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THE LIPIDS CONTENT IN BLOOD AND LIVER OF DAIRY COWS DURING THE TRANSITIONAL PERIOD

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SUMMARY: The objective of the present investigation was to determine the correlation in the blood concentrations of lipids and content in the liver in dairy cows (n=40) in transitional period. The cows were divided into four groups: the first group included late pregnant cows (n=10) from the 10th to 4th day before calving, the second group included late pregnant cows (n=10) from the 4th to 1th day before calving, the third group included puerperal healthy cows (n=10) whereas the fourth group included puerperal ketotic cows (n=10). Samples of liver and blood tissues were taken from all cows. Pathohistological examination of liver samples showed statistically significant higher lipid infiltration (P<0,01) in ketotic cows compared to healthy cows. Biochemical examination of blood serum showed significantly higher values (P<0,01) of free fatty acids in ketotic cows such as significantly lower concentrations of glucose (P<0,01), triacylglycerols (P<0,01) and total cholesterol (P<0,05) compared to the groups of healthy cows. Significant increase of the concentrations of free fatty acids, such as positive correlation (r=0.51, P <0.05) between the free fatty acids in the blood and the content of lipids in liver in ketotic cows compared to healthy ones, suggests that during intensive lipomobilization newly synthetized triacylglycerols accumulated in the hepatocytes.

***Key words** : cows, fatty liver, ketosis, lipids, glucose.*

INTRODUCTION

The transitional period in dairy cows included 3 weeks before and 3 weeks after calving when metabolic processes were adapted to provide energy and precursors required for synthesis of milk compounds (Grummer 1995; Overton and Waldron, 2004). Consequently, such a state caused negative energy balance, a high mobilization of lipids from bodily fat reserves as well as hypoglycaemia (Veenhuizen et al.1991; Gaál 1993;

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Vazquez-Anon et al. 1994). Lipomobilization, characterized by high concentrated free fatty acids in the blood, starts in late pregnancy, reaching its maximum in the early lactation. Free fatty acids are re-esterified and accumulated in the form of triacylglycerols in the liver, primarily due to a decrease capacity of hepatocytes for transport lipids by very low density lipoproteins (VLDL). As a result, lipomobilization intense ketogenosis and lipogenesis in the liver and as consequence lower concentrations of glucose, triacylglycerols and total cholesterol in the blood was manifested (Herdt et al. 1983; Holtenius 1989; Veenhuizen et al.1991; Vazguez-Anon et al. 1994; Sevinc et al. 2003; Đoković et al 2007, 2009, 2010). Primary homeoretic adaptation of glucose metabolism in the early lactation leads to an increased gluconeogenesis in the liver to direct glucose to the mammary gland for lactose synthesis. Metabolic conditions of negative energy balance (fasting, parturition and lactation) lead to an increased uncontrolled rate of mobilization of body fat and its increased accumulation in liver cells, resulting in disturbance of the morphological and physiological integrity of the liver (Grummer et al. 1995; Vazguez-Anon et al. 1994; Bobe et al. 2004). Mild fatty infiltration of liver cells in dairy cows during transition and maximum lactation is considered to be almost physiological. 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 (Gaal,1993). The objective of the present investigation was to determine the correlation in the blood concentration of lipids and content lipids in the liver in dairy cows in transitional period.

MATERIAL AND METHODS

The late pregnant and calved cows (n=40) were chosen from a Holstein dairy herd (PIK-Bečej) and divided into four groups: in the first group (A) included late pregnant cows (n=10) from the 10^{th} to 4^{th} day before calving; the second group (B) included late pregnant cows (n=10) from the 4^{th} to 1^{th} day before calving; third group (C) included 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.The diagnosis of ketosis was based on the clinical symptoms and determined high concentrations of urinary ketone (Lestradet test). The cows were 650 kg by weigt, had 3 lactations and 7625.2 ± 329.17 l milk of average milk production. The trial cows were kept in tie-up stalls in a barn housing. Diet and feeds were in conformity with purpose and animal utilisation. The blood samples were collected by punction of jugular vein into disposable test tubes from 10 h a.m. to noon or from 4 to 6 h after milking and feeding. The blood samples were allowed to clot spontaneously at room temperature. The serum was than centrifuged at 3000 rot/min.and preserved at -18 °C until analyzed. Glucose concentration was determined from fluoride plasma. The values for blood serum parameters were determined by photometric methods using an automatic analyser Cobas Mira and the following tests: glucose (Cat. No. 11803), triacylglycerols (TAG) (Cat. No. 11828), free fattty acids (FFA) (Cat No.FA 115) and total cholesterol (Cat. No. 11828). Shortly after blood sampling the liver tissue was sampled through liver percutaneus biopsy using a biopsy instrument following a modified method of Gaál after Hojovcava-Kacafirek (1967). The biopsy was performed at the right 11^{th} intercostal region, approximatelly 2 cm below the horizontal line through the tuber coxae, with 3-5 cm long and 3-4 mm wide liver specimen. The liver specimens were fixed in neutral 10% formaldehyde solution. Cryostat sections were stained with hemotoxylin and eosin and Sudan-III. Lipid conteined in the hepatocytes was determined through com-

puter image analysis (Software Q Win) made on aplliance (Leica Q 500 MC).The significance of differences of lipids, and glucose concentrations in the blood serum and the content of lipids in the liver between the animal groups used in experiment were determined by ANOVA procedure. Data are expressed as means ± standard deviation (x ± SD). Correlation coefficients were obtained using linear regression models. Differences with $P<0,05$ and $P<0,01$ were considered statistically significant (microsoft STATISTICA ver.5.0, Stat. Soft. Inc.1995).

RESULTS

The results of selected metabolic parameters in the groups of the cows in transitional period are shown in Table 1.

Table 1. Selected metabolic profile parameters in the groups of the cows in transitional period (means ± standard deviation)
Tabela 1. Vrednosti određenih metaboličkih parametara kod ispitivanih grupa krava u tranzicionom period (srednja vrednost ± standardna devijacija).

Groups / *Grupe*	Late pregnancy / *Visoki graviditet*		Puerperium / *Puerperium*	
	A	B	C	D
n	10	10	10	10
Glucose / *Glukoza* (mmol/l)	2.94 ± 0.32^{A}	3.12 ± 0.42^{B}	2.71 ± 0.35^{bc}	1.80 ± 0.43^{ABCD}
FFA / *SMK* (mmol/l)	0.27 ± 0.14^{A}	0.54 ± 0.26^{ABc}	0.46 ± 0.10^{AC}	0.74 ± 0.12^{ACD}
Triacylglycerols *Trigliceridi* (mmol/l)	0.32 ± 0.04^{A}	0.41 ± 0.03^{ABC}	0.35 ± 0.04^{C}	0.27 ± 0.03^{ABCD}
Total cholesterol *Ukupni holesterol* (mmol/l)	1.75 ± 0.20^{a}	1.71 ± 0.30^{b}	1.86 ± 0.62^{c}	1.39 ± 0.29^{acd}
Content of lipids in liver *Sadržaj masti u jetri* (%)	5.30 ± 1.10^{A}	$6.31 \pm 1,18^{B}$	8.37 ± 1.24^{C}	32.91 ± 13.23^{ABCD}

^{a,b,c,d} – Values with different superscripts are significantly different; values market by small superscripts differ significantly ($P<0,05$); values market by capital superscripts differ high-significantly ($P<0,01$).
^{a,b,c,d} – *Vrednosti sa različitim superskriptima su statistički različite; vrednosti obeležene malim superskriptima označavaju značajnost ($P<0,05$); vrednosti obeležene velikim superskriptima označavaju značajnost ($P<0,01$).*
(A - group of cows from 10^{th} to 4^{th} days before calving, B- group of cows from 4^{th} to 1^{st} days before calving; C- group of puerperal healthy cows; D- group of puerperal ketotic cows).
(A- grupa visoko gravidnih krava u periodu od 10. do 4.dana pre teljenja, B - grupa visoko gravidnih krava u periodu od 4. do 1. dana pre teljenja, C- grupa klinički zdravih tek oteljenih krava, D- grupa oteljenih krave obolelih od ketoze).

From Table 1 it can be seen that significant changes of the most parameters in blood in group of ketotic i.e. the group of cows with fatty liver. Biochemical examination in the blood serum showed significantly higher values ($P<0,01$) of free fatty acids in ketotic cows such as significantly lower concentrations of glucose ($P<0,01$), triacylglycerols ($P<0,01$) and total cholesterol ($P<0,05$), compared to values in the blood serum in the groups of healthy cows. The free fatty acids concentration was significantly higher ($P<0,01$) among groups of cows in late pregnancy. In the group of ketotic cows, the

content of lipids in the liver was higher (P<0,01) compared to groups of healthy cows before and after calving

DISCUSSION

In dairy cows, fatty liver occurs primarily in the first 4 weeks after calving, when up to 50 % of all cows have some accumulation of lipids in liver (Grummer et al. 1995; Bobe et al. 2004). In the group of puerperal ketotic cows the content of lipids in the liver was significantly higher (P<0,01) compared to groups of healthy cows. That ketosis and fatty liver are closely in association were obtained by other authors (Gröhn 1985; Veenhuizen et al.1991; Vazguez-Anon et al. 1994; Đokovic et al. 2007). Glucose is a blood parameter defining the energy metabolism in lactating cows. In the group of ketotic cows has determined hypoglycaemia which was statistically lower (P<0,01) compared with groups of healthy cows. Needs for glucose in early lactation in dairy cows is higher than the amount which the body can provide in the condition of high milk production and that it is a important factor in development of hypoglycaemia and ketosis. In ketotic cows there is a decreased ability of hepatocytes to synthesize glucose by gluconeogenesis which causes fatty liver development (Gröhn 1985; Veenhuizen et al.1991; Vazguez-Anon et al. 1994). Energy metabolism in dairy cows in transitional period is closely linked to lipid metabolism. The best indicator of negative energy balance and the degree of mobilization of lipids from bodily fat reserves in the transitional period is increased of FFA concentrations in the blood. (Veenhuizen et al.1991; Vazquez-Anon et al. 1994; Overton and Waldron 2004). The FFA concentrations have been significantly increasing (P<0,01) (among groups A and B) as the calving day was approaching that shows that lipomobilization begins immediately before calving, respectively from four to one days before calving. Similar results were obtained by *Dyk et al.*(1995). Significantly higher (P<0,01) FFA concentrations have been determined in the blood of ketotic cows than in the groups of healthy cows. In accordance, in this experiment has been determined significant positive correlation (r=0.51. P<0,05) among the FFA in the blood and content of lipids In liver in the group of ketotic cows. This agrees with previous studies (Gröhn 1985: Veenhuizen et al. 1991; Gaál 1993; Vazguez-Anon et al. 1994). At ruminants relatively low TAG concentrations in the blood serum and further decline of their concentrations could be the consequence of lipid infiltration of liver cells. In this experiment has been determined significantly lower (P<0,01) TAG concentrations in the blood in the group of ketotic cows than to the values in groups of healthy cows, such as significant negative correlation (r=-0.55, P<0,05) between the TAG concentrations in blood and amount of lipids in the liver in the group of ketotic cows. The indicated that the blood TAG concentrations decreased and proportionally to that increases their amount in the liver cells in which they accumulated. These results are in accordance with observation (Herdt et al. 1983, Gerloff et al.1986; Holtenius 1989, Sevinc et al. 2003). In this study the were determined significantly lower (P<0,05) level of the total cholesterol in the blood in ketotic cows than of healthy groups of cows, which indicate that at condition of ketosis and fatty infiltration of liver cells in dairy cows, their ability to synthesize and transport cholesterol and TAG are decreased (Gerloff et al.1986; Gaál 1993, Sevinc et al. 2003).

CONCLUSION

Pathohistoligical examination of liver samples shows statistically significant higher lipid infiltration (P<0,01) in ketotic cows compared to groups of healthy cows .

Biochemical examination of blood serum showed significantly higher values (P<0,01) of free fatty acids in ketotic cows such as significantly lower concentrations of glucose (P<0,01), triacylglycerols (P<0,01), total cholesterol (P<0,05) compared to the groups of healthy cows.

Significant increase of the concentrations of free fatty acids, such as positive correlation (r=0.51, P<0,05) between the free fatty acids in the blood and the content of lipids in liver in ketotic cows compared to healthy ones, suggest that during intensive lipomobilization newly synthetized triacylglycerols accumulated in the hepatocytes.

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SADRŽAJ LIPIDA U KRVI I JETRI KOD MLEČNIH KRAVA U TRANZICIONOM PERIODU

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Izvod

Cilj ovog rada je bio da se odrede korelacije između koncentracija lipida u krvi i sadržaja lipida u jetri kod mlečnih krava (n=40) u tranzicionom periodu. Ispitivane krave su podeljene u četiri grupe. U prvoj grupi (n=10) nalazile su se visoko gravidne krave u periodu od 10. do 4.dana pre teljenja, u drugoj grupi visoko gravidne krave (n=10) u periodu od 4. do 1. dana pre teljenja, u trećoj grupi (n=10) su se nalazile klinički zdrave tek oteljene krave, a u četrvroj grupi (n=10) ketozne krave. Od svih ispitivanih krava uzeti su uzorci krvi i jetre. Patohistološka ispitivanja uzoraka tkiva jetre pokazala su statistički značajne veće ($P<0,01$) vrednosti sadražaja masti u jetri kod grupe ketoznih krava u odnosu na sadržaj masti u jetri kod grupa zdravih krava. Biohemijska ispitivanja krvnog seruma pokazala su statistički veće ($P<0,01$) koncentracije slobodnih masnih kiseliina, kao i statistički manje vrednosti glukoze ($P<0,01$) triglicerida ($P<0,01$) i ukupnog holesterola ($P<0,05$) kod grupe ketoznih krava u odnosu na vrednosti kod zdravih krava. Značajno veće vrednosti ($P<0,01$) slobodnih masnih kiselina, kao i pozitivna koleracija ($r=0.51$, $P<0,05$) između koncentaracijai slobodnih masnih kiselina u krvi i sadržaja masti u jetri kod grupe ketoznih krava, ukazuje da za vreme intenzivne lipomobilizacije novosintetisani trigliceridi se nakupljaju u ćelijama jetre.

Ključne reči : krave, masna jetra, ketoza, lipidi, glukoza.

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