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SERUM ENZYME ACTIVITIES IN BLOOD AND MILK IN THE DIFFERENT STAGE OF LACTATION IN HOLSTEIN DAIRY COWS

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Abstract: The objective of this study was to determine correlation between serum blood and milk enzyme activities of aspartate-aminotransferase (AST), alanine- aminotransferase (ALT), alkaline-phosphatase (ALP in the 36 dairy Holstein cows divided into three groups according to production period. Group 1 consisted cows in the start of lactation (n = 12); Group 2 -consisted of early lactation cows (n=12) and Group 3 included mid lactation cows (n=15). Statistically significant higher ($P<0.01$) activity of AST in blood serum was established in early lactation groups of cows as compared to mid lactation group of cows. ALT activity showed a lower ($P<0.01$) serum activities in early lactation groups of cows than in the mid lactation cows. Higher values ALP in blood and milk are determined in early lactation groups of cows as compared to mid laltation cows, but without statistical significance ($P>0.05$). Research results showed possibility of mild degree of hepatic lesions, probably due to fat infiltration in early lactation cows. No significant difference ($P>0.05$) was observed in milk serum value for AST, ALT and ALP between the three groups of cows. No significant correlations among AST, ALT and ALP activities in blood and milk serum were determined ($P>0.05$) and shows that activity of these enzyme in the milk are not used as markers for early diagnosis of subclinical metabolic disease.

Keys words: dairy cows, enzymes activities, blood, milk, early lactation periods, mid lactation

Introduction

The metabolic profile, a series of specific blood analytical tests, is routinely used to reveal metabolic problems in dairy cattle (*Oetzel, 2004*, *Stengärde et al*., *2008*; *Gross et al., 2011*). Evaluation of the blood and milk biochemical parameters to assess the animal health and milk yield has always been interested by authors and the various discrepancies have been observed in both blood and milk yield results (*Nozad et al. 2011; Jozwik et al., 2012*). Milk parameters originate from blood and food component and clarifying the appropriate relationships among these parameters individually in food blood and milk are useful in understanding the health and production status in animals (*Jozwik et al.,2012; Liu et al., 2012; 2013*). Major health disorders in high-yielding cows occur around parturition and during lactation. Metabolic conditions of negative energy balance (fasting, parturition and lactation) lead to an increased uncontrolled rate of mobilization of body fat and its increased accumulation in liver cells, resulting in disturbance of the physiological and morphology integrity of the liver (*Vazquez-anon et al.,1994; Overton and Waldron, 2004; Bobe, 2004*). Fatty liver and diffuse infiltration of hepatocytes involve cell membrane damage and hepatocyte destruction accompanied by the release of cytoplasmic enzymes (AST, GGT, LDH), the activity there of in the blood being considerably elevated (*Oezel, 2004; Stojevic et al., 2005; Lubojacka et al., 2005*). Blood serum ALT, AST, ALP and GGT activities were reported to be useful indicator of liver function for postpartum dairy cows (*Bobe, 2004; Stojević et al., 2005*). While little information is available concerning about the activity changes of ALT, AST, GGT and ALP in milk. The activities of these enzymes were monitored in milk and blood serum of cows and results of correlation analysis and regressive models showed a close relation between them (*Liu et al., 2012, 2013; Ghadaa 2014*). More practical attention has been given to detection of enzyme activity in milk and many enzymes have been proposed and listed a reliable markers for early diagnosis of subclinical disease (*Babae et al., 2007; Katsoulos et al., 2010; Liu et al., 2012; 2013).*

The objective of this study was to determine correlation between serum blood and milk enzyme activities in the different stage of lactation in the dairy cows.

Material and methods

Animals, diets and protocol design: A total of 36 dairy cows were randomly selected from the same Holstein herd containing 445 cows (FARM: Šarulja, Knić, Central Serbia). The cows were high-yielding with a preceding lactation of about

8500 L. Three groups of clinically healthy cows were chosen from the herd. Group 1 consisted cows in the start of lactation (n = 12) in period of 5 ± 3 days after calving; Group 2 -consisted of early lactation cows (n=12) in the first month of lactation (22±15 days), and Group 3 included mid lactation cows (n=15) between 90 to 150 days of lactation (133±75 days). The experimental cows were free in open-stall barns. Diet and housing facilities were adapted to research purposes, with diet suited to the energy requirement of late pregnancy, early and mid-lactation cows. Diet in early lactation consisted of of 4 kg grass hay, 10 kg corn silage (30% Dry Matter, DM), 20 kg sweet corn silage, 12 kg beet nodle silage, 4 kg concentrate (18% crude protein, CP) and 1 kg molasses. Diet in mid lactation consisted of 4.5 kg lucerne hay, 19 kg corn silage (30% Dry Matter, DM), 16 kg beet nodle silage, 9 kg concentrate (18% crude protein, CP) and 1.2 kg soybean expeller.

The chemical composition of total mixed rations offered to early lactation and mid lactation dairy cows are given in Table 1.

Table 1. Chemical composition of total mixed rations offered to early lactation and mid lactation dairy cows.

	Early lactation	Mid lactation
Dry Matter (DM) (kg)	15.60	19.58
Net Energy of Lactation (NEL) (MJ)	95.52	128.65
Crude Protein (CP) (% of DM)	11.31	16.88
Rumen undegradable protein (RUP) (% of CP)	33.91	26.33
Fat (% of DM)	3.47	4.68
Fibre (% of DM)	22.17	18.85

Sample collection: Blood and milk samples were taken simultaneously from each lactating cow during morning milking. Blood samples (10 ml) were taken by jugular puncture into a sterile tube from each animal, and the blood serum was separated by centrifugation at room temperature (1,800×g, 15 min). Milk samples were collected in sterile tube and centrifuged at 12,000×g for 30 min at 4°C and the supernatant was transferred into the new sterile tubes. Blood plasma and milk were stored at -20°C until being used for biochemical measurements.

Biochemical analysis: The blood and milk serum activities of aspartate-transaminase (AST) alanine-aminotransferasese (ALT) and alkaline-phosphatase (ALP) were measured in the biochemical laboratory „OXUS“ (Kragujevac, Serbia) by spectrophotometric techniques using a BT 1000, (Biotehnica Italia) and the corresponding commercial kits (DIALAB, YUNICOM).

Statistical analysis: The statistical analysis of the obtained data was carried out by ANOVA-procedure (Statgraphic Centurion, Statpoint Technologies Inc. Warrenton, Va, Virginia, USA). The analysis of variance were used to evaluate the

probability of the significance of the statistical differences between mean serum enzyme activities in each group and the Pearson test was performed for evidencing significant correlations. Differences were considered as significant when P values were below 0.05 or 0.01.

Results and 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 (*Oezel, 2004; Overton and Waldron, 2004*). The present study compared the serum enzyme activities in blood and milk serum in dairy cows during early and mid-lactation period.

The results of the serum blood and milk activities of AST, ALT, ALP in cows in the early and mid-lactation period and correlations among blood an milk serum enzyme activities are given in Tables 2 and 3.

Table 2. Blood and milk serum enzyme activities in the start of lactation (Group 1), early (Group 2) and mid lactation (Group 3) dairy cows (n=12 in each group). Results are expressed as mean ± standard deviation (SD).

	Group 1	Group 2	Group 3
AST (blood) IU/l	90.81±21,98 ^{A}	84.18±16.19 ^{A}	59.72± 10.95 ^{B}
ALT(blood) IU/l	28.00±8.46 ^{A}	28.54 ±3.96 ^{A}	36.45±9.62 ^{B}
ALP(blood) IU/l	162.36±193.25 ^{a}	117.64±22. 28 ^{a}	96.81 ±31.94 ^{a}
AST (milk) IU/l	33.82±23.76 ^{a}	33,27±9.65 ^{a}	25.36±11.87 ^{a}
ALT(milk) IU/l	20.05±12.47 ^{a}	20.27±14.66 ^{a}	29.55±19.83 ^{a}
ALP(milk) IU/l	199.23±186.23 ^{a}	241.11±109.31 ^{a}	121.64±32.56 ^{a}

Legend: Mean values within a row with no common superscript differ significantly, values marked by small letter differ significantly ($p < 0.05$); values marked by capital letter differ high-significantly ($P < 0.01$).

Table 3. Correlation coefficients among the biochemical parameters in the blood and milk calculated for all cows in the present study.

	AST (blood)	ALT (blood)	ALP (blood)
AST (milk)	$r=0.14^{NS}$	$r=0.16^{NS}$	$r=-0.09^{NS}$
ALT(milk)	$r=-0.23^{NS}$	$r=-0.03^{NS}$	$r=0.17^{NS}$
ALP(milk)	$r=-0.06^{NS}$	$r=0.11^{NS}$	$r=0.15^{NS}$

Legend: NS - non-significant ($p>0.05$)

Lactation has a great impact on biochemical parameters in the blood of cows, reflecting on metabolic demands. The activity of AST in blood is very important.

AST act as a catalyst in connecting the metabolism of amino-acids 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. AST is considered as the most sensitive indicator in the diagnosis of fatty liver in cows (*Pechova et al., 1997; Bobe, 2004; Lubojacka et al., 2005; Stojević et al., 2005*). In this study, statistically significant higher ($P<0.01$) activity of AST in blood serum was established in early lactation groups of cows as compared to mid lactation groups of cows No significant difference ($P>0.05$) was observed in milk serum value for AST between the three groups of cows.

ALT 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. The author considers that the role of ALT in predicting liver damage in ketosis is not significant (*Tainturier et al., 1984; Stojević et al., 2005*). Our results confirm this because in the period of mid lactation (third period) we measured the highest ($P<0.01$) concentration of ALT. No significant difference ($P>0.05$) was observed in milk serum value for ALT between the three groups of cows.

ALP are used as biochemical marker in diagnosis of osteoporosis, hepatobiliar disease and fatty liver in the dairy cows. The activity of ALP in blood serum are increased in periods from puerperium to mid lactation in the dairy cows, especially in cows with liver lipidosis. (*Bobe et al.,2004; Stojevic et al., 2005*). In this study, higher values ALP in blood and milk are determined in early lactation groups of cows as compared to mid laltation cows, but without statistical significance ($P>0.05$) as consequence high individual variabilites. On the basis changes in blood and milk AST, ALT and ALP activities in the different stage of lactation, our result suggested that early lactation cows had mild degree of hepatic lesions, probably due to fat infiltration.

Significant correlations among AST, ALT and ALP activities in blood an milk serum are not determined ($P>0.05$) in this study (Table 3), and shows that activity of these enzyme are not used as markers for early diagnosis of subclinical disease. These results are in opposite with results (*Liu et al., 2012; 2013; Ghadaa 2014*), who found a strong correlation between them. Further investigations will confirm or not these statements.

Conclusion

Biochemical examination of blood serum showed higher activities of AST (P<0.05) and ALP (P>0.05), in groups of early lactation cows, and lower activity of ALT (P>0.05) compared to the group of mid lactation cows. No significant difference (P>0.05) was observed in milk serum value for AST, ALT and ALP between the three groups of cows. On the basis of changes in blood AST, ALT and ALP activities in the different stage of lactation, our result suggested that early lactation cows had mild degree of hepatic lesions, probably due to fat infiltration. No significant correlation among AST, ALT and ALP activities in blood an milk serum are determined (P>0.05), showing that activity of these enzyme in the milk are not used as markers for early diagnosis of subclinical metabolic disease.

Serumske enzimske aktinosti u krvi i mleku u različitim stadijumima laktacije

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Rezime

Cilj ovog rada je bio da se utvrde korelacije između serumskih aktivnosti aspartat-amino transferaze (AST), alanin-aminoreasferaze (ALT) i alkalne-fosfataze (ALP)) u krvi i mleku kod 36 mlečnih krava rase Holštajn, koje su podeljene u tri grupe u zavisnosti od laktacionog perioda. Grupu 1 (n=12) činile su krava na samom početku laktacije, Grupu 2 (n=12) krave u ranoj laktaciji i Grupu 3 (n=12) krave na sredini laktacije. Statistički značajno (P<0,01) veće aktivnosti u krvnom serumu AST su utvrđene kod grupa krava u ranoj laktaciju u odnosu na grupu krava tokom sredine laktacije. ALT aktivnosti u krvnom serumu su bile značajno manje (P<0,01) kod grupa krava na početku laktacije u odnosu na grupu krava na sredini laktacije. Najviše vrednosti za aktivnosti ALP u krvi su utvrđene kod grupa krava u ranoj laktaciji u odnosu na grupu krava u sredinu laktacije, ali bez statističke značajnosti (P>0,05). Dobijeni rezultati ukazuju na mogućnost pojave masne infiltracije jetre blagog stepena kod grupa krava na početku laktacije. Nisu utvrđene stistički značajne razlike (P>0.05) za aktivnosti AST,ALT i ALP u mleku između ispitivanih grupa krava. Nisu utvrđene statistički značajne korelacije (P>0.05) između aktivnosti AST, ALT i ALP u krvi i mleku, što ukazuje da

aktivnosti ovih enzima u mleku se ne mogu koristiti kao markeri za ranu dijagnostiku subkliničnih metaboličkih oboljenja.

Ključne reči: mlečne krave, enzimske aktivnosti u krvi i mleku, rana laktacija, sredina laktacije

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