Reproduction recovery of cows with postnatal liver damage using a liver hydrolysate drug

Alexey Zelenkov1, 2, Alexey Ermakov2, Galina Zelenkova2, Tatyana Derezina2, Ivan Gorlov3, Vladimir Evstropov2, Alexander Pakhomov4

1 Ministry of Agriculture and Food of the Rostov region, Krasnoarmeyskaya St., 33, Rostov-on-Don, 344010, Russia
2 Don State Technical University, sq. Gagarina, 1, Rostov-on-Don, 344010, Russia
3 The Volga region research institute of manufacture and processing of meat-and-milk products, Rokossovsky St., 6, Volgograd, 400131, Russia
4 Don State Agrarian University, o. Persianovsky, 346493, Russia

E-mail: zelenkovalex@rambler.ru

Abstract. Assessment of the effectiveness of treatment and recovery of reproductive functions of cows with fatty liver disease using a drug based on a healthy liver hydrolysate (the enzyme structures of the protein after acid treatment and centrifugation remain in the sediment of the tissue substrate, and their released amino acids become constituent components of the oversediment solution of the hydrolysate). Two groups of animals of 15 heads each were formed: first-calf heifers entered the first group on the first day after calving, liver pathology was indicated by an increase in its borders during percussion, and animals with clinical signs of deep fatty liver disease were included in the second group a month after calving. The dose was 40 ml daily (20 ml intramuscularly and 20 ml subcutaneously), the course of treatment was 5 days. After the 1st stage, the remaining 25 cows of both groups were considered as one experimental group of the 2nd stage, which was periodically monitored until the next calving and insemination; the control was 25 animals not subjected to hepatoprotective therapy. Cows that underwent even one treatment procedure were better adapted to the newborn period and subsequent insemination. They have faster involution of the uterus and the resumption of the sexual cycle (P <0.001), higher fertility, and a greater number of healthy calves.

1. Introduction
Fatty [1-7] and acute yellow atrophy of the liver are in the first place among liver pathologies in fresh cows and first-calf heifers in dairy complexes, while the economic damage consists not only of obvious losses, but also due to the fact that it seems impossible to determine the intensity of the distribution of these pathologies in a herd of highly productive animals, which entails hidden losses [2, 4, 5, 8].

The intensity of dystrophic processes depends on the individual characteristics of the organism [5] and on the season of the year: in the winter period, the manifestation of these pathologies occurs much more often than in the summer. In addition, the degree of their severity in first-calf heifers and cows is not the same.

Non-compliance with the technological conditions of feeding and keeping, non-compliance with sanitary hygiene requirements and the use of poor-quality feeds cause a general metabolic disorder in
animals. At the same time, the resistance of the body decreases, the functions and all physiological activity of the body change. Deviations in the metabolism of highly productive cows, which are more sensitive to adverse effects, occur primarily in the liver. Ultimately, milk yield and fertility are reduced [2, 3, 8, 9, 11, 12].

At the beginning of lactation in cows, an increase in feed intake (i.e., the development of a feed intake function) usually lags behind an increase in milk yield (i.e., from a development of a lactation function). As a result, a fresh cow, especially a highly productive one, for the first time for 6-10 weeks may experience a mismatch between the amount of energy and nutrient consumption from the diet and their expenditure on physiological needs, primarily milk formation. In response to this state, excessive spending of tissue reserves of the body can lead to metabolic disorders (ketosis, liver disease) and violation of reproductive function.

High dairy cows with intensive metabolism are sensitive even to insignificant disturbances of feeding and keeping conditions. Moreover, they react with more pronounced metabolic disorders affecting their immunobiological status. The most critical period of time that determines the health and productivity of cows during lactation is the so-called “near-calving period” (perinatal), which includes 3 weeks before calving and 2-3 weeks after calving. Three weeks before calving is a short, but most important period in the life of a cow, which determines the health and productivity of subsequent lactation, and the safety of the herd as a whole. As calving approaches, the concentration of estrogen in the blood remains high or even increases. High level of estrogen in the blood is a leading regulator that reduces appetite. In the last 3 weeks of pregnancy, there is an increased consumption of nutrients for fetal growth, an increase in the placenta and mammary gland. In the last week before calving, cows have a physiologically reduced appetite.

During the first 2-3 weeks of lactation, the cow needs energy for the normal functioning of the uterus, the activation of the ovaries functioning and the milk formation. During this period, the feed intake of the cow often lags behind its nutritional needs, when the animal is especially in need of a sufficient amount of nutrients, especially energy. During milking, highly productive dairy cows often suffer from a significant lack of energy caused by a lack of substrate for glucogenesis. The deficit of energy and plastic substances is compensated by the breakdown of the substances of one's own body, which occurs against the background of an increase in the influence of regulatory biologically active compounds.

The main diseases of lactating cows are manifested in the first two months of lactation, that is, at the time of reaching the peak of productivity. They are due to changes in the metabolism during the transition period, which are not supported by adequate changes in the body due to feeding and sufficient supply of nutrients.

In highly productive cows, during a period of intense milking, a regular deviation from the norm of some biochemical parameters is observed. Subclinical chronic disorders of carbohydrate, lipid, protein, mineral metabolism and acid-base balance in highly productive cows gradually cause dystrophic changes in the liver.

This problem concerns, first of all, new-calf heifers, who feel an increased need for nutrients and energy not only for milk production, but also for the completion of their own growth.

When a cow does not receive enough energy, the body uses fats from the reserves of its body and the liver turns these fats into an easily accessible source of energy - glucose. This is due to a mismatch between the amount of feed consumed and the actual milk productivity in connection with the active use of body reserves for the synthesis of milk components.

With a lack in diets of energy and easily digestible carbohydrates (sugars and starch), protein and amino acids are spent on energy needs, which far exceeds the need for them. All this leads to disruption of energy and carbohydrate-fat metabolism, as well as to problems with reproduction.

In animals with metabolic disorders, along with obesity of the liver, dystrophic phenomena in the uterus and ovaries, a decrease in the percentage of fertilized eggs are noted. The death of embryos occurs at the morula stage. With fatty liver disease in highly dairy cows, the hairline loses its shine, and its hair grows bristled. Animals are depressed, refuse to feed and water, move little and lie more,
weight loss, hypotension, atony and acidosis of the scar, laminitis and purulent-necrotic lesions of the distal parts of the extremities, delay in the placenta and endometritis, dystrophic phenomena in the uterus and ovaries are noted.

In unfavorable conditions in the liver of first-calf heifers, the destruction of hepatocytes occurs more intensively than in cows. The specific features of the microscopic structure of the first-calf heifer liver include pronounced variability in the shape and size of hepatocytes [9].

The deamination of amino acids in the liver is accompanied by the formation of ammonia, its neutralization occurs through the synthesis of urea with a large expenditure of energy. Therefore, when metabolic processes are disturbed, urea synthesis decreases and ammonia begins to act as a strong cellular poison, which causes toxic liver dystrophy. Fatty liver disease of the liver is caused by a change in trophism [5] and morphology of hepatocytes due to violation of energy metabolism in the body and infiltration of liver tissue by lipids [4, 9, 10].

Fatty liver disease liver in cows is detected by percussion along the increased borders of hepatic dullness. Diagnostic measurement of percussion boundaries is carried out in a horizontal line. Along with this, it should also be noted that hepatic changes occur with concomitant changes in the motility of the pancreas (detected by the generally accepted method of auscultation), general exhaustion (by visual examination) and metabolic disorders (by biochemical analysis of blood and samples of a biopsy liver) [2].

Since the lipidous liver causes a delay in normal estrus after calving at the beginning of cyclic ovarian activity, this increases the number of inseminations before fertilization. The immediate reasons for this are a lack of glucose formed in the liver and a violation of the metabolism of sex steroids - a process that occurs directly in the liver. In these cases, there is a pronounced carbohydrate (glucose) deficiency and excessive mobilization of lipids, which can impair liver function, primarily glucose-synthesizing and steroid-metabolizing. With a low concentration of glucose in the blood, its accessibility to peripheral tissues decreases, and the hypothalamic-pituitary-gonadal connections necessary for reproductive function are disrupted. On the other hand, due to decreased functional activity, lipid liver cells cannot provide the necessary metabolism and/or catabolism of sex steroids, which leads to impaired reproductive function.

However, the liver tissue easily regenerates. While maintaining physiological processes in the body, the liver is able to carry out physiological regeneration, while it is possible to restore blood vessels and bile ducts. Therefore, the urgent task is to improve the methods of pharmacocorrection and pharmacoprophylaxis of impaired liver function and the occurrence of fatty and acute yellow atrophy of the liver [9].

The aim of our research was to study the effectiveness of the fatty degeneration treatment of the liver and recovery of reproductive functions in cows using a drug based on a healthy liver hydrolysate.

2. Materials and methods
Scientific research was carried out in the conditions of the dairy complex Aksayskoye Milk LLC, Aksaysky district, Rostov Region on 2.5-year-old black-motley breed cows with a live weight of 500 kg above average fatness and a planned milk yield for lactation of over 8000 kg. In the process of research, an analysis of the primary zootechnical and veterinary documentation on calving and disposal of animals in the winter-summer period (from January to June inclusive) was carried out. Biochemical studies of urine, clinical methods for diagnosing the condition of the liver in animals before and after treatment were carried out.

During the observation period (Table 1), the first-calf cows were departed mainly within two to three weeks after calving. The highest percentage of departing of calves after calving was observed in January. During forced slaughter, all animals at the external examination noted exhaustion, dehydration (eyeballs falling in), ruffled hair, and dry and brittle hair to the touch. During pathological autopsy and internal examination, changes in the liver were observed in all animals. Characteristic changes in the liver were as follows - an increase in size, clay color, when the incision is not made, the edges do not converge, oily plaque remains on the knife, and liver tissue easily breaks [9,10].
Table 1. Departing of first-calf cows for the first half of the year (January-June).

| Period | Calving of heifers, heads | Departing (cause - fatty liver disease) |
|--------|---------------------------|----------------------------------------|
|        |                           | heads | % departed to calving |
| January| 19                        | 8     | 42                   |
| February| 18                       | 4     | 22                   |
| March  | 16                        | 1     | 6                    |
| April  | 17                        | 3     | 18                   |
| May    | 24                        | 5     | 21                   |
| June   | 41                        | 11    | 27                   |
| Total  | 135                       | 32    | Average value 22.6   |

For the treatment and prevention of hepatosis in animals, a method was used [7, 13], which consists in the use of a drug made from the liver of healthy animals by hydrolysis. The standard procedure for the complete hydrolysis of peptides or protein is to heat this peptide or protein with an excess of 6 mole HCl at 100-120°C for 10-24 hours in a container made of resistant glass, from which air is preliminarily evacuated. The hydrolysate obtained in this way contains amino acids in the form of hydrochlorides, mineral salts of isotonic concentration, pH 5.7-7.0. The suspension obtained by repeated pipetting is washed by centrifugation on the principle of precipitation of higher structural compounds and formations. To exclude protein-peptide substances in it (short peptides, as well as regeneration stimulants, which are protein structures, are excluded by repeated pipetting and centrifugation), not peptides (representatives of lipid formations are separated from liver homogenate by extraction to hydrolysis of peptide structures), not amino acids (representatives of carbohydrate nomenclature, after all the steps and procedures for preparing the hydrolysate, practically and theoretically cannot be part of this solution (a preparation from liver tissue) and hepatic protein regenerates of the liver (which does not include protein and peptide structures (up to the primary bonds)). Then a solution of mineral salts is introduced into the composition of this agent to an isotonic concentration [10, 13].

Therapeutic and prophylactic efficacy is achieved directly with the introduction of a hepatoprotective agent by injection subcutaneously, intramuscularly or intravenously once a day. For therapeutic purposes, to first-calf cows with a live weight of 350-550 kg, in which hepatic changes occur with concomitant changes in the motility of the pancreas, general exhaustion and metabolic disorders, the dose is 20.0-40.0 ml, and 0.5 doses are administered subcutaneously and 0.5 dose intramuscularly at the same time for 5-6 consecutive days. For the purpose of prevention, dry cows weighing 350-550 kg are administered at a dose of 10-20 ml with an interval of 7-10 days, a total of 5-7 injections [10, 13].

Taking into account the therapeutic measures, two groups of animals were formed with 15 animals each: the first group included first-calf heifers on the first day after calving, liver pathology was indicated by an increase in its borders during percussion, and the second group, one month after calving, included animals with clinical signs of deep dystrophy of the liver, including cachexia, refusal of feed, loss of milk productivity. The course of treatment was 5 days, the dose of the drug was 40 ml (20 ml intramuscularly and 20 ml subcutaneously).

All animals underwent percussion of the liver before and after treatment, in order to determine its boundaries, as well as auscultation of the scar in order to determine its contractile activity. The technique of percussion of the borders of the liver: along the 12th intercostal space, the border of the liver dullness is at the level of the middle of the scapula, and in the 10th intercostal space, the blunt sound of the liver passes sharply into the tympanic sound of the lung. At auscultation of the scar in the middle of the hungry fossa in patients with animals, atony is noted, a weak sound of “rustling” is heard for a short time [9].

3. Results and discussion
The criterion for a positive result was considered to be an improvement in the general condition of the animal, a decrease in the boundaries of the liver to normal limits (Table 2).
Table 2. The results of a percussion study of the liver in first-calf cows before and after treatment (M±m, n=15).

| Group | Area of liver dullness before treatment, cm | Area of liver dullness after treatment, cm |
|-------|--------------------------------------------|------------------------------------------|
| 1     | 18.90±0.33***                             | 11.00±0.22***                            |
| 2     | 19.60±0.51***                             | 12.70±0.18***                            |

Note: *** - P <0.001

In the first group, the boundaries of liver dullness during percussion decreased by the 5th day of treatment by 41.8%, in the second group, the sizes of the liver dullness decreased by 35.2%. A positive result of treatment is confirmed by a significant improvement in the general condition of the animals. Improving appetite, active chewing, indicates the resumption of scar motility.

A comparative analysis of the results of the performed measures was also carried out, taking into account the culling of animals due to loss of productivity, departing as a result of forced slaughter, average daily milk yield before and after treatment, the number of animals that came to the hunt. The data are presented in Table 3.

Table 3. The results of the fatty liver disease treatment of the liver in first-calf cows, (M±m, n=15).

| Group | Culling | Departing | Yield, l/day | Inseminated |
|-------|---------|-----------|--------------|-------------|
|       | heads   | %        | heads        | %           | before treatment | after treatment | heads | % |
| 1     | 1       | 6        | 0            | 0           | 24.5±0.50       | 29.5±0.52***   | 13    | 86 |
| 2     | 4       | 26       | 1            | 6           | 17.5±0.51       | 24.5±0.51***   | 7     | 46 |

Note: *** - P <0.001 according to the t-criterion when compared with milk yield before treatment

The advantage of the proposed method is that it allows using the method of liver percussion to more quickly monitor the clinical condition of its size, as the results of subclinical (laboratory) analysis, and especially histological studies, as production practice shows, go to the farm at least in 7-14 days [10].

The essence of specific symptomatic metabolite therapy is that it contains amino acids of the liver tissue of clinically healthy animals obtained by hydrolysis, and therefore, its injection into the body allows the liver to selectively use the drug, since the body has a genetically determined dependence on the use of compound structural ingredients derived from similar tissues. In the hydrolytic cleavage of the protein structures of the liver extract, a break in the long polypeptide chains of the protein to amino acids occurs. In this regard, amino acids lose their protein specificity, colloidal properties and no longer have individual toxicity, neither teratogenic, nor antigenic, nor allergic and anaphylactic properties characteristic of protein tissue incompatibility. However, amino acids retained their bipolar affiliation with liver tissue [10, 13].

An injection method for the treatment and prevention of hepatosis, including fatty liver disease in cows, is used due to the fact that in case of liver disease the intake of a therapeutic and prophylactic medicine together with food and its absorption will be ineffective because of the digestive characteristics of ruminants, in addition in a sick animal, as a rule, there is no appetite. As for the digestive characteristics of ruminants (cows, sheep, goats, camels, etc.), the solution of a therapeutic and prophylactic medicine containing amino acids that enters the digestive system can be used by the microorganisms and the animal organism itself as nutritional components, in addition it is impossible to precisely control the dose of the drug received in the body together with the feed. Injection administration of a liver hydrolysate solution promotes faster and better diffusion (penetration) into the liver than when taken together with food, as a result of which regenerative processes and the binding of toxic substances are more active [10].

After the 1st stage, the remaining 25 cows of both groups were considered as one experimental group of the 2nd stage, which was periodically monitored until the next calving and insemination; control was 25 animals not subjected to hepatoprotective therapy.
It can be seen from the presented data that cows that underwent even one treatment procedure are better adapted to the previous calving and calving period (Table 4). They had faster uterine involution and the onset (renewal) of the sexual cycle. Moreover, experimental animals had three times higher fertility, fewer days of infertility before fruitful insemination.

**Table 4.** Veterinarian data for cows that underwent treatment after the previous calving (experience) and analogues that did not undergo hepatoprotective therapy (control), (M±m, n=25).

| Criterion                          | Experience          | Control           |
|-----------------------------------|---------------------|-------------------|
| Uterus involution, days           | 26.8±0.37***        | 34.7±0.16         |
| Beginning of the sexual cycle, days | 49.65±0.24***      | 56.45±0.29        |
| The number of inseminations per head | 1.4                | 2.8               |
| Fruitful insemination, days       | 73.50±0.37***       | 79.70±0.47        |
| Insemination Index                | 1.5                 | 2.7               |
| Fertilized, heads                 | 17                  | 6                 |
| Infertility, days                 | 52.64±0.27***       | 64.60±0.46        |
| Pathology, number                 | 1 abortion          | 1 abortion        |
|                                   | 1 stillborn fetus   |                   |
| Received healthy calves, heads    | 16                  | 4                 |

Note: *** - P <0.001 by t-test when compared with control

The results could be much higher if the cows that underwent a treatment course after calving had previously received preventive therapy before calving [14] at a dose of 10-20 ml with an interval of 7-10 days (5-7 injections in total), instead of 5 days at a dose of 40 ml [9, 10, 14].

With this liver pathology, the acute form lasts from 4 to 7 days, subacute lasts from seven days to three weeks, and without active pathogenetic therapy can lead to the death of the animal or forced slaughter and the departing of highly productive cows from the herd [14]. The chronic course of liver lipidosis does not lead to death in ruminants, but without therapeutic measures they can become the object of culling by productivity and reproductive ability [4]. Recently, in first-calf cows, even with a subclinical form of liver obesity, an extension of the service period has been established [2, 3, 4, 5, 12]. Since the lipidous liver causes a delay in normal estrus after calving (the resumption of cyclic ovarian activity), this increases the number of inseminations before fertilization. The main metabolic cause in this case is the lack of glucose formation in the liver [1, 6] and a violation of the sex steroid metabolism - a process that occurs in the liver. In this context, one can find an explanation of why reproductive function, as a rule, is more often disturbed with high productivity of cows. In these cases, there is a pronounced carbohydrate (glucose) deficiency and excessive mobilization of lipids [1, 6], which can impair liver function, primarily glucose-synthesizing and steroid-metabolizing. With a low concentration of glucose in the blood, its availability to peripheral tissues decreases [1, 6] and the hypothalamic-pituitary-gonadal connections necessary for reproductive function are violated. On the other hand, due to decreased functional activity, lipid liver cells [1, 6] cannot provide the necessary metabolism and/or catabolism of sex steroids, which leads to impaired reproductive function [5, 10, 11].

4. Conclusion
Maintaining the normal functioning of the liver [2, 3, 5] to effectively ensure not only metabolism and productivity, but also the reproductive function of animals [5], is one of the important priorities in the milking and exploitation of cows in the early phase of lactation. At the same time, we believe that the use of a liver hydrolysate with a preventive purpose before calving will reduce gynecological diseases and impaired reproductive cycles. This hepatoprotective drug can be successfully used not only for the treatment of fatty liver disease, but also with other types of hepatitis disorders [7]. In addition, the applied drug has never shown any side effects during its use and in other households.

References
[1] Nikitin V, Belugin N, Pisarenko N and others 2015 Effective animal husbandry 2 112 pp 24-26
[2] Klimenko A, Dushkin Ye and others 2015 *Scientific Almanac of the Black Sea Countries* 3 3 pp 10-19

[3] Weber C, Losand B, Tuchscherer A, Rehbock F 2015 *Journal of Dairy Science* 98 3 pp 1772-1785

[4] Kreipe L, Deniz A, Bruckmaier R, van Dorland H 2011 *Journal of Dairy Science* 94 10 pp 4904-4914

[5] Gross J, Schwarz F, Eder K, van Dorland H, Bruckmaier R 2013 *Journal of Dairy Science* 96 8 pp 5008-5017

[6] Dushkin E, Mikulets Yu 2008 *Scientific-theoretical journal Agricultural Biology. A series of animal biology* 2 pp 63-65

[7] Dushkin E, Paraponov S, Mundyak I 2008 *Livestock of Russia* 1 pp 42-43

[8] Dushkin E 2010 *Agricultural biology. A series of animal biology* 2 pp 18-24

[9] Dushkin E, Konobeysky A, Pianov B 2013 *Effective livestock* 12 98 pp 32-33

[10] Dushkin E, Zelenkov A, Dushkin V 2014 *Genetically determined fatty liver in dairy cows* Textbook (Persianovsky) p 20

[11] Belugin N, Pisarenko N, Skripkin V 2016 *The collection: Innovations and modern technologies in the production and processing of agricultural products. Materials of the international scientific-practical conference* (Stavropol) pp 561-568

[12] Dushkin E, Derezina T, Firsov N, Zelenkov A 2014 *Veterinary pathology* 3-4 49-50 pp 44-48

[13] Dushkin E 2010 *Method for the treatment and prevention of hepatosis in animals. Patent for invention No. 2385728. Application No. 2008113942/13 Bull. No. 10*

[14] Bobe G, Young J, Beitz D 2004 *Journal of Dairy Science* 87 10 pp 3105-3124