Opportunities of complex hepatosis therapy in cattle

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Abstract. The paper discusses the possibility of using a comprehensive treatment regimen for hepatosis in postpartum cows with the help of preparations with various mechanisms of hepatoprotective and anti-toxic effects. It was proved that enteral administration of Thionontrit-S against the background of intramuscular administration of Livazen to cows with clinical signs of hepatosis has a positive effect on the course of the disease and the recovery timing of animals. The use of a complex treatment regimen contributes to the manifestation of positive dynamics of the main hepatosis syndromes - hepatocellular insufficiency and impaired hepatocyte integrity, which is manifested by an increase in the level of total protein by 8.1\%, albumin - by 20.9\%, glucose - by 31.6\%, triglycerides - by 21.4\% and cholesterol - by 27.5\% in comparison with the basic treatment. The use of Thionontrit-S as part of the complex treatment of bovine hepatosis made it possible to reduce the activity of the inflammatory process in the liver, which was manifested by a decrease in enzyme activity: AST - by 47.5\%, ALT - by 19.9\%, alkaline phosphatase - by 1.59 times, and correction of the mineral component of blood serum.

1. Introduction

According to modern research, liver diseases, including hepatoses, occupy from 5 to 25\% of the entire non-infectious pathology of animals, and in cattle they are recorded up to 60\% of the total population [1]. More often than others, high producing animals with intense metabolic processes in the body are affected by hepatosis [1, 2].

Features of feeding with high-concentrate feeds, environmental degradation, and technological stresses (unbalanced feeding, feeding poor-quality feeds, including those containing mycotoxins, violation of the conditions for keeping and exploitation of animals, excessive use of drugs, etc.) lead to an increase in liver diseases in animals that occur against the background of metabolic disorders and are accompanied by a decrease in resistance, productivity, reproductive ability and increased culling of animals [3, 4].

In cows during down calving period, costs of energy and plastic substances are determined by the growth rate of the fetus, and in the post-pregnancy period, by the activity of biosynthesis and secretion of milk components, on the one hand, and on the other hand, by inadequate exogenous intake of nutrients and energy. In the genesis of liver pathology in high producing animals, there is a physiological and genetic predisposition to pathological mobilization of the body’s reserves for dairy products, which is the cause of fatty, and later, protein and toxic liver dystrophy [5, 6].
Toxins of exogenous and endogenous origin, together with bile or through the portal vein, penetrate the liver, causing profound changes in the structure of its cells. Under the influence of lipid peroxidation products, hepatocyte membranes, cell organelles, and nuclear membranes are damaged. An increase in the permeability of hepatocyte membranes leads to the release of proteolytic enzymes that stimulate further proteolysis and autolysis increase of the liver parenchyma, as evidenced by an increase in the activity of transamination enzymes (ALT and AST) [7].

As a result of autolysis of the liver parenchyma, the decay products of hepatocytes turn into antigens that stimulate the formation of autoantibodies, further decay and autolysis of liver cells; detoxification function of the liver is disturbed, ketone bodies and other toxic substances accumulate in the blood. Autoantibodies that are fixed on the hepatocyte membranes cause the effect of sensitized lymphocytes that provoke them. This autoimmune reaction proceeds according to the principle of delayed hypersensitivity. In addition, changes occur in the morphogenesis of hepatocytes, and, accordingly, in the structure of the liver itself [8].

As a result, the structure and function of hepatocyte cell membranes is disrupted, regeneration processes change, dystrophic changes, necrosis develop, and subsequently – hepatitis and hepatoses, while the liver barrier function is disrupted and the body is intoxicated [9].

Liver diseases mainly occur without pronounced clinical manifestations, which greatly complicates their timely diagnosis and effective therapy [10, 11].

And as practice shows, the search and use in animal husbandry of drugs aimed at correcting the impaired functional state of the liver, increasing resistance, normalizing metabolic processes in the animal’s body, is an urgent task of veterinary science and practice.

In connection with the foregoing, in the treatment of hepatosis, it is advisable to combine pathogenetic agents that have a complex hepatoprotective, adsorbing and detoxifying effect, affecting various pathological processes in the animal body, allowing to reduce the damaging effect of xenobiotics on the liver and stabilize metabolic processes [12].

The purpose of the research is to develop and implement a therapeutic regimen for cows with hepatosis during postnatal period using the two new drugs with different mechanisms of hepatoprotective and antitoxic effects.

2. Materials and methods

Objects of the study are Livazen and Thionontrit-S drugs.

Livazen is an injectable drug, which is a clear solution, odorless, slightly oily consistency. The mechanism of action of diisopropylammonium dichloroacetate, which is part of the preparation, is due to its antioxidant and membrane-stabilizing effect. It inhibits the processes of lipid peroxidation, increases the activity of superoxide oxidase, the ratio of lipid to protein, reduces the viscosity of the membrane, increases its fluidity. It modulates the activity of membrane-bound enzymes, receptor complexes, which enhances their binding ability, helps preserve the structural and functional organization of biomembranes. Diisopropylammonium dichloroacetate exerts a lipotropic effect, favorably affects the antioxidant and pigment function of the liver, and increases resistance to hypoxia and various types of intoxications [13].

Thionontrit-S is a complex preparation that includes the natural aluminosilicate mineral from the montmorillonite group - nontronite and hyposulfite. The optimal ratio of the components of the drug determines such pharmacodynamic effects as: adsorption, antitoxic, enveloping (gastroprotective), hepatoprotective, growth-promoting. In addition, nontronite, which is part of the drug, acts as a source of easily exchanged essential macro- and microelements.

An experiment to determine the therapeutic efficacy of the complex regimen was carried out on cows of the postnatal period (on the tenth day after calving), formed on the basis of experimental and clinical studies in two groups - experimental and control (n = 20) with clinical manifestation of hepatosis.

For hepatosis, in addition to the clinical manifestations (general depression, loss of appetite, decreased belching and cud, tachycardia, increased respiratory movements, upset forestomach - hypotension and atony, dyspeptic manifestations - diarrhea alternating with constipation, liver pain
during percussion and palpation and an increase in posterior percussion boundaries, dullness and fragility of the coat, anemia of the visible mucous membranes), is a change in the biochemical blood homeostasis.

Biochemical values of blood serum were characterized by an increase in transaminases (alanine aminotransferase - by 36.2%, aspartate aminotransferase - by 32.3%), bilirubin (2.03 times). Against the background of dysfermentonemia, hypoproteinemia (75.4 ± 5.18 g / l) was established. Violations of the protein spectrum of blood serum were characterized by moderate hypoalbuminemia. The level of γ-globulins remained within physiological boundaries. Relative to α- and β-globulins, certain fluctuations were observed in animals in the direction of an increase in these fractions.

A decrease in the functional activity of liver hepatocytes led to a weakening in them of other synthesis-forming processes. Analysis of blood parameters in animals revealed a decrease in the level of urea, glucose, and triglycerides.

Similar changes in the biochemical profiles of animals are characterized by syndromatics caused by hepatocellular insufficiency (impaired synthetic function of the liver) and impaired hepatocyte integrity (cytolysis, impaired permeability of the hepatocyte membranes) with an increase in the enzymatic activity of the liver - ALT, AST and bilirubin, which is characteristic of hepatosis.

Ultrasound diagnostics of the liver of cows, taken for the experiment, revealed changes in 36 animals, characterized, to one degree or another, by an increase in the organ with rounding of the lower edge, increased echogenicity, the presence of possible foci of lipomatosis, and a granular parenchyma structure.

The research scheme is presented in table 1.

| Group       | Structure of the experiment                                                                 |
|-------------|---------------------------------------------------------------------------------------------|
| I - experimental | Livazen - intramuscularly in a ratio of 1: 5 to sterile saline (4 ml of the drug and 20 ml of saline)  
Basic diet (BD) + 150 g of Thionontrit – S in feed for 21 days |
| II - control | Hepatoject - intramuscularly at a dose of 50 ml                                             |

Thionontrit-S was added daily enteral (per os) to the feeding diets of the animals of the first (experimental) group, against the background of intramuscular injection of Livazen. The animals of the second group were injected intramuscularly with the Hepatoject preparation once a day at a dose of 50 ml for two weeks (according to the instructions for use).

Hepatoject (“API-SAN” LLC, Moscow), is a complex hepatoprotective drug, which includes (with the following ratio of components, ml / mg): L-ornithine - 15 mg, L-citrulline - 10 mg, L-arginine - 40 mg, as well as excipients: betaine - 15 mg, sorbitol - 200 mg, lidocaine hydrochloride - 1 mg, methylparaben - 0.5 mg, propylparaben - 0.2 mg and water for injection - up to 1 ml.

The conditions of keeping and diets of the cows under the experiment were similar (keeping – loose, outdoor; feeding – compound feed, haylage, silage, hay, drinking water from car drinkers).

The effectiveness of comparative therapy was evaluated according to the clinical condition of cows, data from a biochemical blood test and ultrasonography.

Blood biochemical values were determined on an automatic chemical analyzer - Vitalab Selectra Junior with software version 1.0. (open system for conducting photometric tests, manufactured by VitalScientific N. V. Netherlands) using reagents from ELITech Clinical Systems (France) and Analyticon biotechnologies AG (Germany). Protein fractions of blood serum were determined by nephelometric method.

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 ARCADA, Microsoft Excel XP and Statistical for Windows. The study of quantitative traits was
evaluated by comparing the mean values of two sample sets with the determination of Student’s criterion and significance level (p).

3. Results

The proposed complex scheme of therapeutic measures contributed to the normalization and stabilization of the biochemical homeostasis of animals against the background of the leveling of pathogenetic processes arising from hepatosis, which was comparable with the improvement in the clinical condition of experimental cows.

In the experimental group, the clinical manifestations of the disease in 70% of the cows disappeared by the 9th–11th day of the experiment, and complete recovery was observed in 18 animals from the 15th–16th day of therapy with the symptoms of the disease in two animals from the group not completely disappearing. The improvement of the clinical condition and physiological parameters was manifested by an increase in appetite, the disappearance of pain during palpation of the liver area and a decrease in its percussion borders, an increase in the reduction of the rumen to normal (2-5 contractions in 2 minutes), restoration of the functions of the gastrointestinal tract (disappearance of dyspeptic phenomena).

In the control group, the restoration of clinical indicators began to appear from the 13th day of therapy, mainly due to increased appetite and recovery of stool. While the signs of normalization of motility of the forestomachs, cud and burping, the disappearance of ictericity of the mucous membranes and a decrease in the percussion borders of the liver in animals of this group were noted only by 20-22nd day of research. At the same time, on the 18th day of research, one cow from the group was removed for emergency slaughter, while the survival rate in the experimental group was 100%.

The prescribed therapeutic regimen had a more pronounced effect on the biochemical status of the experimental cows (table 2).

The combined administration of Livazen and Thionontrit-S contributed to the restoration of the main metabolic processes in the liver, causing an increase in its protein synthesizing function, which, in comparative aspect, allowed increasing the level of total protein in experimental cows by 8.1% relative to the control group, as well as eliminate dysproteinemia in protein spectrum of blood serum. By the end of the study, the content of albumins in the experimental group increased by 20.9%, while in animals of the control group this indicator was still physiologically low. The differences between the groups in the albumin fraction amounted to 13.8% in favor of the experimental cows. In terms of the level of globulins, a positive correction was noted in both groups, however, the shifts in the proteinograms of the experimental cows were closer to the values of the species norm, which was confirmed by the test of the integral assessment of the protein spectrum of blood serum - albumin / globulin coefficient (A / G).

The complex treatment regimen for hepatosis in the experimental group of animals showed a more pronounced effect on the synthesis-forming functions of the liver, which was manifested by the activation of carbohydrate and lipid metabolism. So, the glucose level in cows relative to the background indicators by the end of the experimental period increased in 1.56 times, relative to the control analogues – by 31.6%. In the lipid spectrum of the blood serum of cows of the experimental and control groups, by the end of the course of therapy, changes were clearly observed towards an increase in the concentration of triglycerides and cholesterol. Moreover, the dynamics of increasing these parameters in experimental animals was significantly higher. Intergroup differences were 27.5% (for cholesterol) and 21.4% (for triglycerides), respectively. A similar effect is due to the lipidotropic effect of dipromonium, which is part of Livazen, which helps to reduce the degree of fatty infiltration in liver cells and activates the energy-synthesizing function of mitochondria.

Table 2. Biochemical blood parameters of cows during the treatment of hepatosis (M±m; n=20).

| Indicators          | Group                  |
|---------------------|------------------------|
|                     | I – experimental       | II – control          |
| Total protein, g/l  | 82.7±4.24*             | 76.5±4.53             |
The use of Thionontrit-S as part of the complex therapy of hepatosis in cows allowed reducing the activity of the inflammatory process in the liver, which was manifested by a decrease in the transamination enzymes - AST and ALT, reflecting the activity of the cytolytic syndrome. In the experimental group, the level of aspartate and alanine aminotransferase had significantly (p<0.01) lower values in comparison with the control cows (by 47.5% and 19.9%, respectively). A tendency toward a decrease in the activity of the cytolytic syndrome was also recorded in the control group, however, the studied parameters in these cows as opposed to experimental animals did not reach physiological values.

In the studied groups, a decrease in the total bilirubin and alkaline phosphatase levels of the blood serum was noted. In this case, the concentration of total bilirubin in the experimental group decreased in 1.8 times, and in the control – in 1.28 times. Differences in alkaline phosphatase indices between groups amounted to 1.59 times in the direction of its decrease in experimental animals.

The optimal-correct mineral complex of the aluminosilicate component of Thionontrit-S had a corrective effect on the phosphorus-calcium metabolism in cows, which was manifested by an increase in the total calcium content in blood serum by 22.7% while reducing the level of inorganic phosphorus by 13%, whereas control cows calcium values did not reach the limits of the lower bounds of the reference values.

A liver ultrasonography performed on the 21st day of the study showed the presence of regenerative processes in the organ parenchyma of the experimental animals. The edges of the liver were smooth, the structure was homogeneous, granular, echogenicity was not changed. In most cases, the size and configuration of the liver were normal. In some cases, in the control group of cows hyperechocicity was observed, which was confirmed by the presence of granular inclusions of different sizes in the liver parenchyma. Sometimes small foci of fatty infiltration were visualized.

Thus, it is possible to note the superior effectiveness of the complex therapy of hepatosis with Livazen with the additional prescription of Thionontrit-S in comparison with the basic use of only one hepatoprotector. The noted pronounced effect in cows is due to the high resorption of various dosage forms of drugs and the summation of their cytoprotective, membrane-stabilizing and reparative effects due to the simultaneous effect on different links of the pathogenesis of the disease.

4. Conclusion
Thus, the results of the studies showed that the comprehensive scheme of pharmacotherapy is aimed at the effective correction of various pathogenesis links of combined mycotoxicoses with the use of adsorbing preparations that can reduce the toxic load on the body on the first line of defense due to its high sorption activity and the additional use of the feed additive with a number of specific pharmacological properties. Based on the results, it can be noted that the combined use of pathogenetic agents in the treatment of hepatosis in high producing dairy cattle has a positive effect on the course of
the disease and the timing of recovery. The use of a comprehensive regimen contributes to the manifestation of positive dynamics of the main hepatosis syndromes with a decrease in clinical manifestations, statistically significant correction of disorders of the functional state of the liver and stabilization of metabolic processes in animals.

The proposed scheme will allow veterinarians to improve the methodology of the system of therapeutic and preventive measures for hepatopathies in animals.

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