

## REVIEW ARTICLE

**(Open Access)****Ultrasound findings of hepatic lipidosis in cows with high milk production**EMILIAN SHABANI<sup>1</sup>, AVNI ROBAJ<sup>2\*</sup>, EGLANTINA XHEMOLLARI<sup>1</sup>, KASTRIOT BELEGU<sup>1</sup>; ROMEO BOÇI<sup>3</sup><sup>1</sup>Faculty of Veterinary Medicine, Agricultural University, Tirana, Albania.<sup>2</sup>Faculty of Agricultural and Veterinary Prishtina, Kosovo.<sup>3</sup>PAZA project, Tirana, Albania.Corresponding author email: [avni.robaj@uni-pr.edu](mailto:avni.robaj@uni-pr.edu)**Abstract**

Cows are always regarded as an essential source of animal products (meat, milk, leather, bones) and as natural land fertilizers. One of the permanent tasks of mankind has been and remains the improvement of the productive and reproductive performance of dairy cows. This exaggerated obsession is related to increased demand for products and by-products originating from cows. Such levels requirements tend to exceed all genetic capabilities of animals and constitute the major ubiquitous cause of metabolic problems [31]. The liver is an organ of great importance in the body. It actively participates in the synthesis of glucose, in plasma protein formation, in formation and excretion of bile salts, in pigment excretion, in the formation of prothrombin, in detoxification and excretion of many substances including photodynamic agents. Ruminants liver has a remarkable functional reserve. Clinical manifestations of liver diseases becomes apparent only if 70% of parenchyma is totally in functional inactivity. Using imaging methods especially ultrasound for diagnosing of hepatic diseases in cattle is indicated because other diagnostic methods including the determination of hepatospecific enzymes, in most cases are insufficient [31]. Hepatic ultrasound examination is rewarding because it defines the hepar size, position, shape, condition, dimension of vessels and different types of hepatic disorders [31]. Hepatic lipidosis is the most important metabolic disorder of dairy cows during early lactation and is responsible for ill-health and poor reproductive performance of the animals. Efficient application of diagnostic and preventive strategies for this syndrome has great economic importance. Transitional period between late pregnancy and early lactation is a situation where creates an excessive drainage of different nutrients. Hepatic lipidosis is a condition that usually develops in the period near of calving and in early lactation [20]. Hepatic lipidosis develops when hepatic lipid intake exceeds the level of oxidation and excretion of lipids from liver. This condition is characterized by high concentration of free fatty acids metabolized by adipose tissue. Lipid excess is deposited as triglycerol who is primarily responsible for reducing the liver's metabolic functions. Liver can be categorized as normal or average, moderate or severe hepatic lipidosis, encephalopathic lipidosis, and hepatic incefalopatia [3] and [6] and [17]. Syndrome of hepatic lipidosis affects almost half of herd immediately after calving. The amount of fat accumulated in liver in the first 10 days after calving ranges from 60 to 120 grams per day. Lipids accumulated in liver occupy 12 to 25% of liver wet weight. In some cases, hepatic lipidosis followed by severe inflammation resulting in patient's death. If patients who suffer from this syndrome are not treated, mortality captures the values 25% [3].

**Review of literature**

Liver is the biggest solid organ in abdomen. Changes in shape, size, opacity and hepatic localization are used to assess liver physiological deviations. Hepatic lipidosis is a condition caused by different situations of physiological disorders in the body. Is multifactorial pathological condition that occurs in dairy cattle after calving [1]. The syndrome is characterized by progressive depression, inadequacy responses to treatment and great predisposition for

many other diseases. Hepatic lipidosis is an excessive fat metabolism in the liver in animals with good condition [2] and [3]. The metabolism of fat causes a negative energy balance and highlighted hormonal changes but not only.

**Causes of hepatic lipidosis***Design of the farm*

Results of recent studies show an interesting correlation among the unfavorable breeding conditions (bad hygiene, inadequate ventilation,

inappropriate layer, density of animals above the permitted levels, poor lighting, poor approach to water and food) with the frequency of metabolic diseases. Studies show that on farms where the design of the building was carried out in an amateur way, the frequency of metabolic diseases in general and hepatic lipidosis in particular is very high.

#### *Energy defects*

Cows with high milk production experience a period of energy scarcity in early lactation. In this period the cows mobilize body reserves to cope milk production. These facts constituting the real causes of moderate hepatic lipidosis which affects the best individuals in the herd. Fat is deposited in all tissues of the body, especially in skeletal muscles. Statistical processing of data from different studies shows that hepatic lipidosis syndrome is part of the general fats mobilization and not just a specific tissue or organ [19] and [23] demonstrated that hepatic lipidosis happens in such circumstances when the energy distribution in animals at late pregnancy is insufficient. Animals with double pregnancy are extremely vulnerable and sensitive to energy deficiencies in recent months of pregnancy. Recent studies shows very clearly the importance of nutrition of animals before and after calving on the etiology of ketosis/hepatic lipidosis syndrome. [10] states that transitional period (3 weeks before and 3 weeks after calving) is the most critical moment in the biological life of dairy cows. During this period modern dairy cows suffer an extraordinary metamorphosis. Spectacular displacement related to the fact that individual excels in a short period of time, from drying cows with low nutritional requirements, at lactating cows with massive metabolic needs. This period of metamorphosis has a spectacular permanent impact in health status, in production and reproduction. In a liver with fatty infiltration, the level of gluconeogenesis is not optimal. This means extension of lipidosis process, especially during the first weeks after calving [27]. Different food regimens during drying period does not influence the

composition of fatty acids in adipose tissue. Intensive Lipidosis results in increased blood concentration of palmitic acid, stearic, oleic and linoleic. Omnipresent consequence of this phenomenon is the excessive accumulation of stearic acid in the liver. This suggests that stearic acid is used by the liver (oxidation) or significantly secreted with milk. During the mobilization of fat it is observed competitive loss of physical condition and subcutaneous adipose tissue. The level of general mobilization of fats depends on the level of obesity and energy immunodeficiency of cow. In early lactation, cows with a thin condition and those who are obese have different metabolic answers and demands. Fatty cows have less ability to use fatty acids mobilized resulting in increased fat accumulation in tissues esterified. Skeletal muscle mass and subcutaneous fat mass get decreased a lot, therefore fatty cows lose 2.5 times more muscle fiber compared to skinny cows in body condition. Loss of body condition is due to the total mobilization of tissue (proteins and fats) and not just fat. The level of mobilization of proteins in fatty cows is higher than in skinny cows in body condition [9]. Hepatic lipidosis develops when hepatic lipid intake exceeds the level of oxidation and excretion of lipids from liver [4]. Energy deficits cause moderate increase in the mobilization of non-esterified fatty acids from adipose tissue. Most of the non-esterified fatty acids diffuse in the liver where the energy is provided by oxidizing carbon dioxide for hepatic function. If the arrival of non-esterified fatty acids in the liver, is greater than the energy needed, excess is oxidized in an incomplete manner, generate ketone bodies which are used as energy precursors of all skeletal muscles. Excesses of non-esterified fatty acids in the liver convert into triglycerol and deposited there. In normal conditions, triglycerol is secreted by the liver in the form of lipoproteins with very low density. Inadequate secretion of VLDL give significant contribution to the development of hepatic lipidosis [3] and [5] and [7]. Food consumption is insufficient to fulfill the increased energy requirements to maintain lactation. In these conditions, the animals experience a negative

energy balance, they mobilize the adipose stores of the organism, with final result of fat accumulation in the liver. Peak milk production is reached 4/7 weeks after calving but the highest level of voluntary food consumption by animals is not reached until 8/10 weeks after calving [30]. Metabolic adaptation during the period of the energy deficiency is related to the fact that amino acids, (Dietary and mobilized) that serve as precursors for the synthesis of glucose, become limiting for the synthesis of lipoprotein with very low density, thereby contributing to the further development of hepatic lipidosis. [8] and [29]

#### *Inflammatory conditions*

Always it has been reported a correlation between mastitis and metabolic diseases. Hyperglycemia followed by hypoglycemia it has been reported as part of the inflammatory phase. It is believed that the hypoglycemia phase is associated with the reduction of the level of glucose released from the liver. [18] reported a transitional growth of plasma concentration in NEFA after intravenous injection of E.coli lipopolysaccharide in heifers. Inflammatory responses are part of the etiology of hepatic lipidosis. Hepatic lipid accumulation is stimulated by the growing of concentration of proinflammatory cytokines and tumor necrosis factor alpha [7] and [21]. Inflammatory conditions and the response of the acute phase activate the macrophages that release a wide variety of products known as cytokines. The most important cytokines are TNF alpha, interleukin 1 and interleukin 6 that promote the production of a large range of proteins in the liver. These proteins are known as SAA and haptoglobina which reach high concentrations in the plasma of dairy cows immediately after calving. The levels are similar with the situations when we inject endotoxin intravenously [3] and [28]. Endotoxins are components of cell membranes in all gram negative bacteria and play a very important role in the development of many metabolic diseases among them and hepatic lipidosis [3] and [5] and [26]. Administration of TNF alpha in cows causes

significant decrease of appetite and increase the release of NEFA in the blood from fatty deposits. The condition of animals with reduced appetite and decreased food consumption, in a situation with a negative energy balance and hepatic lipidosis, about the calving time, is related also with the transfer of endotoxins in the blood [3]. Reduction of plasma glucose concentration and growth of NEFA level in plasma, in inflammatory situations in cows before calving, is related with all metabolic disorders that are energy dependent. The immune system in dairy cows in early lactation which suffer from mastitis, has significant quantitative changes due to changes in energy metabolism, and that's why it is thought that they are not only companion but also cause of hepatic lipidosis. Intravenous infusion of lipopolysaccharide causes significant reduction of the rumen peristaltic, decrease its absorption capacity and loss of appetite. All together they affect and enhance the state of the body's energy deficit [29].

#### *Phosphorus deficiency*

[16] concluded that phosphorus deficiency after calving plays an important role in the pathogenesis of hepatic lipidosis. The same author recommended phosphorus supplements as a useful prophylactic and therapeutic measure for postpartum hepatic lipidosis. This scientific facts were emphasized even more by [28] who described post-mortem examination in cattle with haemoglobin deficiency after calving. The liver of this animals appears enlarged and with great fatty infiltration. Changes of degenerative fatty infiltration were found also in histopathological examinations. [30] studied 180 cattle of Holshtein breed 40 days after calving. From this study, the author reported that hypophosphatemia in 10.7% of cases was associated with hyperacetonemia. [24] suggested that hypophosphatemia comes as a secondary metabolic event, as a result of subclinical condition of ketoacidosis in the period before calving.

#### *Other causes*

Hepatic lipidosis it is a pathological change which is not yet very clear on the causality, but many authors suggest and give as the main problem disorder of the intrahepatic blood circulation. [2]. [6] showed that degenerative local changes of hepatic lipidosis can be identified with special diagnostic techniques (ultrasound). Hepatic accumulation of triglycerides due to increased level of hepatic intake of NEFA stimulate an increased activity of diacylglycerol acyltransferases [22] Recent studies have managed to explain why the liver has a limited capacity for oxidation of fatty acids. Lack of oxalacetate which is needed to keep in view the tricarboxylic acid cycle, lack of carnitine which is necessary for mitochondrial transport and oxidation of acetyl coenzyme A, lack of niacin, disturbed endocrine factors of patient, are the correct answers of the question WHY [16]. Hepatic lipidosis occurs when the unesterified fatty acid concentrations reach levels 1000  $\mu\text{Eq}$  per L [25]. Hepatic triglyceride level is negatively correlated with plasma level of alfatoopherol. Microsomal hepatic triglycerides transfer the protein activity and the body mass index it is not affected by nutritional status of cows that are not in lactation [2]. Increased level of estrogen at the time of calving has a dramatic effect on the cow's energy balance. Estrogen stimulates the storage of triglycerides in the liver when unesterified fatty acids are in exaggerated levels in plasma [1] and [17] and [28] and [30]. Recent studies affirm unanimously that ruminants are the property of hepatic lipidosis due to reduced capacity of the liver for exporting lipoproteins with very low density. The evidences of scientific studies highlighted the role of estrogen in the development of hepatic lipidosis due to his lipolytic function [28] and [30]. Insufficiency of methionine at the time before calving it is associated with the development of hepatic lipidosis [19]. Correlation between fats in various tissues and those in liver in developing hepatic lipidosis it is identified with the role of some hormones unleashed by the fat called adipocytokine. These hormones prolong in time, the negative effect of negative energy balance, meanwhile cows continue to lose the fat from their

body [24]. [16] proved that hepatic lipidosis can diagnosed in dairy cattle herds by the presence of the high incidence of clinical and subclinical ketosis situation in the first two weeks of lactation. Other very important clinical factors that help in the diagnosis of these cases include obesity, persistent ketonic situation, the high level of cases with abomas displacement and high level of mortality in early lactation. [27] found that non esterified fatty acids (NEFA) betaoxidized form acetylcoenzyme A which than is oxidized in tricarboxylic acid cycle from the connection with oxalacetate acid. In early lactation acetylcoenzyme A does not enter in the Krebs cycle being directed in ketogenesis and consequently in the development of ketonic situation. To adapt the accelerated appearance of ketogenesis during hepatic lipidosis, aminoacids such as aspartic acid are mobilized profusely from skeletal muscles (the most important source of amino acids). Excessive amounts of amino acids that are lost from skeletal muscles induces the lying cow syndrome. On 30% of cows with high milk production hepatic fatty infiltration is very bad, but reversible process with significant effects on the structure and function of the liver [28].

### Clinical findings

[24] described hepatic lipidosis as a specific clinical condition that occurs in obese dairy cows. This illness situation causes serious health problems at the time of calving. The same author noted that the level of morbidity was 82% and mortality 25%. Cows with a very good condition at the calving period are more likely to be affected from hepatic lipidosis and cows with hepatic lipidosis are very likely to develop the ketosis condition [29]. Hepatic lipidosis syndrome may be developed within 24 hours and lasts for a long time. By histological, cows classified into 3 groups based on the level of fat content in the hepatic level at individuals one week after calving

1. Less than 20% lipids corresponds to less than 50 mg per gram of liver weight.
2. 50 to 100 mg lipids per gram of liver.

3. More than 40% is more than 100 mg lipids per gram of liver.

These concentrations correspond to the average rates, moderate and severe of hepatic lipidosis. Cows with less than 20% lipids in liver, in a week after calving are considered normal, individuals with over 20% are considered with hepatic lipidosis syndrome. Clinical evidences of liver disease do not pull the attention of a doctor or a farmer until such time as concentrations of lipids in liver do not reach the values 35%, 45% or more [28]. The total of hepatic lipids, mainly triglycerides, come to a climax on the day of calving or between 1 and 5 week after calving. This situation persists until the 12th week of lactation [28, 3] in the total of affected animals of a herd, generally 25%, fatality level in affected individuals is 90% [28] and [31]. The vital indicators (body temperature, cardiac and respiratory frequency) are within values of the norm. Rumen peristaltic is weak or absent and faeces are generally scarce. Periods of lying for a long time are common in patients who suffer from this illness condition. Heavy ketosis condition which does not answers in ordinary treatment is frequent. Patients demonstrate full anorexia. This fact makes the animals physically weaker and forces them to stay lying. Animals with lying syndrome die within 7 or 10 days [28]. Clinical findings in patients with serious hepatic lipidosis are yellowing of the mucous membranes, anorexia, ketosis, frequent movement and unintentional of the head, the left shift of abomasum, lying and coma. However it should be noted that this illness condition it is not characterized by specific clinical signs. A clinical moment which should never be forgotten is that the hepatic lipidosis is a physiological deviation that occurs at the beginning of lactation. Special attention should be shown to animals in early lactation because other diseases as ketoza, paresis, mastitis, metritis, displacement of abomasum and the rest of the placenta are more frequent in animals with hepatic

lipidosis. Almost in all cases hepatic lipidosis is coexistent with the above diseases [28].

### Ultrasound examination of liver

The complete ultrasound examination of the liver must provide detailed information on shape, size, position, ultrasound model of hepatic parenchyma, size of the gall bladder, size of the internal and external liver duct and topography of the large blood vessels. Ultrasound allows doctors to accurately trial hepatic pathological processes and helps setting the correct diagnosis [2] and [6]. Ultrasound diagnosis methods are safe and do not cause damage to the liver cells [6]. The best region for ultrasound examination of the liver in cows is the area between the ribs 7 and 12 on the right side. Care should be taken in patients with right abomasal displacement or with diaphragmatic hernia or different congenital malformations which relocate liver and make it invisible on ultrasound examination. Normal pattern of normal cow's liver during the ultrasound examination consists of a poor echoic homogeneity distributed throughout the liver area. The lumen of the portal and hepatic vein is anechoic. The normal liver ultrasonogram consists in a number of weak echo distributed homogeneously throughout the liver area, with a thin longitudinal anechoic line, veins and wall of the arteries are thick and hyperechoic [29] Hepatic ultrasonogram is rewarding in assessing the degree of hepatic fatty infiltration in dairy cows [4]. Ultrasound diagnosis of liver using liver/kidney contrast has only limited use in dairy cows [1]. [5, 6] indicated that the breed and the cow's age does not influence the liver ultrasound appearance. Different echo models of bright patterns, light stains in deep hepatic vessels and different angles can be used to distinguish varying or diffuse hepatocellular disorders in dairy cows. Ultrasound methods can also be used as a screening test before using other invasive techniques [1]. Ultrasound findings of hepatic lipidosis is associated with hepatomegaly appearance, round borders of liver, hyperechoic parenchyma close to the abdominal wall,

the weak echoic view with increasing distance from the abdominal wall and poor appearance of the liver blood vessels [6]. Local hepatic lipidosis not cause displacement of adjacent blood vessels [17]. By increasing the fat content in the liver observed reduction in the diameter of the portal vein and increase the size of the gallbladder [19]. Digital ultrasound tests have the potential to classify the level of triglycerides in the liver infiltration and evaluate the hepatic triglyceride content. These analyzes are appropriate and safe to be realized in a large group of animals on the farm. The diagnosis of hepatic lipidosis makes successful treatment and significantly lowers the level of mortality in patients suffering from this pathological condition [4].

### Conclusions

From this study of literature were some important moments which should be always in consideration during the management of herds of dairy cows, in order to successfully avoid the productive and reproductive health problems.

- The application of biochemical examining methods for animals during negative energy balance is rewarding for the diagnosis and prevention of hepatic lipidosis.
- Ultrasound diagnostic techniques are safe, noninvasive and rewarding in early diagnosis of hepatic lipidosis.
- The combined application of biochemical examining and ultrasound leads in early and correct diagnosis of hepatic lipidosis and helps prevent and treatment of this syndrome.

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