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Fatty liver disease in dairy cattle – risk factors, symptoms and prevention

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ABSTRACT

Fat cow syndrome (fatty liver disease) is a common metabolic problem in dairy cow production during the transition period, from the dry period to lactation. This syndrome occurs when due to liver uptake of non-esterified fatty acids (NEFAs), they are stored in excess as triacylglycerols (TAGs) in the liver. Currently, diagnosis of this disorder must be confirmed by a liver biopsy to determine the fat content of liver cells, as well as blood tests for increased levels of liver enzymes (GGT, SDH, AST, and bilirubin). Fat cow syndrome is associated with the occurrence of clinical diseases, decreased immunity, and problems with reproduction. It is a major problem in the herd health due to the high cost of veterinary services, a longer calving interval, and reduced longevity of cows, causing enormous economic losses. The best means of prevention is good nutrition and routine blood tests in the herd.

KEY WORDS: dairy cattle, metabolic disease, fatty liver

INTRODUCTION

Fatty liver is defined as the accumulation of fat (mainly triacylglycerols) in the hepatocytes. They are formed by long-chain fatty acids initially released from adipose tissue and then absorbed by the liver. Depending on the fat content of the liver, the condition is classified as mild, moderate, or severe liver steatosis (Kirovski and Sladojevic, 2017). Due to a lack of energy, fat metabolism is increased, and excess fat is deposited in the liver. This syndrome is the major metabolic disease occurring during early lactation in dairy cows. Fats released from the adipose tissue are transported with the blood to the liver and stored in the liver cells as triacylglycerols (TAGs). This condition occurs due to the sudden increase in the need for energy in early lactation. In beef cows, it can decrease appetite in pregnant, obese animals, especially in twin pregnancy (Radostits et al., 2007).

It can lead to metritis, mastitis, ketosis, down cow syndrome, retained placenta, and infertility (Andrew et al., 2008). Changes in the activity of enzymes in the blood may result from their increased

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activity in cells, but also from cell damage. Research has shown serum AST activity in cows to be significantly ($P < 0,05$) higher in early lactation than in late pregnancy, confirming damage to hepatocytes by steatosis and release of the enzyme into the blood. Activity of liver enzymes in the blood is correlated with liver damage due to fat infiltration. AST is the most sensitive indicator of liver condition during the transition period. An increase in AST activity in early lactation has been demonstrated. Research indicates metabolic problems and mild fatty liver due to the mobilization of adipose tissue in early lactation (Djoković et al., 2013).

Risk factors

Obesity in pregnant cows is an important factor in the development of postpartum liver steatosis. Fat cows after parturition show a decrease in appetite, which causes a negative energy balance in early lactation, leading to increased lipolysis of adipose tissue (Kirovski and Sladojevic, 2017). Appetite loss in excessively fat cows after calving results in even more fat mobilization, causing more severe fatty liver. Up to three weeks before and after calving, liver fat levels increase (Andrew et al., 2008). AST shows slight irregular changes during pregnancy and early lactation. In late pregnancy, levels of this enzyme are higher than in the first weeks after calving. Dry cows generally have higher AST levels than lactating cows due to metabolic changes during pregnancy (Kubkomawa et al., 2015). The nutritional factors that cause obesity are very important in the development of a fatty liver. Fat cows show a greater decrease in feed consumption than healthy cows in the perinatal period (3 weeks before and 3 weeks after parturition), leading to a loss of energy in early lactation. Loss of appetite and energy deficiency lead to increased lipolysis (Kirovski and Sladojevic, 2017). Higher AST levels have been found in early lactation compared to the dry period and mid-lactation. This may be due to damage to the hepatocytes and the release of this enzyme into the blood. Research indicates that cows in early lactation have morphological and physiological abnormalities in liver function, possibly as a result of a mild form of fatty liver (Krsmanović et al., 2016). The peak of milk production is 4-7 weeks after calving, but the highest feed intake occurs 8-10 weeks after parturition. As a result of energy deficiency, the cow releases fat stored in adipose tissue to meet the energy demand for milk production. Body condition at the time of calving has a very important impact on the health, milk production and fertility of cows. Dairy cows with a very long dry period have problems with obesity and a tendency towards fatty liver in the post-calving period. The disease is rare in beef cows (as pregnancy poisoning), occurring at a rate of about 1%, but has a 100% mortality rate. Heifers are more vulnerable than older cows. The disease is most common in late pregnancy (7-9 months) or immediately after calving (Radostits et al., 2007). In a study conducted in southern Iraq, the AST level in cattle averaged $54 \pm 9,53$ U/l in males and $53,12 \pm 8,26$ U/l in females (Al-Fartosi et al., 2010). The authors found no aberrations in AST activity during various stages of lactation or during pregnancy in dairy cows with a yield of up to 6000 l of milk per year (Klebaniuk and Rocki, 2011). Mean AST and ALP values have been shown to vary (Mohamed, 2014). AST was higher in early lactation than in mid-lactation, while ALP was increased in mid-lactation compared to early lactation. There were no significant differences in the levels of ALT and GGT (Mohamed, 2014). The study compared two groups: cows in early lactation and in late pregnancy. The results of NEFA and BHB were statistically higher ($P < 0,05$) in early lactation than in late pregnancy. AST was higher in early lactating cows. Although the mean GGT value was higher in late pregnancy, no statistically significant differences were found ($P > 0,05$). NEFA content > 0,4 mmol/L indicates problems with the energy balance, which results in the intense mobilization of fat from adipose tissue. Elevated

NEFA is a good indicator of energy balance and may indicate increased fat mobilization. Almost 50% of calving cows are at risk of developing metabolic disorders. When fatty liver occurs and changes appear in the liver tissue, the activity of the enzymes AST, GGT and GLDH will increase. Biochemical results show that in early lactation cows have metabolic problems related to ketosis. Changes in liver tissue and the activity of BHB, NEFA, and AST can help with assessment of metabolic status in pregnancy and lactation (Djoković et al., 2013).

Pathogenesis

In the case of energy deficiency, stored fat is released in the form of free fatty acids, which can be used as a source of energy or are oxidized to triglycerides in the liver, where they are accumulated or transported as very-low-density lipoproteins (VLDL). Due to the limited possibilities of synthesis of triglycerides and their transport as VLDL, a large amount of released fats results in fatty liver as fat accumulation in liver cells. Serum fatty acid levels increase from about two weeks before calving, peaking up to two days after calving, and return to normal levels in the third week of lactation (Nowak et al., 2006). Fatty liver is associated with a negative energy balance, which is normal during the first few weeks after calving. Fatty liver occurs when the metabolism cannot be adjusted to the body's requirements. Under normal physiological conditions, the level of fat in the liver rises a few weeks before calving to about 20% in the first week after calving and slowly decreases to less than 5% by week 26 postpartum. However, the difference can range from almost 0% to 70% in the first week after calving. Fat mobilization begins 2-3 weeks before calving, most likely initiated by hormonal changes caused by parturition, not by energy deficiency. The changes in the liver are functional, reversible and dependent on metabolic requirements in late pregnancy and early lactation. In cows with experimentally induced fatty liver, the intensity of liver glycogenesis during the perinatal period was higher than in cows without steatosis. Low glucagon content successively leads to low blood glucose concentration, low insulin levels and high fatty acid mobilization, causing severe fatty liver disease. In a subclinical fatty liver, low blood glucose and high blood NEFA levels are closely linked to the triacylglycerol level in the liver. Fatty liver occurs when the concentration of lipids exceeds the ability of the liver to oxidize and excrete them. The excess lipids are stored as triacylglycerols (TAGs) in the liver, which is associated with a decrease in liver metabolism (Kirovski and Sladojevic, 2017). In dairy cattle, fatty acids are mainly synthesized in adipose tissue, not in the liver. The released NEFAs provide energy to the liver cells, where they are well absorbed. If more NEFAs reach the liver than is needed, the excess is either oxidized to produce ketone bodies or converted to TAGs and stored (Kirovski and Sladojevic, 2017), causing fatty liver. The degree of fatty liver and AST activity vary depending on physiological status (pregnancy or lactation). In cows 15-5 days before parturition, liver hepatic fat was 5,3%, and AST was 70,23 U/l. In cows 5-1 days before parturition, hepatic fat was 6,31% and AST 75,42 U/l. In healthy post-calving cows, liver fat was 8,37% and AST 92,8 U/l (Đoković et al., 2012). AST shows slight, irregular changes during pregnancy and early lactation. AST activity has been shown to be lower during lactation, especially in the first few weeks, than in dry cows in late pregnancy, due to metabolic preparation for milk production (Kubkomawa et al., 2015). Research (Issi et al., 2015) has shown AST activity in the range of 58-74 U/l ~ 66,2 ± 1,54 in the control group, 63-97 U/l ~ 80,7 ± 3,96 in the group with subclinical ketosis, and 96-147 U/l ~ 116,2 ± 5,86 in the group with clinical ketosis. There were also changes in the blood urea level. A low blood urea level may be a sign of a protein deficiency in the diet, hunger, or liver damage. In the study cited, however, it may have been increased due to reduced feed consumption, which is

associated with ketosis, liver degeneration, and protein deficiency. Increases in AST and bilirubin are used to monitor liver damage (Issi et al., 2015). AST activity in the blood of cows (Radkowska, 2015) was found to vary widely, ranging from a low level before calving (53 and 55 U/l) to levels exceeding the normative values after calving (113 and 106 U/l). The increase in postpartum AST activity compared to the dry period was statistically significant ($P \leq 0,05$). The slow increase in NEFAs in the blood during the last stage of pregnancy may explain the gradual decrease in dry matter intake, contributing to the accumulation of triacylglycerols in the liver. During this period the blood glucose level is elevated and the level of BHBA (β-hydroxybutyric acid) is reduced. The high energy demand of high-yielding dairy cows immediately after calving, or in beef cows in twin pregnancy, results in an increase in mobilization of fat, usually subcutaneous. It is released into the blood and transported mainly to the liver, but also to the muscles and kidneys. The degree of fat loss will depend on the fatness of the cow and the energy deficiency. Subcutaneous fat and skeletal muscles are reduced after calving, with fat cows losing 2,5 times more muscle mass than thin cows. The body condition loss is due to the overall mobilization of the body (fat and protein). In severe cases, the accumulation of triglycerides in the cytoplasm of the hepatocytes is accompanied by a disturbance of the structure and functioning of the liver, causing hypoglycaemia (low blood glucose level) and ketonaemia (presence of ketone bodies in the blood). Cows that are not initially fat do not show symptoms of fatty liver (Radostits et al., 2007). Liver cells in cows two weeks after calving have a greater ability to esterify NEFAs than on day 50 (in the same animals) (Kirovski and Sladojevic, 2017). Changes in enzyme activity in the blood may be a consequence of an increase in their activity in cells (mainly hepatocytes), but may also result from cell damage. Djoković et al. (2013) found that AST activity was significantly ($P < 0,05$) higher in early lactation than in late pregnancy, confirming damage to the hepatocytes by steatosis and release of the enzyme into the blood. The range of AST activity in late pregnancy and early lactation was 78-132 U/l. Blood activity is correlated with liver damage through fatty infiltration. AST is the most sensitive indicator for diagnosing liver condition during the transition period. During their experiment the authors established a higher level of AST activity in early lactation compared to late pregnant cows. This condition may be due to body fat released at the beginning of lactation and can cause metabolic problems in the form of a mild fatty liver (Djoković et al., 2013).

The relationship between fat reserves and the ovarian cycle

In dairy cattle, an increase in adipose mobilization is associated with the prolongation of the anoestrus phase after parturition. Additionally, very thin cows generally have a delayed first oestrus after calving compared to cows with good body condition (Radostits et al., 2007). Cows with fatty liver cannot sustain pregnancy due to disturbed internal hormonal signalling, which reduces fertility (Kirovski and Sladojevic, 2017). Oestrogen levels are hundreds of times higher immediately after calving than during oestrus and can increase synthesis of TAG in the liver (Kirovski and Sladojevic, 2017).

Clinical symptoms - blood results

The biochemical changes associated with fatty liver disease have been described based on blood and liver tissue samples taken from cows during abdominal surgery, by skin biopsy of the liver, or in the abattoir immediately after slaughter. Differences in biochemical markers will depend on the degree of fatty liver. Their activity increases with fat content in the liver (Kirovski and Sladojevic, 2017). Assessment of the condition of the liver requires blood tests, especially of liver enzyme levels.

When liver cells are damaged during steatosis, the activity of these enzymes is increased (Gerspach et al., 2016). With a significant fatty liver, the amounts of enzymes produced are reduced, which leads to a reduction in blood glucose, total protein, albumin, globulin, cholesterol, triglycerides and urea. In addition, the secretory functions of hepatocytes are reduced, which leads to an increase in total bilirubin, ammonia and bile acid in the blood (Đoković et al. 2012). Free fatty acids, bilirubin, and AST increase, while glucose, cholesterol, albumin, magnesium, insulin, and leukocyte levels decrease (Andrew et al., 2008). In a study on 59 cows, 50% of them required treatment for secondary ketosis and 50% had biochemical results indicating a fatty liver, with levels at least twice the norm for two or more of the following: GGT (gamma-glutamyltransferase), AST (transaminase aspartate), SDH (succinate dehydrogenase), and total bilirubin. The authors showed that in severe fatty liver disease, serum levels of sorbitol dehydrogenase activity or bilirubin concentration were abnormally elevated in only 8%, while AST levels were 83% sensitive and 62% specific (Cebra et al. 1997). Higher AST levels have been found (Krsmanović et al., 2016) during early lactation compared to the dry period and mid-lactation. This may be due to fatty liver, damage to the hepatocytes, and the release of AST into the blood. The study indicates that early lactating cows have morphological and physiological changes in liver function, possibly as a result of a mild form of fatty liver. The AST enzyme level was 78-132 IU/L. The results indicate that AST is closely linked to the degree of fatty degeneration of the liver. The authors point to AST as the most sensitive determinant of liver condition and functioning in dairy cows (Krsmanović et al., 2016). At 15 days before calving, NEFA was significantly lower than in other groups ($P < 0,05$), with the highest NEFA level noted on the 15th day of lactation (0,41 mmol/L), which was higher than on the day of calving (0,32 mmol/L) and on day 45 of lactation (0,3 mmol/L). High NEFA content is a consequence of changes in the energy balance and intensification of lipolysis of adipose tissue due to energy deficiency. It often appears in the second month of lactation, although other authors have shown an increase between the 17th and 2nd day before calving. AST was 91,69 ± 8,90 to 96,21 ± 7,75 U/L; ALT was 29,69 ± 5,91 to 31,14 ± 5,84 U/L, and GGT was 21,89 ± 2,47 to 23,00 ± 2,75 U/L, which is within the normal range. This confirms the results of other studies, in which high-yield cows producing more than 25 l/day had AST up to 97.1, compared to 69,3 U/L in cows with lower productivity. In early lactation, GGT was 22,7 ± 3,11 U/L (Joksimović et al., 2012). GGT is a membrane cell enzyme found in cells with high activity (absorption or secretion). High GGT activity has been noted in the liver, kidneys, pancreas, intestine and spleen. An increase in GGT is associated with damage to the liver and kidneys (Kubkomawa et al., 2015). The following results (Djoković et al., 2013) were reported for lactation (L) and the dry period (D): NEFA – 0,38 mmol/L (L), 0,17 mmol/L (D); BHB 1,59 mmol/L (L), 1,14 mmol/L (D), and AST 69,46 IU/L (L), 33,55 IU/L (D). No significant changes in the GGT level were found during the study. High-yielding cows in the first months of lactation can have metabolic problems, with 5-10% developing severe fatty liver disease and 30-40% having a mild fatty liver. Activity of the enzymes AST, GGT, GLDH is increased due to reversible changes in liver tissue (Djoković et al., 2013). The reference values for ALP are very broad and two consecutive results cannot be compared, making it useless in liver disease diagnosis (Kubkomawa et al., 2015). Samples of liver tissue and blood were taken during standard displaced abomasum surgery. AST was significantly higher in cows with any degree of fatty liver, compared to the control group with normal fat content, without fatty liver. Other biochemical parameters were not elevated and there were no statistically significant differences between the groups. AST activity was as follows: control group

82,3 U/L; mild fatty liver 195,4 U/l; moderate fatty liver 180,1 U/l; and severe steatosis 201,8 U/l (p-value 0,003) (Gerspach et al., 2016). The highest correlation was noted between fatty liver and AST (r = 0,69). The other correlations between fatty liver and individual enzymes were as follows: NEFA r = 0,51; BHB r = 0,58, TG r = -0,55; cholesterol r = -0,34; albumin r = -0,53; glucose r = -0,69; bilirubin r = 0,5 (Đoković et al., 2012).

Liver biopsy

A liver biopsy can be used to identify the degree of fatty liver and triglyceride concentration. It is the most reliable method for describing this condition. The concentration of triacylglycerols in the liver of a healthy cow is between 10-15% of the liver weight. The lipid content of the liver tissue can be determined by biopsy using biochemical or histological methods. Both provide equally reliable results for liver fat content (Radostits et al., 2007). Depending on the degree of degeneration and fat infiltration in the hepatocytes, the following are distinguished: normal liver or mild steatosis (0-20% lipids), moderate fatty liver (20-40%), and severe fatty liver (over 40%). Fatty liver causes the degeneration of hepatocytes by damaging the cell membrane and leads to the release of cytoplasmic enzymes (AST, GGT and LDH) and an overall increase in their activity in the blood (Đoković et al. 2012).

Control and prevention

For disease prevention, the potential risk factors of the disease must be reduced or eliminated. Early diagnosis and treatment that affect voluntary feed intake in late pregnancy and shortly after calving are essential for reducing fat mobilization to cover the energy requirements during periods of negative energy balance and to maintain or increase liver glycogenesis. Diseases such as ketosis, abomasum displacement, retained placenta, mastitis, hypocalcaemia, and down cow syndrome must be treated as soon as possible to avoid fatty liver (Radostits et al., 2007; Kirovski and Sladojevic, 2017). The main element of prevention is a restrictive diet during the dry period to ensure adequate body condition at calving time, i.e. BCS: 2,5-3 (Andrew et al., 2008). A fatty liver is one of the most important metabolic diseases in high-yielding cows in early lactation. It usually begins 10 days after calving. Untreated, it leads to other metabolic disorders, infectious diseases, or reproductive problems. Cows with a fatty liver commonly do not show clinical signs of disease, but only health or reproductive issues. The disease causes substantial economic losses for the dairy farm, but it is often overlooked due to difficulties in diagnosis (Kirovski and Sladojevic, 2017). Many studies have shown that an increase in serum AST can be a very sensitive indicator of liver cell damage caused by fatty liver, even of a subclinical nature. AST has been shown to be closely linked to the degree of fatty liver degeneration and can be used in diagnostics (Tracz et al., 2012; Joksimović et al., 2012; Kirovski and Sladojevic, 2017; Đoković et al.2012; Krsmanović et al., 2016). In cows with diagnosed ketosis after calving, liver fat was 32,91% and AST 131,6 U/L (Đoković et al., 2012). AST activity was increased in cows with ketosis compared to the control group ($P < 0,05$) (Ghanem et al., 2017). The authors found that AST varied depending on the physiological state (pregnancy or parturition). Cows 15-5 days before parturition had 5,3% liver fat and AST levels of 70,23 U/l. In cows 5-1 days before parturition, liver fat was 6,31% and AST 75,42 U/l. In healthy post-calving cows, liver fat was 8,37% and AST 92,8 U/l (Đoković et al., 2012). Increased postpartum AST level can be caused by damage to muscles and mobilization of the body's fat reserves, as well as by protein mobilization during muscle gluconeogenesis (Mohamed, 2014). During the dry period, AST was significantly lower than during lactation. It attains high levels in early lactation until day 45 and then drops, but does not reach

the level from the dry period. In studies with moderately productive cows, the AST level ranged from 53,96 to 55,92 U/L, while in high-yielding cows it was 75,36-76,59 U/L. The authors showed no significant correlation between the level of GGT in the blood and milk parameters (Kirovski and Sladojevic, 2017). Milk production has a huge impact on the intensity of metabolic changes, which explains the increased level of AST in early lactation. In a study conducted in 1997, the AST value of healthy cows was 105 (±27) U/L, but research conducted in 2005 found that AST activity was 57,79 (±16,49) in early lactation, 45,82 (±7,39) in late lactation, and 44,91 (±6,93) before drying off, which gives an average of 43,35 (±13,56) for the whole group. This is a lower level than in a study from 1984, in which an AST level of 65,05 U/l (±31,31) was obtained in healthy cows (Mohamed, 2014).

CONCLUSIONS

Fatty liver in dairy cattle is a disease that causes large economic losses in the herd. Difficult to detect and treat, it creates problems in modern cattle husbandry. Clinical symptoms usually occur with severe liver damage when treatment is no longer effective. Prevention involving an appropriate diet during every stage of dairy production is essential. Routine tests, including blood tests, are needed to reveal the scale of the problem in the herd.

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