

DETERMINATION OF THE ACTIVITY OF SPECIFIC ENZYMES OF BLOOD IN THE PERIPARTUM PERIOD AND DURING THE FULL LACTATIONS

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Abstract: This study examined the activities of aspartate-amino-transferase (AST), gamma-glutamyl-transferase (GGT) and lactate-dehydrogenase (LDH) in the blood serum of 45 dairy Simmental cows divided into three groups according to production period. The first group (n=15) consisted of late pregnant dairy cows, the second group (n=15) cows in the early lactation, and the third group (n=15) cow in mid lactation. The significant higher activity ($P<0.05$) of AST, GGT and LDH were determined in the early lactation period than in dry period and during full lactation. Research results showed possibility of mild degree of hepatic lesions, probably due to fat infiltration in early lactation cows. Serum AST enzyme activities were significant correlated ($P<0.05$) with GGT and LDH activities and may be most sensitive indicator.

Keys words: dairy cows, serum enzymes activities

Introduction

Priorities intensive dairy production is the prevention of the generation of metabolic and other diseases. Metabolic diseases cows are closely associated with poor diet and management. The most important disease of dairy cows are: fatty liver, ketosis, lameness, mastitis, puerperal paresis, retained placenta and endometritis. Determination of metabolic profile in dairy cows is considered as a routine diagnostic method in detecting metabolic diseases. This involves taking blood samples from at least 8 to 12 cows, 4 times a year, in the dry period, during early, full and mid-lactation (*Gross et al., 2001; Oetzel, 2004; Stengårde et al.*

2008). A purpose of these tests is to determine a blood serum enzyme activity, such as aspartate amino-transferase (AST), alanine amino-transferase (ALT), γ -glutamyl transferase (GGT) and lactate dehydrogenase (LDH). The activities of these enzymes in the blood are very significant when assessing the degree of damage to liver cells. Fatty infiltration and degeneration of liver cells in dairy cows in early lactation, usually leads to destruction of cell membrane of liver cells (hepatocytes), which releases enzymes (AST, ALT, GGT, LDH) and a significant increase in their activity in the circulation (Pechova et al. 1997; Lubojacka et al. 2005; Stojević et al. 2005). Increased activity of AST in blood serum is a very sensitive indicator in assessing damage liver cells, especially in the infiltration mass and degeneration of hepatocytes (fatty liver) (Kauppinen, 1984; Pechova et al., 1997; Meyer and Harvey, 1998; Lubojacka et al. 2005). GGT is a microsomal membrane and the enzyme-linked highest in cells of the liver, kidneys and small intestine. Increased activity of this enzyme in the blood can indicate damage to the cellular structure of hepatocytes (Kupczyński et al. 2002; Lubojacka et al. 2005). Tainturier et al. (1984) find that the activities of AST and GGT enzymes showed irregular changes during pregnancy and early lactation, while activity. LDH is not for organ-specific enzyme, since it is in large concentrations in the muscles, heart, kidneys and liver and is released during acute inflammation of these organs. Also, the activities of LDH in the blood are closely correlated with the degree of fatty liver cells infiltration (Pechova et al. 1997). The aim of this study was to evaluate the functional state of liver cells, through changes in AST, GGT and LDH in the blood serum with the Simmental dairy cows during the peripartum period and full lactation.

Materials and Methods

The experiment was conducted on a farm of Simmental cows with frequent occurrence of metabolic and reproductive disorders (Farma Farmad, Vrdila-Kraljevo). The cows had an average lactation of about 6,200 l. Three groups of clinically healthy cows were chosen for the tests as follows: Group of cows in the dry period ($n = 15$), or the time of 15 ± 2 days before calving, another group of cows ($n = 15$), in early lactation, 14 ± 5 days of lactation and the third group ($n = 15$) in full lactation, 112 ± 25 days of lactation. Cows were kept in loose housing in an open barn. Feeding cows has been adapted to the energy needs in late pregnancy and lactation. Blood samples were taken at around 10:00 in the morning, or 4 to 6 hours after milking and feeding, puncture of the jugular vein. Was allowed to clot for 3 hours at 4°C and centrifugation (1500g, 10 minutes, 4°C), the serum was frozen at -20°C until analysis. Serum AST, GGT and LDH were determined using a variety of methods spectrophotometer (Cobas Mira plus) and commercial kits. Statistical analysis was performed ANOVA procedure (Statgraphic Centurion, StatPoint Technologies Inc. Warrenton, Va, Virginia, USA).

Results and Discussion

High milk production often poses a major risk for the development of metabolic disorders. For the purpose of diagnosis of fatty liver, it is important to determine the value of the important liver enzyme levels (Stojević *et al.* 2005). Average AST, GGT and LDH in the blood serum are shown in Table 1.

Table 1. Mean values (x±SD) of AST, GGT and LDH activities in dairy cows during transition period and full lactation.

	Late pregnancy	Early lactation	Full lactation
AST (IJ/l)	43.78±15.18 ^a	64.41±18.08 ^b	39.47±17.36 ^a
GGT (IJ/l)	9.37±4.16 ^a	14.64±4.24 ^b	8.45±1.86 ^a
LDH (IJ/l)	1250.73±482.54 ^a	1850.33±586.78 ^b	1167.40±336.12.95 ^a

Legend: Mean values within a row with no common superscript differ significantly (P<0.05)

Table 2. Correlation coefficients for testing the activity of enzymes in the blood of dairy cows in peripartum and during full lactation

	GGT	LDH
AST	0.32*	0.43*
GGT		0.18

Legend: Significant correlations (P<0.05) are marked with *.

AST is considered as the most sensitive indicator in the diagnosis of fatty liver in cows (Pechova *et al.* 1997; Kupczyński *et al.* 2002; Lubojacka *et al.* 2005). AST is located in the cytoplasm and mitochondria of different tissues and organs, but the maximum activity determined in skeletal muscle, heart and liver in cows (Lubojacka *et al.* 2005). Accordingly, changes in activity of this enzyme in the blood may be due to damage to the cellular structure of the body (primarily the liver). In this study, serum AST were significantly higher (P<0.05) in cows in early lactation in relation to activities in pregnant cows and cows during a full lactation, which may indicate the development of fatty infiltration of the liver cells, damage to hepatocytes and release of the intracellular enzymes into the circulation. GGT is microsomal and membrane-bound enzyme (Lubojacka *et al.* 2005). Increased activity of this enzyme is the result of liver cell destruction (Kupczyński *et al.* 2002; Lubojacka *et al.* 2005). GGT activity also depends on the observed period. In this study, a statistically significantly higher activity in blood serum were found in cows in early lactation compared to the peak of high pregnancy and lactation (P<0.05). Similar results have been found by Bobe *et al.* (2004). LDH is not organ specific enzyme, as it has a high concentration in muscle, heart, kidneys and liver (Pechova *et al.* 1997; Lubojacka *et al.* 2005). Its activity is increased in acute

damage to these organs (*Lubojacka et al., 2005*). In this research, the activity of LDH was significantly higher ($P < 0.05$) in cows in early lactation, in relation to the gestating cows and cows in full lactation. The results suggest that cows in early lactation have disrupted morphological and physiological condition of the liver, probably as a result of a mild degree of fatty liver cells infiltration. In this paper, AST, GGT, and LDH levels were mostly within the normal range (AST: 78-132 IU / l; GGT: 10-25 IU / l; LDH: 692-1445 IU / l) (*Stojić, 1996*), and were significantly higher after calving, which may indicate the development of a mild degree of fatty infiltration of cows' liver cells in early lactation. *Pechova et al. (1997)* showed that serum liver enzymes, AST in particular, are closely correlated with the degree of fatty infiltration and degeneration of liver cells. According to this paper, only the serum activities of AST significantly correlated ($P < 0.05$) with the activities of GGT and LDH. Based on the results AST can be considered as the most sensitive indicator in the assessment of the functional state of the liver in dairy cows.

Conclusion

Biochemical tests of blood serum showed a statistically significantly greater activity for AST ($P < 0.05$), GGT ($P < 0.05$) and LDH ($P < 0.05$) of cows in early lactation, compared to values blood in the other two groups. Serum AST activity was significantly correlated with GGT and LDH and may be considered as the most sensitive indicator to evaluate the morphological and functional state of the liver in dairy cows. Results indicate that early lactating cows have reduced functional capacities of hepatocytes, which is probably associated with a mild degree fatty infiltration of the liver cells.

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Određivanje aktivnosti specifičnih enzima krvi u peripartalnom periodu i tokom pune laktacije

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Rezime

U ovom radu je određivana aktivnost aspartat-aminotransferaze (AST), gama-glutamat-transferaze (GGT) i laktat dehidrogenaze (LDH) u krvnom serumu kod 45 simentalških mlečnih krava, podeljenih u tri grupe u zavisnosti od produktivnog perioda. Prvu grupu (n=15) su činile visoko gravidne krave, drugu grupu (n=15) krave u ranoj laktaciji, a treću grupu (n=15) krave tokom pune laktacije. Statistički značajno veće aktivnosti AST ($P<0.05$), GGT ($P<0.05$) i LDH ($P<0.05$) u krvnom serumu su utvrđene kod krava u ranoj laktaciji u odnosu na aktivnosti ovih enzima u serumu kod zasušenih krava i krava u punoj laktaciji. Dobijeni rezultati ukazuju na mogućnost blagog stepena oštećenja ćelija jetre, odnosno masnu infiltraciju hepatocita kod krava na početku laktacije. Serumske aktivnosti AST su bile u značajnoj korelaciji ($P<0.05$) sa aktivnostima GGT i LDH u krvnom serumu pa se AST može smatrati pouzdanim indikatorom u tvrdivanju funkcionalnog stanja jetre kod mlečnih krava tokom peripartalnog perioda i pune laktacije.

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