

The functional state of liver cells in dairy cows during transition and lactation

R. Đoković

Faculty of Agronomy, Čačak, Serbia

Z. Ilić

Faculty of Agriculture, Zubin Potok, Serbia

V. Kurćubić, S. Jevtić

Faculty of Agronomy, Čačak, Serbia

Abstract: The objective of this study was to determine characteristic blood parameters, i.e. indicators of the functional state of liver in the following groups of cows: in cows (n=15) from day 15 to day 1 prior to parturition, in cows (n=15) from day 1 to day 15 after parturition and in those (n=15) from day 90 to day 100 of lactation. Blood glucose levels were statistically significantly lower in the early lactation cows than in the preparturient cows and those examined during the maximum lactation period, which suggested an increased glucose uptake by the mammary gland at the beginning of lactation and decreased gluconeogenesis in the liver as induced by fatty infiltration and liver cell degeneration. The significantly lower blood triglyceride levels as well as the low total blood cholesterol levels in the postparturient cows reflected their accumulation in liver cells, as opposed to the cows before parturition and those during maximum lactation. Significantly lower blood levels of total protein, albumin and urea were recorded in the postparturient cows, which suggested the reduced synthetic capacity of liver cells in the early lactation cows. Blood bilirubin levels in the early lactation cows were significantly higher as compared to the preparturient and maximum lactation cows, which clearly indicated the reduced excretory capacity of the liver. Significantly increased AST, GGT and LDH activities in the blood in the postparturient cows clearly evidenced the disturbed morphological and functional integrity of liver cells and the release of these intracellular

enzymes into the blood due to the fat accumulation in the hepatocytes. The obtained results suggested that fatty infiltration and different degrees of liver cell degeneration were recorded in the early lactation cows, as opposed to the preparturient cows and those during maximum lactation showing preserved morphological and functional capacities of hepatocytes.

Key words: cows, fatty liver, proteins, lipids, bilirubin, enzymes

Introduction

During the transition period, from immediately before to after parturition, and with the establishment of lactation, the organism in high-yielding dairy cows is pushed to its physiological limits, reaching maximum until day 120 of lactation, resulting in a substantial load on the organism, specifically on the digestive organs, liver, udder and the reproductive organs.

Major health disorders in high-yielding cows occur around parturition. They include sudden changes in energy metabolism that are likely to induce severe uncontrolled disorders of organic matter metabolism (Grummer 1995, Overton and Waldron 2004).

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 physiological integrity and morphology of the liver (Veenhuizen *et al.* 1991, Reist *et al.* 2002, Djoković *et al.* 1998, 2007).

Moderate fatty infiltration of liver cells in dairy cows during transition and maximum lactation is considered to be almost physiological. The fat content of liver can range from 10-60%, as dependent on the degree of pathology (Gaal 1993).

Increased metabolic load on the dairy cows' organism and fat accumulation in liver cells induce disturbances in the morphological and functional integrity of hepatocytes, resulting in decreased blood levels of individual liver-synthesized indicators of liver function (glucose, total protein, albumin, globulin, cholesterol, triglycerides, urea). Furthermore, the excretory function of hepatocytes is reduced and, accordingly, the levels of certain metabolic products in the blood (bilirubin, ammonia, bile acids) are generally increased (Herdt *et al.* 1983, Holtenius 1989, Veenhuizen *et al.* 1991, Vazquez-anon *et al.* 1994, Reynolds *et al.* 2003, Sevinc *et al.* 2003, Lubojacka *et al.* 2005, Đoković *et al.* 1998, 2007).

Blood bilirubin concentration is among major indicators of the functional capacity of hepatocytes (Vasilev 1979, Bobe 2004).

Severe fatty liver and diffuse infiltration of hepatocytes involve cell membrane damage and hepatocyte destruction accompanied by the release of cytoplasmic enzymes (AST, GGT, LDH), the activity thereof in the blood being considerably elevated (Jovanović *et al.* 1993, Pechova 1997, Lubojacka *et al.* 2005).

Considering the increased metabolic loads on the liver in lactating dairy cows, the objective of the present study was to determine blood parameters, i.e. indicators of the functional condition of the liver, being the following: glucose, triglyceride, total cholesterol, total proteins, albumins, urea, bilirubin and activities

of liver-specific enzymes (AST, GGT, LDH) in both transition and lactating dairy cows.

Material and Method

Animals used in the experiment

Preparturient cows, freshly calved cows and those from day 90 to day 100 of lactation (n=45) were randomly selected from the Simmental herd (Farm-Farmad-Vrdila-Kraljevo) for examination. The cows were divided into three groups. The first group (group A) included clinically healthy cows from day 15 to day 1 before parturition (n=15). The second group (group B) comprised clinically healthy cows from day 1 to day 15 after parturition (n=15), the third one (group C) being composed of clinically healthy cows from day 90 to day 100 of lactation (n=15). The examined cows were four to six years old. Their average weight was 650 kg pre during the prepartum period and 580 kg after parturition and during lactation. There were three lactations on average with an average milk yield of 6,825 l of milk over the period of 305 days of lactation. The examinations were conducted during the same season, in mid July. The cows were kept in free stalls in a closed barn. The diet and the housing facilities were adapted to research purposes. The diet suited the energy needs of the cows in late pregnancy and lactation.

Blood sampling

Blood was sampled from all examined cows by puncture of vena jugularis, from 10:00 to 12:00 a.m, i.e. four to six hours after milking and feeding. Two test tubes of blood (approximately 20 ml) were taken per puncture. Serum separation after spontaneous coagulation at room temperature was performed by centrifugation at 3000 revolutions/min. The serum samples were kept refrigerated at -18 °C until analysis.

Biochemical examination of the blood serum

An enzymatic spectrophotometric assay was used to determine the levels of glucose (cat. No. 11803), triglyceride (cat. No. 11828) and urea (cat. No. 11536). The levels of total cholesterol (cat. No. 11828), total protein (cat. No. 11500), albumin (cat. No. 11547) and bilirubin (cat. No. 11515) and the activities of the liver-specific enzymes: AST- aspartate aminotransferase (cat. No. 11830), GGT – gamma-glutamyl transferase (cat. No. 11584), LDH- lacto-dehydrogenase (cat. No. 11552), were determined spectrophotometrically. All biochemical blood parameters were assayed using a Cobas Mira device at the biochemical laboratory Medicus in Kraljevo.

Statistical analysis of the obtained data

The statistical analysis of the obtained data was carried out by ANOVA-procedure. The analysis of variance and LSD test were used to evaluate the probability of the significance of the statistical differences of mean blood parameter values between the groups of cows used in the experiment at $p < 0.05$ and $p < 0.01$. (Microsoft STATISTICA ver.5.0 Stat.Soft.Inc.1995).

Results

Table 1 shows the research results on the blood levels of glucose, triglyceride, total cholesterol, total proteins, albumin, urea and bilirubin as well as those on the activities of AST, GGT and LDH in dairy cows during transition and lactation.

Tab. 1. Blood levels of glucose, triglyceride, total cholesterol, total protein, albumin, urea and bilirubin and the activity of AST, GGT and LDH in the preparturient cows (group A), in the postparturient cows (group B) and in those during maximum lactation (group C) and the statistical significance of the obtained means

Group	A	B	C	P<0.05	P<0.01
n	15	15	15		
Glucose (mmol/l)	2.73 ± 0.70	2.21 ± 0.48	3.11 ± 0.46	A:B	B:C
Triglycerides (mmol/l)	0.29 ± 0.14	0.18 ± 0.08	0.27 ± 0.10	A:B;B:C	
Total cholesterol (mmol/l)	2.66 ± 0.58	2.26 ± 0.59	2.75 ± 0.84		
Total proteins (g/l)	70.26 ± 10.54	63.51 ± 7.70	72.71 ± 6.65	A:B	B:C
Albumins (g/l)	33.98 ± 4.63	29.54 ± 3.89	35.43 ± 3.83		A:B;B:C
Urea (mmol/l)	4.47 ± 1.60	3.85 ± 0.98	5.50 ± 1.30	A:B	B:C
Bilirubin (μmol/l)	2.92 ± 0.83	4.44 ± 1.11	2.73 ± 0.95		A:B;B:C
AST (IJ/l)	43.78 ± 15.18	64.41 ± 18.08	39.47 ± 7.36		A:B;A:C
GGT (IJ/l)	9.37 ± 2.98	14.65 ± 4.25	8.45 ± 1.86		A:B;A:C
LDH (IJ/l)	1250 ± 382.5	1805 ± 386.8	1167 ± 336.1		A:B;A:C

Table 1 shows that blood levels of glucose and total proteins were statistically significantly lower in the postparturient cows than in the preparturient ones ($p<0.05$) and in those during maximum lactation ($p<0.01$). Blood urea values in the postparturient cows were statistically significantly lower as compared to those in the cows before parturition ($p<0.05$) and during lactation ($p<0.01$). Statistically significantly lower blood levels of triglyceride ($p<0.05$) were found in the postparturient cows than in the cows before parturition and in those during lactation. Total cholesterol values were lower in the postparturient cows than in the other two groups of cows, but no statistical significance was recorded ($p>0.05$).

Blood bilirubin values and the activity of the AST, GGT and LDH enzymes were statistically significantly higher ($p < 0.01$) in the postparturient cows than in the cows before parturition or those during lactation.

Discussion

Glucose is a blood parameter defining the energy metabolism in late pregnancy and lactating cows. Blood glucose levels in all examined groups of cows was within the physiological limits (2.2-4.0 mmol, Jovanović 1984). The postparturient cows showed statistically significantly lower blood glucose values as compared to the preparturient and lactating cows. The above results are in agreement with the literature data (Veenhuizen *et al.* 1991, Grummer 1995, Reist *et al.* 2002, Đoković *et al.* 2007) indicating that physiological glycemia in early lactation cows is at the lower physiological limit due to the sudden activity of the mammary gland and increased lactose synthesis. Furthermore, the negative energy balance, lipomobilization and increased fat accumulation in hepatocytes induce a considerable reduction in glucose synthesis by gluconeogenesis in the liver.

Lipid metabolism parameters include the blood levels of triglyceride and total cholesterol. Significantly lower blood triglyceride levels were determined in the postparturient cows, the total cholesterol values being lower but statistically insignificant as compared to those in the other two groups of cows. The results suggested an increased accumulation of triglyceride and total cholesterol in liver cells in the early lactation cows. The data are in agreement with the results obtained by other authors (Pechova *et al.* 1997, Veenhuizen *et al.* 1991, Vazquez-Anon 1994, Sevinc *et al.* 2003, Đoković *et al.* 1998, 2007) showing that the triglyceride and cholesterol transport from the liver into blood by the very low-density lipoproteins was significantly reduced due to lipomobilization, the development of fatty infiltration and hepatocyte degeneration in early lactation.

Nitrogen metabolism parameters include determination of the blood levels of liver-synthesized total proteins, albumin and urea, the values thereof decreasing in cases of liver cell damage (Jovanović *et al.* 1993, Lubojacka *et al.* 2005). Albumin is an indicator of the synthetic capacity of the liver, its decrease in the blood to values as low as 20% being induced by chronic liver diseases (Sevinc *et al.* 2003). The values of the above blood parameters were within the physiological limits (total proteins 60-80 g/l; albumins 30-40 g/l; urea 1.66-6.66 mmol/l) in all examined groups of cows (Jovanović 1984). They were statistically significantly lower in the early lactation cows than in the other two groups of cows, suggesting the reduced synthetic capacity of the liver cells in the early lactation cows. The reduced synthesis of total proteins, albumin and urea at the beginning of lactation in dairy cows is induced by the development of fatty infiltration and degeneration of liver cells (Pechova *et al.* 1997, Sevinc *et al.* 2003, Overton and Walton 2004, Lubojacka *et al.* 2005).

Blood bilirubin value is a highly sensitive indicator of the functional capacity of liver cells. Reynolds *et al.* (2003) reported positive correlation between the blood bilirubin values and the liver fat content. The blood bilirubin values recorded in the present study were within physiological limits (0.85- 5.60 $\mu\text{mol/l}$) in all examined groups of cows (Jovanović, 1984). Significantly higher

values were determined in the early lactation cows suggesting the disturbance in the excretory capacity of the liver cells due to fat accumulation in the hepatocytes. Similar results were obtained by other authors (Vasilev 1979, Herdt et al. 1983, Holtenius 1989, Bobe 2004). According to Rosenberger (1979), the level of blood bilirubin of up to 8.55 $\mu\text{mol/l}$ reflects a liver function disturbance or a hemolytic process, the levels above 8.55 $\mu\text{mol/l}$ always suggesting liver pathology.

The activities of enzymes are highly important blood parameters used in evaluating the degree of hepatocyte damage. The study focused on determining the blood activities of AST, GGT and LDH enzymes. AST is located in the cytoplasm and mitochondria of different tissues and organs, its highest activities being detected in the heart and skeletal musculature as well as in the liver. The AST activity is found to be the most sensitive indicator in diagnosing fatty liver in cows (Pechova *et al.* 1997, Lubojacka *et al.* 2005). Kupczynski *et al.* (2002) determined an increase in blood AST activity in the postparturient cows during the high metabolic load, resulting in pathological changes occurring after parturition, disturbed reproduction, damages in the parenchymatous organs and the energy metabolism disturbance. Blood AST activities in this study were found to be statistically highly significantly higher in the postparturient cows than in the preparturient and lactating ones, which suggested the development of fatty infiltration of hepatocytes, cell membrane damages and the release of the intracellular enzyme into the blood.

Blood GGT activity was above physiological limits in the postparturient cows 3.5-10 IU/l (Jovanović 1984), being statistically significantly higher as compared to the other two groups of cows, indicating the increased activity of the enzyme in the postparturient cows. GGT is a microsomal and membrane-bound enzyme found mostly in the liver, kidneys and small intestines. The increase in the activity of this enzyme results from damages of the cellular structure of hepatocytes (Jovanović *et al.* 1993, Kupczynski *et al.* 2002, Lubojacka *et al.* 2005).

LDH is not an organ-specific enzyme, being found at high concentrations in the muscles, heart, kidneys and the liver. It is released into the blood in cases of acute cell damage of the above organs. The study revealed a statistically significantly higher activity of LDH in the postparturient cows, clearly indicating hepatocyte damage and the enzyme release into the blood. According to Pechova *et al.* (2007), the blood activity of LDH is correlated with the degree of fatty infiltration of hepatocytes.

Conclusion

Blood glucose levels were statistically significantly lower in the early lactation cows than in the preparturient and maximum lactation ones, suggesting an increased glucose uptake by the mammary gland at the beginning of lactation and a reduced degree of gluconeogenesis in the liver, as induced by the development of fatty infiltration and liver cell degeneration.

Significantly lower blood triglyceride values and low values of total cholesterol in the postparturient cows suggest their being accumulated in the liver

cells, as opposed to the preparturient cows and those examined during the maximum lactation period.

Significantly lower blood levels of total proteins, albumin and urea were found in the postparturient cows, which suggested the decreased synthetic capacity of liver cells in the early lactation cows.

Blood bilirubin levels were significantly higher in the cows at the beginning of lactation than in the preparturient cows and those during maximum lactation, which clearly indicated the decreased excretory capacity of the liver.

Significantly increased activities of AST, GGT and LDH in the blood of the postparturient cows clearly reflected the disturbed morphological and functional integrity of liver cells and the release of the intracellular enzymes into the blood as induced by fat accumulation in hepatocytes.

The analysis of the blood parameters as indicators of the functional capacity of liver cells suggested that early lactation cows revealed fatty infiltration and different degrees of liver cell degeneration, as opposed to the cows before parturition and those during maximum lactation, in which the morphological and functional capacity of hepatocytes was preserved.

References

- Bobe G. (2004): Pathology, etiology, prevention, treatment of fatty liver in dairy cows. *J. Dairy Sci.*, **87**: 3105-3124.
- Doković R., Šamanc H., Kurćubić V. (2007): Changes in Lipid Content of the Blood and Liver of Cows in Periparturient Period. *Acta Agriculturae Serbica*, **3** (6): 9-16.
- Doković R., Šamanc H., Jovanović M., Nikolić Z. (2007): Blood concentrations of thyroid hormones and lipids in the liver in dairy cows in transitional period. *Acta Vet. Brno*, **76**: 525-532.
- Gaal T. (1993): Sindrom masne jetre u mlečnih krava. *Vet. Glasnik*, **47** (4-5): 311-317.
- Grummer R.R. (1995): Impact of changes in organic nutrient metabolism on feeding the transition dairy cows. *J. Anim. Sci.*, **73**: 2820-2833.
- Herd T.H., Leisman J.S., Gerloff B.J., Emery R.S. (1983): Reduction of serum triacylglycerol-rich lipoprotein concentrations in cows with hepatic lipidosis. *Am. J. Vet. Res.*, **44**: 293-296.
- Holtenius P. (1989): Plasma lipids in normal cows around partus and in cows with metabolic disorders with and without fatty liver. *Acta Vet. Scand.*, **30**: 441-445.
- Jovanović M. (1984): *Fiziologija domaćih životinja*. Beograd –Zagreb.
- Jovanović M.J., Šamanc H., Damnjanović Z., Marković S., Doković R. (1993): Funkcionalno stanje jetre krava u visokom graviditetu i ranoj laktaciji. *Vet.glasnik*, **47** (4-5): 295-310.
- Kupczynski R., Chudoba-Drozdowska B. (2002): Values of selected biochemical parameters of cows blood during their drying-off and the beginning of lactation. *Electronic journal of Polish Agricultural Universities*, **55**: 225-231.
- Lubojacka V., Pechova A., Dvorak R., Drastich P., Kummer V., Poul J. (2005): Liver steatosis following supplementation with fat in dairy cows diets. *Acta Vet. Brno*, **74**: 217-224.
- Overton T.R., Waldron M.R. (2004): Nutritional management of transition dairy cows: Strategies to optimize metabolic health. *J. Dairy Sci.*, **87**: 105-119.
- Pechova A., Llek J., Halouzka R. (1997): Diagnosis and control of the development

- of hepatic lipidosis in dairy cows in the periparturient period. *Acta Vet. Brno*, **66**: 235-243.
- Reist M., Erdin D., Von Euw D., Tschuemperlin K., Leunberger H., Chilliard H., Hammon M., Morel C., Philopona C., Zbinder Y., Kuenzi N., Blum J.W. (2002): Estimation of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. *J. Dairy Sci.*, **85**: 3314-3327.
- Reynolds C.K., Aikman P.C., Lupoli B., Humpheirs D.J., Beever D.A. (2003): Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. *J. Dairy Sci.*, **86**: 1201-1217.
- Rosenberger G. (1979): *Clinical examination of cattle*. Verlag Paul Parey. Berlin and Hamburg.
- Sevinc M., Basoglu A., Guzelbekta H. (2003): Lipid and lipoprotein levels in dairy cows with fatty liver. *Turk. J. Vet. Anim.Sci.*, **27**: 295-299.
- Vasilev B. (1979): Bilirubin v krvia serum na kravi, klinički zdravi bolni atketozat is hepatopati. *Vetrinarmedicinski Nauki*, **2**:7-11.
- Vazquez-Anon M., Bertics S., Luck M., Grummer R. (1994): Peripartium liver triglyceride and plasma metabolites in dairy cows. *J. Dairy Sci.*, **77**: 1521-1528.
- Veenhuizen J.J., Drackley J.K., Richard M.J., Sanderson T.P., Miller L. D., Joung J.W. (1991): Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. *J. Dairy Sci.*, **74**: 4238-4253.

FUNKCIONALNO STANJE ĆELIJA JETRE KOD MLEČNIH KRAVA U TRANZICIONOM PERIODU I TOKOM LAKTACIJE

- originalni naučni rad -

R. Djoković

Agronomski fakultet, Čačak

Z. Ilić

Poljoprivredni fakultet, Zubin Potok

V. Kurćubić, S. Jevtić

Agronomski fakultet, Čačak

Rezime

Cilj ovog rada je bio da se odrede odgovarajući parametri krvi, indikatori funkcionalnog stanja jetre kod grupe krava (n=15) od 15. dana do 1. dana pre telenja, kod grupe krava (n=15) od 1. do 15. dana posle telenja i kod grupe krava (n=15) u periodu od 90 do 100. dana laktacije. Koncentracije glukoze u krvi su bile statistički značajno manje kod grupa krava na početku laktacije u odnosu na vrednosti u krvi kod grupa krava pre telenja i tokom maksimalne laktacije što ukazuje na povećanu potrošnju glukoze od strane mlečne žlezde na početku laktacije i smanjeni stepen glukoneogeneze u jetri usled razvoja masne infiltracije i degeneracije ćelija jetre. Značajno manje vrednosti triglicerida u krvi kao i niske vrednosti ukupnog holesterola u krvi kod grupe krava posle telenja ukazuju na njihovo nakupljanje u ćelijama jetre, za razliku od grupa krava pre telenja i tokom maksimalne laktacije. Kod grupe krava posle telenja utvrđene su značajno manje vrednosti ukupnih proteina, albumina i uree u krvi što ukazuje na smanjenje sintetske sposobnosti ćelija jetre kod krava na početku laktacije. Koncentracije bilirubina u krvi kod krava na početku laktacije su bile značajno veće u odnosu na vrednosti kod krava pre telenja i tokom maksimalne laktacije, što jasno ukazuje na smanjenu ekskrecionu sposobnost jetre. Značajno povećane aktivnosti AST, GGT i LDH u krvi kod grupe krava posle telenja jasno ukazuju na narušen morfološki i funkcionalni integritet ćelija jetre i oslobađanje ovih intracelularnih enzima u krv usled nakupljanja masti u hepatocitima. Na osnovu dobijenih rezultata može se zaključiti da kod krava na početku laktacije je prisutna masna infiltracija i degeneracija ćelija jetre različitog stepena, za razliku od grupa krava pre telenja i tokom maksimalne laktacije kod kojih je očuvana morfološka i funkcionalna sposobnost hepatocita.