

Relationship among Blood Indicators of Hepatic Function and Lipid Content in the Liver during Transitional Period in High-Yielding Dairy Cows*

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ABSTRACT

Background: Production diseases, such as those associated with improper nutrition or management are common in transitional high-yielding dairy cows. The diseases listed in this include: the fat liver syndrome, ketosis, oxidative stress, laminitis, mastitis, milk fever, retained placenta, metritis and infertility. The diseases occur mainly around calving. They are all interrelated and form the so-called periparturient disease complex. The term transition is to underscore the important physiological, metabolic, nutritional and endocrine changes occurring in this time frame. Diagnosing liver lipidosis and susceptibility of 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 metabolites. The objective of the present study was to determine a relationship among blood indicators of hepatic function and lipid content in the liver during transitional period in high-yielding dairy cows.

Materials, Methods & Results: Late pregnant and calved cows ($n = 40$) were selected from a Holstein dairy herd and allocated to four groups: a late pregnant cows ($n = 10$) from day 15 to day 5 before calving; late pregnant cows ($n = 10$) from day 4 to day 1 before calving; clinically puerperal healthy cows ($n = 10$) and clinically ketotic puerperal cows ($n = 10$). Liver and blood samples were taken from all cows. The blood metabolites concentrations were determined by photometric methods using a Cobas Mira automatic analyzer. Liver specimens were histopathologically analyzed for lipid contents using a freezing microtome. The statistical analysis of the obtained data was carried out by ANOVA-procedure. The results of present investigation have shown that the lipid content in the liver and the blood non-esterified fatty acids, β -hydroxybutyrate, total bilirubin concentrations and the AST activities were significantly higher ($P < 0.05$) as well the blood glucose, total cholesterol, triglycerides and albumin concentrations were significantly lower ($P < 0.05$) in puerperal ketotic cows as compared to the values of these parameters in the blood of healthy cows in the transition period.

Discussion: Liver biopsy is the only reliable method to determine severity of fatty liver in dairy cattle in the transitional period. Blood, urine and milk metabolites or blood enzyme activity have been proposed as diagnostic tools. This investigation demonstrated that in healthy transitional cows a mild fatty infiltration occurred in liver during the late pregnancy and early lactation. The histopathological examination showed a moderate to severe degree of fatty liver in ketotic cows. The lipomobilisation markers, serum BHB and NEFA concentrations, were markedly enhanced in puerperal ketotic cows. However, liver steatosis compromised hepatocyte metabolism, leading to significantly weaker circulating concentrations of glucose, TG and total cholesterol, and induced some cellular lesions as evidenced by significant increases in the serum bilirubin concentrations and in the AST enzyme activities in puerperal ketotic cows. All these biochemical metabolites may be used as important biochemical indicators in the determination of the functional status of the liver in high-yielding dairy cows during the transition period.

Keywords: cows, fatty liver, ketosis, blood metabolites, transition period.

INTRODUCTION

Major health disorders in dairy cows occur around parturition. They include sudden changes in energy metabolism that can induce severe uncontrolled metabolic disturbances [1,3,5,11]. Negative energy balance in early lactation causes a high mobilisation of lipids from body fat reserves as well as hypoglycaemia [5,7,19]. Lipomobilisation characterized by high blood non-esterified fatty acids concentrations which are preferentially and greatly accumulated as triglycerides in the liver, primarily because of a decrease in the very low density lipoproteins (VLDL) synthesis [1,4,8,9,17-19]. The increased fatty acids accumulation in hepatocytes and blood ketone bodies, resulting in disturbances of the morphological and physiological liver integrity [7,18,19].

However, when an important steatosis occurs, the endogenous liver syntheses are lowered leading to decreases in blood concentrations of glucose, total proteins, albumins, globulins, cholesterol, triglycerides 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 [12,13,15,20]. 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) [10,12,13,15].

The objective of the present study was to determine a relationship among blood indicators of hepatic function and lipid content in the liver during transitional period in high-yielding dairy cows.

MATERIALS AND METHODS

Animals

Late pregnant and calved cows (n = 40) were selected from a Holstein dairy herd (PIK Bečej, Serbia) and allocated to four groups:

Group A - included late pregnant cows (n = 10) from day 15 to day 5 before calving;

Group B - included late pregnant cows (n = 10) from day 4 to day 1 before calving;

Group C - included clinically puerperal healthy cows (n = 10) and

Group D - comprised clinically ketotic puerperal cows (n = 10).

Liver and blood samples were taken from all cows. The late pregnant cows were selected during a certain period on the basis of the time of the artificial and after detection of conception. Calved cows were selected as single selection in calving stalls. The diagnosis of ketosis was based on clinical symptoms (decreased appetite, rumen atony, behaviour changes), including high concentrations of β -hydroxybutyrate in the blood (>2.6 mmol/L) [14] and ketone bodies in the urine. The presence of ketone bodies in the urine was examined using the Lestradet test. The cows were aged 4-6 years on average, weighing 6852.8 ± 55.52 kg in groups of cows in late pregnancy and 5852.1 ± 48.27 kg in groups of cows in early lactation (body score condition, BSC, 3.5-4.0). The average milk yield was 7795 ± 655 kg (calculated over 305 days) in the previous lactation. The experimental cows were kept in tie-stall barns. Both diet and feed complied with the intended use of animals.

Late pregnant cows were fed a diet consisting of 1.5 kg grass hay, 0.60 kg wheat straw, 10 kg corn silage (33% DM), 2.50 kg alfalfa haylage (51.79% DM), 0.98 kg corn grain, 0.50 kg barley grain, 0.30 kg soybean grits, 1.10 kg soybean meal (44% N), 0.50 kg wheat flour, 0.10 dextrofat SC.

Early lactation cows were fed a diet consisting of 3.43 kg alfalfa hay, 9.50 kg corn silage (44% DM), 9.0 kg corn silage (33,94% DM), 5.0 kg alfalfa haylage (47.40% DM), 5.0 kg brewers grain (21.0% DM), 2.50 kg corn grain, 1.50 kg barley grain, 1.30 kg soybean grits, 1.13 kg soybean meal (44% N), 1.30 kg wheat flour, 1.82 kg sugar beet pulp, 0.40 kg dextrofat SC. The dietary nutrient content for dairy cows in late pregnancy and in early lactation is given in Table 1.

Table 1. Nutrient composition of daily rations for late pregnant and dairy cows in early lactation yielding 35 kg of milk.

	Late pregnancy	Puerperium
Dry matter (DM), kg	9.82	23.63
Net energy of lactation (NEL), MJ	65.50	163.03
Crude protein (CP), % DM	15.03	16.05
Rumen undegradable protein (RUP), %	5.09	5.06
Fat, % DM	3.82	4.78
Fibre, % DM	25.12	17.2
Acid detergent fibre (ADF), % DM	25.31	22.08
Neutral detergent fibre (NDF), % DM	40.59	35.48

Biochemical analysis

Blood samples were collected in the morning from 4 to 6 h after milking and feeding by puncture of the jugular vein into sterile disposable test tubes without anticoagulant. After clotting for 3 h at 4°C and centrifugation (1500 g, 10 min), sera were carefully harvested and stored at -20°C until analysis. Blood samples collected with fluoride were immediately centrifuged according to the same modality and plasmas were assessed for glucose concentrations. The circulating concentrations of glucose, non-esterified fatty acids (NEFA), β -hydroxybutyrate (BHB) total cholesterol, triglycerides (TG), albumin, bilirubin and serum aspartate aminotransferase (AST) activities were determined at the Biochemical Laboratory¹, by photometric methods using a Cobas Mira automatic analyzer and corresponding commercial kits^{2,3}.

Histopathological analysis

Shortly after blood collection, the liver was sampled through percutaneous biopsy. Liver specimens were fixed in neutral 10% formaldehyde solution and histopathologically analyzed for lipid contents at the Pathological Department of the Faculty of Veterinary Medicine in Belgrade. Sections obtained using a freezing microtome⁴ were specifically stained with Sudan III. The liver lipid contents were semi-quantified through computer image analysis (Software Q Win) made using the appliance (Leica Q 500 MC).

Statistical analysis

The statistical analysis of the obtained data was carried out by ANOVA-procedure (Microsoft STATISTICA, ver.5.0, Stat.Soft.Inc.1995). 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

The results of the selected metabolic parameters in cows in the transition period are given in Table 2.

The liver lipid content in healthy cows before and after calving was within the physiological range

(around 5%), being $5.90 \pm 1.10\%$ (Group, A.), $7.21 \pm 2.18\%$ (Group B) and $8.56 \pm 3.24\%$ (Group, C). In ketotic cows (Group, D), the lipid content in the liver was $34.55 \pm 13.23\%$ and it was significantly higher ($P < 0.01$) as compared to groups of healthy cows before and after calving. No significant change in lipid content in the hepatocytes was determined in healthy cows before and after calving.

Table 2 shows significant changes in most blood metabolites in ketotic cows i.e. in cows with fatty liver. Blood glucose concentration was significantly lower in ketotic cows as compared to healthy cows ($P < 0.01$). The biochemical examination of lipids and ketone bodies in the blood serum showed significantly higher values ($P < 0.01$) of NEFA and BHB in ketotic cows as well as significantly lower concentrations of TG ($P < 0.01$) and total cholesterol ($P < 0.05$), as compared to the values of these parameters in the blood sera of healthy cows. In addition, albumin concentrations were markedly depressed in the ketotic group as compared to healthy groups of cows ($P < 0.01$). By contrast, the total bilirubin concentrations and the AST activities were markedly increased ($P < 0.05$) in the puerperal ketotic cows compared to the healthy pregnant and puerperal females.

Furthermore, as reported in Table 3, the intensity of fatty infiltration of the liver correlated positively and significantly with the lipomobilization markers NEFA: ($r = 0.510$, $P < 0.05$) or BHB: ($r = 0.580$, $P < 0.05$) concentrations, and AST: ($r = 0.690$, $P < 0.05$) activities in sera, but negatively and significantly with the circulating concentrations of compounds synthesized in the liver: glucose ($r = -0.690$, $P < 0.05$), TG ($r = -0.550$, $P < 0.05$), albumin ($r = -0.530$, $P < 0.05$) and total cholesterol ($r = -0.340$, $P > 0.05$). Likewise, serum NEFA and BHB concentrations were positively correlated ($r = 0.720$, $P < 0.05$), whereas NEFA concentrations were negatively correlated with glycaemia ($r = -0.380$, $P > 0.05$) and TG: ($r = -0.640$, $P < 0.05$), and BHB concentrations showed a negative correlation with triglyceride concentrations ($r = -0.500$, $P < 0.05$) and glucose concentrations ($r = -0.610$, $P < 0.05$). Finally, AST activities in sera were positively correlated with NEFA: ($r = 0.540$, $P < 0.05$) and BHB: ($r = 0.590$, $P < 0.05$), but negatively with the TG: ($r = -0.600$, $P < 0.05$) concentrations in sera.

Table 2. Selected metabolic profile parameters in dairy cows during the transition period (means ± standard deviation). [A-group of cows from day 15 to day 5 before calving, n=10; B-group of cows from day 4 to day 1 before calving, n = 10; C-group of puerperal healthy cows, n = 10; D-group of puerperal ketotic cows, n = 10].

Groups	Late pregnancy		Puerperium	
	A	B	C	D
Liver lipid (%)	5.90 ± 1.10 ^A	7.21 ± 2.18 ^B	8.56 ± 3.24 ^C	34.55 ± 13.23 ^{ABCD}
Glucose (mmol/L)	2.94 ± 0.32 ^A	3.12 ± 0.42 ^{aBC}	2.71 ± 0.35 ^C	1.80 ± 0.43 ^{ABCD}
NEFA(mmol/L)	0.27 ± 0.14 ^A	0.54 ± 0.26 ^{ABc}	0.46 ± 0.10 ^C	0.84 ± 0.12 ^{ACD}
BHB(mmol/L)	0.78 ± 0.42 ^A	0.89 ± 47 ^B	0.98 ± 0.35 ^C	3.30 ± 0.65 ^{ABCD}
TG(mmol/L)	0.32 ± 0.04 ^A	0.41 ± 0.03 ^{ABC}	0.35 ± 0.04 ^C	0.27 ± 0.03 ^{ABCD}
T. chol. (mmol/L)	1.75 ± 0.20 ^a	1.71 ± 0.30 ^b	1.86 ± 0.62 ^c	1.39 ± 0.29 ^{abcd}
Bilirubin(μmol/L)	5.25 ± 1.13 ^a	4.83 ± 1.70 ^b	5.80 ± 1.05 ^c	6.79 ± 1.85 ^{abcd}
Albumin(g/L)	35.04 ± 2.85 ^A	34.65 ± 2.17 ^B	34.39 ± 2.70 ^C	31.73 ± 3.15 ^{ABCD}
AST (U/L)	70.23 ± 44.05 ^a	75.42 ± 48.81 ^b	92.80 ± 29.50 ^c	131.60 ± 58.05 ^{abcd}

a)- Values marked by letters (a,b,c,d) in one row describe significant differences; b)- Values marked by small letter differ significantly ($P < 0.05$); c)- Values marked by capital letter differ highly significantly ($P < 0.01$).

Table 3. Correlations between blood biochemical and histological (lipid infiltration in liver) parameters in puerperal ketotic cows during the transition period. Significant correlations ($P < 0.05$) are indicated in bold letters.

	NEFA	BHB	TG	T.chol.	Albumin	AST	Glucose	Bilir.
Fatty liver	r = 0.510*	r = 0.580*	r = -0.550*	r = -0.340	r = -0.530*	r = 0.690*	r = -0.690*	r = 0.500*
NEFA		r = 0.720*	r = -0.640*	r = -0.310	r = -0.200	r = 0.540*	r = -0.380	r = 0.400
BHB			r = -0.500*	r = -0.310	r = -0.340	r = 0.590*	r = -0.610*	r = 0.520*
TG				r = -0.420	r = 0.350	r = -0.600*	r = 0.540*	r = -0.270
T chol.					r = 0.140	r = -0.290*	r = 0.170	r = -0.350
Albumin						r = -0.320*	r = 0.530*	r = -0.320
AST							r = -0.350	r = -0.210
Glucose								r = -0.540*

Values marked by asterix (*) describe significant correlations ($P < 0.05$).

DISCUSSION

In dairy cows, it was observed that up to 50% of females exhibited some lipid accumulation in liver in the first 4 weeks after calving and that fatty liver occurs primarily in this period [5,10,11]. Liver can be categorized into normal liver or mild (0-20% of lipids), moderate (20-40% of lipids) and severe fatty liver (more than 40% of lipids) as dependent on the degree of pathology [6]. In agreement with that, the mean liver lipid content in late pregnant and puerperal healthy cows was within the physiological range (around 5%), but the histological parameter was significantly ($P < 0.01$) increased in puerperal ketotic cows. Similar results were obtained by other authors [6,11,13,18,19].

In the present study, glycaemia measured both in pregnant females and in puerperal cows was

included into the physiological limits, e.g. from 2.5 to 4.2 mmol/L [16]. Nevertheless, glycaemia was significantly depressed ($P < 0.01$) in puerperal ketotic cows compared to healthy pregnant and puerperal cows and was under of the physiological values (hypoglycemia). This decrease in the glucose concentrations in puerperal dairy cows previously reported in different studies [3,4,10,19] may be related to the sudden activity of the mammary gland and the increased lactose synthesis. Furthermore, the negative energy balance associated with lipomobilisation and increased fat accumulation in hepatocytes may induce a considerable reduction in the liver gluconeogenesis, also contributing to reduce glycaemia.

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 lipomobilisation [2,14,19]. In the present study, although the BHB concentrations have remained within the physiological ranges (< 1.2 mmol/L) [2,14] in the healthy groups of dairy transitional cows, lactating puerperal ketotic cows exhibited significantly ($P < 0.01$) higher BHB concentrations than the pregnant cows, suggesting ketonemic pathological state and a very intensive mobilisation of fat stores. In the same way, the blood concentration of NEFA, considered as the best indicator of negative energy balance and of the lipomobilisation intensity during the transition period [2,5,7,19] was also significantly ($P < 0.01$) increased in the group of ketotic cows in early lactation compared to the groups of transitional healthy cows. Additionally, blood BHB and NEFA concentrations were found highly and positively correlated together in the current study and these 2 parameters were also significantly and positively associated with the liver steatosis intensity. The simultaneous and parallel variations observed between the extent of the fat infiltration in liver and the serum BHB and NEFA concentrations in puerperal ketotic cows clearly indicated that the intense lipomobilisation in the post-partum period has induced lipid overloading in the liver.

On the other hand, it was observed significant decreases in the serum triglyceride ($P < 0.01$), total cholesterol ($P < 0.05$) and albumin ($P < 0.01$) concentrations in puerperal ketotic cows compared to the late pregnant and puerperal healthy females. In addition, these biochemical parameters positively correlated together and with the glycaemia but were negatively correlated with the BHB or the NEFA concentrations and with the liver steatosis intensity. These results suggested an increased accumulation of triglycerides and total cholesterol in hepatocytes in the puerperal ketotic cows, probably linked to a depleted liver synthesis of VLDLs as previously evoked [1,8,9,17]. The albumin concentration was lowered ($P < 0.01$) in puerperal ketotic cows compared to the transitional healthy females, confirming the reduction of the liver syntheses induced by the development of fatty infiltration in liver [10,12,15].

By contrast, liver damage induces an increase in the serum total bilirubin, and the haemic compound is considered as a sensitive indicator for liver injury [13,15]. West [20] 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 posi-

tively correlated not only with liver steatosis intensity but also with the BHB and NEFA concentrations here. In addition, the mean bilirubin concentration was significantly and markedly increased ($P < 0.05$) in the puerperal ketotic cows compared to the late pregnant and puerperal healthy ones. As bilirubin concentrations, high serum activities of some enzymes highly expressed in liver in ruminants such as AST, GGT and LDH are observed in liver injury and highly contribute to evaluate the degree of tissue damage [12,13,15]. AST is located in the cytoplasm and mitochondria of different tissues and organs, and the highest activities are detected in heart and skeletal musculature, as well as in liver in cows [13]. The serum AST activity is considered as the most sensitive indicator for diagnosing fatty liver in this species [12,13,15]. In the present study, the serum AST activities measured in late pregnant and in early lactating cows were significantly higher ($P < 0.05$) in ketotic cows, corroborating that the development of fatty infiltration in liver has led to cell disruption and release of the intracellular enzymes into the blood flow. Moreover, according to Pechova *et al.* [15], the blood activities of liver enzymes are correlated with the degree of fatty infiltration in the organ. In agreement, AST activities were highly positively associated with the fat proportions in liver and with the serum NEFA and BHB concentrations, but they negatively correlated with the circulating triglyceride, total cholesterol and albumin concentrations,

As a conclusion, this investigation demonstrated that in healthy transitional cows, a mild fatty infiltration occurred in liver during the late pregnancy and early lactation which could be considered as almost physiological. The histopathological examination showed a moderate to severe degree of fatty liver in ketotic cows. The lipomobilization markers, serum BHB and NEFA concentrations, were markedly enhanced in puerperal ketotic cows. However, liver steatosis compromised hepatocyte metabolism, leading to significantly weaker circulating concentrations of glucose, TG and total cholesterol, and induced some cellular lesions as evidenced by significant increases in the serum bilirubin concentrations and the AST enzyme activities in puerperal ketotic cows. All these biochemical parameters may be used as important biochemical indicators in the determination of the functional status of the liver in high-yielding dairy cows during the transition period.

SOURCES AND MANUFACTURES

¹INEP - Institute for the Application of Nuclear Energy, Zemun, Serbia.

²Fortress Diagnostics, Antrim, United Kingdom.

³Randox Clinical Diagnostics, Crumlin, United Kingdom.

⁴Leica 1850, Jung Tissue Freezing Medium, Biosystem, Barcelona, Spain.

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