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Diagnostic tests for fatty hepatosis in high-yielding cows

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Abstract. The research relevance is determined by problems in the dairy farming sector related to the development of diseases in high-yielding cows during the transition period. Early diagnosis of metabolic disorders during the transition period is necessary for the timely prevention of complications and the development of irreversible changes in the body of cows and the subsequent culling of animals from the herd. The study aimed to confirm the development of fatty hepatosis in dairy cows during the transition period, identify its possible causes, and determine effective measures for the prevention of this disease and its consequences. For this purpose, clinical diagnostic methods and biochemical studies of cow blood serum were used as early markers of metabolic changes in the animals' bodies. The study determined that clinical signs of pathology are manifested in only 12-20% of cows from each physiological group. Changes in the biochemical parameters of cows' blood serum depending on the physiological period had unique characteristics. During the study, changes were found in such blood serum parameters as total protein, albumin, globulin, protein coefficient, urea, urea nitrogen, aspartate aminotransferase activity, glucose, total calcium, inorganic phosphorus, calcium-phosphorus ratio, carotene, lipoproteins, thymol test, non-esterified fatty acids, and vitamin E. The study established that for timely detection of metabolic disorders in cows, it is necessary to conduct regular medical examinations of the herd covering all physiological groups of animals, including blood tests, and, based on the results obtained, to apply correction methods in a timely manner. Identifying the causes of metabolic disorders and pathological changes in the

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liver and calcium-phosphorus metabolism in cows during different physiological periods will make it possible to select appropriate prevention methods and prevent economic losses from milk yield reduction and culling of cows in the conditions of a particular farm

Keywords: hepatolipidosis; hyperglobulinaemia; non-esterified fatty acids; lipid metabolism; triacylglycerols; hypoglycaemia

Introduction

Cattle breeding is substantial in animal husbandry, especially in terms of milk and meat production. A highly productive cow can produce the maximum amount of milk by utilising its genetic potential only if it is in excellent health. However, health of each cow directly depends on many factors, including feeding, housing and operating conditions, proper management, lactation/gestation period, etc. As noted by S. Göncü & M.G. Sucak (2024), deterioration of health among cows decreases quantity and quality of milk produced, increases costs and reduces farm profitability. Therefore, monitoring and timely treatment of sick cows are vital for dairy farms. Diseases that occur in cows on farms can be caused by various factors and are substantial causes of economic losses. To prevent spread of these diseases in cows, livestock enterprises must apply methods of care, feeding and herd management, vaccination, hygiene, regular testing and biosecurity control. However, each farm must also conduct internal investigation into root causes, identifying common problems in unique circumstances. Based on above, instead of treating cows for common diseases on farms, permanent solution should be to identify preventive measures with an emphasis on the causes of these diseases.

In contrast, G. Buonaiuto *et al.* (2023) considered that high culling rates in dairy farming indicate poor animal welfare, suboptimal farming conditions and inefficient use of animal resources, which undermines the sustainability of dairy sector. High replacement rates lead to reduced herd productivity and increased replacement costs. In Holstein-Friesian cows, maximum milk production occurs only in third lactation, and a full return on initial investment in their rearing usually occurs at the end of the second lactation. Thus, culling cows during first two lactations negatively affects the profitability of the farm. M.E. Elmhadi *et al.* (2022) noted that it is common practice in the modern intensive management system to feed ruminants with large amounts of highly concentrated feed. As a result, these animals suffer from metabolic diseases. In dairy herds, one or more metabolic disorders can affect every second cow.

V. Tufarelli *et al.* (2024) argued that during perinatal period, dairy cows are usually in a state of negative energy balance (NEB), during which they mobilise fat reserves in the body to provide themselves with the energy they need at this stage. According to Y. Mekuriaw (2023), feeding and housing methods, as well as breed differences, can lead to different NEB statuses in dairy cows. Holstein breeds mobilise significantly more body energy at the beginning of lactation than Danish Red and Jersey breeds. First-calf heifers are more prone to metabolic stress during the transition period than cows with repeated lactations. Therefore, the duration and severity of NEB in dairy cows depend on how the cows are managed. Although NEB is common in high-producing dairy cows, with consequences ranging from mild to severe, its extent and severity can be minimised through proper feeding and the addition of feed supplements. Cows fed a diet with a higher energy value had a better energy balance. In general, NEB is associated with adverse effects on cow health, milk production and fertility, which in turn affects farm profitability.

P. Melendez & P. Pinedo (2024) argued that in this complex scenario, the liver is considered a key organ in regulating overall metabolism, especially in high-yielding dairy cows, which can exceed its normal function, leading to a series of local and systemic inflammatory and degenerative processes that jeopardise the overall health of the animal. M. Holzhauer & J.F. Valarcher (2024) believed that in the early stages of lactation, cows usually develop fatty hepatosis, which is associated with various health problems. Development of this disease is associated with a higher susceptibility to inflammation and infections such as mastitis and endometritis due to decreased immunity. In addition, cows with fatty hepatosis are more likely to develop ketosis, displaced abomasum, hoof health problems, and decreased productivity and reproductive capacity in first weeks/months after calving. Therefore, fatty hepatosis problems in herd must be diagnosed early in transition period to prevent such disorders. The study aimed to determine health status of herd of high-yielding cows at all stages of lactation period, to identify clinical and metabolic disorders in their bodies, and to take timely preventive measures to prevent development of pathologies and their complications.

Literature Review

The state of dairy farming worldwide is characterised by continued growth in cow productivity and a reduction in feed costs per unit of production. However, increased production inevitably leads to a deterioration in herd health. According to C. Zhang *et al.* (2023), during the first month of lactation, 5-10% of high-yielding cows suffer from severe fatty hepatosis, and 30-40% of cows develop mild fatty hepatosis, indicating that almost 50% of these cows are at risk of metabolic disorders. Cows with fatty hepatosis usually do not show clinical signs, but this condition is associated with other health and production problems and, as a result, with huge economic losses on dairy farms. Despite the economic impact of this disease, it is often misidentified or ignored due to diagnostic difficulties.

D. Kirovski & Z. Sladojevic (2017) concluded that the high energy requirements of high-yielding dairy cows immediately after calving lead to increased mobilisation of fat, usually subcutaneous fat. It is released into the blood and transported mainly to the liver, but also to the muscles and kidneys. The degree of fat loss depends on the cow's body condition and energy deficit. Subcutaneous fat and skeletal muscle decrease after calving, with well-fed cows losing 2.5 times more muscle mass than thin cows. The loss of physical condition is due to the general mobilisation of the body (fat and protein).

During lactation, energy expenditure in cows increases significantly, which, in the context of reduced dry matter intake, leads to the development of a negative energy balance. The mobilisation of fats from depots leads to increased concentrations of non-esterified fatty acids in the blood. Their excess can cause the development of metabolic disorders. In particular, the liver of cattle has a limited ability to convert non-esterified fatty acids. They can be oxidised or excreted from hepatocytes as very low-density lipoproteins. When the functional capacity of the liver is reached, triacylglycerols and acetyl-CoA begin to accumulate in it. The synthesis and accumulation of triacylglycerols in the liver determine the high risk of developing "fatty liver syndrome" in cows with lipolysis syndrome. This contributes to the accumulation of triacylglycerols and a deficiency of lipotropic substances in the diet, since, according to J.J. Gross (2022), microbial fermentation products from the rumen alone, especially at the beginning of lactation, are insufficient to meet the needs of high-yielding dairy cows. A deficiency of methionine, lysine, histidine and B vitamins synthesised by microorganisms may be the primary limiting factor not only in milk production but also a significant cause of fatty hepatosis in cows. In addition, an apparent deficiency of lipases for the hydrolysis of triacylglycerols from the cytosolic storage pool may limit their incorporation into very low-density lipoproteins (VLDL), thereby reducing the removal of triacylglycerols from hepatocytes.

P. Melendez & P. Pinedo (2024) believed that the transition period is usually characterised by a curvilinear decline and then an increase in dry matter intake, with the lowest point occurring around calving. In addition, the energy requirements for lactogenesis in cows increase sharply immediately after calving. As a result, cows develop a typical postpartum negative energy balance characterised by hypoglycaemia, as glucose is used for lactose synthesis during the milk production process. These metabolic reactions are the result of increased synthesis of IGF-I and growth hormone, decreased synthesis and release of insulin, and a surge of glucagon and catecholamines, which in turn activate hormone-sensitive lipase and other enzymes in adipose tissue, triggering the typical breakdown of triglyceride ester bonds, releasing glycerol and non-esterified fatty acids into the bloodstream. In contrast, V. Yefimov *et al.* (2023) noted that the development of fatty hepatosis reduces glucagon levels and the intensity of gluconeogenesis in the liver, which leads to a decrease in blood glucose levels and a reduction in insulin secretion. As a result, lipid mobilisation in tissues increases, fatty infiltration of the liver occurs, and ketogenesis intensifies. This leads to the development of a "circle" and the deepening of liver pathology and, as a result, the removal of the most productive cows from the herd.

C. Zhang *et al.* (2023) divided fatty hepatosis into mild, moderate, and severe fatty liver dystrophy. The latter is divided into non-encephalopathic severe fatty hepatosis and hepatic encephalopathy. The pathology of mild and moderate fatty liver disease is less studied than the pathology of severe fatty liver disease. In severe cases, the accumulation of triacylglycerols in the cytoplasm of hepatocytes is accompanied by a disruption in the structure and functioning of the liver, causing hypoglycaemia and ketonaemia (Radostits, 2000). D. Kirovski & Z. Sladojevic (2017) noted that serum glucose levels are a good indicator of liver function, and a decrease in its concentration may reflect fat infiltration in animals with high lipomobilisation. In the subclinical form of fatty hepatosis, low glucose levels and high levels of non-esterified fatty acids in the serum of animals are closely related to the level of triacylglycerol in the liver. Fatty hepatosis occurs when the concentration of lipids exceeds the liver's ability to oxidise and excrete them. Excess lipids are stored as triacylglycerols in the liver, which is associated with a decrease in metabolism.

Given the large number of biomarkers that may indicate functional changes in the liver, biochemical abnormalities are usually non-specific, as there is no single metabolite that can be considered reliable for the diagnosis of fatty liver disease in dairy cows. Only a comprehensive and combined analysis of all available serum metabolites, combined with clinical symptoms or their absence, can provide a more accurate diagnosis of fatty liver disease in dairy cows. The final confirmation of the diagnosis of fatty hepatosis can only be made by taking a biopsy to determine the lipid content in liver cells. However, this method is not suitable for monitoring the health of cows during routine herd screening on industrial farms.

Materials and Methods

The research was conducted in 2024 in industrial farming conditions in eastern Ukraine on Holstein cattle. The research was conducted during routine medical examinations of the farm, which is free from infectious and invasive diseases. All animals were routinely vaccinated against anthrax. Twice a year, routine deworming of all adult cattle and routine diagnostic tests for tuberculosis, leukaemia and brucellosis were conducted. Cows were kept in typical tethered housing. Lighting in the room where cows were kept was 200 lux. Floor for cows was covered with rubber mats. Feeding was conducted at a feeding table at the level of front legs. Animals were provided with water from the water supply through automatic drinkers.

Depending on the physiological period of lactation or dry period on the farm, cows were divided into 5 physiological groups: 3 lactation periods and 2 dry periods. For the experiment, 30 animals were selected for each group. Each physiological group received a unique diet. The diet of cows on the farm consisted of following components: hay, straw, corn silage, haylage, compound feed, which includes sunflower seed meal, wheat, chalk, salt, dry matter premix, milk premix (for cows in the relevant groups), buffer, absorbent, and bypass. Components of the diet were calculated and added to diet in amounts necessary for corresponding physiological period of cows and their needs during this period. Medical examinations were conducted to identify pathologies at an early stage and prevent further development of diseases and culling of cows. Clinical condition of the cows and results of their blood biochemical analysis were examined. For biochemical blood test, five cows were selected from each physiological group. For this purpose, 5 groups of animals were formed, corresponding to each physiological period, of which 2 groups of cows were in dry period, 60 and 20 days before calving, and 3 groups of cows were in lactation period, 5 days, 70-80 days and 175-185 days of lactation.

Clinical condition of cows was determined using standard methods – during examination, physique was assessed, and mucous membranes, condition of coat and skin, lymph nodes, organ systems and individual organs were examined. Blood was collected from cows in sterile disposable vacuum tubes with a clotting activator. During transport, blood samples were stored in a cooler bag with ice packs, separated by a layer of cotton wool to inhibit biochemical processes in whole blood cells and, as a result, changes in parameters. During medical examination at dairy farm, all bioethical requirements regarding animals were ensured, following the Law of Ukraine No. 249 (2012) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986).

During the study, biochemical parameters of blood were determined, including total protein, albumin, globulin, protein coefficient, urea, creatinine, aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity, and alkaline phosphatase, glucose, total calcium, inorganic phosphorus, carotene, lipoproteins, thymol test, non-esterified fatty acids, vitamins A and E. Biochemical parameters were determined using an automatic biochemical analyser Biochem-200, HTI (USA), and vitamins on a high-performance liquid chromatograph LC 300, PerkinElmer, (USA) at Biosafety Centre for Biosafety and Environmental Control of Agricultural Resources of Dnipro State Agrarian and Economic University. Statistical processing of research results was performed using Microsoft Excel 2016 computer program.

Results and Discussion

During the diagnostic stage of medical examination, the study determined that clinical symptoms of the disease manifested themselves in only 12-20% of cows from each physiological group. The symptoms observed included slight depression, low mobility, pale pink conjunctiva and pulse rate at the upper limit of physiological fluctuations or slightly increased. Appetite in cows is reduced, taste distortion is noted, and rumen contraction is weak, in some cows below normal. Body temperature within physiological limits. The area of liver dullness in some cows is enlarged and extends beyond the last rib. Lymph nodes accessible for clinical examination are normal. To obtain the most complete picture of the level and state of metabolism in lactating cows, laboratory blood tests were performed. During the analysis of blood biochemical parameters, a range of changes in metabolism were found in cows of all physiological groups. An increase in the total protein content in the blood serum of cows at 70-80 days and 170-185 days of lactation (Table 1) compared to the maximum indicator of physiological fluctuations occurs due to an increase in the content of globulin fraction proteins. According to V.V. Sakhniuk *et al.* (2017), hyperproteinemia is a substantial indicator of hepatodystrophy, diagnosed in 60-80% of sick cows. An increase in the content of globulin fraction proteins above the upper normal range by 9.7%–40% was determined in the blood serum of cows of all physiological groups, with the exception of the late dry period group (20 days before calving). Hypergammaglobulinaemia occurs during all immunological reactions, accompanied by increased globulin synthesis and caused by an increase in the content of almost all classes of immunoglobulins and non-specific antibodies in many bacterial infections, chronic hepatitis, hepatodystrophy, cirrhosis of the liver, etc. In fatty hepatodystrophy, an increase in the globulin fraction in the blood serum of cows develops as a result of irritation of the immunocompetent cells of the mononuclear system. On the other hand, a decrease in the globulin content in the blood serum of cows during late dry period (Table 2) to normal levels is the result of the transfer of blood immunoglobulins to the mammary gland tissues during the preparation of the cow for calving and the formation of a pool of colostral antibodies in them for secretion in colostrum in the first hours after calving.

Table 1. *Biochemical indicators of cows' blood during lactation, M ± m, n = 5*

No.	Indicators	5th lactation day	70-80th lactation day	170-185th lactation day	Norm
1	Total protein, g/l	67.60 ± 3.40	80.00 ± 1.10	77.00 ± 1.38	55-75
2	Albumin, g/l	29.20 ± 1.02	31.00 ± 0.63	32.20 ± 0.20	30-35.5
3	Globulins, g/l	38.40 ± 2.86	49.00 ± 1.00	44.80 ± 1.28	30-35
4	Protein coefficient, units	0.76 ± 0.05	0.64 ± 0.02	0.74 ± 0.02	06-1.1
5	Thymol test, unit S-H	4.40 ± 0.55	5.38 ± 0.55	4.32 ± 0.50	0-4
6	Urea, mmol/L	3.32 ± 0.57	1.96 ± 0.25	2.32 ± 0.25	2.8-5.8
7	Urea nitrogen, mg%	6.36 ± 1.08	3.78 ± 0.49	4.46 ± 0.46	8-14
8	Creatinine, μmol/L	64.40 ± 3.33	62.40 ± 5.10	72.20 ± 1.93	45-140
9	AST, units per litre	112.60 ± 16.27	149.40 ± 20.69	96.60 ± 7.00	10-50
10	ALT, units per litre	19.40 ± 3.64	26.00 ± 1.52	27.00 ± 1.67	10-40
11	De Ritis index (AST/ALT), units	6.06 ± 0.64	5.88 ± 0.98	3.58 ± 0.30	1.0-3.4
12	Alkaline phosphatase, units per litre	93.00 ± 9.68	128.20 ± 15.31	99.40 ± 10.96	20-150
13	Glucose, mmol/L	2.26 ± 0.09	2.34 ± 0.08	2.58 ± 0.12	2.5-4.16
14	Vitamin E, mcg/ml	1.48 ± 0.15	1.79 ± 0.35	2.18 ± 0.41	3-14
15	Vitamin A, mcg/100 ml	21.04 ± 3.41	34.32 ± 6.36	43.49 ± 2.59	12.5-50.0
16	Carotene, mcg%	353.40 ± 38.68	367.80 ± 55.48	394.40 ± 64.75	275-965
17	Total lipoproteins, mg%	627.20 ± 35.35	925.60 ± 77.97	796.00 ± 73.19	400-800
18	Total calcium, mmol/l	2.16 ± 0.05	2.20 ± 0.03	2.36 ± 0.04	2.43-3.1
19	Ca/P, units	1.52 ± 0.04	1.38 ± 0.02	1.60 ± 0.07	1.2-1.6
20	Inorganic phosphorus, mmol/l	1.46 ± 0.07	1.60 ± 0.03	1.46 ± 0.04	1.45-1.94

Source: compiled by the authors

Table 2. *Biochemical blood parameters of cows 2 months and 20 days before calving, M ± m, n = 5*

No.	Indicators	60 days before calving	20 days before calving	Norm
1	Total protein, g/l	73.20 ± 2.44	63.60 ± 1.54	55-75
2	Albumin, g/l	33.80 ± 0.58	35.00 ± 0.32	30-35.5
3	Globulins, g/l	39.40 ± 2.91	28.60 ± 1.63	30-35
4	Protein coefficient, units	0.88 ± 0.09	1.22 ± 0.09	0.6-1.1
5	Thymol test, unit S-H	4.04 ± 0.63	3.58 ± 0.81	0-4
6	Urea, mmol/L	2.02 ± 0.18	3.74 ± 0.17	2.8-5.8

***Table 2.** Continued*

No.	Indicators	60 days before calving	20 days before calving	Norm
7	Urea nitrogen, mg%	3.86 ± 0.32	7.14 ± 0.30	8-14
8	Creatinine, μmol/L	51.80 ± 6.49	47.80 ± 0.58	45-140
9	AST, units per litre	84.60 ± 9.00	78.00 ± 6.27	10-50
10	ALT, units per litre	23.20 ± 1.71	20.60 ± 1.21	10-40
11	De Ritis index (AST/APT), units	3.70 ± 0.29	3.84 ± 0.37	1.0-3.4
12	Alkaline phosphatase, units per litre	91.60 ± 11.73	80.80 ± 8.49	20-150
13	Glucose, mmol/L	2.26 ± 0.11	2.24 ± 0.04	2.5-4.16
14	Non-esterified fatty acids, mmol/L	-	0.59 ± 0.17	up to 0.25
15	Vitamin E, mcg/ml	7.01 ± 0.72	4.22 ± 0.53	3-14
16	Vitamin A, mcg/100 ml	27.20 ± 3.64	29.68 ± 5.22	22.5-80.0
17	Carotene, mcg%	264.60 ± 30.30	318.80 ± 38.60	275-965
18	Total lipoproteins, mg%	720.20 ± 32.23	909.00 ± 48.12	400-800
19	Total calcium, mmol/l	2.12 ± 0.04	2.02 ± 0.06	2.43-3.10
20	Ca/P, units	1.06 ± 0.24	0.86 ± 0.11	1.2-1.6
21	Inorganic phosphorus, mmol/l	2.26 ± 0.30	2.44 ± 0.27	1.45-1.94

Source: compiled by the authors

Five days after calving, a decrease in albumin content below the lower limit of physiological fluctuations was found in the blood serum of cows. This is a consequence of liver damage caused by lipomobilisation syndrome. According to V.V. Sakhniuk *et al.* (2017), in the blood serum of 85-90% of cows with hepatodystrophy, the proportion of albumin (11-36%) decreases with a simultaneous increase in the relative and absolute content of globulins (beta and gamma fractions). Hypoalbuminaemia in cows can develop as a result of protein starvation and is a typical sign of liver disease, since albumin synthesis occurs only in hepatocytes. Hypoalbuminaemia also occurs in various diseases, including hepatitis, hepatosis, ketosis, liver abscesses, etc. In addition, it indicates chronic liver pathology, since only about one-third of the total amount of albumin is found in blood plasma, with the rest located in the extravascular space.

Elevated thymol test values in the blood serum of cows of all physiological groups by 1-34.5% compared to the upper limit of physiological fluctuations, with the exception of the late dry period group (20 days before calving), indicate dysproteinemia and confirm pathological changes in the liver of the studied animals. The thymol test has high sensitivity and the ability to detect even minor disturbances in liver function. It determines the level of protein fractions in blood serum, which change when liver function is impaired, and can be used to detect damage and inflammatory processes in liver tissue at an early stage, which is necessary for the timely treatment and prevention of diseases in productive animals.

The urea content in the blood serum of cows 70-80 days after the start of lactation, 170-185 days of lactation and 60 days before calving was lower by 30%, 17.1% and 27.9%, respectively, compared to the minimum physiological fluctuation. Impaired urea synthesis may also indicate liver damage in these animals. According to V.V. Vlizlo *et al.* (2021), indicators of liver function are the levels of total protein, albumin and urea in blood serum, and a decrease in their concentration may reflect fatty infiltration in animals with high lipomobilisation. In particular, the urea content in the blood serum of cows with fatty hepatosis is lower than in clinically healthy animals. It is noted that the urea-forming function of hepatocytes is quite stable; its violation indicates significant changes in the liver parenchyma and may be accompanied by syndromes of hepatic encephalopathy and hepatic coma. Urea is the product of protein metabolism. During urea synthesis, ammonia, which is quite toxic to animals, especially to the cells of the nervous system, is neutralised. A decrease in the urea content in the blood serum of animals is observed in cases of alimentary exhaustion and severe liver pathology. Even when 85% of the liver parenchyma is affected, urea synthesis is maintained. Since the cows studied were not clinically exhausted, significant liver damage was implied. R. Djoković *et al.* (2019) indicated that nitrogen metabolism indicators in their studies decreased in cows after calving compared to lactating cows, which is a consequence of the development of fatty hepatosis.

For cattle, the study of AST activity in blood serum is indicative, since AST has high activity in the cells of large animals, and ALT has high activity in small ones. Therefore, the de Ritis index increases by 5-78% in the blood serum of cows in all study groups compared to the upper limit of physiological fluctuations. According to E. Bombik *et al.* (2020), liver enzyme activity in the blood correlates with liver damage due to fatty infiltration. AST is the most sensitive indicator of liver condition during the transition period. Fatty infiltration of the liver causes damage to liver tissue and a general increase in enzyme activity, in particular AST and others, indicating damage to hepatocytes (Andjelić, 2022). With the development of fatty hepatosis in cows, an increase in AST activity above the upper limit of physiological fluctuations occurs already with ultramicroscopic changes in the organ, when the activity of other enzymes is still changing little. V.I. Levchenko *et al.* (2002) found that hyperfermentaemia depends on the amount of fat deposited in the liver parenchyma. During the medical examination of cows, an increase in AST activity was recorded in all physiological groups compared to the upper limit of physiological fluctuations, with the highest indicator in the group of cows on the 70th-80th day of lactation, almost three times higher, 2.25 times higher 5 days before calving, at 170-185 days of lactation by 93.2%, 60 days before calving by 69.2% and by 56% in the group of cows 20 days before calving. Data from V. Yefimov *et al.* (2023) confirm the assumptions of the current study and suggest that the development of cytolysis syndrome is determined by the anatomical features of the liver: pronounced hyperfermentaemia of cytosolic enzymes develops immediately after damage to the plasma membrane of hepatocytes and quickly reaches high levels. The highest AST activity increases during the acute process, while in the chronic process, this hyperfermentemia is less pronounced. Changes in serum enzyme activity may be the result of increased enzyme activity in cells (mainly hepatocytes), but may also be the result of cell damage. When fat infiltrates the liver, damage occurs in its tissues, and the levels of enzymes indicating liver damage (AST) usually increase. R. Djoković *et al.* (2019) found that AST activity in their studies was significantly ($P < 0.05$) higher in the blood serum of cows at the beginning of lactation than at the end of gestation, confirming damage to hepatocytes by steatosis and the release of this enzyme into the blood. AST is the most sensitive biomarker for diagnosing liver conditions during the transition period. This condition may be associated with fat deposits released at the beginning of lactation and may cause metabolic problems in the form of mild fatty liver dystrophy. This fact is confirmed by data from other scientists, C.K. Cebra *et al.* (1997), who claimed that in severe fatty hepatosis, AST activity was 83% sensitive and 62% specific, while only 8% of animals had abnormally elevated serum bilirubin levels. M. Krsmanović *et al.* (2016) stated that AST activity is closely related to the degree of fatty hepatosis, which can be used in the diagnosis of this disease in cows, even in subclinical cases.

Hypoglycaemia in cows at the beginning of lactation was observed throughout the lactation period, except for cows at 170-185 days of lactation. Hypoglycaemia may be caused by insufficient gluconeogenesis in the liver, as well as mammary gland activity and increased lactose synthesis in lactating cows. Hypoglycaemia is also often recorded in liver pathology, especially in cows with fatty hepatosis. Therefore, a decrease in this indicator below the reference values is another confirmation of the development of fatty hepatosis in the studied cows. L.S. Caixeta & B.O. Omontese (2021) believed that the body attempts to eliminate hypoglycaemia by activating the process of gluconeogenesis through an increase in glucocorticoid levels. Hypersecretion of glucocorticoids causes the mobilisation of fats from depots, which, when they enter the liver, cause fatty infiltration of hepatocytes. In addition, the development of fatty hepatosis reduces the intensity of gluconeogenesis in the liver, which leads to a decrease in serum glucose levels and a decrease in insulin secretion. A decrease in the level of these metabolites contributes to the increased mobilisation of lipids from adipose tissue, causing the development of fatty liver infiltration and increased ketogenesis.

A 14% higher level of non-esterified fatty acids compared to the upper limit of physiological fluctuations in the last dry period (20 days before calving) indicates the intensive development of lipomobilisation syndrome, which is characteristic of the transition period in cows. According to I. Lakić *et al.* (2018), the level of non-esterified fatty acids in the blood of cows is the most reliable indicator of negative energy balance and lipomobilisation during lactation. Elevated levels of circulating non-esterified fatty acids result from the remodelling of adipose tissue, which leads to their absorption by the liver in proportion to their levels in blood plasma. The simultaneous lack of oxaloacetate, which is largely used for gluconeogenesis, along with elevated levels of non-esterified fatty acids, leads to the accumulation of acetyl-CoA in the liver. Subsequently, instead of utilising acetyl-CoA through the complete Krebs cycle, incomplete oxidation (ketogenesis) and/or re-esterification occurs, followed by storage of triglycerides in the liver. If the concentration of non-esterified fatty acids exceeds the processing capacity of hepatocytes, such as during excessive lipid mobilisation, liver function is impaired due to the accumulation of triglycerides, causing fatty liver dystrophy and excessive production of ketone bodies such as β-hydroxybutyrate. A massive influx of unesterified fatty acids causes lipotoxicity in the liver of dairy cows. As a result, the balance between oxidation and antioxidant capacity in the liver is disrupted due to the formation of excess lipid peroxides and reactive oxygen species, leading to oxidative stress (Konvičná *et al.*, 2015). In addition to oxidative stress, apoptosis and inflammation can also be triggered by lipotoxicity induced by non-esterified fatty acids (Li *et al.*, 2020).

Thus, lipolysis becomes excessive, and glucose balance is seriously disrupted. Therefore, the studied changes in the content of non-esterified fatty acids and glucose in the blood serum of cows during the transition period (20 days before calving, 70-80 days of lactation) are key and trigger the mechanism of fatty hepatosis, the consequences of metabolic changes that are also observed in other physiological periods (5 days after calving, 70-80 and 175-180 days of lactation). In addition, increased concentrations of non-esterified fatty acids in the blood serum of cows affect inflammatory reactions and enhance the production of active oxygen species in cows during the transition period. According to L.M. Sordillo & W. Raphael (2013), oxidative stress can cause additional lipolysis, thus entering a vicious circle of lipolysis and the production of reactive oxygen species. High concentrations of non-esterified fatty acids and the production of reactive oxygen species are characteristics of metabolic stress and are recognised risk factors for the development of diseases in cows during the transition period, such as mastitis, retained placenta, ketosis and fatty hepatosis. Since vitamin E is considered an important antioxidant, sufficient levels of it in the blood serum should prevent the development of these diseases in cows, especially during the critical period of increased non-esterified fatty acids. According to fundamental research conducted by L. Pinotti *et al.* (2020) based on disease risk and immune function in dairy cows, the concentration of vitamin E in blood serum should be greater than approximately 3 μg/ml during the perinatal period. Below this threshold, vitamin deficiency occurs, specifically hypovitaminosis E.

A decrease in vitamin E content in cow blood serum 5 days after calving by more than 2 times compared to the lower limit of physiological fluctuations (1.48 ± 0.15 μg/ml) may be the result of its release in large quantities along with colostrum. A. Abuelo *et al.* (2015) noted that the cause of the decrease in vitamin E content after calving may also be inflammation in the liver of dairy cows during the transition period. This reduces the production of various vitamin-carrying proteins by the liver and, as a result, lowers the level of vitamin E in the blood plasma of animals. On the other hand, low vitamin E levels in the blood serum of cows during the 70-80 and 175-180 days of lactation (1.79 ± 0.35 and 2.18 ± 0.41 μg/ml, respectively) indicate a deficiency of this vitamin in the bodies of the studied cows throughout the entire lactation period. According to S. Haga *et al.* (2015), a study of vitamin E distribution in cattle tissues showed that the liver is central in regulating its distribution, as evidenced by the high hepatic expression of six genes associated with this vitamin. Therefore, low vitamin E levels in the blood serum of the studied cows may indicate significant changes in liver function, particularly antioxidant function.

An increase in the content of total lipoproteins in the blood serum of cows of two physiological groups by 13.6% and 15.7% (20 days before calving and 70-80 days of lactation, respectively) compared to the maximum physiological fluctuation may indicate the most critical periods for metabolic changes and the development of diseases in the animals' bodies. A study of the total calcium content in the blood serum of the cows studied showed a decrease in cows of all physiological groups by 2.9-16.9% compared to the lower limit of physiological fluctuations (Tables 1, 2). On the one hand, this may indicate an insufficient content of this macroelement in the feed, and on the other hand, the reason may be a violation of its absorption and metabolism in the animal's body. O.M. Chekan & V.V. Stryzhyus (2025) indicated that the reasons for the decrease in calcium content in the blood serum of cows in the postpartum period are disturbances in its absorption from the gastrointestinal tract, as well as its assimilation in the body of cows as a result of a lack of vitamin D and parathyroid hormone. P.R. Aziz *et al.* (2025) believed that a strong negative energy balance is a common cause affecting adaptation mechanisms, which can lead to increased cortisol levels in cows' blood and calcium homeostasis disorders. According to I. Horalska & A. Pavliuk (2021), calcium deficiency in the blood serum of cows is considered a risk factor for the development of fatty hepatodystrophy or its consequence. In addition, hypocalcaemia disrupts the functional state of the kidneys and heart, characterised by signs of acute inflammation in cardiomyocytes, typical of myocarditis. Therefore, low serum calcium levels in cows are a significant problem, especially during the transition period.

An increase in inorganic phosphorus content in the blood serum of cows 60 and 20 days before calving by 16.5% and 25.8%, respectively, is a negative factor, especially in the context of low total calcium content. This imbalance disrupts the Ca:P ratio in the blood serum of cows in the last trimester of pregnancy, which can negatively affect foetal growth and skeletal formation during this period. The assessment of biological markers and their correlation with each other can provide additional insight into the mechanisms underlying the development of fatty hepatosis. Changes in the liver parenchyma can be both functional and reversible. Therefore, it is necessary to address preventive measures to avoid the progression of pathological processes in the liver of lactating cows and the development of secondary diseases, including dietary adjustments, the use of hepatoprotectors, minerals, vitamins, lipotropic and other biologically active substances. These actions will primarily be aimed at maintaining homeostasis, stimulating the development of regenerative processes in the liver, positively affecting intracellular metabolism, and contributing to an increase in the quantity and improvement in the quality of products produced.

Conclusions

Examination of high-yielding cows during herd screening revealed significant changes in their health status. The cows examined showed signs of chronic and/or subacute liver disease and calcium-phosphorus metabolism disorders, characterised by mild clinical symptoms that were only evident in some animals (12-20%), in particular: slight depression, low mobility, decreased appetite, distorted taste, weak rumen contraction, enlarged liver dullness extending beyond the last rib. Depending on the period of lactation or dryness, changes in the biochemical parameters of cow blood serum have unique characteristics. Cows' blood parameters 5 days after calving are characterised by increased aspartate aminotransferase activity, positive thymol test, hyperglobulinaemia, decreased albumin, urea nitrogen, glucose, vitamin E and hypocalcaemia. Cows' blood parameters at 70-80 days of lactation are characterised by an increase in total protein, total lipoproteins, aspartate aminotransferase activity, a positive thymol test, hyperglobulinaemia, and a decrease in urea, urea nitrogen, glucose, vitamin E, and hypocalcaemia. Blood parameters in cows at 170-185 days of lactation are characterised by an increase in total protein, aspartate aminotransferase activity, a positive thymol test, hyperglobulinaemia and a decrease in urea, urea nitrogen, vitamin E and hypocalcaemia. Blood parameters in cows 60 days before calving are characterised by increased aspartate aminotransferase activity, positive thymol test, hyperglobulinaemia, hyperphosphataemia, decreased urea, urea nitrogen, glucose, carotene, hypocalcaemia and decreased Ca: R ratio. Blood parameters in cows 20 days before calving are characterised by an increase in total protein, total lipoproteins, non-esterified fatty acids, aspartate aminotransferase activity, hyperphosphataemia and a decrease in urea nitrogen, glucose, hypocalcaemia and the Ca: P ratio.

For timely detection and prevention of clinically pronounced symptoms in cows, it is necessary to conduct regular medical examinations of the herd, including blood tests, and, based on the results obtained, apply corrective measures in a timely manner. A promising area for future research is the correction of diets for cows of all physiological groups and the development of effective preventive measures that include macro- and microelements, vitamins, hepatoprotectors, and other substances that affect the correction of metabolic processes in the body of animals.

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Conflict of Interest

None.

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Діагностичні дослідження за жирового гепатозу у високопродуктивних корів

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Анотація. Актуальність дослідження зумовлена проблемами в секторі молочного тваринництва пов'язаними з розвитком захворювань високоудійних корів у транзитний період. Рання діагностика метаболічних розладів в транзитний період необхідна для своєчасного попередження ускладнення і розвитку незворотних змін в організмі корів та послідуючому вибракуванні тварин із стада. Метою цього дослідження було підтвердити розвиток у молочних корів транзитного періоду жирового гепатозу, визначити можливі його причини і визначити дієві кроки для профілактики цього захворювання та його наслідків. Для цього були використані клінічні методи діагностики та біохімічні дослідження сироватки крові корів, як ранні маркери зміни метаболізму в організмі тварин. Встановлено, що клінічно ознаки патології проявляються лише у 12-20 % корів із кожної фізіологічної групи. Зміни біохімічних показників сироватки крові корів залежно від фізіологічного періоду мали свої особливості. Під час дослідження були виявлені зміни в таких показниках сироватки крові, як загальний білок, альбуміни, глобуліни, білковий коефіцієнт, сечовина, азот сечовини, активність аспартатамінотрансферази, глюкоза, кальцій загальний, фосфор неорганічний, кальцій-фосфорне співвідношення, каротин, ліпопротеїди, тимолова проба, неестерифіковані жирні кислоти, вітамін Е. Встановлено, що для своєчасного виявлення метаболічних порушень у корів необхідно проводити регулярну диспансеризацію стада охоплюючи всі фізіологічні групи тварин, включаючи аналіз крові та, на основі отриманих результатів вчасно застосовувати методи корекції. Визначення причин порушення обміну речовин та патологічних змін в печінці і кальцій-фосфорному обміні в організмі корів у різні фізіологічні періоди дасть можливість підібрати належні методи профілактики та запобігти зменшенню економічних втрат від недоотримання молока і вибракування корів в умовах конкретного господарства

Ключові слова: гепатоліпідоз; гіперглобулінемія; неестерифіковані жирні кислоти; обмін ліпідів; триацилгліцероли; гіпоглікемія