the reference group. A more significant increase in the leukocyte count toward the end of the preweaning period was observed in the calves of group 1, which reached 15.42% ($P<0.01$). The difference between groups was of 9.37%. Apparently, this can be explained by different intensity of growth in the reference and experimental animals. Increased leukocyte count is usually observed in weakly growing animals.

It should be noted that increased leukocyte count in the calves of group 1 was observed almost throughout the entire scientific and economic experiment and, compared to the previous age, reached 18.59% in the age of 9 months, 15.09% in the age of 12 months, and it was only by 15 months of age that their slight quantitative reduction (1.12%) was observed in calves. However, statistical processing of the data did not confirm veracity of reduction.

The erythrocyte count in calves in the reference group during the preweaning period showed no significant difference in the studied age periods, and ranged between $8.14\pm1.28\times10^{12}$g/l at the age of 6 months and $7.62\pm0.57\times10^{12}$g/l by the end of the fattening period.

In group 3, erythrocyte count in calves in the age of 15 months was $8.30\pm0.52\times10^{12}$g/l, and in the age of 6 months - $8.82\pm2.10\times10^{12}$g/l, and was above the initial level by 6.14%.

In the subsequent age periods, this pattern remained. Thus, while in the age of 9 months the superiority of calves over their counterparts in the reference group reached 6.11% ($P<0.05$), by the age of 12 months this excess with a high degree of veracity reached as much as 9.60% ($P<0.01$). By 15 months of age, when the calves in group 3 had higher live weight, their blood contained more erythrocytes, and the difference in their favor for this indicator reached 7.32% ($P<0.05$).

This fact is a confirmation of the above conclusion about activation of the redox processes in the organisms of calves who received Vitartil. The authors believe that it is a positive aspect, since the primary function of erythrocytes is transporting respiratory gases. Substances are transported through the membrane by diffusion and by binding with carrier molecules. The ATP required for these processes is formed as a result of glycolysis. It should be noted that in case of significant changes in red blood, the change in the erythrocyte count and amount of haemoglobin (respiratory blood pigment) may occur to a different extent. Generally, all this indicates the need to determine biochemical parameters of blood in experimental calves.

In this respect, the dynamics of lipid peroxidation under the influence of the Vitartil biologically active additive was studied.

The content of primary and secondary lipid peroxidation products soluble in isopropanol fraction, the content of primary and secondary oxidized phospholipids in blood serum decreased by 22% ($p<0.01$) and 29% ($p<0.05$), in the heart muscle - by 11% ($p<0.01$) and 16% ($p<0.001$), in the liver tissue - by 17% ($p<0.01$) and 22% ($p<0.001$), compared to the reference group.

In 15-month-old calves that did not receive the Vitartil additive, the content of primary and secondary lipid peroxidation products in blood serum that were soluble in the heptane fraction (primary and secondary overoxidized neutral lipids) increased, compared to experimental groups 2 and 3, in blood serum by 15% ($p<0.01$) and 21% ($p<0.001$), in the heart muscle – by 4% ($p<0.05$) and 13% ($p<0.01$), in the liver tissue – by 8% ($p<0.05$) and 14% ($p<0.01$), respectively.

The content of primary and secondary products of lipid peroxidation in blood serum that were soluble in the heptane fraction in 15-month-old animals who received the Vitartil additive at the age of 6 months slightly increased: by 7% (U) and 15% ($p<0.05$), in the heart muscle – by 10% (U), in the liver tissue – by 8% (U) and 10% (U), respectively, compared to the reference group.

For more complete understanding of the dynamics of lipid peroxidation formation in blood serum, the authors determined the secondary product of lipid peroxidation – MDA.

MDA content in blood serum of 15-month-old animals that did not receive the additives increased by 17% ($p<0.001$), in the heart muscle - by 12% ($p<0.001$), in the liver tissues - by 10% ($p<0.001$), compared to the reference group. In the animals that had received Vitartil for 30 days at the age of 6 months, the content of MDA in blood serum decreased by 32% ($p<0.01$), in the heart muscle - by 30% ($p<0.001$), in the liver tissues - by 26% ($p<0.05$), compared to the reference group.

Thus, the maximum MDA content was observed in 9- and 15-month-old animals that did not receive additives. Blood serum of these animals contained more MDA by 24% than blood serum of 15-month-old animals that had received the additive. In the tissues of heart and liver, the increase reached 17% and 14%, respectively.

Catecholamines in the tissues were inactivated with participation of specific enzymes, mainly monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT), which were mainly localized in the liver.

At the same time, MAO inhibitors indirectly slow down inactivation of biogeneous amines, and are used in practice.

In the calves at the age of 6 months that had received Vitartil for 15 days, activity of the enzyme increased in the heart muscle by 35% ($p<0.01$), and in the liver tissue almost remained at the background level.

In the calves at the age of 6 months that had received the biologically active additive Vitartil for 30 days, activity of MAO increased by 50% ($p<0.001$) in the heart muscle, and by 38% ($p<0.001$) in the liver tissues, compared to the reference group.

In the calves at the age of 15 months that had not received Vitartil, a decreased MAO activity was observed in the heart muscle - by 12% ($p<0.05$), in liver tissues - by 9% (U), compared to the reference group.

In the calves at the age of 15 months that had received the Vitartil additive, MAO activity increased in the heart muscle - by 22% ($p<0.001$), and in the liver tissues - by 12.3% ($p<0.05$), compared to the reference group.

Table 1. The dynamics of lipid peroxidation under the influence of the Vitartil active biological additive in blood serum, heart muscle and liver tissues of calves (M±m, n=6).

Indicators / Calves' life period	Heptane fraction					
	E 232/E220 Primary products of lipid peroxidation			E 278/E220 Secondary products of lipid peroxidation		
The object of study	blood serum	heart muscle	liver tissues	blood serum	heart muscle	liver tissues
1	2	3	4	5	6	7
1st series of experiments						
Reference	0.415± 0.005	0.332± 0.050	0.468± 0.010	0.128± 0.004	0.183± 0.004	0.226± 0.001
6 months + Vitartil 15 days	0.378± 0.003 91%	0.329± 0.005 99%	0.450± 0.030 96%	0.119± 0.010 93%	0.176± 0.006 97%	0.202± 0.004 89%
6 months + Vitartil 30 days	0.371± 0.004 89%	0.326± 0.030 98%	0.380± 0.040* 81%	0.106± 0.003 83%	0.172± 0.001 96%	0.200± 0.005 88%
2-d series of experiments						
Reference	0.434± 0.040*	0.358± 0.006	0.495± 0.050	0.143± 0.002	0.207± 0.003	0.238± 0.002
9 months, received Vitartil at the age of 6 months (for 15 days)	0.385± 0.050 89%	0.345± 0.010 96%	0.486± 0.030 98%	0.138± 0.001* 97%	0.200± 0.001* 97%	0.218± 0.050U 92%
9 months, received Vitartil at the age of 6 months (for 30 days)	0.380± 0.003 88%	0.329± 0.030 92%	0.477± 0.070U 96%	0.121± 0.010 85	0.181± 0.006 87%	0.206± 0.004 87%
4-th series of experiments						
Reference	0.484± 0.043	0.414± 0.010	0.573± 0.040	0.158± 0.005	0.244± 0.005**	0.287± 0.006
15 months, received Vitartil at the age of 6 months (for 15 days)	0.410± 0.032U 85%	0.396± 0.010U 96%	0.530± 0.060U 92%	0.140± 0.020* 89%	0.237± 0.010U 97%	0.230± 0.004 80%
15 months, received Vitartil at the age of 6 months (for 30 days)	0.381± 0.034 79%	0.360± 0.040 87%	0.490± 0.061 86%	0.122± 0.010 77%	0.203± 0.004 89%	0.223± 0.063U 78%

Note: veracity of the difference from the appropriate reference: *-p<0.05, ** -p<0.01, ***p<0.001.

1) The table shows the level of primary (acylhydroperoxides and carbohydrate conjugates) and secondary (ketodien and conjugated trienes) lipid peroxidation products;

2) The level of lipid peroxidation products was calculated in conditional units of the oxidation index, which was calculated as the ratio of optical densities E_{232}/E_{220} for primary and E_{278}/E_{220} to secondary lipid peroxidation products.

Inhibition of the MAO enzyme activity was observed in 9- and 15-month-old calves that did not receive the biologically active additive, and increase - in 15-month-old calves that received Vitartil.

Thus, cellular activity is regulated by free radical oxidation. However, under certain conditions of the organism (stress, inflammation, physical inactivity, obesity, ionizing radiation, carcinogens), excessive number of free radicals is observed, i.e. the process of lipid peroxidation accelerates, increasing viscosity of the membranes, the number of hydrophilic areas, and reducing the number of liquid lipid areas.

Antioxidant enzymes that inhibit peroxides' activity in the organism of calves have been studied. The objects of study were catalase, glutationreductase, HC, tocopherol, and SOD.
In the calves at the age of 6 months that had received the Vitartil biologically active additive for 15 days, the content of tocopherol increased by 6.3% (U), HC – by 2.67%. In the calves at the age of 6 months that had received the Vitartil biologically active additive for 30 days, the content of tocopherol increased by 14.5% ($p<0.01$), and HC – by 10.7% ($p<0.05$).
In the serum of 9-month-old calves that had not received biologically active additive, SOD activity, catalase in blood serum and globulin (GL) were lower than those in the reference group. In the calves that had received Vitartil at the age of 9 months for 30 days, SOD activity increased by 10.47% ($p<0.05$), catalase activity - by 3.7%, and GL – by 6% (U).
In blood serum of 15-months old calves that had not received the additive, SOD activity decreased by up to 37% ($p<0.01$), catalase activity decreased by 18% ($p<0.01$), and GL – by 22% ($p<0.01$), compared to the reference group. In the animals that had received the additive, enzyme activity increased and reached 109%. Thus, the activity of SOD enzymes, catalase and GL was the highest only in 6-month-old calves that had received Vitartil for 30 days along with the fodder.
In 6 months old calves that had received the Vitartil additive along with fodder for 15 days, SOD activity in the liver increased by 5% (U), catalase – by 15% ($p<0.01$), and HC content - by 22% ($p<0.01$). In the 6-month-old calves that had received the Vitartil biological additive for 30 days, SOD activity increased by up to 12% ($p<0.05$), catalase - by 24%, 14% ($p<0.01$), and HC – by 33% ($p<0.001$).
SOD in the liver tissues of 15-month-old calves that had not received the additives was inhibited by 7% (U), catalase activity decreased slightly - by 2% (U), and the content of HC decreased by 13% ($p<0.05$).
In the animals that had received the Vitartil additive, SOD activity was within the reference values - 104% (U), catalase, content of HC in the liver increased by 12% ($p<0.05$).
Thus, development of the overall adaptation syndrome and its outcome depend on the severity of stress-realizing and stress-limiting systems and the nature of their interaction, contributing to stimulation of the antioxidant defensive mechanisms of the organism for limiting free radical oxidation under stress.

4 Discussion

The main objective of transamination enzymes is providing the most complete set of amino acids to the organism, which are essential for vital functions: synthesizing the deficient amino-acids from the ones obtained with the fodder. Therefore, the more complete the amino acid composition of the fodder is, the lower the activity of aminotransferases is. During the studies, in the animals of groups 2 and 3, oscillations of enzyme activity corresponded to intensity of calves' growth in the experimental groups, but while activity of serum glutamic pyruvic transaminase by the end of the experimental period increased by 12.46% in calves in group 2, and by 21.96% - in group 3, in the reference group serum glutamic pyruvic transaminase activity increased 1.43 times.
The nature of changes in the concentrations of total lipids and phospholipids in 15-month-old calves may be the indication of more complete lipid metabolism in the organism of the calves that received Vitartil. This is confirmed by the values of lipid index, which amounted to 0.49 and 0.52, respectively, for the 1st and 3rd groups of calves. Lower values of this ratio in calves in the experimental groups indicate intensive recovery of phospholipids with the aim of providing the energy of intensified anabolic processes in protein metabolism during intensive growth.
Studying metabolism, one cannot omit considering the state of mineral metabolism. The results of the studies performed at phase 1 of the experiment have shown that in the feed the concentration of iron exceeded the feeding norm by 7.17%, of copper – 1.59 times, of cobalt – 1.69 times on the background of low zinc content, the lowest concentration of which was found in milk replacer – 0.34 μmol/l, with the standard value for whole milk of 0.08 μmol/l. The disbalance of iron, copper, cobalt, zinc, and partly manganese (103.28 mg/kg, with the norm of 80.0 mg/kg) was exacerbated by the presence of elements like lead, nickel and cadmium in the fodder; with that, while lead content in all of them was within the maximum permissible level, the cadmium content exceeded this indicator in hay by 20.0%.
Analysis of the bioelemental status of calves has shown that the geochemical situation that has formed in the agricultural landscapes affects the state of animals via the food chain. According to the obtained data, the content of inorganic phosphorus and calcium in calves at the beginning of the experiment exceeded the upper level of the norm on the average by 66%, 86%, and 31.32%, respectively. The content of iron and copper also exceeded the norm by 70%, 55%, and 26.20%, respectively. Particularly, in the blood of the calves in all groups, one should note the reduction of zinc - the element responsible for the immune system formation in young animals, on the average, by 25.77%.
At the time of the scientific and production experiment start, the blood of calves contained elements that were environmental pollutants exhibiting mainly the anti-metabolite role. Thus, according to the data obtained, the content of lead ranged between 0.22 and 0.25 μmol/l; that of cadmium – between 0.28 and 0.31 μmol/l; that of nickel – between 1.69 and 1.84 μmol/l, and were within permissible values.
The use of Vitartil influenced the bioelement status of calves. It was manifested in the veracious decreases in the concentrations of lead - 3.0 times; nickel – 2.06 times, and cadmium – 3.10 times in the blood of calves that received Vitartil. In the second group of calves, on the background of using Vitartil, this reduction amounted to 5.50, 2.73, and 3.22 times, respectively. In the reference group of calves, lead concentration during the period of study increased by 41.56%, while that of nickel and cadmium decreased by 10.17% and 27.27%, respectively, by elements.
In our opinion, high metabolic effect of Vitartil is mainly due to its chemical nature.
Summarizing the blood analysis data, it should be noted that the Vitartil biologically active additive had a metabolic effect, with the most significant effect on intermediate metabolism.
The data obtained during the study of certain hematological parameters have shown that the use of Vitartil is aimed at forming anabolic processes in intermediate metabolism, normalization of the bioelemental status, improving the functional state of organs and the entire organism.

5 Conclusion

Thus, the research and the nature of the obtained results confirm the appropriateness of using the Vitartil biologically active additive for activating growth and development of animals, contributing to exhibiting the antioxidant properties and reducing lipid peroxidation in the technology of cattle growing and fattening.

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