Biotechnology in Animal Husbandry 28 (4), p 705-714 , 2012
Publisher: Institute for Animal Husbandry, Belgrade-Zemun

ISSN 1450-9156
UDC 636.034
DOI: 10.2298/BAH1204705D

RELATIONSHIP AMONG BLOOD METABOLITES AND LIPID CONTENT IN THE LIVER IN TRANSITIONAL DAIRY COWS

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Original scientific paper

Abstract: 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 dairy cows. 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 5 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 results of present investigation have shown that the lipid content in the liver and the blood non-esterificed 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. The fat proportions in liver were positively (P<0.05) associated with the AST activities and with the serum NEFA and BHB concentrations, but negatively correlated (P<0.05) with the circulating triglyceride, total cholesterol and albumin concentrations, All these biochemical metabolites may be used as important biochemical indicators in the determination of the functional status of the liver in dairy cows during the transition period.

Key words: cows, fatty liver, ketosis, blood metabolites, transitional period

Introduction

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 (*Jorritsma et al. 2000 and 2001; Drackley et al., 2001; Bobe et al., 2004; Dann 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 (*Veenhuizen et al., 1991; Drackley et al., 2001***;** *González et al., 2011*). Lipomobilization characterized by high blood non-esterificed fatty acids concentrations starts within high pregnancy and reaches a maximal intensity in the early lactation (*Veenhuizen et al., 1991; Vazquez-Anon et al., 1994***;** *Jorritsma et al., 2000***;** *Drackley et al., 2001*). Non-esterificed fatty acids are preferentially and greatly accumulated as triglycerides in the liver, primarily because of a decrease in the very low density lipoproteins (VLDL) synthesis by hepatocytes (*Herdt et al., 1983; Holtenius, 1989; 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 acid accumulation in hepatocytes and blood ketone bodies, resulting in disturbances of the morphological and physiological liver integrity (*Vazquez-Anon et al., 1994; González et al., 2011*).

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 (*Gaál et al., 1983*). However, when an important steatosis occurs, the endogenous liver syntheses are lowered leading to decreases in blood concentrations of glucose, total proteins, albumin, 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 (*West, 1990; Pechova et al., 1997; Kupczynski et al., 2002; Lubojacka 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; Jorritsma et al., 2001; Kupczynski et al., 2002; Lubojacka et al., 2005*).

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 dairy cows on the basis changes of blood concentrations of various metabolites and lipid contents in the liver.

Materials and Methods

Late pregnant and calved cows (n= 40) were selected from a Holstein dairy herd 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 5 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 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, Oetzel, 2004) 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 675.28± 55.52 kg in groups of cows in late pregnancy and 585.21± 48.27 kg in groups of cows in early lactation. The average milk yield was 7695 ± 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 3 kg lucerne hay, 3 kg wheat straw, 10 kg maize silage (30%DM), 4 kg lucerne haylage, 2 kg maize ear silage (68%DM), 0.5 kg dry sugar beet pulp, 1.5 kg concentrate (30%CP). Early lactation cows were fed a diet consisting of 4 kg lucerne hay, 15 kg maize silage (30%DM), 8 kg lucerne haylage, 4 kg maize ear silage (68%DM), 2 kg dry sugar beet pulp, 2 kg extruded soybean grains, 4.5 kg concentrate (30%CP).

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	12.1	21.5
Net energy of lactation (NEL), MJ	66.2	153.2
Crude protein (CP), % DM	12.1	18.3
Rumen undegradable protein (RUP), %	35.82	39.69
Fat, % DM	3.09	4.92
Fibre, % DM	25.12	17.2
Acid detergent fibre (ADF), % DM	32.33	22.6
Neutral detergent fibre (NDF), % DM	49.08	37.16

Blood samples were collected in the morning from 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), 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-esterificed fatty acids (NEFA), β-hydroxybutyrate (BHB) total cholesterol, triglycerides (TG), albumin, bilirubin and serum aspartate aminotransferase (AST) activities were determined by photometric methods using a Cobas Mira automatic analyzer and corresponding commercial kits.

Shortly after blood collection, the liver was sampled through percutaneous biopsy. Liver specimens histopathologically analyzed for lipid contents at the Pathological Department of the Faculty of Veterinary Medicine in Belgrade. Sections obtained using a freezing microtome (Leica 1850, Jung Tissue Freezing Medium) 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).

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

Results and discussion

The results of the selected metabolic parameters in cows in the transition period are given in Tables 2 and 3.

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 5 to day 1 before calving, n=10; C- group of puerperal healthy cows, n=10; D -group of puerperal ketotic cows, n=10).

	Late pregnancy		Puerperium	
Groups	A	B	C	D
Liver lipid (%)	5.30 ± 1.10^{A}	6.31 ± 2.18^{B}	8.37 ± 1.24^{C}	32.91 ± 13.23^{ABCD}
Glucose (mmol/l)	2.94 ± 0.32^{A}	3.12 ± 0.42^{B}	2.71 ± 0.35^{bc}	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)	$35\ 04 \pm 2.85^{a}$	34.65 ± 2.17^{b}	34.39 ± 2.70^{c}	31.73 ± 3.15^{abcd}
AST (U/l)	70.23 ± 44.05^{a}	$75\ 42\pm 48.81^{b}$	92.80 ± 29.50^{c}	131.60 ± 58.05^{abcd}

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

Table 3: Correlations among blood biochemical and histological parameters in puerperal ketotic cows. Significant correlations (P<0.05) are indicated with *.

	NEFA	BHB	TG	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
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*

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 (*Jorritsma et al. 2000 and 2001; Drackley, 2001*). 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 (*Pechova et al., 1997***;** *Veenhuizen et al., 1991; Vazquez-Anon et al., 1994; Jorritsma et al., 2000 and 2001*)

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 (*Radostits et al., 2000*). 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 (*Veenhuizen et al., 1991; Drackley et al., 2001;, Dann et al., 2005*) may be related to the sudden activity of the mammary gland and the increased lactose synthesis. Furthermore, the negative energy balance associated with lipomobilization and increased fat accumulation in hepatocytes may induce a considerable reduction in the liver gluconeogenesis, also contributing to reduce glycaemia.

The serum BHB concentration is another indicator of energy metabolism disruptions which is more sensitive than glycaemia and which fluctuates in parallel to lipomobilization (*Veenhuizen et al.,1991; Oetzel, 2004*). In the present study, although the BHB concentrations have remained within the physiological ranges (<1.2 mmol/l) (*Oetzel, 2004; Civelek et al., 2011*) 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 lipomobilization intensity during the transition period (*Veenhuizen et al., 1991; González et al., 2011; Civelek et al., 2011*), 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 (Table 3). 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 lipomobilization in the post-partum period has induced lipid overloading in the liver.

On the other hand, it was observed significant decreases (P<0.05) in the serum triglyceride, total cholesterol and albumin 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 (Table 3). These results suggested an increased accumulation of triglycerides and cholesterol in hepatocytes in the puerperal ketotic cows, probably linked to a depleted liver synthesis of VLDLs as previously evoked (*Herdt et al., 1983; Holtenius, 1989; Sevinc et al., 2003*). 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 (*Pechova et al., 1997; Jorritsma et al., 2001*; *Kupczynski et al., 2002; Lubojacka et al., 2005*).

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 (*Pechova et al., 1997; Lubojacka et al., 2005*). 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 not only with liver steatosis intensity but also with the BHB and NEFA concentrations here (Table 3). 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 (*Pechova et al., 1997; Kupczynski et al., 2002; Lubojacka et al.; 2005*). The serum AST activity is considered as the most sensitive indicator for diagnosing fatty liver in *this species (*Pechova et al., 1997; Kupczynski et al., 2002; Lubojacka et al., 2005). 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 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. 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 (Table 3).

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 dairy cows during the transition period.

Acknowledgment

This work was financed by Ministry of Education, Science and Technological Development, Republic of Serbia, projects TR 31001 and TR 31086.

Korelacije između metabolita krvi i sadržaja masti u jetri kod mlečnih krava za vreme tranzicionog perioda

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Rezime

Visoko-gravidne i tek oteljene krave (n= 40) izabrane su iz zapata Holštajn krava i podeljene su u 4 grupe: grupa visoko-gravidnih krava (n=10) u periodu od 15 do 5 dana pre teljenja; grupa visoko-gravidnih krava (n=10) u periodu od 5 do1 dana pre teljenja; grupa klinički zdravih krava u puerperijumu (n=10) i grupa klinički ketoznih krave u peuerperijumu (n=10). Uzorci tkiva jetre i krvi uzeti su od svih ispitivanih krava. Rezultati ispitivanja pokazuju da sadržaj mastu u jetri, vrednosti slobodnih masnih kiselina, β- hidroksi buterne kiseline, ukupnog bilirubina i aktivnosti AST u krvnom serumu su bile statistički značajno veće ($P<0.05$) dok su istovremeno vrednosti glukoze, ukupnog holesterola, triglicerida i albumina u krvnom serumu bile statistički značajno manje ($P<0.05$) kod grupe ketoznih krava u odnosu na vrednosti ovih parametara kod grupa zdravih krava pre i posle teljenja. Takođe, sadžaj masti u jeti je bio u pozitivnoj korelaciji ($P<0.05$) sa aktivnošću AST u krvi, kao i sa koncentracijama slobodnih masnih kiselina i β-hidroksi buterne kiseline, a u negativnoj korelaciji ($P<0.05$) sa koncentracijama glukoze, ukupnog holesterola, triglicerida i albumina u krvnom serumu ketoznih krava. Svi ovi metaboliti krvi mogu se koristiti kao važni biohemijski indikatori u određivanju funkcionalnog stanja jetre kod mlečnih krava u tranzicionom periodu.

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Received 24 April 2012; accepted for publication 15 September 2012