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METABOLIC STATUS IN SIMMENTAL DAIRY COWS DURING TRANSITION PERIOD

R. Đoković[1] , V. Kurćubić[1], Z. Ilić[2], M. D. Petrović[1], J. Stojković[2], B. Milošević[2], M. Cincović[3]

[1]Department of Animal Science, Faculty of Agronomy-Čačak, University of Kragujevac, Cara Dušana 34, Čačak.
[2]Department of Animal Science, Faculty of Agronomy, University of Priština, Kopaonička bb. Lešak,
[3]Department of Veterinary Medicine Faculty of Agriculture, University of Novi Sad, Trg D.Obradovića 8, Novi Sad.
Corresponding author: djokovici@ptt.rs
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Abstract: The objective of the present study was to determine metabolic status in late pregnant (n = 15) and puerperal (n = 15) Simmental dairy cows. The various blood metabolites and serum enzyme activities were determined by photometric methods. The early lactation cows had the indicative values of the beta-hydroxybutyrate (BHB) (> 1.20 mmol/l) but did not display any clinical signs, which means that they had a typical subclinical condition. The lipomobilization markers, serum BHB and non-esterified fatty acids (NEFA) concentrations, were markedly enhanced ($P<0.05$) in early lactation cows. Liver steatosis compromised hepatocyte metabolism, leading to significantly weaker ($P<0.05$) circulating concentrations of glucose, triglyceride (TG) and urea, and induced some cellular lesions as evidenced by significant increases ($P<0.05$) in the serum bilirubin concentrations and theaspartate transaminase (AST) enzyme activities in early lactation cows. On the basis biochemistry estimation, early lactation cows had metabolic disturbances which were associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration.

Key words: dairy cows, transition period, liver steatosis, ketosis, blood metabolites, enzymes.

Introduction

Transitional period in dairy cows included 3 weeks before and 3 weeks after calving when metabolic processes were adapted to providing energy and nutrients required for synthesis of milk compounds (*Overton and Waldron, 2004*). Major health disorders in high-yielding cows occur around parturition. They include sudden changes in energy metabolism that can induce severe uncontrolled

disorders related to the organic matter metabolism (*Drackley et al., 2005*). As a consequence, such a state caused negative energy balance, a high mobilization of lipids from body fat reserves as well as hypoglycaemia in early lactation (*Djoković et al., 200;, Civlelek et al., 201;, Gonzales et al., 2011*). Lipomobilisation characterized by high blood non-esterified fatty acids (NEFA) concentrations starts within high pregnancy and reaches a maximal intensity in the early lactation (*Veenhuizen et al., 1991; Vazquez-Anon et al., 1994; Dann et al., 2005; Djoković et al., 2007*). NEFA are preferentially and greatly accumulated as triglyceride (TG) in the liver, primarily because of a decrease in the very low density lipoproteins (VLDL) synthesis by hepatocytes (*Herd et al., 1983; Jorritsma et al., 2001**;** Sevinc et al., 2003*). Consequently, physiological situations leading to a negative energy balance (fasting, parturition and lactation) are coupled to an increased uncontrolled rate of body fat mobilisation and the increased fatty acids accumulation in hepatocytes, resulting in disturbances of the morphological and physiological liver integrity (*Veenhuizen et al., 1991; Vazquez-Anon et al., 1994; Djokovic et al., 2007*). However, when an important steatosis occurs, the endogenous liver syntheses are lowered leading to decreases in blood concentrations of glucose, total proteins (TP), albumin and globulins, cholesterol, TG and urea. Furthermore, the excretory function of hepatocytes is reduced and accordingly, the blood concentrations of some compounds such as total bilirubin, ammonia and bile acids are generally increased (*West, 1990; Herd et al., 1983; Sevinc et al., 2003; Bobe et al., 2004; Drackley et al., 2005*). The fatty liver infiltration and the hepatocyte degeneration involve cell membrane damage and hepatocyte destruction coupled to the release of cytoplasm enzymes (AST, GGT, LDH) and marked increases in the circulating activities (*Pechova et al.,1997, Lubojacka et al.,2005*).

The objective of the present study was to determine metabolic status in transitional dairy cows on the basis of blood concentrations of various metabolites.

Materials and Methods

This experiment was carried out in the January 2012 in dairy herd (119 Simmental cows) with several metabolic and reproductive disorders (Farms: Ćurcić, Mrsać, Kraljevo). The cows were mid-yielding with a preceding lactation 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 herd. One group consisted of late pregnant cows (n = 15) in period from 25 to 1 (13.7 ± 9.3) days to partus and a second group included early postpartum cows (n = 15) in the first month of lactation (16.1 ± 9.2 days). The estimated cows had body score condition among 3.5 and 4.0. 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 necessary for cows in late pregnancy and early lactation.

The cows in late pregnancy were fed with a diet consisting of 6 kg lucerne hay, 15 kg maize silage (30% dry mater,DM) and 3 kg concentrate (30% crude proteins, CP). The cows in early lactation were fed with a diet consisting of 7 kg lucerne hay, 20 kg maize silage (30% DM) and 5 kg concentrate (30% CP). Dietary nutrient contents for dairy cows in late pregnancy and in early lactation are given in Table 1.

Table 1. Nutrient contents in daily ration for dairy cows in the late pregnancy and in the 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

The 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 according to the same modalities and plasmas were assessed for glucose concentrations. The beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglyceride (TG), glucose, total proteins (TP), albumin, urea, total bilirubin and serum aspartate transaminase (AST) and gamma-glutamyl transferase (GGT) were measured in the biochemical laboratory Kvarklab. (Kragujevac, Serbia) by different colorimetric techniques using a spectrophotometers (Cobas Mira and Gilford Stasar) and the corresponding commercial kits.

The statistical analysis of the obtained data was carried out by ANOVA-procedure (Statgraphic Centurion, Statpoint Technologies Inc.Warrenton, Va). The analysis of variance and LSD test were used to evaluate the probability of the significance of the statistical differences between mean parameter values 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

The present study compared the metabolic status in dairy cows during transition period. The results of the selected blood metabolites in cows in the transition period and correlations among blood metabolites are given in Tables 2 and 3.

Table 2. Blood metabolites 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)	20.61 ± 4.16	25.05 ± 4.91	NS

Legend:NS: not significant

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

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

The blood glucose values in the late pregnant cows were within physiological range 2.5 - 4.2 mmol/L (*Radostis et al., 2000*), whereas in early lactation cows hypoglycemia was determined. Nevertheless, glycaemia was significantly depressed (P<0.05) in puerperal cows compared to pregnant cows. This decrease in the glucose concentrations previously reported in different studies (*Veenhuizen et al., 1991, Drackley et al., 2001, Djokovic et al., 2007*) may be related to the sudden activity of the mammary gland and the increased lactose synthesis. In such situations, the serum BHB concentration is another indicator of energy metabolism disruptions which is more sensitive than glycaemia and which fluctuates in parallel to lipomobilization (*Civlelek et al., 2011, Gonzales et al., 2011*). In the present study, the lactating cows exhibited significantly higher

(P<0.05) BHB concentrations than the pregnant cows, suggesting a strong mobilisation of fat stores. Subclinical ketosis 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*). The early lactation cows had the indicative values of the BHB (1.59±0.25mmol/l) but did not display any clinical signs, which means that they had a typical subclinical condition. In the same way, the blood concentration of NEFA, considered as the best indicator of negative energy balance and of the lipomobilization intensity during the transition period (*Oetzel, 2004, Civlelek and al., 2011, Gonzales et al., 2011*) was also significantly increased (P<0.05) in the group of cows in early lactation compared to the group of late pregnant cows. Additionally, blood BHB and NEFA concentrations were found highly and positively correlated (r=0.39, P<0.05) together in the current study. The serum BHB and NEFA concentrations in puerperal cows clearly indicated that the intense lipomobilization in the post-partum period has induced ketogenesis and lipid overloading in the liver.

On the other hand, it was observed significant decreases (P<0.05) in the serum TG, urea and albumin concentrations in puerperal cows compared to the late pregnant females and TP were also decreased, although not significantly (P>0.05), during the post-partum period. In addition, all these biochemical parameters positively and some of them significantly (P<0.05) correlated together and with the glycaemia but were negatively correlated with the BHB and NEFA concentrations (Table 3). These results suggested an increased accumulation of TG in hepatocytes in the puerperal cows, probably linked to a depleted liver synthesis of VLDLs as previously evoked (*Herd et al.,1983; Jorritsma et al., 2001; Sevinc et al., 2003*). In the same way, the uraemia, proteinemia and albuminemia were lowered in puerperal cows compared to the late pregnant females, confirming the reduction of the liver syntheses induced by the development of fatty infiltration in liver (*West, 1990, Herd et al., 1983; Sevinc et al.,2003; Bobe et al., 2004; Drackley et al., 2005*).

By contrast, liver damage induces an increase in the serum total bilirubin, and the hemic compound is considered as a sensitive indicator for liver injury (15, 18). *West (1990)* reported a positive and significant correlation between the lipid amounts in the liver and the serum total bilirubin concentrations. In the same way, bilirubin concentrations significantly and positively correlated (r=0.63; P<0.05) with the NEFA concentrations here. In addition, the mean bilirubin concentration was significantly and markedly increased (P<0.05) in the puerperal cows compared to the late pregnant ones. As bilirubin concentrations, high serum activities of some enzymes highly expressed in liver in ruminants such as AST and GGT are observed in liver injury and highly contribute to evaluate the degree of tissue damage (*Pechova a et al.,1997; Lubojacka et al.,2005*). In the present study, the serum AST activities were significantly higher (P<0.05), and GGT activities were higher, but without significant difference (P>0.05) in early lactation cows,

corroborating that the development of fatty infiltration in liver has lead to cell disruption and release of the intracellular enzymes into the blood flow. Moreover, according to *Pechova et al. (1997),* the blood activities of liver enzymes are correlated with the degree of fatty infiltration in the organ. A positive correlation between AST activity and lipomobilization (NEFA values) was observed by the significant coefficient ($r= 0.34$; $P<0.05$). In the present study, all data concerning liver enzymes suggested that the process of lipomobilization was enough to cause liver lesions in the early lactating cows.

Conclusion

- This investigation demonstrated that the early lactation cows had the indicative values of the BHB (>1.20 mmol/l) but did not display any clinical signs, which means that they had a typical subclinical condition.
- The lipomobilization markers, serum BHB and NEFA concentrations, were markedly enhanced in early lactation cows. Liver steatosis compromised hepatocyte metabolism, leading to significantly weaker ($P<0.05$) circulating concentrations of glucose, TG and urea, and induced some cellular lesions as evidenced by significant increases ($P<0.05$) in the serum bilirubin concentrations and the AST enzyme activities in early lactation cows.
- On the basis of biochemistry estimation, early lactation cows had metabolic disturbances which were associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration.

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Metabolički status mlečnih krava simentalske rase za vreme tranzicionog perioda

R. Đoković, V. Kurćubić, Z. Ilić, M. D. Petrović, J. Stojković, B. Milošević, M. Cincović

Rezime

Cilj ovog rada je bio da se proceni metabolički status kod visoko gravidnih mlečnih krava (n=15) i mlečnih krava na početku laktacije (n=15) Simentalske rase. Metaboliti i enzimska aktivnost krvnog seruma su određivani fotometriskom metodom. Krave na početku laktacije su imale indikativan nivo beta-hidroksi buterne kiseline (BHB) (>1.20 mmol/l) u krvi, karakteristčnu za subkliničku ketozu. Markeri lipomobilizacije, beta-hidroksi buterna kiselina (BHB) i neesterifikovane masne kiseline (NEFA), bili su statističko značajno veći ($P<0.05$) kod krava na početku laktacije u odnosu na visoko gravidne krave. Masna infiltacija ćelija jetre uzrokuje značajno nižu ($P<0.05$) vrednost glukoze, triglicerida, albumina i ureje u krvi, kao i ćeliska oštećenja koje se manifestuju značajnim povećanjem ($P<0.05$) koncentracije ukupnog bilirubina i aktivnosti aspartat-transaminaze (AST) u krvnom serumu kod krava na početku laktacije. Na osnovu rezultata biohemijskih ispitivanja može se zaključiti da kod krava na početku laktacije postoje metabolički poremećaji koju su povezani sa ketozom, kao i oštećenja hepatocita koji su verovatno nastali kao posledica masne infiltracije ćelija jetre.

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