

SERUM ACTIVITIES OF AST, ALT, GGT AND LDH IN CLINICALLY HEALTHY DAIRY COWS DURING TRANSITIONAL PERIOD AND MID LACTATION*

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SUMMARY: This study examined the activities of aspartate-aminotransferase (AST), alanine- aminotransferase (ALT), gamma-glutamyltransferase (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 was determined in the early lactation period than in dry period, while enzyme activity in the mid lactation period was higher (P>0.05) than in the dry period. ALT activity showed a lower (P>0.05) serum activities in early lactation cows than in the late pregnant and mid lactation cows. The higher serum activities of GGT (P>0.05) and LDH (P<0.05) were determined in early lactation cows than in the late pregnant and mid lactation cows. 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 ALT, GGT and LDH activities and may be most sensitive indicato in determining the functional liver state in dairy cows during transition period and mid lactation.

Keys words: dairy cows, serum enzymes activities, transition period, mid lactation.

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INTRODUCTION

The priority for intensive milk production is prevention of metabolic diseases and other disorders. Production diseases i.e. diseases associated with improper nutrition or management are common in dairy cows. The diseases listed in this include: the fat liver syndrome, ketosis, laminitis, mastitis, milk fever, retained placenta, metritis and infertility. The metabolic profile, a series of specific blood analytical tests is routinely used to reveal metabolic problems in dairy cattle. It involves collecting blood samples from eight to twelve cows at 4 time periods relative to calving (dry, early lactation, peak lactation and mid lactation) and measuring selected blood metabolites (Gross et al., 2001; Oetzel, 2004; Stengarde et al., 2008). A part of that test is determining enzyme activities in the serum: aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), γ -glutamyl-transferase (GGT) and lactate-dehydrogenase (LDH). The activities of enzymes are highly important blood parameters used in evaluating the degree of hepatocyte damage. The fatty liver infiltration and the hepatocyte degeneration in dairy cows involve cell membrane damage and hepatocyte destruction coupled to the release of cytoplasm enzymes (AST, ALT, GGT, LDH) and marked increases in the circulating activities (Pechova et al., 1997; Lubojacka et al., 2005; Stojević et al., 2005). Increased AST activity in the serum is a sensitive marker of liver damage, even if the damage is of a subclinical nature (*Kauppinen, 1984; Meyer and Harvey, 1998*). The AST activity is found to be the most sensitive indicator in diagnosing fatty liver in cows (Pechova et al., 1997; Lubojacka et al., 2005). GGT is a microsomal and membrane-bound enzyme found mostly in the liver, kidneys and small intestines. The increase in the activity of this enzyme results from damages of the cellular structure of hepatocytes (Kupczynski et al., 2002; Lubojacka et al., 2005). According to Tainurier et al. (1984) the activity of AST and GGT enzymes shows occasional irregular, small changes during pregnancy and early lactation, while the activity of ALT decreases significantly in the seventh and eighth month of pregnancy and at the beginning of lactation. LDH is not an organ-specific enzyme, being found at high concentrations in the muscles, heart, kidneys and the liver. It is released into the blood in cases of acute cell damage of the above organs. The blood activity of LDH is correlated with the degree of fatty infiltration of hepatocytes (Pechova et al., 1997).

The aim of this study was to determine the activity of AST, ALT, GGT and LDH and their relations in the blood plasma in Simmental dairy cows during transitional period and the mid lactation, in order to identify the pathological processes and changes in the metabolic status.

MATERIAL AND METHODS

This experiment was carried out at Simmental dairy herd with several metabolic and reproductive disorders. The cows were mid-yielding with a preceding lactation about 6,500 l. Three groups of clinically healthy cows were chosen from herd. One group consisted late pregnant cows (n=15) in period from 25 to 1 (13±9) days to calving, a second group included early post-partum cows (n=15) in the first month of lactation (16 ± 9 days) and third group (n = 15) in mid lactation (115±29 days). The experimental cows were kept in tie-stall barns. The diet and the housing facilities were adapted to

research purposes. The diet suited the energy necessary for cows in late pregnancy, early and mid lactation.

The blood samples were collected at 10:00 h or 4 to 6 hours after milking and feeding, by puncture of the jugular vein into sterile disposable test tubes. After clotting for 3 hours at 4°C and centrifugation (1500g, 10 minutes, 4°C), sera were carefully harvested and stored at -20°C until analysis. Serum AST, ALT, GGT and LDH were measured in the biochemical laboratory by different colorimetric techniques using a spectrophotometer (Cobas Mira plus) and the corresponding commercial kits.

The statistical analysis of the obtained data was carried out by ANOVA-procedure (Statgraphic Centurion, Statpoint Technologies Inc. Warrenton, Va, Virginia, USA).

RESULTS

The mean values of AST, ALT, GGT and LDH activities in the blood plasma of dairy cows are shown for each period in Table 1. In this study highest serum activities of AST, GGT and LDH were determined in group of early lactation cows than in late pregnant and mid lactation cows, and showed significant variations ($P < 0.05$) for AST and LDH activities. The lowest serum ALT activities were determined in early lactation cows, without significant differences ($P > 0.05$) compare to other two groups of cows.

Correlation coefficients for the serum enzyme activities in dairy cows during transition period and mid lactation are shown in Table 2.

Table 1. Mean values ($\bar{x} \pm SD$) of AST, ALT, GGT and LDH activities in dairy cows during transition period and mid lactation (average \pm SD)

	Late pregnancy	Early lactation	Mid lactation
AST (U/l)	26.45 \pm 8.97 ^a	33.55 \pm 9.35 ^b	32.61 \pm 8.90 ^b
ALT (U/l)	72.47 \pm 24.16 ^a	66.87 \pm 12.96 ^a	96.38 \pm 80.46 ^a
GGT (U/l)	20.61 \pm 4.16 ^a	25.51 \pm 4.91 ^a	23.03 \pm 9.94 ^a
LDH (U/l)	715.69 \pm 160.72 ^a	1058.15 \pm 205.45 ^b	670.59 \pm 134.95 ^a

^{a,b,c} Values with different superscripts, within the row, significant differ ($p < 0.05$).

Table 2. Correlation coefficients for the serum enzyme activities calculated for all cows in the present study.

	ALT	GGT	LDH
AST	0.33*	0.32*	0.43*
ALT	-	0.07	0.28
GGT	-	-	0.18

*Significant correlations ($P < 0.05$).

DISCUSSION

Modern milk production often puts the production capabilities of cows at risk, which can result in metabolic disorders. In order to predict such disorders and eventual subclinical diseases it is necessary to determine physiological ranges of biochemical

parameters in a clinically healthy herd. (Stojević et al., 2005).

The serum AST activity is considered as the most sensitive indicator for diagnosing fatty liver in this species (Pechova et al., 1997; Kupczynski et al., 2002; Lubojacka et al., 2005). AST is located in the cytoplasm and mitochondria of different tissues and organs, and the highest activities are detected in heart and skeletal musculature, as well as in liver in cows (Lubojacka et al., 2005). Accordingly, changes in their activity in the blood can be a consequence of their increased activity in cells (primarily liver), but also a reflection of cell structure damage. In the present study, the serum AST activities were significantly higher ($P < 0.05$) in early lactating cows than in late pregnant cows, corroborating that the development of fatty infiltration in liver has led to cell disruption and release of the intracellular enzymes into the blood flow. ALT activity in cows differs during certain production periods. The lowest ALT activity was measured during early lactation, while activity increased ($P > 0.05$) in the mid lactation cows. In the dry period enzyme activity decreased, but it was not statistically higher ($P > 0.05$) than in the early lactation cows. Similar result was obtained by Kauppinen (1984). The author considers that the role of ALT in predicting liver damage in ketosis is not significant. Tainturier et al. (1984) in their study presented information that ALT activity decreased in the seventh and eighth months of pregnancy and that it remained stable until the end of pregnancy, and in the first month of lactation. Our results confirm this only partially because in the period of mid lactation we measured the highest concentration of ALT.

GGT is a microsomal and membrane-bound enzyme (Lubojacka et al., 2005). The increase in this enzyme activity results from disruption of the cellular structure of hepatocytes (Kupczynski et al., 2002, Lubojacka et al., 2005). GGT activity also depended on the observed period. Higher values were measured in early lactation compared to late pregnancy and mid lactation period, without statistically differences ($P > 0.05$). El-Ghoul et al. (2000) found that GGT activity in late pregnancy is much lower than in the first week after calving, and 6 weeks after delivery the activity increased. LDH is a not specific organ enzyme, found at high concentrations in muscles, heart, kidneys and liver (Pechova et al., 1997; Lubojacka et al., 2005). It is released into the blood flow in cases of acute cell damage of the above organs (Lubojacka et al., 2005). In this study, LDH activities was significant higher ($P < 0.05$) in early lactation cows than in late pregnant and mid lactation cows. This result suggested that early lactation cows had metabolic disturbances, and mild degree of hepatic lesions, probably due to fat infiltration.

In the present study, the serum AST, ALT, GGT and LDH activities measured in late pregnant in early and mid lactating cows were included inside the physiological limits (AST: 78-132 U/l, ALT: 7-35 U/l, GGT: 10-25 U/l and LDH: 692-1445 U/l), (Stojić, 1996), but they were higher after calving, except ALT activities, corroborating that the development of fatty infiltration in liver has led to cell disruption and release of the intracellular enzymes into the blood flow. Moreover, according to Pechova et al. (1997), the blood activities of liver enzymes are correlated with the degree of fatty infiltration in the organ. In agreement, only the serum AST enzyme activities were significant correlated ($P < 0.05$) with ALT, GGT and LDH activities and may be most sensitive indicator in determining the functional liver state in dairy cows during transition period and mid lactation.

CONCLUSION

Biochemical examination of blood serum showed higher activities of AST, GGT, LDH in early lactation cows such as lower concentrations of ALT compared to the groups of late pregnant and mid lactation cows. The serum AST enzyme activities were significant correlated with ALT, GGT and LDH activities and may be most sensitive indicator in determining the functional liver state in dairy cows during transition period and mid lactation. This result suggested that early lactation cows had metabolic disturbances, and mild degree of hepatic lesions, probably due to fat infiltration.

REFERENCES

- EL-GHOUL, W., HOFMANN, W., Y. KHAMIS, Y., A. HASSANEIN A.: Beziehungen zwischen Klauenerkrankungen und peripartalen Zeitraum bei Milchrindern. *Prakt. Tierarzt*, 82:862-868, 2000.
- GROSS, J., VAN DORLAND, H.A., BRUCKMAIER, R.M., SCHWARZ, F.J.: Performance and metabolic profile of dairy cows during a lactation and deliberately induced negative energy balance with subsequent realimentation. *J. Dairy Sci.*, 94:1820-1830, 2001.
- KAUPPINEN, K.: ALAT, AP, ASAT, GGT, OCT activities and urea and total bilirubin concentrations in plasma of normal and ketotic dairy cows. *Zbl. Vet. Med. A.*, 31:567-576, 1984.
- KUPCZYNSKI, R., CHUDOBA-DROZDOWSK, B.: Values of selected biochemical parameters of cows blood during their drying-off and the beginning of lactation. *Electr. J. Polish Agric. Univ.*, 55:225-231, 2002.
- LUBOJACKA, V., PECHOVA, A., DVORAK, R., DRASTICH, P., KUMMER, V., POUL, J.: Liver steatosis following supplementation with fat in dairy cows diets. *Acta Vet. Brno*, 74:217-224, 2005.
- OEZEL, G.R.: Monitoring and testing dairy herds for metabolic diseases. *Vet. Clin. North Am. Food Anim. Pract.*, 20:651-674, 2004.
- PECHOVA, A., LLEK, J., HALOUZKA, R.: Diagnosis and control of the development of hepatic lipidosis in dairy cows in the peri-parturient period. *Acta Vet. Brno*, 66:235-243, 1997.
- MEYER, D.J., HARVEY J.W.: Evaluation of hepatobiliary system and skeletal muscle and lipid disorders. In: *Veterinary Laboratory Medicine. Interpretation and Diagnosis*. (Meyer, D.J., Harvey, J.W., Eds.) 2nd ed., W.B. Saunders company Philadelphia, London, Toronto, Montreal, Sydney, Tokyo. pp. 157-187, 1998.
- STOJEVIĆ, Z., PIRSLJIN, J., MILINKOVIC-TUR, S., ZDELAR-TUK M., LJUBIC B.B.: Activities of AST, ALT and GGT in clinically healthy dairy cows during lactation and in the dry period. *Vet. Arhiv*, 75:67-73.2005.
- STOJIC V.: *Physiology of domestic animals*. IP "Naučna knjiga", Faculty of Veterinary Medicine, Belgrade, pp.23-67, 1996.
- STENGARDE, L., TRAVEN, M., EMANUELSON, U., HOLTENIUS, K., HULTGREN, J., NISKANEN, R.: Metabolic profile in five high-producing Swedish dairy herds with a history of abomasal displacement and ketosis. *Acta Vet. Scand.*, 50:31, 2008.
- TAINTURIER, D., BRAUN, J.P., RICO, A.G., THOUVENOT J.P.: Variation in blood composition in dairy cows during pregnancy and after calving. *Res. Vet. Sci.*, 37:129-131, 1984.

SERUMSKE AKTIVNOSTI AST, ALT, GGT I LDH KOD KLINIČKI ZDRAVIH MLEČNIH KRAVA TOKOM TRANZICIJSKOG PERIODA I SREDINE LAKTACIJE

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Izvod

U radu je procenjivana aktivnosti aspartat-aminotransferaze (AST), alanin-amino-transferaze (ALT), gama-glutamat-transferaze (GGT) i laktat dehidrogenaze (LDH) u krvnom serumu kod 45 Simentalskih mlečnih krava, podeljenih u tri grupe u zavisnosti od stadijuma produkcijonog perioda. Prvu grupu (n=15) su činile visoko gravidne krave, drugu grupu (n=15) krave u ranoj laktaciji, a treću grupu (n=15) krave u sredini laktacije. Statistički značajno više ($P<0.05$) aktivnosti AST su utvrđene kod krava u ranoj laktaciji u odnosu na zasušene krave, dok su enzimske aktivnosti AST kod krava u sredini laktacije bile veće ($P>0.05$) u odnosu na krave u zasušenju. ALT aktivnosti su pokazale niže vrednosti ($P>0.05$) kod krava na početku laktacije u odnosu na grupe krava u zasušenju i u sredini laktacije. Veće aktivnosti 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 i krava u sredini laktacije. Dobijeni rezultati ukazuju na mogućnost blagog stepena oštećenja hepatocita, odnosno masnu infiltraciju hepatocita kod krava na početku laktacije. Serumske aktivnosti AST su bile u značajnoj korelaciji ($P<0.05$) sa aktivnostima ALT, GGT i LDH u krvnom serumu i AST može biti najosteljiviji indikator u utvrđivanju funkcionalnog stanja jetre kod mlečnih krava tokom tranzicionog perioda i sredine laktacije.

Ključne reči: mlečne krave, serumske enzimske aktivnosti, tranzicioni period, sredina laktacije.

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