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LIPIDEMIA AND LIPID CONTENTS IN THE LIVER IN HOLSTEIN COWS DURING TRANSITION PERIOD

LIPIDEMIJA I SADRŽAJ LIPIDA U JETRI KOD HOLSTEIN KRAVA U PERIPARTALNOM TRANZIVIONOM PERIODU

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ABSTRACT

The aim of the present study was to determine a correlation between blood concentration of lipids and the content of lipids in the liver of dairy cows in the transitional period. The Holstein dairy cows (n = 40) were divided into four groups: the first group (A) included late pregnant cows (n = 10) from the 10th to 4th day before calving; the second group (B) included late pregnant cows (n = 10) from the 4th to 1st day before calving; the third group (C) included clinically puerperal healthy cows (n = 10), whereas the fourth group (D) included puerperal ketotic cows (n = 10). The liver and blood samples were taken from all the cows. Pathohistological examination of liver samples showed statistically significantly higher ($p < 0.01$) lipid infiltration in ketotic cows compared to healthy cows in late pregnancy and puerperium. Biochemical examination of blood serum showed significantly higher values ($p < 0.01$) of nonesterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) in ketotic cows, such as lower blood concentrations of glucose ($p < 0.01$), triacylglycerols (TG) ($p < 0.01$), and total cholesterol TChol. ($p > 0.05$) compared to the values obtained in the blood serum in the groups of healthy cows before and after calving. The significantly positive correlations were determined between the content of lipids in the liver and blood concentration of NEFA ($r = 0.67$; $p < 0.05$) and BHB ($r = 0.55$; $p < 0.05$) as well as the negative ones between the content of lipids in the liver and blood concentrations of glucose ($r = -0.45$; $p < 0.05$), TG ($r = -0.55$; $p < 0.05$) and TChlol. ($r = -0.39$; $p < 0.05$). Our investigations suggested that changes in the blood concentrations of NEFA, BHB, TG, TChol. and glucose served as major biochemical indicators in determining ketosis and liver steatosis in the dairy cows in the transitional period.

Key words: cows, fatty liver,ketosis, transitional period, biochemical indicator.

SAŽETAK

Cilj ovog istraživanja bio je da se utvrdi korelacija između koncentracije lipida u krvi i sadržaja lipida u jetri mlečnih krava u prelaznom periodu. Holštajn mlečne krave (n = 40) podeljene su u četiri grupe: prva grupa (A) obuhvatala je kasno steone krave (n = 10) od 10. do 4. dana pre teljenja; druga grupa (B) obuhvatala su kasno steone krave (n = 10) od 4. do 1. dana pre teljenja; treća grupa (C) obuhvata klinički puerperalne zdrave krave (n = 10), dok četvrta grupa (D) uključuje puerperalne ketotične krave (n = 10). Od svih krava uzeti su uzorci jetre i krvi. Patohistološki pregled uzoraka jetre pokazao je statistički značajno veću ($p <$

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0,01) lipidnu infiltraciju kod ketotičnih krava u odnosu na zdrave krave u kasnoj gravidnosti i puerperijumu. Biohemijsko ispitivanje krvnog seruma pokazalo je značajno veće vrednosti ($p < 0,01$) neesterifikovanih masnih kiselina (NEFA) i beta-hidroksibutirata (BHB) kod ketotičnih krava, kao što su niže koncentracije glukoze u krvi ($p < 0,01$), triacilglicerola (TG) ($p < 0,01$), i ukupni holesterol TChol. ($p > 0,05$) u poređenju sa vrednostima dobijenim u krvnom serumu u grupama zdravih krava pre i posle teljenja. Utvrđene su značajno pozitivne korelacije između sadržaja lipida u jetri i koncentracije NEFA u krvi ($r = 0,67$; $p < 0,05$) i BHB ($r = 0,55$; $p < 0,05$), kao i negativne između sadržaja lipida. u jetri i koncentraciji u krvi glukoze ($r = -0,45$; $p < 0,05$), TG ($r = -0,55$; $p < 0,05$) i TChlola. ($r = -0,39$; $p < 0,05$). Naša istraživanja su pokazala da promene u koncentraciji NEFA, BHB, TG, TChol u krvi. a glukoza je služila kao glavni biohemijski indikatori u određivanju ketoze i steatoze jetre kod mlečnih krava u prelaznom periodu.

Ključne reći: krave, masna jetra, tranzicioni period, biohemijski indikatori.

INTRODUCTION

Production diseases i.e. diseases associated with improper nutrition or management are common in dairy cows and mostly occurred during transition period. (1-3). Ketosis is a common disease in high producing dairy cows during the early lactation period. Ketoss can be categorized into three types: ketosis type I, (spontaneous, underfeeding ketosis, 3-6 weeks after calving), ketosis type II or fatty liver (1-2 weeks postpartum), and ketosis type III, butyric acid silage (4-6). Subclinical ketosis may be diagnosed when serum BHB concentrations are above 1.2 mmol/l , and clinical ketosis with blood BHB level above 2.6 mmol/l (4,5). The 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 the synthesis of milk compounds (1). Early lactation in dairy cows resulted in negative energy balance, high mobilization of lipids from bodily fat reserves as well as hypoglycaemia, ketonemia and ketouria (7-9). The main blood markers of lipomobilization in dairy cows are BHB, the most important ketone body,and NEFA (9-11). Lipid mobilisation characterised by highly concentrated NEFA in blood starts in a high degree of pregnancy, reaching its maximum in early lactation. NEFA are reesterified and accumulated in the form of TG in the liver, primarily due to the decreasing capacity of hepatocytes for transport of lipids by very low density lipoproteins (VLDL), (12). As a result, lipid mobilisation intense ketogenosis and lipogenesis in the liver and consequently lower concentrations of glucose, TG and T.Chol. in blood were manifested (13,12,11,14). Primary homeorhetic adaptation of glucose metabolism in early lactation leads to increased gluconeogenesis in the liver to direct glucose into the mammary gland for lactose synthesis (2). If the degree of gluconeogenesis in the liver does not meet the increased needs of glucose in dairy cows in early lactation, hypoglycaemia, lipidemia, ketonaemia and ketonuria are likely to occur (9,15). 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) (16,17,14) and a mild fatty infiltration of liver in dairy cows during transition and maximum lactation is considered to be almost physiological. However, when an important steatosis occurs, the endogenous liver syntheses are lowered, leading to decreases in blood concentrations of glucose, proteins, lipids and urea (12,18). The aim of the present study was to determine a correlation between blood concentration of lipids and the content of lipids in the liver of dairy cows in the transitional period.

MATERIALS AND METHODS

Late pregnant and calved cows (n = 40) were chosen from a Holstein dairy herd and divided into four groups: the first group (A) included late pregnant cows (n = 10) from the 10th to 4th day before calving; the second group (B) included late pregnant cows (n = 10) from the 4th to 1st day before calving; the third group (C) included clinically healthy puerperal cows (n = 10), whereas the fourth group (D) included ketotic cows. (n = 10). The liver and blood samples were taken from all the cows. The late pregnant cows were selected during a certain period on the basis of the time of artificial insemination and after detection of conception. Calved cows were selected as single selection in calving stalls. The diagnosis of ketosis was based on the clinical symptoms (reduced appetite, rumen atony, behavioural changes) and determined high

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concentrations of blood BHB (more than 1.2 mmol/l). Healthy cows before and after calving did not show clinical symptoms of ketosis. The experimental cows were kept in tie-up stalls in barn housing. The meal was prepared in a manner to suit the energy needs of animals in late pregnancy and early lactation. 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 circulating concentrations of glucose, beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), total cholesterol (T.chol), triglycerides (TG), were determined by photometric methods using an automatic analyser Cobas Mira and the corresponding commercial kits. Shortly after blood collection, the liver was sampled through liver percutaneous biopsy using a biopsy instrument according to the Gaal's method (19) modified by Hajavcova and Kacafirek (20). The biopsy was performed at the right 11th intercostal region, approximately 2 cm below the horizontal line through the tuber coxae, with 3-5 cm long and 3-4 mm wide liver specimens. Liver tissues were histopathologically analysed for lipid contents at the Pathological Department of the Faculty of Veterinary Medicine in Belgrade. Liver specimens were fixed in neutral 10% formaldehyde solution and routinely processed. 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 on 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). 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 blood biochemistry and content of lipids in the liver performed in cows during the transition period was summarized in Table 1. As shown in Table 1, the mild liver fatty infiltration observed in healthy cows during the late pregnancy end puerperium, whereas in ketotic cows were determined moderate liver lipidosis (32.91±13.23% of lipids) and these liver lipid contents were significantly higher ($p<0.01$) than in healthy cows during transition period. The serum concentrations of NEFA and BHB were significantly higher ($p<0.01$) in the ketotic cows compared to healthy ones, whereas the mean TG a concentration was significantly lowered ($p < 0.01$) and the cholesterolemia was not significantly ($p > 0.05$) altered although this parameter was slightly decreased in the puerperal ketotic cows. In addition, the glucose concentrations was markedly depressed in the ketotic group of cows ($p < 0.01$).

The intensity of the liver fatty infiltration correlated positively and significantly ($p < 0.05$) with the markers of lipomobilisation (NEFA or BHB concentrations), but negatively and significantly ($p < 0.05$) with the circulating concentrations of compounds synthesised in liver (glucose, TG and TChol.). In the same way, the serum NEFA and BHB concentrations were positively coupled together whereas the NEFA and BHB concentrations were negatively correlated with glycaemia and the TG (Table 2).

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Table 1. Blood biochemistry and lipid contents in liver in transitional dairy cows in late pregnancy (from day 10th to day 4th before parturition, (group A); from the 4th to1st day before calving, (group B) and in early lactation (clinically healthy puerperal healthy cows, (group C); and ketotic cows, (group D) (n = 10 in each group). Results are expressed as mean ± standrad deviation.

	Group A	Group B	Group C	Group D
Liver lipids(%)	5.30 ±1.10^{A}	6.31± 1.18^{A}	8.37± 1.24^{A}	32.91± 13.23^{B}
NEFA(mmol/l)	0.27± 0.14^{A}	0.54± 0.26^{B}	0.56± 0.10^{B}	0.92± 0.12^{C}
BHB (mmol/l)	0.32± 0.14^{a}	0.41± 0.18^{b}	1.02± 0.35^{C}	2.42± 0.75^{D}
Glucose(mmol/l)	2.94± 0.32^{A}	3.12± 0.42^{A}	2.71± 0.35^{A}	1.80± 0.43^{B}
TG (mmol/l)	0.32± 0.11^{A}	0.21± 0.08^{A}	0.17± 0.06^{A}	0.12± 0.03^{B}
TChol. (mmol/l)	1.65± 0.40^{a}	1.61± 0.30^{a}	1.76± 0.62^{a}	1.39± 0.29^{a}

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 a high significance ($p < 0.01$).

Table 2. Correlation coefficients between biochemical indicators in blood and the content of lipids in liver, calculated for all groups of cows.

	NEFA	BHB	glucose	TG	T.Chol.
Fatty liver	r = 0.67**	r = 0.55**	r = -0.45 **	r = -0.55 **	r = -0.39 **
NEFA		r = 0.57 **	r = -0.52 **	r = -0.63 **	r = -0.20
BHB			r = -0.50 **	r = -0.55 **	r = -0.15
glucose				r = 0.44 **	r = 0.12
TG					r = 0.47 **

Significant correlations (*p< 0.05 or ** p< 0.01) are with asterix.

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 (8,16,17). 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 lipid content in the liver was significantly increased ($p < 0.01$) (moderate fatty liver) in puerperal ketotic cows selected in this study. Similar results were obtained by other authors (7,8,17,14,18).

In general, circulating NEFA levels of 0.9 mmol or greater are considered to place the cow at a high risk of developing clinical ketosis and fatty liver disease. As fatty liver disease and ketosis are closely related, early detection of elevated ketone body levels can give an indication of herd risk levels. Beta-hydroxybutyrate (BHB) is commonly measured in blood or milk to assess the risk of ketosis. Levels of BHB in excess of 2.5 mmol are considered to place the cow at a high risk of suffering from fatty liver disease (8,3,18).

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 pospartal period (16,10,6), was also significantly increased ($p < 0.01$) in the group of ketotic cows in early lactation compared to the other groups of cows. However, the NEFA concentrations were significantly increasing ($p < 0.01$) among the groups cows before calving, which showed that lipid mobilisation intensive begining from four days to one day before calving. Similar results were obtained by Veenhuizen et al. (7) and Djokovic et al. (17). 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 (8,4,5,10). In the present study, ketotic cows exhibited significantly higher ($p < 0.01$) BHB concentrations than the healthy transitional cows, suggesting a strong mobilisation of fat stores.

Additionally, blood BHB and NEFA concentrations were found highly and positively correlated together ($p < 0.05$) in the current study and these 2 parameters were also significantly and positively associated ($p < 0.05$) 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 the fatty liver in the cows (16,10,11,6).

In the present study, glycaemia as indicator of energy metabolism was significantly ($p < 0.01$)

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depressed (hypoglycaemia) in ketotic cows compared to healthy ones. This decrease in the glucose concentrations previously reported in different studies (6,7,15) 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 (8,9).

On the other hand, it was observed significant decreases ($p < 0.01$) in the serum TG concentrations in puerperal ketotic cows compared to the healthy females and other biochemical parameter, at least partially synthesised in the liver, such as TChol. was also decreased ($p > 0.05$), although not significantly, during the post-partum period. In addition, these biochemical parameters positively correlated ($p < 0.05$) together, but were negatively correlated ($p < 0.05$) with the BHB or the FFA concentrations and with the liver steatosis intensity. These results suggested an increased accumulation of TG and T.chol. in hepatocytes in the puerperal ketotic cows, probably linked to a depleted liver synthesis of VLDLs as previously evoked (7,12,16,9).

CONCLUSION

This investigation demonstrated that in ketotic cows were occured a moderate degree of fatty liver (ketosis type II), whereas in healthy transitional cows were a mild fatty infiltration in liver. In parallel, the lipomobilisation markers, such as the serum BHB and NEFA concentrations, were markedly enhanced. The liver steatosis has compromised the hepatocyte syntheses leading to significantly weaker circulating concentrations of glucose and TG. All these biochemical parameters may be important biochemical indicators in determining the ketosis and fatty liver in dairy cows during transition period.

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