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Evaluation of metabolic status in Simmental dairy cows during late pregnancy and early lactation

Radojica Djoković[1]*, Vladimir Kurćubić[1], Zoran Ilić[2], Marko Cincović[3], Milun Petrović[1], Natalija Fratrić[4], and Boban Jašović[2]

[1]*Department of Animal Science, Faculty of Agronomy Čačak, University of Kragujevac, Čačak, Serbia*

[2]*Department of Animal Science, Faculty of Agriculture, University of Priština, Lešak, Serbia*

[3]*Faculty of Agriculture, Department of Veterinary Medicine, University of Novi Sad, Novi Sad, Serbia*

[4]*Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia*

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ABSTRACT

The objective of this experiment was to evaluate the metabolic status of late pregnant and early lactation dairy cows based on changes in characteristic blood metabolites. Blood samples were collected from 15 late pregnant cows and 15 early lactation cows to measure beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglycerides (TG), glucose, total protein (TP), albumin, total bilirubin, urea and the activity of aspartate transaminase (AST) and gamma-glutamyl transferase (GGT). Cows in early lactation had significantly higher ($P<0.05$) levels of serum BHB and NEFA, and lower ($P<0.05$) glycaemia compared to the late pregnant cows. High lipomobilization (NEFA>0.4 mmol/L) was detected in 6 (40%) of early lactation cows but in none of the late pregnant cows, while subclinical ketosis (BHB>1.2 mmol/L) was detected in 14 (94.4%) of the early lactation cows and 4 (26.6%) of the late pregnant cows. AST activities above 100 U/L were detected in 2 early lactation cows and in none of the late pregnant cows. TG levels below 0.12 mmol/L and glucose below 2.5 mmol/L were found in 7 (44%) and 10 (66.6%) of the early lactation cows, respectively, and in none of the late pregnant cows. Early lactation cows were found to have lower blood serum levels of TG ($P<0.05$), albumin ($P<0.05$) urea ($P<0.05$) and GTT ($P>0.05$) activities and higher concentrations of total bilirubin ($P>0.05$) TP ($P>0.05$) and AST activities ($P<0.05$) compared to the late pregnant cows. The results of blood serum levels of glucose, TG, BHB, NEFA and AST in early lactation cows suggest metabolic disorders associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration. These serum parameters may have a key role in evaluating metabolic status in late pregnant and early lactation dairy cows.

Key words: blood metabolites, enzymes, subclinical ketosis, hepatic lipidosis, dairy cows, periparturient period

*Corresponding author:

Full Prof. Radojica Djoković, Cara Dušana 34, 32000 Čačak, Serbia, Phone: +381 32 303 400; +381 64 44 97 952; Fax: +381 32 303 401; E-mail: djokovici@ptt.rs

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Introduction

Pregnancy and lactation are physiological statuses considered to modify metabolism in animals and induce stress (IRIADAM, 2007; TANRITANT et al., 2009; PICCIONE et al., 2011). The periparturient period is important in terms of its influence on health and subsequent performance of dairy cows, since cows develop serious metabolic and physiological changes during these periods (TANAKA et al., 2011; PICCIONE et al., 2012).

Clinical ketosis in dairy cows usually occurs between the second and seventh week of lactation (DUFFIELD et al., 1997, DUFFIELD, 2000). Nevertheless, most cows in this stage of lactation may suffer from subclinical ketosis, defined as increased blood ketone bodies with no symptoms other than a considerable decrease in milk yield and susceptibility to other diseases (DUFFIELD et al., 1997, DUFFIELD, 2000). The prevalence of subclinical ketosis in high-yielding dairy cows in The Netherlands was estimated in 12 to 47% of the herd (NIELEN et al., 1994). This prevalence is considered to be higher during the first month of lactation compared to the second month, with a peak of occurrence at the fourth week (BAIRD, 1982). Dairy cows suffer from negative energy balance (NEB) during the first week of lactation because of energy expenditure due to milk production and limited feed intake. This results in NEB, a high degree of lipid mobilization from body fat reserves and hypoglycaemia in early lactation (VEENHUIZEN et al., 1991; DJOKOVIĆ et al., 2007, DJOKOVIĆ et al., 2011). The main blood indicators of lipomobilization in ruminants include BHB, the most important and abundant ketone body, and NEFA (CIVELEK et al., 2011; GONZALEZ et al., 2011; CINCOVIĆ et al. 2012). NEFA are preferentially and greatly accumulated as TG in the liver, primarily due to a decrease in the very low density lipoproteins (VLDL) synthesis by hepatocytes (HERDT et al., 1983; SEVINC et al., 2003). Fatty liver infiltration and hepatocyte degeneration involve cell membrane damage and hepatocyte destruction, coupled with the release of cytoplasm enzymes (AST, GGT, LDH) and a marked increase in circulating activities (PECHOVA et al., 1997; LUBOJACKA et al., 2005; STOJEVIĆ et al., 2005). However, once steatosis occurs, endogenous liver syntheses are reduced, leading to a decrease in blood concentrations of glucose, TP, albumin and globulins, cholesterol, TG and urea. Furthermore, the excretory function of hepatocytes is reduced and, accordingly, blood levels of some compounds such as total bilirubin, ammonia and bile acids are generally increased (WEST, 1990; VEENHUIZEN et al., 1991; SEVINC et al., 1998; SEVINC et al., 2003; BOBE et al., 2004; PICCIONE et al., 2011, PICCIONE et al., 2012; PICCIONE et al., 2012a).

Diagnosing liver lipidosis and susceptibility to ketosis in dairy cows may include liver biopsy or ecography, but a less invasive and more economical analytical method may be the measurement of blood biochemical indicators (BAIRD, 1982; BOBE et al., 2004). According to blood biochemical indicators, ketosis in cows may be diagnosed when the following values match the clinical signs: BHB>1.2 mmol/L, glucose <2.5 mmol/L, and

TG <0.12 mmol/L, and blood values: NEFA >0.7 mmol/L and AST activity above 100 U/L, which is indicative of hepatic lipidosis (SEVINC et al., 1998; OETZEL, 2004; XU et al., 2008; GONZALEZ et al., 2011). The objective of this experiment was to evaluate the metabolic status of late pregnant and early lactation dairy cows based on changes in characteristic blood metabolites.

Materials and methods

This experiment was conducted in a dairy Simmental herd, diagnosed with a number of metabolic and reproductive disorders. The cows were mid-yielding with a preceding lactation of about 6.500 L (late pregnant cows 6392 ± 1005 L and early lactation cows 6488 ± 980 L in previous lactation). Two groups of clinically healthy cows were chosen from the herd: Group 1, consisting of late pregnant cows (n = 15) at 25 to 1 (13.7 ± 9.3) days to partus, and Group 2, including early post-partum cows (n = 15) in the first month of lactation (16.1 ± 9.2 days). Body condition scores (BCS) were recorded by the same observer, using the 1~5 scale according to FERGUSON et al. (1994), with 1 being too thin and 5 too fat. Late pregnant and early lactation cows had BSC 3.80 ± 0.33 and 3.42 ± 0.55, respectively. 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 requirements for cows in late pregnancy and early lactation. The cows in late pregnancy were fed a diet consisting of 6 kg lucerne hay, 15 kg maize silage (30% DM) and 3 kg concentrate (18% crude proteins, CP). The cows in early lactation received a diet consisting of 7 kg lucerne hay, 20 kg maize silage (30% DM) and 5 kg concentrate (18% CP). Dietary nutrient contents for dairy cows in late pregnancy and early lactation are given in Table 1.

Table 1. Nutrient contents in daily rations for dairy cows in late pregnancy and early lactation.

	Late pregnancy	Early lactation
Dry Matter (DM) (kg)	11.94	16.05
Net energy of lactation (NEL) (MJ)	65.25	87.15
Crude protein (CP) (% of DM)	12.55	13.58
Rumen undegradable protein (RUP) (% of CP)	30.86	35.91
Fat (% of DM)	3.27	3.15
Fibre (% of DM)	25.28	23.26

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. Blood samples collected on fluoride were immediately centrifuged according to the same modalities, and plasmas were assessed for glucose concentrations. The following blood biochemical components were measured at the Kvarklab

Biochemical Laboratory by different colorimetric techniques using spectrophotometers (Cobas Mira and Gilford Stasar): BHB and NEFA levels were measured using kits from Randox (United Kingdom), AST, GGT, glucose and total bilirubin using kits from Human (Germany), albumin and urea using kits from Biosystem (Spain), TP and TG using kits from Elitech (France).

Data were subjected to statistical analysis using the GLM model and t-test for difference of means between the two independent groups (late pregnancy group vs. early lactation group) (software: Statgraphic Centurion, Statpoint Technologies Inc. Warrenton, Va, Virginia, USA). The Pearson test was performed to assess significant correlations between the metabolites selected. Differences were considered significant at P values below 0.05 or 0.01.

Results

The results of serum biochemical analysis for both groups of cows are given in Table 2. Lipomobilization indicators, *i.e.*, NEFA and BHB, were statistically higher ($P<0.05$) in early lactation cows compared to late pregnant cows. Activities of serum AST of early lactation cows were higher ($P<0.05$) than those of late pregnant cows. Although the mean GGT values of late pregnant cows were above those of early lactation cows, no significant difference ($P>0.05$) was observed between the two groups of cows. Serum concentrations of TG, glucose, albumin and urea in early lactation cows were significantly lower ($P<0.05$) compared to late pregnant cows. No significant difference ($P>0.05$) was observed in serum values for TP and total bilirubin between the two groups of cows.

Table 2. Blood biochemistry in transitional dairy cows (n = 15 in each group). Results are expressed as mean standard ± deviation.

Parameter	Late pregnant cows	Early lactation cows	P
Glucose (mmol/L)	3.36 ± 0.30	2.29 ± 0.48	< 0.05
BHB(mmol/L)	1.14 ± 0.36	1.59 ± 0.25	< 0.05
NEFA(mmol/L)	0.17 ± 0.06	0.38 ± 0.29	< 0.05
TG(mmol/L)	0.29 ± 0.07	0.12 ± 0.02	< 0.05
TP(g/L)	77.08 ± 4.57	78.89 ± 4.92	NS
Albumin(g/L)	42.57 ± 7.53	34.61 ± 3.56	< 0.05
Urea (mmol/L)	5.29 ± 1.32	3.60 ± 1.07	< 0.05
Total bilirubin (µmol/L)	3.26 ± 0.49	3.91 ± 2.85	NS
AST (IU/L)	33.55 ± 9.38	69.46 ± 30.89	< 0.05
GGT (IU/L)	25.05 ± 4.91	20.61 ± 4.16	NS

NS: non-significant

Table 3 shows the correlation coefficients among the biochemical parameters calculated for all cows in this experiment. Significant negative correlations (P<0.05) were observed between BHB and glucose, BHB and TG, NEFA and TP, NEFA and glucose, glucose and GGT, AST and urea. Significant positive correlations (P<0.05) were observed between NEFA and BHB, NEFA and AST, NEFA and total bilirubin, glucose and TG, glucose and albumin, albumin and TG, urea and glucose, urea and albumin, urea and TG, and AST and GGT.

Table 3. Correlation coefficients for the biochemical parameters calculated for all cows in the present study. Significant correlations (p<0.05) are indicated in bold letters.

	NEFA	BHB	TG	TP	Albumin	Urea	Bilirubin	AST	GGT
Glucose	**r = -0.35** P< 0.05	**r = -0.47** P< 0.05	**r = 0.65** P< 0.05	r = 0.01 NS	**r = 0.47** P< 0.05	**r = 0.43** P< 0.05	r = -0.03 NS	r = -0.23 NS	**r = -0.34** P< 0.05
NEFA		**r = 0.39** P< 0.05	r = -0.21 NS	**r = -0.34** P< 0.05	r = -0.26 NS	**r = -0.45** P< 0.05	**r = 0.63** P< 0.05	**r = 0.34** P< 0.05	r = -0.17 NS
BHB			**r = -0.36** P< 0.05	r = 0.06 NS	r = -0.23 NS	r = -0.27 NS	r = 0.13 NS	r = 0.15 NS	r = 0.06 NS
TG				r = 0.05 NS	**r = 0.63** P< 0.05	**r = -0.61** P< 0.05	r = -0.28 NS	r = -0.04 NS	r = 0.22 NS
TP					r = 0.11 NS	r = -0.29 NS	r = 0.24 NS	r = 0.30 NS	r = 0.07 NS
Albumin						**r = -0.46** P< 0.05	r = -0.28 NS	r = -0.29 NS	r = -0.19 NS
Urea							r = -0.07 NS	**r = -0.33** P< 0.05	r = -0.14 NS
Bilirubin								r = 0.16 NS	r = 0.01 NS
AST									**r = 0.32** P< 0.05

BHB: β-hydroxybutyrate; NEFA: Non-esterified fatty acids; TG: triglycerides; TP: total proteins; AST: aspartate aminotransferase; GGT: γ-glutamyl transferase; NS: non-significant.

Discussion

This experiment compared the metabolic status of dairy cows during the periparturient period, showing that NEFA and BHB values were significantly higher in early lactation animals (P<0.05) than in late pregnant cows. NEFA concentrations >0.40 mmol/L indicate problems with energy balance and subsequent intensive lipomobilization (OETZEL, 2004). According to this report, 6 early lactating cows (40%) and none of the late pregnant cows in the present study had NEFA concentrations above the value indicative of subclinical ketosis. Given the fact that concentrations of serum NEFA >0.70 mmol/l are associated with ketosis (OETZEL, 2004), 2 early lactating cows (13.3%) and none of the late pregnant cows in the present study had NEFA concentrations above the value indicative of subclinical ketosis. Subclinical ketosis may also be diagnosed at serum BHB concentrations above 1.2 mmol/L, while clinical ketosis is associated with BHB concentrations above 2.6 mmol/L (DUFFIELD, 2000; OETZEL, 2004). In the present experiment, 14 early lactation cows (94.4%) and 4 late pregnant cows (26.6%) had serum BHB concentrations above 1.2 mmol/L. These data suggest that serum NEFA levels are most likely less efficient indicators of subclinical ketosis (13.3%) compared to serum BHB (94.4%) in dairy cows during the periparturient period. This is in agreement with DUFFIELD (2000), who stated that the use of NEFA is a better indicator of energy imbalance in prepartum animals compared to BHB, which is more useful postpartum. In this study, a significant positive correlation ($r = 0.39$, $P<0.05$) was observed between NEFA and BHB in the sera tested, suggesting that both parameters are helpful indicators of energy balance during the periparturient period. In other words, a high degree of lipomobilization (high NEFA values) does not necessarily mean that the cow has subclinical ketosis. In the present study, none of the late pregnant cows had NEFA values >0.70 mmol/L, and 4 (26.6%) of the late pregnant cows had BHB values >1.2 mmol/L. These results are in agreement with the findings of GONZALEZ et al. (2011), who suggested that cows exhibiting low lipomobilization (normal NEFA values) may have ketonemia associated with subclinical ketosis (28% of mid lactation cows). Therefore, the data presented show that serum NEFA may be used for detecting high lipomobilization, but not subclinical ketosis. Additionally, the relationship between BHB and NEFA may be inferred by the significant correlation between BHB and glucose ($r = -0.47$; $P<0.05$) values. Blood glucose values in late pregnant cows were within the physiological range of 2.5-4.2 mmol/L (RADOSTITS et al., 2000), whereas hypoglycemia was detected in early lactation cows. In the present study, 10 early lactation cows (66.6%) and none of the late pregnant cows had blood glucose concentrations below 2.5 mmol/L. According to the serum parameters, ketosis in cows may be diagnosed when the following values match the clinical signs: BHB >1.2 mmol/L, glucose <2.5 mmol/L, and TG <0.12 mmol/L (SEVINC et al.,1998; OETZEL, 2004; XU et al., 2008; GONZALEZ et al., 2011).

Given this criterion, 7 cows in early lactation (44%) and no late pregnant cow had the indicative values, but did not display any clinical signs, suggesting that they had a typical subclinical condition, compared to the results of GONZALEZ et al. (2011), who showed that a typical subclinical condition was detected in 22% of high-yielding cows in early lactation.

Serum TG levels were significantly lower ($P<0.01$) in ketotic cows compared to healthy cows (DJOKOVIĆ et al., 2007). These results showed that TG accumulate in the liver cells of ketotic cows and cause their blood values to decrease. The present experiment showed that 7 early lactation cows (44%) and no late pregnant cow had TG concentrations below 0.12 mmol/L. In addition, no significant correlation (r = -0.21, P>0.05) was found between TG and NEFA, suggesting that TG values may not be considered as an adequate indicator for lipomobilization in dairy cows. However, all cows suffering from subclinical ketosis (BHB >0.12 mmol/L) according to the criterion cited above (SEVINC et al., 1998; OETZEL, 2004; XU et al., 2008; GONZALEZ et al., 2011), 6 cows or 40% of the early lactation cows (NEFA >0.40 mmol/L) and none of the late pregnant cows, had TG values lower than 0.12 mmol/L and glycemia below 2.5 mmol/L.

Hepatic lipidosis is generally preceded by an increase in the concentration of ketone bodies in serum and urine. During the first month of lactation, 5 to 10% of high-yielding dairy cows suffer from severe hepatic lipidosis and 30 to 40% have mild hepatic lipidosis (BOBE et al., 2004). This means that nearly 50% of those cows are at risk for metabolic disorders. When fat infiltrates the liver, a lesion appears in the hepatic tissues and the levels of enzymes indicating liver injury (AST, GGT, and GLDH) are generally augmented (PECHOVA et al., 1997; LUBOJACKA et al., 2005; STOJEVIĆ et al., 2005). The values of AST in the present study were statistically higher (P<0.05) in early lactation cows than in late pregnant cows, and no significant difference (P>0.05) was observed between GGT activities in the two groups of cows. Given that AST activity higher than 100 U/L is indicative of hepatic lesions (GONZALEZ et al., 2011), 2 early lactation cows (13.3%) in our study suffered from some degree of hepatic lesions, probably due to fat infiltration. These animals included 2 out of 7 cows considered to be ketotic according to our criteria and with blood NEFA values above 0.70 mmol/L. Meanwhile, none of the late pregnant cows had AST values higher than 100 U/L. A positive correlation between AST activity and lipomobilization (NEFA values) was observed by the significance coefficient (r = 0.34, P<0.05). In the present study, all data regarding liver enzymes suggested that the process of lipomobilization was sufficient to cause liver lesions in 13.3% of the early lactating cows.

Fat infiltration of the liver may also affect the concentration of some blood components. Glucose levels, as well as concentrations of TP, albumin, urea and total bilirubin may be diminished (WEST, 1990; VEENHUIZEN et al., 1991; SEVINC et al., 2003; DJOKOVIĆ et al., 2011; PICCIONE et al., 2011, 2012, 2102a; CINCOVIĆ et al., 2012). In the present study, glycaemia and serum concentrations of albumin and urea in the early lactation cows were significantly lower (P<0.05) than those in the late pregnant cows. No significant difference (P>0.05) was observed in serum values for TP and total bilirubin between the two groups of cows. Serum levels of glucose, TP, albumin and urea are indicators of hepatic function (BOBE et al., 2004) and decreases in their concentration may imply fat infiltration into the liver. In fact, a significant correlation of NEFA values was observed between glucose (r = -0.35; P<0.05), TP (r = -0.34; P<0.05), urea (r = -0.45; P<0.05) and total bilirubin (r = 0.63; P<0.05). Possible alterations in liver function may have deleterious effects on the metabolism of these animals, and may adversely impact milk production or reproduction.

In conclusion, biochemical evaluation suggested that early lactation cows had metabolic disturbances associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration. Serum levels of BHB, NEFA, TG, glucose and AST activities may have a key role in evaluating the metabolic status in late pregnant and early lactation dairy cows.

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SAŽETAK

Cilj je ovog rada bio procijeniti metabolički status mliječnih krava tijekom kasne gravidnosti i na početku laktacije na osnovi promjena karakterističnih metabolita krvi. Pretraživane su bile dvije skupine krava simentalske pasmine: 15 krava u visokom stupnju gravidnosti i 15 krava na početku laktacije. U uzorcima krvi pretraživanih krava određivane su vrijednosti beta-hidroksimaslačne kiseline (BHM), neesterificiranih masnih kiseina (NEFA), triglicerida (TG), glukoze, ukupnih proteina (TP), albumina, ukupnog bilirubina, ureje, aktivnost aspartat transaminaze (AST) i gama glutamil-transferaze (GGT) u krvi. Krave na početku laktacije imale su statistički značajno veće vrijednosti (P<0,05) BHM i NEFA u krvnom serumu i značajno nižu glikemiju (P<0,05) u odnosu na krave u visokoj gravidnosti. Visoke vrijednosti NEFA (>0,4 mmol/L) utvrđene su u 40% krava na početku laktacije, ali u nijedne visoko gravidne krave, dok je supklinička ketoza utvrđena (BHM>1,2 mmol/L) kod 14 (94,4%) krava na početku laktacije te kod 4 (26,6%) visoko gravidnih krava. Aktivnost AST u krvnom serumu iznad 100 U/L utvrđena je u dvije (13,3%) krave na početku laktacije, a nije utvrđena u krava u kasnoj gravidnosti. Vrijednosti TG u krvi bile su ispod 0,12 mmol/L u sedam (44%), a glukoze ispod 2,5 mmol/L u 10 (66,6%) krava na početku laktacije dok nisu dokazane u krava u visokoj gravidnosti. Uspoređujući funkciju jetre, u krvnom serumu krava na početku laktacije utvrđene su niže vrijednosti TG (P<0,05), albumina (P<0,05), ureje (P<0,05) i aktivnosti GGT (P>0,05), odnosno veće vrijednosti ukupnog bilirubina (P>0,05), TP (P>0,05) i aktivnosti AST (P<0,05) u odnosu na vrijednosti ovih pokazatelja krvi kod krava u visokom stupnju gravidnosti. Vrijednosti glukoze, BHB, NEFA, TG i aktivnosti AST u krvnom serumu upućuju na zaključak da su krave na početku laktacije opterećene metaboličkim poremećajima koji su povezani s ketozom i određenim stupnjem oštećenja hepatocita, vjerojatno, kao posljedica masne infiltracije. Navedeni pokazatelji krvi mogu biti od velikog značenja u procjeni metaboličkog statusa mliječnih krava u peripartalnom razdoblju.

Ključne riječi: metaboliti krvi, enzimi, supklinička ketoza, jetrena lipidoza, mliječne krave, peripartalno razdoblje