

VETERINARSKI ARHIV 75 (1), 67-73, 2005

## Activities of AST, ALT and GGT in clinically healthy dairy cows during lactation and in the dry period

Zvonko Stojević\*, Jasna Piršljin, Suzana Milinković-Tur,  
Maja Zdelar-Tuk, and Blanka Beer Ljubić

*Department of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia*

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**STOJEVIĆ, Z., J. PIRŠLJIN, S. MILINKOVIĆ-TUR, M. ZDELAR-TUK, B. BEER LJUBIĆ: Activities of AST, ALT and GGT in clinically healthy dairy cows during lactation and in the dry period. Vet. arhiv 75, 67-73, 2005.**

### ABSTRACT

This study examined the activities of aspartate aminotransferase (AST; EC 2.6.1.1), alanine aminotransferase (ALT; EC 2.6.1.2) and gamma-glutamyltransferase (GGT; EC 2.3.2.2) in the plasma of 120 dairy Holstein breed cows divided into four groups according to production period. The cows were held in an intensive farm breeding premises. Their meal consisted of ordinary alfalfa, silage, hay and concentrates with energy and protein supplements. The first group consisted of animals from the 10<sup>th</sup> until 45<sup>th</sup> day of lactation, the second from the 46<sup>th</sup> until 90<sup>th</sup> day of lactation, and the third group from the 91<sup>st</sup> day of lactation until the end of milk production. Cows in the dry period comprised the fourth group. The highest activity of AST was determined in the first production period, while enzyme activity in the second and third periods was higher than in the dry period. ALT activity showed a statistically significant increase from the 46<sup>th</sup> day of lactation until the dry period, and activity in the second and third periods was statistically higher than in the dry period. GGT activity in the first production period and in the dry period was statistically higher in comparison with the second and third periods. Research results showed a significant influence of milk production and the dry period on the activities of examined enzymes in the blood plasma, and we can conclude that there is a need for constant monitoring of the described parameters during production.

**Key words:** AST, ALT, GGT, dairy cows, blood plasma, lactation, dry period

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### Introduction

In the higher standards of milk production, the priority in modern breeding is keeping dairy cows in high lactation and healthy. The control of their feeding and metabolic status

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\* Contact address:

Prof. Dr. Zvonko Stojević, Department of Physiology and Radiobiology, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia, Phone: +385 1 2390 172; Fax: +385 1 2441 390; E-mail: stojevic@vef.hr

is equally important for the health of the herd in the health control system. It is known that highly productive animals compared to those who produce less are in a more or less negative energy status. The negative energy status mostly appears during three situations: in late pregnancy, in the first weeks of lactation, and during disease (CEBRA et al., 1997). Therefore, the priority for intensive milk production is prevention of metabolic diseases and other disorders. The test of metabolic profiles by analyzing biochemical parameters in the blood of dairy cows made by PAYNE and PAYNE (1987) is a significant contribution to veterinary medicine. A part of that test is determining enzyme activities in the serum: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT). Such a procedure can be connected with several difficulties: reference animals are difficult to select and because blood reference values and their variations are often unknown, and results are often contradictory (TAINTURIER et al., 1984).

In the liver AST, ALT and GGT show high activity and are most often determined if there is a suspicion of acute and chronic liver disease. Determining AST and GGT activities in dairy cows is most often connected with fatty liver syndrome (CEBRA et al., 1997), low appetite and the appearance of ketosis in dairy cows during early lactation (STEEN, 2001). 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). Unlike AST, horse, pig, and ruminant liver cells do not show high ALT activity, and the increased activity of that enzyme in the serum during liver damage, even in necrosis, is insignificant (FORENBACHER, 1993).

Gamma-glutamyltransferase is a membrane-bound enzyme in organs with emphasized functions in secretion and resorption. In the plasma it is significant as a sign of hepatobiliary system diseases connected with cholestasis and is used in diagnosing liver disease. Its activity is relatively high in livers of cows, horses, sheep and goats (TENANT, 1997).

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. EL-GHOUL et al. (2000) determined significant differences in the activity of GGT and AST. In late pregnancy, GGT showed a significant decrease in activities, while the activity of AST in this period was much higher than in the first week after birth.

The aim of this study was to determine the activity of these enzymes and their relations in the blood plasma in highly productive Holstein breed dairy cows during lactation and the dry period, and to apply systematic health monitoring and the animal breeding status in order to identify changes that occur as a consequence of pathological processes and changes in the metabolic status in good time.

## Material and methods

The research was carried out using 120 Holstein breed dairy cows in second lactation and in the dry period. The animals were held in intensive farm breeding premises, divided into groups according to level of production. The meal was adjusted to production intensity, and consisted of ordinary alfalfa, silage, hay and concentrates with energy and protein supplements. According to the level of production, the animals were divided into four groups, 30 animals in each group. The first group consisted of animals from the 10<sup>th</sup> to 45<sup>th</sup> day of lactation, the second group of cows in lactation from the 46<sup>th</sup> to 90<sup>th</sup> day. The third group consisted of animals from the 91<sup>st</sup> day of lactation until the end of milk production. The fourth group comprised dairy cows in the dry period.

Blood for testing was taken in the morning in order to avoid daily variations in activities. The samples were taken by puncture of the v. jugularis and with heparin as an anticoagulant. The blood plasma was obtained by centrifugation at 1500 g for 15 minutes. The activity of AST, ALT and GGT were determined. AST, ALT and GGT activities were measured on a Helios gamma UV visible spectrophotometer, Thermo spectronic UK, using kits produced by "Herbos dijagnostika" d.o.o., Sisak, Croatia.

Results are presented as a mean value  $\pm$  standard deviation (M  $\pm$  SD). Statistical comparisons were made by Student's *t*-test.

## Results

The mean values of AST, ALT and GGT activities in the blood plasma of dairy cows are shown for each period in Table 1.

Table 1. Mean values of AST, ALT and GGT (U/L) activities in dairy cows during lactation and dry period

Enzyme	(10.-45.days of lactation)	(46.-90. days of lactation)	(91.-until the end of lactation)	(dry period)
AST	57.79 $\pm$ 16.49 <sup>2, 3, 4</sup>	45.82 $\pm$ 7.39 <sup>1,4</sup>	44.91 $\pm$ 6.93 <sup>1,4</sup>	32.90 $\pm$ 7.06 <sup>1,2,3</sup>
ALT	8.91 $\pm$ 2.23 <sup>2, 3, 4</sup>	18.90 $\pm$ 4.61 <sup>1,4</sup>	20.08 $\pm$ 3.74 <sup>1, 2, 4</sup>	11.84 $\pm$ 3.26 <sup>1,2,3</sup>
GGT	19.00 $\pm$ 4.23 <sup>2,3</sup>	15.14 $\pm$ 3.94 <sup>1,4</sup>	14.72 $\pm$ 3.73 <sup>1,4</sup>	19.56 $\pm$ 4.09 <sup>2,3</sup>

Values denote the mean  $\pm$  standard deviation of measurements from n=30 animals. <sup>(1-4)</sup>Means within a row with common superscript differ significantly (P<0.001).

Aspartate aminotransferase activities in animals during the three lactation periods and in the dry period show significant differences. The measured activities showed a constant decrease of AST values in the blood plasma of observed animals from the beginning of

lactation until the dry period. In the first 45 days of lactation, AST activity in the blood plasma was statistically much higher than in the second, third and fourth observed periods ( $P < 0.001$ ). Results obtained from animals which were in the second and third period of lactation showed a statistically significant decrease of AST activities comparing to the results during early lactation ( $P < 0.001$ ), but the values were much higher compared to the dry period ( $P < 0.001$ ) (Table 1). A much lower AST activity was determined during the dry period.

Alanine aminotransferase activity in the blood plasma differs significantly between production periods. In the second and third periods there was an increase of the tested enzyme activity values (Table 1). Through interactive comparison of results for each period, we noticed a statistically significant increase of activities after 46 days of lactation until the dry period ( $P < 0.001$ ). The activities of this enzyme in the second and third periods of lactation were much higher and statistically significant compared with those measured in the dry period ( $P < 0.001$ ).

Comparing GGT activities in the blood plasma during different production periods, results were statistically different. Mean values of GGT activities measured in the beginning of lactation and during the dry period were much higher than activities obtained from the 46<sup>th</sup> day of lactation until the end of milk production ( $P < 0.001$ ). During the dry period, values were insignificantly higher than at the beginning of lactation.

The range and mean values of AST, ALT and GGT activities on a model of 120 cows are shown in Table 2.

Table 2. Ranges and mean of AST, ALT and GGT (U/L) activities in blood plasma of dairy cows.

Enzyme	M $\pm$ SD	M $\pm$ 2SD	M $\pm$ 3SD	MIN-MAX
AST	45.35 $\pm$ 13.56	18.24-72.47	4.67-86.03	19.21-84.97
ALT	14.89 $\pm$ 5,88	3.14-26.64	0-32.52	5.24-29.68
GGT	17.11 $\pm$ 4,51	8.10-26.14	3.55-30.67	8.11-27.79

The lowest AST activity on a model of 120 cows was 19.21 U/L, while the highest measured value was 84.97 U/L. The mean value of all obtained data was 45.35 U/L  $\pm$  13.56 (table 2). Alanine aminotransferase activities were in the range of 5.24-29.68 U/L, and mean value was 14.89  $\pm$  5.88 U/L. Activity of GGT was in the range of 8.11-27.79 U/L, and the mean value calculated from 120 dairy cow blood plasma samples was 17.11  $\pm$  4.51U/L.

## 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. Lactation has a great impact on biochemical parameters in the blood of cows, reflecting on metabolic demands. The need for their systematic monitoring and the role of each individual parameter were shown by PAYNE and PAYNE (1987), STOJEVIĆ et al. (2002), KIDA (2003). The activity of aminotransferases in blood is very important. Aminotransferases act as a catalyst in connecting the metabolism of aminoacids and carbohydrates. 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 this research, AST, ALT and GGT activities in dairy cows during lactation and the dry period showed significant variations. The highest AST activity was recorded during early lactation and as lactation progressed the activity of this enzyme decreased. TAINTURER et al. (1984) found that AST activity in dairy cows changes irregularly and occasionally during pregnancy and lactation, but that these changes were not statistically significant. EL-GHOUL et al. (2000) established a significant increase in AST activity 6 weeks before parturition, which is contrary to our finding of lowest activity during the dry period. KANEKO et al. (1997) mention the value of AST activity in cows as  $105 \pm 27$  U/L, while the value in this study was  $43.35 \pm 13.56$  U/L, which is 132% less. In KAUPPINEN's study (1984) values were shown for a group of healthy cows from  $65.05 \pm 31.31$  U/L, which is 43% greater than the values we obtained.

Alanine aminotransferase activity in cows differs during certain production periods. The lowest ALT activity was measured during early lactation, while activity increased in the second and third periods of lactation. In the dry period enzyme activity decreased, but it was still statistically much higher than in the first period of lactation. If we compare the mean value of activity found as  $14.89 \pm 5.88$  U/L in present study with values of  $27 \pm 14$  U/L (KANEKO et al., 1997), activities recorded in this study were 81% lower. Compared to KAUPPINEN's values (1984) of  $17.82 \pm 11.51$  U/L, the recorded values in our test are 20% lower. 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 late lactation (third period) we measured the highest concentration of ALT.

Gamma-glutamyltransferase activity also depended on the observed period. Higher values were measured in early lactation and the dry period, and not from the 46<sup>th</sup> day

of lactation until the dry period. 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. On the total sample of cows our calculated value was  $17.11 \pm 4.52$  U/L. If we compare that value with values obtained by KANEKO et al. (1997)  $15.7 \pm 4.0$  U/L, the activity measured in present study is 8% higher. KAUPPINEN (1984) presented a value of  $15.78 \pm 6.18$  U/L, which is 7% less than in our study.

Seasons, circadian changes, age of the animal and energy status have an influence on tested values. We therefore tried to avoid the impact of these factors as much as possible. Blood was taken always at the same time (morning); all cows were in second lactation and nearly of the same age. Cows were clinically healthy and in good production capability, so we consider that the described changes are a reflection of metabolic events. We can assume that the statistically significant difference of enzyme activity in blood per certain production phase arises from the increased or decreased activity of these enzymes in cells. Because of the large differences found in some studies, and of our own results, we made a sum of activities of tested enzymes throughout all production periods. According to the usual procedure used by other authors, we defined a mean value of  $M \pm 2SD$  and  $M \pm 3SD$  (PAYNE and PAYNE, 1987). We determined referent values in clinically healthy cows from a farm where the research was conducted, with possible variations. These referent values were determined based on a sufficient number of animals under investigation (120) and represent a starting point for a clinical evaluation of the herd's health.

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Received: 23 January 2003

Accepted: 14 January 2005

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**STOJEVIĆ, Z., J. PIRŠLJIN, S. MILINKOVIĆ-TUR, M. ZDELAR-TUK, B. BEER LJUBIĆ: Aktivnost AST, ALT i GGT u klinički zdravih mliječnih krava u laktaciji i suhostaju. *Vet. arhiv* 75, 67-73, 2005.**

**SAŽETAK**

U radu je istražena aktivnost aspartat aminotransferaze (AST; EC 2.6.1.1), alanin aminotransferaze (ALT; EC 2.6.1.2) i gama-glutamilttransferaze (GGT; EC 2.3.2.2) u krvnoj plazmi 120 mliječnih krava holštajnske pasmine podijeljenih u četiri skupine prema proizvodnom razdoblju. Krave su držane u intenzivnom farmskom uzgoju, slobodno bez ispusta. Obrok se sastojao od sjenaže lucerke, silaže, sijena i koncentrata uz energetske i proteinski dodatak. Prvu skupinu činile su životinje od 10. do 45. dana laktacije, drugu od 46. do 90. dana, a treću od 91. dana laktacije do kraja proizvodnje mlijeka. Krave u suhostaju činile su četvrtu skupinu. Najviša aktivnost AST utvrđena je u prvom proizvodnom razdoblju, dok je aktivnost enzima u drugom i trećem razdoblju bila značajno viša u odnosu na suhostaj. Aktivnost ALT pokazala je statistički značajan porast od 46. dana laktacije do suhostaja tako da je aktivnost u drugom i trećem razdoblju bila statistički značajno veća od one u suhostaju. Aktivnosti GGT izmjerene u prvoj fazi laktacije i suhostaju bile su statistički značajno veće od onih u drugom i trećem razdoblju. Rezultati istraživanja pokazali su značajan utjecaj proizvodnje mlijeka i suhostaja na aktivnost istraživanih enzima u krvnoj plazmi pa se na toj osnovi zaključuje o potrebi stalnog praćenja opisanih pokazatelja tijekom proizvodnje.

**Ključne riječi:** AST, ALT, GGT, mliječne krave, krvna plazma, laktacija, suhostaj

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