Biotechnology in Animal Husbandry 32 (1), p 1-8 , 2016
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.23'619
DOI: 10.2298/BAH1601001D

DETERMINATION METABOLIC AND NUTRITIONAL STATUS IN DAIRY COWS DURING EARLY AND MID LACTATION

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Original scientific paper

Abstract: The objective of the present study was to investigate nutritional and metabolic status in Simmental cows during early and mid-lactation. Fifteen early lactating cows and 15 mid lactating cows were chosen for the investigation. Blood samples were collected to measure beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglycerides (TG), glucose and the activity of aspartate transaminase (AST). Early lactation as compared to mid lactating cows were found to have significantly higher ($P<0.05$) blood serum concentrations of NEFA, BHB and AST and lower blood serum concentrations of glucose ($P<0.05$) and TG ($P>0.05$). Significantly negative correlations were observed between BHB and glucose ($P<0.01$), BHB and TG ($P<0.05$), NEFA and glucose ($P<0.05$). Significantly positive correlations were observed between NEFA and BHB ($P<0.05$), NEFA and AST ($P<0.05$), glucose and TG ($P<0.01$). The results suggest that these parameters can serve as useful indicators of the nutritional and metabolic status of dairy cows during lactation.

Key words: blood metabolites, dairy cows, early lactation, mid lactation

Introduction

Production diseases, those associated with improper nutrition or management are common in dairy cows. Dairy cows suffer from negative energy balance (NEB) during the first weeks of lactation due to energy expenditure associated with milk production and limited feed intake, resulting high mobilization of lipids from body fat reserves, and hypoglycaemia (*Veenhuizen et al., 1993; Drackley, 1999; Oetzel, 2004*). Nutrition, age, heredity, body condition score (BCS), management and energy imbalance are various risk factors which possibly play a role in NEB, periparturient fatty liver and ketosis (*Morrow et al.,*

1990; Pechova et al., 1997; Duffield et al., 1997). Clinical ketosis in dairy cows usually occurs between the second and seventh week of lactation. Nevertheless, most of cows in this stage of lactation may suffer a subclinical form of ketosis defined as increased blood ketone bodies without any other symptoms but accompanied by considerable decrease in milk yield and susceptibility other diseases (*Duffield et al., 1997*). Consequently, stressors and poor nutritional management causing reduction in dry matter intake will result in large increases in NEFA around calving (*Drackley, 1999*). NEFA are preferentially and greatly accumulated as TG in the liver, primarily because of a decrease in the very low density lipoproteins (VLDL) synthesis by hepatocytes (*Herdt et al., 1982; Sevinc et al., 2003*). However, when steatosis occurs, endogenous liver synthesis decreases, leading to a reduction in blood glucose, total proteins, albumins and globulins, cholesterol, TG and urea. (*Veenhuizen et al., 1993; Drackley, 1999; Sevinc et al., 2003; Djokovic et al., 2007; Djokovic et al., 2011*). Fatty liver infiltration and hepatocyte degeneration involve cell membrane damage and hepatocyte destruction (15) coupled with the release of cytoplasmic enzymes (aspartate aminotransferase (AST), gamma-glutyml transferase (GGT), lactate dehydrogenase (LDH)) and marked increases in their circulating activities (*Lubojacka et al., 2005; Pechova et al., 1997*).

The objective of the present study was to investigate nutritional and metabolic status in Simmental cows during early and mid lactation.

Materials and methods

Animals, diets and milk production

This experiment was carried out in a dairy herd (166 Simmental cows) suffering from several metabolic and reproductive disorders (Farm: Miličić-Ćurćić, Mrsać, Kraljevo). Two groups (n=15 cows) of clinically healthy cows were chosen from the herd. Group 1 consisted of early lactation cows, in the first month of lactation (16.1±9 days), and Group 2 included mid lactation cows between 3 to 5 months of lactation (124.8±27 days). The cows were mid-yielding with a preceding lactation of about 6500 l. The body condition scores (BCS) of the test cows were 3.42 ± 0.55 (early lactation) and 3.27 ± 0.74 (mid lactation) *(Ferguson et al., 1994).* The experimental cows were kept in tie-stall barns. Diet and the housing facilities were adapted to research purposes, with diet suited to the energy requirement of early and mid lactation cows. Early lactating cows were fed a diet consisting of 7 kg lucerne hay, 20 kg maize silage (30% Dry Matter, DM), 5 kg concentrate (18% crude proteins, CP). Mid lactating cows received a diet consisting of 5 kg meadow hay, 7 kg lucerne hay, 30 kg maize silage (30% DM), 8 kg concentrate (18% CP).). Dietary nutrient contents for dairy cows in early and

mid lactation are given in Table 1. The chemical analysis of the feed was performed by Weende methodology (*Givens et al., 2000*).

Table 1. Nutrient contents in daily ration for early lactation and mid lactation dairy cows

	Early lactating cows	**Mid lactating cows**
Dry Matter (DM) (kg)	16.05	24.82
Net Energy of Lactation (NEL) (MJ)	87.15	130.23
Crude Protein (CP) (% of DM)	13.58	13.38
Rumen undegradable protein (RUP) (% of CP)	35.91	28.33
Fat (% of DM)	3.09	3.14
Fiber (% of DM)	23.26	24.33

Biochemical analysis of blood

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, without anticoagulant. 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 in the same manner and plasmas were assessed for glucose concentrations. The following biochemical blood components were measured by different colorimetric techniques using spectrophotometers (Cobas Mira, Roche, Belgium and Gilford Stasar III, Gilford, USA): BHB and NEFA levels were measured by Randox (United Kingdom) kit, AST and glucose by Human (Germany) kit, and TG by Elitech (France) kit.

Statistical analysis

Difference between metabolic adaptation in early and mid lactation was confirmed by difference in concentration of metabolic parameters, by t-test. Pearson's test was performed to evaluate significant correlations between biochemical metabolites in pooled sample including cows in early and mid lactation. For this purpose was used statistic software Statgraphic Centurion (Statpoint Technologies Inc.Warrenton, Va, Virginia, USA).

Results and Disscusion

Blood biochemical metabolites in early lactation and mid-lactation cows were compared in this study. Homeostasis induces intense lipid mobilization and ketogenesis, and the liver has been adapted to metabolic changes in dairy cows (*Drackley, 1999*). Intensive postpartum lipid mobilization and ketogenesis are

sufficient for a series of compensatory metabolic processes with changes in blood metabolic profile during early lactation in healthy cows (*Drackley, 1999; Cincovic et al., 2012*). Results of blood biochemical metabolites, for both groups of cows are shown in Table 2.

Table 2. Blood metabolites in early and mid-lactating dairy cows (n=15 in each group). Results are expressed as mean ± standard deviation (SD). NS: non-significant

	Early lactating cows	Mid-lactating cows	P
Glucose (mmol/l)	2.29±0.48	2.76±0.43	< 0.05
BHB (mmol/l)	1.59±0.25	0.91±0.16	< 0.05
NEFA (mmol/l)	0.38±0.29	0.13±0.04	< 0.05
TG (mmol/l)	0.12±0.02	0.15±0.04	NS
AST (U/l)	69.46±27.54	39.31±18.90	< 0.05

The correlation coefficients among the biochemical parameters calculated for all cows in this experiment are summarized in Table 3.

Table 3. Correlation coefficients for the biochemical metabolites calculated for all cows in the present study. Significant correlations are marked with asterix (* P<0.05; ** P<0.01).

	NEFA	BHB	TG	AST
Glucose	r= -0.35*	r=-0.47**	r=0.65**	r=-0.23
NEFA		r=0.39*	r=-0.21	r= 0.34*
BHB			r=-0.36*	r=0.15
TG				r=-0.04

In early lactating cows, NEFA and BHB values were significantly higher (P<0.05) than in mid-lactating cows. NEFA concentrations > 0.40 mmol/l indicate problems with energy balance and subsequent intensive lipomobilization *(Oetzel, 2004)*. According to this report, in early lactating cows, NEFA values in blood were 0.38 ± 0.29 mmol/l, showing evidence of high lipomobilization in the present study. Given the fact that serum NEFA concentrations > 0.70 mmol/l are associated with ketosis *(Oetzel, 2004)*. These are the result of some early lactating cows in the present study having NEFA concentrations above the values indicative of subclinical ketosis. Subclinical ketosis also may be diagnosed when serum BHB concentrations are above 1.2 mmol/l, while clinical ketosis is associated with BHB concentrations above 2.6 mmol/l *(Oetzel, 2004; Duffield, 2000)*. The results of early lactating cows in the present study showed BHB concentrations above the value indicative of subclinical ketosis (1.59±0.25 mmol/l). The data presented show that serum NEFA may be used for detecting high lipomobilization, but not subclinical ketosis. This is in agreement with *(Duffield, 2000)*, who stated that the

use of NEFA is a better indicator of energy imbalance in prepartum animals than BHB, but BHB is more useful at postpartum. In the present study, a significant positive correlation was established between NEFA and BHB ($P<0.05$) in the sera, suggesting that both parameters are helpful indicators of EB during lactation.

Blood glucose values in mid-lactation cows were within the physiological range 2.5 - 4.2 mmol/l *(Radostis et al., 2000*), whereas hypoglycemia (2.29 ± 0.48 mmol/l) was detected in early lactating cows. Taking this criterion into account, early lactating cows had indicative values, but did not display any clinical signs, suggesting that they had a typical subclinical condition. In fact, a significant correlation was observed between NEFA values and glucose ($P<0.05$) and BHB and glucose ($P<0.01$). Similar correlations were observed by other authors (*Bobe et al., 2004; Djokovic et al., 2011).*

Fat infiltration into the liver may also affect the concentration of some blood components. *(Morrow et al., 1990; Lubojacka et al., 2005).* Serum level of TG, is an indicator of hepatic functionality, and decreases in their concentration may suggest fat infiltration in the liver *(Lubojacka et al., 2005; Djokovic et al., 2007).* The concentration of serum TG was significantly lower ($P<0.05$) in ketotic cows compared to healthy cows *(Djokovic et al., 2007).* These results may show that TG accumulate in the liver cells of ketotic cows and causes blood TG to decrease. In the present study, TG in the blood was lower (0.12 ± 0.02 mmol/l vs 0.15 ± 0.04 mmol/l) in both groups of cows, but without significant difference. This study has shown a possibility of the development a fat infiltration of the liver in early lactation cows which was confirmed by a significant correlation between TG and glucose, ($P<0.01$) and TG and BHB ($P<0.05$). When fat infiltrates the liver, a hepatocyte degeneration involve cell membrane damage and hepatocyte destruction, and the levels of enzymes that indicate liver injury (AST, GGT, and LDH) are generally augmented (*Pechova et al., 1997; Lubojacka et al., 2005; Djokovic et al., 2011).* AST values in the present study were statistically higher ($P<0.05$) in early lactation cows than in mid-lactating cows. AST activity higher than 100 U/l is indicative of hepatic disorders (*González et al., 2011).* These are result, early lactation cows in our study showed a changes in the morphological and functional state of liver cells, probably due to mild fat infiltration. Also, a positive correlation ($P<0.05$) was observed between AST activity and NEFA values. Mild fatty infiltration of liver in dairy cows during transition and maximum lactation is considered to be almost physiological (*Bobe et al., 2004).* In the present study, all data concerning serum AST activities suggested that the process of lipomobilization was sufficient to cause mild fat infiltration of liver cells in of the early lactating cows.

Conclusion

In conclusion**,** on the basis of changes of blood biochemical metabolites, this study suggests that early lactation cows showed physiological adaptive changes, which were associated with subclinical ketosis and mild fat infiltration of liver cells. They can serve as useful indicators of the nutritional and metabolic status of dairy cows during lactation.

Acknowledgment

This study was financially supported by the Ministry of Education and Science, Republic of Serbia, Project TR 31001.

Određivanje metaboličkog i hranidbenog statusa kod mlečnih krava tokom početka i sredine laktacije

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Rezime

Cilj ovog rada je bio da se ispita matabolički i hranidbeni status mlečnih krava simentalske rase za vreme rane laktacije i tokom sredine laktacije. 15 mlečnih krava na početku laktacije i 15 tokom sredine laktacije je odabrano za ispitivanje. Uzorci krvi su uzeti i merene su vrednosti za beta-hidroksi-buternu kiselinu (BHB), slobodne masne kiseline (NEFA), trigliceride (TG), glukozu i aktivnosti aspartat-amino transaminaze (AST). Krave na početku laktacije su imale statistički značajno veće koncentracije ($P<0.05$) NEFA, BHB i AST u krvi i značajno niže vrednosti za glukozu i trigliceride ($P<0.05$). Statistički značajne negativne korelacije su utvrđene između vrednosti BHB i glukoze ($P<0.01$), BHB i TG ($P<0.05$), NEFA i glukoze ($P<0.05$). Statistički značajne korelacije su utvrđene između NEFA i BHB ($P<0.05$), NEFA i AST ($P<0.05$), glukoze i TG ($P<0.01$). Rezultati ukazuju da ovi parametri krvi mogu biti korisni pokazatelji metaboličkog i hranidbenog statusa kod mlečnih krava za vreme laktacije.

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Received 11 February 2016; accepted for publication 15 March 2016