

EVALUATION OF METABOLIC STATUS IN DAIRY COWS DURING TRANSITION PERIOD

Radojica DJOKOVIC^{1,*}, Marko CINCOVIC², Vladimir KURCUBIC¹, Zoran ILIC³, Milun PETROVIC¹, Miroslav LALOVIC⁴, Boban JASOVIC³

¹Department of Animal Science, Faculty of Agronomy, University of Kragujevac, Čačak, Serbia

²Department of Veterinary medicine Faculty of Agriculture, University of Novi Sad, Novi Sad, Serbia

³Department of Animal Science, Faculty of Agronomy, University of Priština, Lešak, Serbia

⁴Faculty of Agriculture, University of East Sarajevo, Bosnia and Herzegovina

*Corresponding author: djokovici@ptt.rs

Abstract

The objective of this study was to evaluate organic and inorganic blood parameters, i.e. indicators of metabolic status in dairy cows (n=30) during the transition period. The cows were divided into two groups. The first group (n=15) comprised clinically healthy prepartal cows, and the second one included clinically health postpartal cows (n=15). Blood samples were taken from all the examined dairy cows. Blood glucose levels were statistically significantly lower ($p<0.05$) in the postpartum cows, which suggested an increased glucose uptake by the mammary gland and decreased gluconeogenesis in the liver. The significantly lower ($p<0.05$) blood triglyceride levels as well as the low total blood cholesterol levels ($p>0.05$) in the postpartum cows indicated their accumulation in liver cells. Significantly lower ($p<0.05$) blood levels of total protein, albumin and urea were recorded in the postpartum cows, which suggested the reduced synthetic capacity of liver cells. Blood bilirubin levels in the postpartum cows were significantly higher ($p<0,01$), which clearly indicated the reduced excretory capacity of the liver. Blood calcium, phosphorus and magnesium levels in the postpartum cows were lower, but they were not statistically significant ($p>0.05$), which most likely resulted from the increased uptake of these blood macroelements by the mammary gland. Biochemical evaluation suggested that early lactation cows had metabolic disturbances associated with some degree of hepatic lesions, probably due to fat infiltration.

Key words: *cows, metabolic status, blood metabolites*

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 and morphology integrity of the liver (Veenhuizen et al., 1991; Reist et al., 2002; Bobe, 2004; Djoković et al., 2007, 2011). 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 resulting in decreased blood levels of

individual liver-synthesized indicators of liver function (glucose, total protein, albumin, globulin, cholesterol, triglyceride, 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; Bobe, 2004; Lubojacka et al., 2005; Djoković et al., 2007, 2011). The blood levels of calcium, inorganic phosphorus and magnesium in cows during peripartal period, reflect the intake of intake if these macroelements through diet and their utilization by the mammary gland. Any reduction in these blood parameters as compared to the physiological values in cows in the beginning of lactation, or their deficiency, as well as an abnormal relationship between them most commonly lead to subclinical and clinical manifestations which adversely affect cow health and fertility (Ivanov et al., 1993; Sevinc et al., 1997; Kupczynski et al., 2002). The objective of this study was to evaluate organic and inorganic blood parameters, i.e. indicators of metabolic status in dairy cows during the transitional period.

Materials and methods

This experiment was conducted in a dairy Simmental herd diagnosed with a number of metabolic and reproductive disorders. The cows were mid-yielding with a preceding lactation of about 6.500 l. Two groups of clinically healthy cows were chosen from the herd: Group 1, consisting of late pregnant cows (n = 15) at 25 to 1 (13 ± 9) days to partus, and Group 2, including early post-partum cows (n = 15) in the first month of lactation (16 ± 9 days). Body condition scores (BCS) were recorded by the same observer using the 1~5 scale according to Ferguson et al. (1994), with 1 being too thin and 5 too fat. Late pregnant and early lactation cows had BSC 3.80 ± 0.33 and 3.42 ± 0.55, respectively. The experimental cows were kept in tie-stall barns. The diet and the housing facilities were adapted to research purposes. The diet suited the energy requirements for cows in late pregnancy and early lactation. The cows in late pregnancy were fed a diet consisting of 6 kg lucerne hay, 15 kg maize silage (30% DM) and 3 kg concentrate (18% crude proteins, CP). The cows in early lactation received a diet consisting of 7 kg lucerne hay, 20 kg maize silage (30% DM) and 5 kg concentrate (18% CP). Dietary nutrient contents for dairy cows in late pregnancy and early lactation are given in Table 1.

Table 1. Nutrient contents in daily ration for dairy cows in late pregnancy and early lactation.

	Late pregnancy	Early lactation
Dry Matter (DM) (kg)	11.94	16.05
Net energy of lactation (NEL) (MJ)	65.25	87.15
Crude protein (CP) (% of DM)	12.55	13.58
Rumen undegradable protein (RUP) (% of CP)	30.86	35.91
Fat (% of DM)	3.27	3.15
Fiber (% of DM)	25.28	23.26

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. 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 following blood biochemical components were measured at the Biochemical laboratory by different colorimetric techniques using spectrophotometers (Cobas Mira and Gilford Stasar): glucose, total cholesterol, total bilirubin, magnesium and inorganic phosphorus using kits from Human (Germany), albumin, urea and calcium using kits from Biosystem (Spain), total protein and triglyceride using kits from Elitech (France). Data were subjected to statistical

analysis using the GLM model and t-test for difference of means between two independent groups (late pregnancy group vs. early lactation group) (software: Statgraphic Centurion, Statpoint Technologies Inc. Warrenton, Va, Virginia, USA). Differences were considered significant at p values below 0.05.

Results and discussion

Blood metabolic organic and inorganic parameters in late pregnant and early lactation cows were compared in this study. Table 1 shows the research results on the blood levels of glucose, triglyceride, total cholesterol, total protein, albumin, urea and total bilirubin as well as blood levels calcium, magnesium and inorganic phosphorus in dairy cows during periparturient period.

Table 2. Metabolic parameters in the late pregnant cows and in the early lactation cows and significant difference

Group	Late pregnancy	Early lactation
n	15	15
Glucose (mmol/l)	3.36 ± 0.30 ^a	2.29 ± 0.48 ^b
Triglyceride (mmol/l)	0.29 ± 0.07 ^a	0.12 ± 0.02 ^b
Total cholesterol (mmol/l)	3.48 ± 1.07 ^a	3.24 ± 0.81 ^a
Total protein (g/l)	78.89 ± 4.92 ^a	75.27 ± 4.49 ^b
Albumin (g/l)	42.57 ± 7.53 ^a	34.61 ± 3.56 ^b
Urea (mmol/l)	5.29 ± 1.32 ^a	3.60 ± 1.07 ^b
Bilirubin (µmol/l)	3.91 ± 1.85 ^a	5.22 ± 1.05 ^b
Calcium (mmol/l)	2.25 ± 0.26 ^a	2.16 ± 0.29 ^a
Magnesium (mmol/l)	0.91 ± 0.16 ^a	0.85 ± 0.19 ^a
Inorganic phosphorus (mmol/l)	1.59 ± 0.31 ^a	1.46 ± 0.18 ^a

Legends: Mean values within a row with no common superscript differ significantly ($P < 0.05$).

Glucose is a blood parameter defining the energy metabolism in late pregnancy and early lactating cows. Blood glucose values in late pregnant cows were within the physiological range of 2.5 - 4.2 mmol/l (Radostits et al., 2000), whereas hypoglycemia was detected in early lactation cows. The postparturient cows showed statistically significantly lower ($p < 0.05$) blood glucose values as compared to the preparturient cows. The above results are in agreement with the literature data (Veenhuizen et al., 1991; Grummer, 1995; Reist et al., 2002; Djoković et al., 2007, 2011) 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 ($p < 0.05$) blood triglyceride levels were determined in the postparturient cows, the total cholesterol values being lower, but statistically insignificant ($p > 0.05$) as compared to those in the other group 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 et al., 1994; Sevinc et al., 2003; Djoković et al., 2007, 2011) showing that the triglyceride and cholesterol transport from the liver into blood by the very low-density lipoproteins was 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 protein, albumin and urea, the values there of decreasing in cases of liver

cell damage (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 protein 60-80 g/l; albumin 30-40 g/l; urea 1.66-6.66 mmol/l; Radostits et al., 2000) in all examined groups of cows. They were statistically significantly lower ($p < 0.05$) in the early lactation cows than in the other group of cows, suggesting the reduced synthetic capacity of the liver cells in the early lactation cows. The reduced synthesis of total protein, 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; Djoković et al., 2011). 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 $\mu\text{mol/l}$; Radostits et al., 2000) in all examined groups of cows. Significantly higher ($p < 0.05$) 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 (Herdt et al., 1983; Holtenius, 1989; Bobe, 2004).

In this study, blood levels of calcium, magnesium and inorganic phosphorus were lower, but no statistical significance ($p > 0.05$) in postparturient cows than in preparturient cows. The obtained results on the blood level of macroelements showed that the calcium and inorganic phosphorus values (Ca: 2.0-3.0 mmol/l; P: 1.6-2.3 mmol/l; Radostits et al., 2000) in the blood serum were under or at the lower physiological limit in both groups of cows, which resulted from the sudden activity of the mammary gland and its utilization of these macroelements. The obtained results suggested the possible development of subclinical puerperal paresis in early lactation cows. Similar results were obtained by other authors (Ivanov et al. 1993, Sevinc et al. 1997). Magnesium homeostasis depends on an optimum supply from alimentary sources and, hence, magnesium levels depend on ruminal resorption. Magnesium resorption is insufficient in diets rich in potassium and proteins, but lacking cellulose. Magnesium values in the early lactation cows were low within physiological range (0.7-1.2 mmol/l; Radostits et al., 2000) and in accordance with results of other authors (Ivanov et al., 1993; Sevinc et al., 1997; Kupczynski et al., 2002) suggesting a reduced supply from alimentary sources and/or increased utilization by mammary gland at the beginning of lactation.

Conclusion

Significantly lower ($p < 0.05$) blood levels of glucose, total protein, albumin and urea were found in the postparturient cows, which suggested the decreased synthetic capacity of liver cells. Significantly lower ($p < 0.05$) blood triglycerides values and low values of total cholesterol ($p > 0.05$) in the postparturient cows suggest their being accumulated in the liver cells, as opposed to the preparturient cows. Blood bilirubin levels were significantly higher ($p < 0.05$) in the cows at the beginning of lactation than in the preparturient cows, which clearly indicated the decreased excretory capacity of the liver. The analysis of the blood parameters as indicators of the functional capacity of liver cells suggested that early lactation cows had metabolic disturbances associated with some degree of hepatic lesions, probably due to fat infiltration, as opposed to the cows before parturition, in which the morphological and functional capacity of hepatocytes was preserved. Blood calcium, phosphorus and magnesium levels in the postpartum cows were lower, but they were not statistically significant ($p > 0.05$), which most likely resulted from the increased uptake of these blood macroelements by the mammary gland.

Acknowledgment

This study was financially supported by the Ministry of Education and Science, Republic of Serbia, Projects TR31001 and TR31062.

References

- Bobe G. 2004. Pathology, etiology, prevention, treatment of fatty liver in dairy cows. *Journal of Dairy Science*, vol. 87, 3105-3124.
- Djoković R., Šmanc H., Jovanović M., Nikolić Z. 2007. Blood concentrations of thyroid hormones and lipids in the liver in dairy cows in transitional period. *Acta Veterinaria. Brno*, vol.76, 525-532.
- Djoković R., Ilić Z., Kurćubić V., Petrović M., Dosković V. 2011. Functional and morphological state of the liver in Simmental dairy cows during transitional period. *Revue de Medecine Veterinaire* 162, vol.574-579.
- Gaal T. 1993. Fatty liver syndrome in dairy cows. *Veterinarski glasnik*, vol.47, No 4-5, 311-317.
- Ferguson J.D., Galligan D.T., N. Thomsen N. 1994. Principal descriptors of body condition score in Holstein cows. *Journal of Dairy Science*, vol. 77, 2695-2703.
- Grummer R.R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cows. *Journal of Animal Science*, vol. 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. *American Journal of Veterinary Research*, Vol. 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 Veterinaria Scandinavica*, vol. 30, 441-445.
- Ivanov I., Damnjanović Z., Radojčić S. 1993. Metabolism of macro-elements in high gravidity and early lactation. *Veterinarski glasnik*, vol.47, No 4-5, 319-329. (in Serbian)
- Kupczynski R., Chodoba-Drozowska B. 2002. Values of selected biochemical parameters of cows blood during their dry-coming-off and the beginning of lactation. *Electronic journal of Polish Agricultural Universities*, vol. 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 Veterinaria Brno*, vol. 74, 217-224.
- Overton T.R., Waldron M.R. 2004. Nutritional management of transition dairy cows: Strategies to optimize metabolic health. *Journal of Dairy Science*, vol. 87, E105-E119.
- Pechova A., Llek J., Halouzka R. 1997. Diagnosis and control of the development of hepatic lipidosis in dairy cows in the periparturient period. *Acta Veterinaria Brno*, vol.66, 235-243.
- Radostits O. M., Blood D.C., Gay C.C., Hinchcliff K.W. 2000. *Veterinary Medicine, A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses*. Ninth Edition W.B. Saunders Company Ltd London, New York, Philadelphia, San Francisco, St. Louis, Sydney
- Reist M., Erdin D., Von Euw D., Tchuemperlin K., Leunberger H., Chillard H., Hammon M., Morel C., Philopona C., Zbinder Y., Kuemzi 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. *Journal of Dairy Science*, vol. 85, 3314-3327.
- Reynolds C.K., Aikman P.C., Luoli B., Humpheirs D.J., Beever D.A. 2003. Splanchnic metabolism of dairy cows during the transition from late gestation through early lactation. *Journal of Dairy Science*, 86,1201-1217.
- Sevinc M., Basoglu A., Birdane F., Gokcen M., Kucukfindik M. 1997. The changes of metabolic profile in dairy cows during dry period and after. *Turkish Journal of Veterinary and Animal Science*, vol.3, 475-478.

- Sevinc M., Basoglu A., Guzelbekta H.2003. Lipid and lipoprotein levels in dairy cows with fatty liver. Turkish Journal of Veterinary and Animal Science, vol.27. 295-299.
- Vazquez-Anon M., Bertics S., Luck M., Grummer R. 1994. Peripartium liver triglyceride and plasma metabolites in dairy cows. Journal of Dairy Science, vol. 77, 1521-1528.
- Veenhuizen J.J., Drackley J.K., Richard M.J., Sanderson TP., 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. Journal of Dairy Science, vol. 74, 4238-4253.