Features of free radical oxidation of lipids in cows with fat liver dystrophy

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Abstract. The mechanisms of the pathogenesis of liver damages of various genesis include destabilization of hepatocyte membranes, depending on the initiation of lipid peroxidation. The article presents research to determine the characteristics of lipid peroxidation in cows with fatty liver dystrophy. It was found out that in highly productive Holstein cows with a diagnosis of fatty hepatosis, the processes of free radical lipid oxidation are activated with an increase in the concentration of all lipoperoxidation products (diene conjugates, ketodienes and malondialdehyde) in blood and milk. Moreover, in sick animals, the level of antioxidant defense indicators such as carotene, vitamin C and glutathione peroxidase decreases.

1. Introduction
At the present time of dairy cattle breeding development, the issue of metabolic disorders in highly productive cows and related liver damages are of significant importance. Breeding cows aimed at high milk productivity has led not only to an increase in their genetic potential, but also created serious problems in health of animals. The physiological features of highly productive dairy cows include their high sensitivity to technological conditions of maintenance and operation, as well as an imbalance in the structure of diets, which often leads to metabolic failures in animals and provokes metabolic disorders and the development of pathological processes in liver. Moreover, liver diseases with dominant fatty hepatosis occupy up to 50% of the total gastroenteric pathology recorded in cattle [1, 7].

The mechanisms of the pathogenesis of liver damages of various genesis include destabilization of hepatocyte biomembranes, which depends on the initiation of free radical lipid peroxidation in cells. Currently, any pathological process in the body is considered from the point of view of the balance in the system of lipid peroxidation (LPO) and antioxidant defense (AOD) of the body, the violation of which leads to the development of oxidative stress [2].

Normally, the processes of formation of lipid peroxidation products are regulated by the AOD system, the basis of which are enzymatic and non-enzymatic antioxidants. However, with oxidative stress, there is an excessive accumulation of lipid peroxidation products, and their concentration carries information about the degree and depth of the pathological process. LPO markers are intermediates such as diene conjugates, ketodienes, malondialdehyde. The antioxidant system of the body is represented by enzymatic antioxidants (glutathione peroxidase, superoxide dismutase, catalase, etc.) and non-enzymatic ones (glutathione, carotenoids, vitamin C, tocopherol, etc.) [4, 6].
Recent scientific studies have revealed that with AOD insufficiency or a decrease in its power due to extreme external factors, LPO processes go beyond physiological limits, excessive accumulation of its products, the development of oxidative stress and free radical pathology with structural changes in organs and tissues, including liver [3, 5].

At the same time, the issues of lipid peroxidation and the state of the AOD system under various pathological conditions of the hepatobiliary system of animals remain unresolved, especially in highly productive dairy cattle.

The aim of the research was to determine the intensity of lipid peroxidation processes and the state of the antioxidant system of the body in highly productive cows with fatty hepatosis.

2. Materials and methods

The studies were carried out in the conditions of the livestock breeding farm of Krasnodar Region (Agro Firm Kuban LLC, Seversky district) on Holstein cows, with an average annual milk productivity of 7.0 thousand kg per herd, in 1-2 months of lactation. The animals were selected in the experiment, ranked by physiological condition, the results of the clinical examination, the biochemical profile of blood, as well as by the ultrasound diagnosis of liver. In total, 20 cows were selected using the paired analogs method and two groups were formed (n = 10): the first experimental one was diagnosed with fatty hepatosis; the second control group was with healthy cows.

Clinical examination was carried out according to the generally accepted scheme, paying particular attention to the color of the mucous membranes, the condition of the skin, the number of scar reductions, as well as palpation and percussion of liver to establish the percussion border of liver, the nature of the surface and the sensitivity of the organ.

Blood was sampled from the jugular vein of animals in the morning. Laboratory biochemical studies were performed on a Vitalab Selectra Junior automated analyzer with software version 1.0. (open system for conducting photometric tests, manufactured by Vital Scientific N. V. Nether-lands) using reagents from ELITech Clinical Systems (France) and Analyticon biotechnologies AG (Germany). The concentration of protein fractions and vitamins was determined in accordance with the “Methodological guidelines for the use of standardized biochemical methods for the study of blood, urine, milk in veterinary laboratories” (1981). At the same time, the level of lipid peroxidation processes and the antioxidant defense system of cows was evaluated using a number of blood parameters such as diene conjugates (DC), ketodienes (KD), malondialdehyde (MDA) and glutathione peroxidase (GPO), in accordance with the methodological recommendations of All-Russian Scientific Research Veterinary Institute of Pathology, Pharmacology and Therapy of the Russian Agricultural Academy (1997). The concentration of MDA in milk was evaluated by the color reaction with thiobarbituric acid.

Ultrasound diagnostics was performed using a PS-380V veterinary ultrasound scanner (Russia, sensor wavelength 5.0 MHz).

Statistical processing of the results was carried out using the packages of statistical programs ARCADIA, Microsoft Excel XP and Statistical for Windows. The study of quantitative signs was evaluated by comparing the average values of two sample sets with the determination of Student’s criterion and significance level (p).

3. Research results and discussion

When making a diagnosis in cows with hepatosis, general depression, decrease in appetite, dystonia of the pancreas with a violation of rhythm and strength of rumination, dullness and fragility of the fur were revealed. In 40% of cows, the mucous membranes were pale; in 60% of cows, the ictericity was recorded. In most cases (70%), a simultaneous increase in the area of percussion border of the liver and an increase in pain sensitivity were revealed. For other cows, only one of the symptoms was recorded, while an increase in body temperature and splenomegaly were not revealed.

The diagnosis of hepatosis was confirmed by biochemical blood tests of cows. Violation of the protein synthesizing function of liver was manifested by hypoproteinemia (a decrease in the level of
total protein to 68.7 ± 2.48 g/l) and hypouremia (the average concentration of urea in the groups was 2.8 ± 0.11 mm/l), as well as a low level of the albumin fraction (35.6 ± 1.32%). A number of selected animals with the diagnosis of hepatitis had a slight manifestation of inflammatory processes in the liver, which led to an average increase in γ-globulins in the group and a positive thymol test (degree +, with a maximum of ++++).

The presence of a cytolytic syndrome was manifested by an increase in hepatocellular enzymes: the quantitative content of AST in the blood of sick cows was 112.6 ± 6.54 U/l, ALT was 38.4 ± 2.44 U/l. The intensity of pigment metabolism in animals with hepatitis showed an increase in the content of total bilirubin to 9.6 ± 2.37 μM/l. Concentrations of glucose, triglycerides, cholesterol were recorded beyond the lower limit of the norm. In mineral metabolism, a violation of the calcium-phosphorus ratio was revealed (because of the low level of calcium).

The ultrasound diagnosis of the hepatobiliary system confirmed the diagnosis of “fatty hepatitis”: the liver was enlarged, the edges of the lobes were rounded, uneven, smeared, the echo-structure was heterogeneous, fine-grained, of increased echogenicity, and foci of fatty dystrophy of hepatocytes were noted.

Liver pathology in cows was manifested by activation of lipid peroxidation processes, which was confirmed by an increase in all detected lipoperoxidation products (table 1).

| Indicator                      | Groups                        |
|-------------------------------|-------------------------------|
| DC, OD units / mg of lipids   | 1 – sick animals              |
|                               | 0.31±0.013                    |
| KD, OD units / mg of lipids   | 0.125±0.005*                  |
| MDA, μM / l of blood          | 1.88±0.021**                  |

Note: significance level *p≤0.05; **p≥0.001 in relation to healthy cows

The most pronounced changes were recorded in the concentration of MDA, which in sick animals significantly exceeded the corresponding indicator of clinically healthy cows by 52.8% (p≥0.001). This intermediate most objectively reflects the final stages of lipid peroxidation in the body, and namely its content in biological objects that is usually used as an indicator of the activity of chemical reactions of lipid peroxidation. The difference in other lipid peroxidation products between the sick and healthy cows was 29.2% in terms of DC and 40.4% in terms of KD (p≤0.05).

At the same time, in sick cows, a decrease in antioxidant protection indicators relative to healthy animals was revealed (table 2).

| Indicator                      | Groups                        |
|-------------------------------|-------------------------------|
| Vitamin C, mg%                | 0.47±0.08*                    |
| Carotene, mg%                 | 0.28±0.03**                   |
| Glutathione peroxidase, mMGS-SH/l min. | 10.8±0.56 | 12.1±0.73 |

Note: significance level *p≤0.05; **p≥0.001 in relation to healthy cows

The maximum changes were recorded in the non-enzymatic link of antioxidant protection. Therefore, the difference in cows with liver pathology with healthy animals for carotene was 39.2% (p≥0.001), and for vitamin C it was 29.8% (p≤0.05). Moreover, in clinically healthy animals, the content of the determined vitamins was recorded at the level of the lower limit of the norm, which was because of their increased consumption during milking.
The pathological process in liver affected the concentration of glutathione peroxidase, which is an enzyme of the second line of antiradical protection. A decrease in its activity between groups was determined without a pronounced manifestation with a difference of 12%. The level of MDA in milk was characterized by similar to blood changes; in sick animals, it significantly (p≤0.05) exceeded the corresponding indicator of clinically healthy cows by 33.9% (figure 1).

![Figure 1. Concentration of MDA in the milk of healthy cows and cows with hepatosis.](image)

4. Conclusion
Thus, the development of fatty hepatosis in cows occurs on the background of activation of lipid peroxidation, with an increase in all lipoperoxidation products. At the same time, the level of MDA in the blood increases significantly, the concentrations of DC and KD increase less pronounced. The level of MDA in milk is characterized by similar to blood changes – there is a significant in-crease in its concentration. In sick cows, a decrease in a number of the antioxidant protection indi-cators relative to healthy animals is revealed with a maximum difference in the non-enzymatic link of anti-radical protection (carotene and vitamin C).

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