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RELATIONSHIPS BETWEEN SERUM ENZYME ACTIVITES IN THE MILK AND BLOOD IN DAIRY COWS DURING DIFFERENT STAGE OF LACTATION PERIOD

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Abstract: This study aimed to determine blood and milk enzyme activites as indicators of liver function and their correlations in dairy cows during different stage of lactation period. Blood and milk samples were collected from 100 Holstein dairy cows during morning milking. The cows were allocated to four groups according to the production period, including cows in early (n = 18), full (n = 26), mid (n = 25) and late (n = 31) lactation. The value of serum enzyme activites of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and lactate dexydrogenase (LDH) in the blood and milk were determined. The activities of blood and milk serum AST, ALT, ALP and LDH were significantly higher (P< 0.01) in early and full lactation cows than in the other two groups of cows, suggesting mild fat infiltration of liver cells. The enzyme activities in milk were positively correlated with those in the blood (P< 0.01). In conclusion, similar changes in blood and milk serum enzyme activites during lactation and milk to blood correlations confirm that milk has great potential in predicting of blood metabolites and metabolic status of cows.

Keywords: cows; blood, milk, enzyme activites, lactation

Introduction

The metabolic profile, a series of specific blood analytical tests, is routinely used to reveal metabolic problems in dairy cattle (Oetzel, 2004; Stengarde et al., 2008; Gross et al., 2011). Evaluation of the blood and milk biochemical

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parameters to assess the animal health and milk yield has always been interesed 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; Ghadaa, 2014).

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 (Jorritsma et al., 2001; Overton and Waldron, 2004; Bobe et al., 2004).

Fatty liver and diffuse infiltration of hepatocytes involve cell membrane damage and hepatocyte destruction accompanied by the release of cytoplasmic enzymes (ALT, AST, GGT, LDH), the activity there of in the blood being considerably elevated (Oezel, 2004; Stojevic et al., 2005; Lubojacka et al., 2005; Djokovic et al., 2018; 2019).

Blood plasma and serum ALT, AST, ALP and GGT activities were reported to be useful indicator of liver function for postpartum dairy cows (Bobe et al, 2004; Stojevic et al., 2005 Mordak et al.,2020). 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 sreliable markers for early diagnosis of subclinical desease (Babaei et al., 2007; Katsoulos et al., 2010; Djokovic et al., 2019).

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

Materials and methods

Animals and study design

A total of 100 dairy cows were randomly selected from the same Holstein herd containing 1200 cows (FARM: Kraisnik, Backa Palanka, Vojvodina, Serbia).

Clinically healthy cows were allocated to four experimental groups: Group 1 – early lactation cows (n = 18), from 1 to 49 days of lactation; Group 2 – full lactation cows (n=26), from 50 to 109 days of lactation; Group 3 – mid-lactation cows (n=25), between 110 and 209 days of lactation; and Group 4 – late lactation cows (n=31), from 210 to 305 and more days of lactation. The cows were high-yielding, aged 4 years on average (with an average of 2.7 lactations), with a preceding lactation of about 8500L (average weekly yield was 26.5 L/cow/day). The average body condition score (BCS) was 3.36±0.55 for all experimental cows. The experimental cows were housed in free-stall barns. Diet and housing conditions were adapted to the purposes of the experiment, with diet tailored to the cows' energy requirements during different periods of lactation using NRC standards (NRC, 2001).

Blood analysis

Blood samples were taken 4 to 6 h after milking and feeding from the coccygeal vein into evacuated serum separator tubes. After clotting for 3 h at 4 °C and centrifugation (1500 G, 10 min), blood sera were analyzed for the following biochemical parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and lactate dexydrogenase (LDH), which were determined by colorimetric kits (Biosystem, Spain and Randox, Carlisle, UK) and a Chemray spectrophotometer (Rayto, Shenzhen, China).

Milk analysis

Milk samples were collected during morning milking into tubes with and without additives on the same day blood was sampled. Upon serum separation, milk was subjected to biochemical tests for the determination of the enzymes (AST, ALT, ALP, GGT, LDH). Milk serum was separated after centrifugation at 10,000g for 30 minutes, and was transferred to new tubes for analysis. The biochemical reagents and apparatus used for milk serum analysis were the same as for blood serum. All analyses were performed at the Laboratory of Pathophysiology, Department of Veterinary Medicine, University of Novi Sad.

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 effect of lactation period on blood and milk serum enzyme activites was examined by an ANOVA analysis coupled with an LSD post hoc

test. Associations between milk and serum enzyme activites were determined by Pearson's coefficient of correlation. The SPSS statistics software (IBM, USA) was used.

Results and discussion

The peripartal and early lactation periods were considered as time periods that have the potential to enhance lactation performance. Modern dairy farming often results in forced milk production, giving rise to metabolic disorders in cows. In order to predict such disorders and related subclinical diseases, it is necessary to establish the physiological ranges of biochemical parameters in a clinically healthy herd (Reist et al., 2002; Radostits et al., 2000; Pires et al., 2022).

Lactation period showed a statistically significant influence on blood and milk biochemical parameters. In most cases in this experiment, blood and milk serum enzyme activites during early lactation were significantly different from those in the other periods of lactation (Tables 1 and 2).

Table 1. Blood metabolites in early (Group 1), mid (Group 2), full (Group 3) and late (Group 4) lactation dairy cows. Results are expressed as mean ± standard deviation (SD).

	Group 1	Group 2	Group 3	Group 4		
AST (IJ/L)	134.8±37.3	100.9±30.1	99±37	95.2±31.7	P<0.001	1:2,1:3,1:4
ALT (IJ/L)	59.16±19.87	36.00±9.46	28.60±5.63	28.64±6.85	P<0.001	1:2,1:3,1:4, 2:3,2:4
LDH (IJ/L)	1795±942	1647±329	1389±224	1312±285	P<0.001	1:3,1:4, 2:4
ALP (IJ/L)	108.17±27.9	85.88±16.7	75.84±15.8	71.00±18.3	P<0.001	1:2,1:3,1:4
GGT (IJ/L)	24.61±6.1	21.19±6.1	23.24±10.8	27.19 ±6.6	P<0.05	2:4

ST – aspartate aminotransferase; ALT – alanine aminotransferase; ALP – alkaline phosphatase; GGT – gamma-glutamyl transferase; LDH – lactate dexydrogenase.

Table 2. Milk metabolites in early (Group 1), mid (Group 2), full (Group 3) and late (Group 4) lactation dairy cows. Results are expressed as mean ± standard deviation (SD).

	Group 1	Group 2	Group 3	Group 4		
AST (IJ/L)	125.7±71.7	62.3±35.1	59.1±32.4	69.6±58.1	P<0.001	1:2,1:3,1:4
ALT (IJ/L)	54.8±24.9	36.3±11.2	28.3±6.9	28.4±7.03	P<0.001	1:2,1:3,1:4, 2:3,2:4
ALP (IJ/L)	947.1±543.3	629.5±338.5	568.2±325.8	670.8±358.7	P<0.05	1:2,1:3,1:4, 2:4,3:4
GGT (IJ/L)	561.9±217.1	446.9±161.7	571.3±325.8	670.8±135.9	P<0.01	2:3,2:4
LDH (IJ/L)	316.4±156.4	205.6±132.6	200.6±123.2	213.6±117.1	P<0.05	1:2,1:3,1:4

AST – aspartate aminotransferase; ALT – alanine aminotransferase; ALP – alkaline phosphatase; GGT – gamma-glutamyl transferase; LDH – lactate dexydrogenase.

Table 3 shows the coefficients of correlation between blood and milk biochemical parameters calculated for all cows in this experiment.

Table 3. Correlation between milk composition and diagnostic blood metabolites

	Milk AST	Milk ALT	Milk ALP	Milk GGT	Milk LDH
Blood AST	**0.450****	0.185	0.035	-0.115	0.029
Blood ALT	**0.266****	**0.649****	**0.262****	-0.004	0.161
Blood LDH	0.06	**0.347****	0.073	-0.135	0.116
Blood ALP	**0.399****	**0.492****	**0.343****	0.01	**0.338****
Blood GGT	0.036	0.107	0.163	**0.211***	-0.017

* – statistically significant correlation (P< 0.05); **– highly significant correlation (P< 0.01)

During the first month of lactation, 5–10% of high-yielding dairy cows suffer from severe hepatic lipidosis and 30–40% of cows develop mild hepatic lipidosis (Bobe et al., 2004), which indicates that almost 50% of these cows are at risk for metabolic disorders. Fatty infiltration of the liver causes lesions in the hepatic tissue and a general increase in the levels of the enzymes indicating

hepatocyte injury, i.e. AST, GGT, and GLDH (Pechova et al., 1997; Lubojacka et al., 2005; Stojevic et al., 2005; Le Blanc, 2010; Djokovic et al., 2019; Mordak et al., 2020).

In this experiment, the activities of blood and milk serum AST, ALT, ALP and LDH were significantly higher ($P< 0.01$) in early and full lactation cows than in the other two groups of cows, suggesting fat infiltration of liver cells and a release of these enzymes in circulation as induced by lipomobilization. Changes in blood and milk AST, ALT, ALP, LDH and GGT activities at different lactation stages indicated a mild degree of hepatic lesions in early lactation cows, probably due to fat infiltration.

This statement was confirmed by strong significant positive correlations between blood and milk serum AST ($r= 0.450$; $P< 0.01$), ALT ($r= 0.649$; $P< 0.01$), ALP ($r= 0.344$; $P< 0.01$) and GGT ($r= 0.211$; $P< 0.05$) activities in this study (Table 3).

These results are supported by the reports of other authors (Liu et al., 2012, 2013; Ghadaa, 2014; Djokovic et al., 2018; Benedet et al., 2019), who showed that milk enzyme activities can be good indicators of lipid mobilization and ketogenesis in cows during lactation for early detection of subclinical disease. The high correlation coefficient of the work agrees with the finding of Liu et al., (2012), and the high significance arises due to the large number of samples examined in this experiment. In the experiment in ewes, the relations between indicators of milk composition, milk production and blood indicators as well as their mutual connections indicate the justification of using the analysis of liver status indicators in ewes (Antunovic et al., 2022).

Conclusion

Based on changes in blood and milk serum enzyme activites metabolites at different stages of lactation, the results of this study indicated a mild degree of hepatic lesions in early lactation cows, probably due to fat infiltration. The results showed that changes in blood and milk serum enzyme activites over the entire course of lactation in dairy cows and their relationships can be used to monitor metabolic status in dairy cows at the herd level, and can serve as excellent milk biomarkers for the early detection of subclinical metabolic disease.

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