Relationships Between Contents of Biochemical Metabolites in Blood and Milk in Dairy Cows During Transition and Mid Lactation

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ABSTRACT

The objective of this study was to determine blood and milk metabolites and their inter-relationships to evaluate the metabolic status in dairy cows for early diagnosis of subclinical metabolic disease at different stages of lactation. For this study, blood and milk samples were taken from 36 Holstein dairy cows during morning milking. The cows were divided into three groups according to the production period.

- Group 1 consisted of cows in late pregnancy (n = 12)
- Group 2 comprised early lactation cows (n=12)
- Group 3 included mid-lactation cows (n=12).

From late pregnant (Group 1) cows' milk samples were collected after calving. The concentrations of glucose, triglycerides (TG), total cholesterol (TChol), total protein (TP), albumin, urea, calcium (Ca), magnesium (Mg) and inorganic phosphorus (iP), and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and

alkaline phosphatase (ALP) in the blood and the contents of fat, protein, urea, Ca, iP, Mg, and activities of AST, ALT and ALP in milk were determined. Statistically significant differences *(P < 0.05)* were observed in blood TG, TChol, urea, AST, ALT, and ALP levels and milk fat and urea concentrations among experimental groups of cows. Significantly negative correlations *(P < 0.05)* were found between TG in blood and fat (r=-0.525) and urea (r=-0.711) in milk; and between TP in blood and urea in milk (r=-0.366). Significantly positive correlations *(P < 0.05)* were observed among TG in blood and Ca (r=0.403), Mg (r=0.430), iP (r=0.353), protein (r=0.381) and ALT (r=0.508) in milk; TChol in blood and fat (r=-0.362) in milk; albumin in blood and ALT (r=0.390) in milk; activity of AST in blood and fat (r=0.429) and urea (r=0.455) in milk. Based on changes in blood and milk metabolites at different stages of lactation, the present results suggested that early lactation cows showed a mild degree of hepatic lesions, probably due to fat infiltration. The results showed that changes in blood and milk metabolite levels and their interrelationships can be helpful in the herd monitoring of metabolic status in dairy cows and for the early diagnosis of subclinical metabolic disease.

INTRODUCTION

A metabolic profile, a series of specific blood analytical tests, is routinely used to reveal metabolic problems in dairy cattle. [1,2] Evaluation of blood and milk biochemical parameters to assess animal health and milk yield has always attracted attention, and various discrepancies have been observed in both blood and milk yield results.[3,4,5] Milk parameters originate from blood and feed components, and clarifying appropriate relationships among these parameters individually in feed, blood, and milk is useful in understanding the health and production status of animals.[5] The metabolic profile test is known as an applicable approach in assessing blood biochemical parameters, such as glucose, protein, urea, TG, cholesterol, BHB, and macro-minerals.

The same test is used for milk parameters including protein, fat, urea, lactose, and macro-minerals as major animal products.[5,6,7] Major health disorders in high-yielding cows occur around parturition and during lactation (ketosis, fatty liver, puerperal paresis, etc). Fatty liver and diffuse infiltration of hepatocytes involve cell membrane damage and hepatocyte destruction accompanied by the release of cytoplasmic enzymes (AST, GGT, LDH), which activity results in the blood being considerably elevated.[8,9,10,11] Blood serum ALT, AST, ALP, and GGT activities were reported to be useful indicators of liver function for postpartum dairy cows , while little information is available concerning changes in ALT, AST, GGT, and ALP activities in milk.[12] The activities of these enzymes were monitored in milk and blood sera of cows and the results of correlation analysis and regressive models showed a close relationship between them.[3,4,12] More practical attention has been given to the detection of enzyme activity in milk, and many enzymes have been proposed and listed as reliable markers for early diagnosis of subclinical disease.[13,14]

The objective of this study was to determine blood and milk metabolites and their interrelationships to evaluate the metabolic status of dairy cows for early diagnosis of subclinical metabolic disease at different stages of lactation.

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: Sarulja, Kragujevac, Central Serbia). Three groups of clinically healthy cows were chosen from the herd.

- Group 1 consisted of late pregnant cows (n = 12) from 30 to 1 day (25 ±15) to partus
- Group 2 comprised early lactation cows (n=12) in the first month of lactation (22±12 days)
- Group 3 included mid-lactation cows (n=15) between 90 to 150 days of lactation (133±75 days).

Table 1. *Chemical composition of total mixed rations offered to late pregnant, early lactation and mid-lactation dairy cows.*

	Late pregnancy	Early lactation	Mid lactation
Dry Matter (DM) (kg)	12.85	15.60	19.58
Net Energy of Lactation (NEL) (MJ)	60.94	95.52	128.65
Crude Protein (CP) (% of DM)	8.25	11.31	16.88
Rumen undegradable protein (RUP) (% of CP)	28.86	33.91	26.33
Fat (% of DM)	2.41	3.47	4.68
Fiber (% of DM)	34.16	22.17	18.85

The cows were high-yielding with a preceding lactation of about 8,500 L (late pregnant cows: 8325 ± 795 L, early lactation cows 8458 ± 920 L, and mid-lactation cows: 8677 ± 1055 L). The experimental cows were housed in open-stall barns. Diet and housing facilities were adapted to research purposes, with diet suited to the energy requirement of late-pregnant, early lactation, and mid-lactation cows. Diet for late-pregnant cows included 7 kg grass hay, 5 kg corn silage (30% Dry Matter, DM), 4 kg sweet corn silage, 6 kg beet noodle silage, 5kg straw, and 1 kg concentrate (18% crude protein, CP). Diet for early lactation cows consisted of 4 kg grass hay, 10 kg corn silage (30% Dry Matter, DM), 20 kg sweet corn silage, 12 kg beet noodle silage, 4 kg concentrate (18% crude protein, CP), and 1 kg molasses. Diet for mid-lactation cows contained 4.5 kg alfalfa hay, 19 kg corn silage (30% Dry Matter, DM), 16 kg beet noodle silage, 9 kg concentrate (18% crude protein, CP), and 1.2 kg soybean expeller. The chemical composition of total mixed rations offered to late-pregnant, early lactation, and mid-lactation dairy cows is shown in Table 1.

Sample Collection

Blood and milk samples were taken simultaneously from each lactating cow during morning milking. From late pregnant (Group 1) cows, milk samples were collected in the period of 5 ± 3 days after calving. Blood samples (10 mL) were taken by jugular vein puncture into sterile tubes from each animal, and blood serum was separated by centrifugation at room temperature (1,800×g, 15 min). Blood samples collected into fluoride-containing tubes were immediately centrifuged in the same manner, and plasmas were assessed for glucose concentrations. Milk samples were centrifuged at 12,000×g for 30 min at 4°C, and the supernatant was transferred into new sterile tubes. Blood and milk sera were stored at -20°C until biochemical analysis.

Biochemical Analysis of Blood

The levels of glucose, triglycerides (TG), total cholesterol (TCh), total proteins (TP), albumin, urea, and serum aspartate transaminase (AST) alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured in the OXUS biochemical laboratory (Kragujevac, Serbia) by spectrophotometric techniques using a BT 1000 spectrophotometer (Biotehnica Italia) and the corresponding commercial kits (DIALAB, YUNICOM). The concentrations of Ca and Mg were determined by atomic absorption spectrophotometry (AAS) and QP-11.09 methods, and iP levels were measured by QP-11.55 methods using a BT 1000 spectrophotometer, Biotehnica, Italy.

Biochemical Analysis of Milk

The content of fat in milk was determined by the acidobutrics method (Gerber), total protein by volumetric method according to Kjeldahl (QP-11.52), and urea concentration by spectrophotometry (QP-11.50). Milk

Table 2. *Blood metabolites in late pregnant (Group 1), early lactation (Group 2) and mid-lactation (Group 3) dairy cows (n=12 per group). Results are expressed as mean ± standard deviation (SD).*

Variables	Group 1	Group 2	Group 3
Glucose(mmol/L)	3.17 ±0.32[a]	2.83±0.53[a]	3.00±0.28[a]
TG(mmol/L)	0.13 ±0.05[A]	0.03±0.03[B]	0.04±0.04[B]
TChol. (mmol/L)	3.63±1.83[A]	2.93 ±0.59[A]	5.93±1.17[B]
TP(g/L)	73.10 ±6.23[a]	68.36±7.62[a]	72.36±5.5[a]
Albumin(g/L)	35.09± 2.25[a]	32.64±4.46[a]	34.63±2.61[a]
Urea (mmol/L)	4.81±1.34[a]	3.67 ±0.71[b]	4.70±1.09[a]
AST (IU/l)	59.72± 10.95[A]	90.81±21.98[B]	84.18±16.19[B]
ALT (IU/l)	28.54 ±3.96[A]	28.00±8.46[A]	36.45±9.62[B]
ALP (IU/l)	96.81 ±31.94[a]	117.64±22. 28[a]	162.36±193.25[a]
Ca (mmol/L)	2.04± 0.17[a]	1.95±0.15[a]	1.99±0.19[a]
iP (mmol/L)	2.33± 0.30[a]	2.13±0.26[a]	2.13±0.13[a]
Mg (mmol/L)	1.02 ±0.14[a]	1.03±0.29[a]	1.20±0.17[a]

Mean values within a row with no common superscript differ significantly; values followed by small letters differ significantly (P < 0.05); values marked with capital letters differ highly significantly (P < 0.01).

serum activities of AST, ALT, and ALP were measured by spectrophotometric techniques using a BT 1000 spectrophotometer (Biotehnica Italy). The concentrations of Ca and Mg in milk were determined by atomic absorption spectrophotometry (AAS) (QP-11.09 method), and iP was evaluated by the QP-11.55 method using a BT 1000 spectrophotometer, Biotehnica Italy.

Statistical Analysis

The statistical analysis of the obtained data was carried out by ANOVA-procedure (Statgraphic Centurion, Statpoint Technologies Inc.Warrenton, VA, USA). The analysis of variance was used to evaluate the probability of the significance of statistical differences between mean blood and milk metabolites and 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

The present study compared organic and inorganic parameters in blood and milk sera and their interrelationships in dairy cows. Results of blood biochemical metabolites for all groups of cows are shown in Table 2.

Biochemical testing for metabolites in the blood serum showed significantly lower values *(P<0.05)* of TG and urea in early and mid-lactation cows than in late-pregnant cows. In peripartal cows, serum concentrations of TChol. were statistically lower *(P<0.01)* than in mid-lactation cows. In addition, serum AST activity was significantly higher (P < 0.01) in lactating cows than in late-pregnant animals. The highest ALP activity in the blood serum was determined in mid-lactation cows, and this value was significantly higher *(P < 0.05)* than in peripartal cows. No significant difference *(P > 0.05)* was observed in the blood values of glucose, TP, albumin, ALP, Ca, Mg, and iP among experimental cows. The results of milk biochemical analysis for all groups of cows are given in Table 3.

Fat contents and urea concentrations in milk were statistically lower *(P<0.05)* in cows in very early lactation (Group 1) compared to the other two groups of lactating cows. Milk enzyme activities showed no statistical difference (P>0.05) across experimental groups of cows as the result of

Table 3. *Milk metabolites in experimental groups of lactating dairy cows (n=12 per group). Results are expressed as mean ± standard deviation (SD).*

Variables	Group 1	Group 2	Group 3
Fat (%)	3.52±0.28[a]	3.80±0.04[b]	3.86±0.03[b]
Protein (%)	3.67±2.72[a]	3.39±0.99[a]	3.52±0.39[a]
Urea (mmol/L)	4.55±0.67[a]	5.68±0.36[b]	5.75±0.32[b]
AST (IU/L)	33.82±23.76[a]	33.27±9.65[a]	25.36±11.87[a]
ALT (IU/L)	29.55±19.83[a]	20.27±14.66[a]	20.05±12.47[a]
ALP (IU/L)	199.23±186.23[a]	121.64±32.56[a]	241.11±109.31[a]
Ca (g/kg)	1.44±0.61[a]	1.21±0.21[a]	1.16 ± 0.20[a]
iP (g/kg)	1.20±0.36[a]	1.05±0.21[a]	1.06±0.15[a]
Mg (g/kg))	0.119± 0.06[a]	0.097±0.019[a]	0.088±0.010[a]

Mean values within a row with no common superscript differ significantly; values marked with small letters differ significantly (P < 0.05); values followed by capital letters differ highly significantly (P < 0.01).

high variability of individual enzymes. No significant difference (P>0.05) was observed in milk levels of protein, Ca, iP, and Mg among the experimental groups of cows.

Correlations between blood and milk biochemical metabolites for all cows in this experiment are given in Table 4, which shows coefficients of correlation among biochemical parameters calculated for all cows in this experiment. Significantly negative correlations *(P < 0.05)* were observed between blood TG and milk fat (r=-0.525) and urea (r=-0.711) levels, and between blood TP and milk urea (r=-0.366) concentrations. Significantly positive correlations *(P < 0.05)* were determined between blood TG and milk Ca (r=0.403), Mg (r=0.430), iP (r=0.353), protein (r=0.381), and ALT (r=0.508) levels, between blood TChol. and milk fat (r=-0.362), between blood albumin and milk ALT (r=0.390), and between AST activity in the blood and milk fat (r=0.429) and urea (r=0.455) levels.

DISCUSSION

Modern milk production often puts the production capabilities of cows at risk, which can result in metabolic disorders. In order to

Table 4. *Correlation coefficients for biochemical metabolites in milk and blood calculated for all cows in the present study.*

	Ca	Mg	iP	fat	urea	protein	AST	ALT	ALP
Glucose	0.216	0.194	0.121	-0.224	-0.318	0.155	-0.036	0.193	0.176
TG	**0.403**	**0.430**	**0.353**	**-0.525**	**-0.711**	**0.381**	**0.303**	**0.508**	**-0.077**
TCho.	-0.125	-0.189	0.029	**-0.362**	0.406	-0.113	-0.330	-0.264	0.238
TP	0.091	0.087	0.179	-0.186	**-0.366**	0.113	0.087	0.119	-0.079
albumin	0.302	0.145	0.102	-0.189	-0.164	0. 107	0.175	**0.390**	0.085
urea	0.075	0.009	-0.003	0.236	0.342	-0.041	0.095	0.134	-0.009
AST	0.014	-0.024	-0.012	**0.429**	**0.455**	-0.065	0.135	0.158	-0.089
ALT	0.026	-0.010	0.114	0.096	0.023	0.066	-0.229	-0.028	0.171
ALP	0.050	0.014	0.019	0.107	0.220	-0.008	0.096	0.115	0.153
Ca	0,1074	0,0610	0,0061	-0,1308	-0,1127	0,0862	-0,0617	0,1028	0,3265
Mg	0,003	-0,173	-0,099	0,092	0,237	-0,170	-0,157	-0,105	0,095

Significant correlations (P < 0.05) are presented in bold.

predict such disorders and eventual subclinical diseases, it is necessary to determine physiological ranges of biochemical parameters in a clinically healthy herd.[1,2,15]

In the present study, glycemia measured both in pregnant females and in lactating cows was within the physiological limits, e.g., from 2.5 to 4.2 mmol/L.[16] Nevertheless, glycemia was depressed in puerperal cows compared to pregnant and mid-lactation cows. This decrease in glucose concentrations previously reported in different studies[6,10,17,18] may be related to the sudden activity of the mammary gland and increased lactose synthesis.

On the other hand, significant decreases *(P<0.01)* in serum TG concentrations were observed in lactating cows compared to late-pregnant females. Other biochemical parameters, at least partially synthesized in the liver, such as glucose, cholesterol, albumin, urea, and total proteins, were also decreased during the puerperal period. These results suggested an increased accumulation of TG and TChol. in hepatocytes in puerperal cows, which was probably associated with depleted liver synthesis of VLDLs. [19,20,21,22,23]

It was reported that, in cases of liver cell damage,[10,11,21] the parameters of nitrogen metabolism, including uremia, proteinemia and albuminemia, declined. Although the values of these three parameters measured in dairy cows during the transitional and mid-lactation period in the present study were within the physiological limits (60–80 g/L for proteinemia, 30–40 g/L for albuminemia and 1.66–6.66 mmol/L for uremia,[16] they were lowered in puerperal cows compared to pre-parturient and mid-lactation females, which confirms the reduction of liver synthesis induced by the development of fatty infiltration in the liver.[10,11,22,23,24]

Lactation has a great influence on blood biochemical parameters in cows, thus affecting metabolic demands. The activity of AST in blood is of high importance. AST acts 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 an indicator of cell structure damage. AST is considered as the most sensitive indicator in the diagnosis of fatty liver in cows.[3,8,11,12] In this study, a significantly higher *(P<0.01)* activity of AST in blood serum was established in early lactation cows compared to late-pregnant and mid-lactation cows. No significant difference (P>0.05) was observed in milk serum values of AST among the three groups of cows.ALT activity in cows differed across production periods.

The lowest ALT activity in the blood serum was measured during transition, while it increased in mid-lactation. In the dry period, the enzyme activity decreased, but it was still statistically much higher than in the first period of lactation. Authors observed that the role of ALT in predicting liver damage in ketosis is not significant.[3,4,8,11,25] This was confirmed by the present research in which the highest *(P<0.01)* concentration of ALT was measured in mid-lactation. No significant difference (P>0.05) was determined in milk serum ALT levels among the three groups of cows.

ALP is used as a biochemical marker in diagnosing osteoporosis, hepatobiliary disease, and fatty liver in dairy cows. The activity of ALP in the blood serum increased during the period from puerperium to mid-lactation in dairy cows, especially in cows with liver lipidosis.[11,12, 25] In this study, higher values of ALP in blood and milk sera were determined in mid-lactation cows compared to the other two groups of cows, but without statistical significance (P>0.05) due to high individual variability.

Based on changes in blood and milk AST, ALT, and ALP activities at different stages of lactation, the present results suggested that early lactation cows showed a mild degree of hepatic lesions, probably due to fat infiltration. No significant correlations among AST, ALT, and ALP activities in blood and milk sera were determined

(P>0.05) in this study (Table 4), These results are inconsistent with the findings of other authors.[3,4,12] who found a strong correlation.

No significant difference *(P > 0.05)* was observed in the blood and milk values of Ca, Mg, and iP among experimental groups of cows. Milk Ca and iP output are directly related to milk yield, as milk iP concentration is constant.[26,27] In fact, as milk production increases, more minerals from the ingested amount are transferred to milk and less are excreted with feces. The decrease in iP and Mg concentrations in mature milk are likely in part due to dilution resulting from increased milk production.[26,27]

Stage of lactation significantly influences the composition of raw milk of dairy cows.[21,28] Factors predisposing to general or mammary gland diseases should be evaluated in combination with major milk components (fat and protein) by measuring urea in milk as indicators of a balanced diet.[29]

In this study, fat contents and urea contents in milk were statistically lower *(P<0.05)* in very early lactation cows (Group 1) compared to the other two groups of lactating cows. No significant difference *(P > 0.05)* was observed in milk protein levels among the experimental groups of cows. Milk fat can increase or decrease depending on ration composition. Early lactation cows have a tendency to mobilize body reserves while ingesting rations that are low in effective fiber will tend to decrease milk fat levels.[21,29,30] Lower milk fat content is frequently used on farms as an indicator of sub-acute ruminal acidosis and to predict the effectiveness of diet structure for chewing. [5,29,31] Low milk fat content is caused by a lack of major precursor, acetic acid in rumen, which is produced in insufficient quantity.[15,21,30] Generally, the increase in milk protein content and decrease in milk fat content lead to sub-clinical acidosis.[15]

The level of urea in milk is an indicator of metabolic nitrogen balance of cows,[32,33] which characterizes their health and reproductive ability. The obtained results on milk urea were within physiological limits (2-6 mmol/l) in all groups of cows.[31] Among the nutritional factors, the energy to protein ratio is the most important in cow rations. Urea concentration increases with increasing intake of rumen degradable protein, but also when energy in rations is lacking, since no optimal amount of protein can be utilized from the ration due to decreased activity of rumen bacteria. As the feed energy supply increases, the concentration of urea in milk decreases.[31,32,33]

At the very beginning of lactation (Group 1), milk urea and fat levels were significantly lower *(P<0.05)* than in the other two lactation periods due to decreased energy supply through diet in the peripartal period, as well as due to increased lipomobilization from body reserves. The significant correlation between milk urea and total protein in the blood (r=-0.366) and between blood triglycerides and milk protein (r=0.381), fat (r=-0.525), and urea (r=-0.711) can indicate a correlation between protein and lipids in the blood and urea in milk, i.e., indicate the nitrogen metabolism of the mammary gland. Similar results were reported elsewhere.[5,31,32,33]

Based on changes in blood and milk metabolites at different stages of lactation, the present results suggest that early lactation cows showed a mild degree of hepatic lesions, probably due to fat infiltration. The results showed that changes in blood and milk metabolites and their relationships can be helpful in the herd monitoring of metabolic status in dairy cows, and for the early diagnosis of subclinical metabolic disease.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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