Bulgarian Journal of Veterinary Medicine, 2019, **22**, No 3, 314–321
ISSN 1311-1477; DOI: 10.15547/bjvm.2074

Original article

EVALUATION OF SOME BLOOD LIVER PARAMETERS IN COWS WITH SUBCLINICAL AND CLINICAL KETOSIS

V. J. MARUTSOVA[1], P. D. MARUTSOV[2] & R. G. BINEV[1]

[1]Department of Internal Medicine; [2]Department of Veterinary Microbiology, Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria

Summary

Marutsova, V. J., P. D. Marutsov & R. G. Binev, 2019. Evaluation of some blood liver parameters in cows with subclinical and clinical ketosis. *Bulg. J. Vet. Med.,* **22**, No 3, 314–321.

The purpose of the present study was to establish the changes in some blood liver parameters in cows in different physiological conditions with subclinical (SCK) and clinical ketosis (CK). The study was performed on 157 Holstein cows with annual milk yield of 9,000–11,000 L. The animals were divided into 3 groups: I group – pregnant cows (from day 15 to day 0 pre-calving); II group – recently calved (from day 0 to day 15 postpartum) and III group – lactating (from day 30 to 45 postpartum). Blood concentrations of β-hydroxybutyric acid (BHBA) (mmol/L), glucose (mmol/L), total protein (g/L), albumin (g/L) and total bilirubin (μmol/L) were assayed in all cows. The animals were divided in groups with SCK and CK according to their blood BHBA levels. It was established, that the concentrations of total bilirubin were statistically significantly elevated vs control groups, while blood glucose, total protein and albumin values decreased, reflecting the impaired liver function in cows with SCK and CK.

Key words: biochemical parameters, dairy cows, ketosis

INTRODUCTION

Ketosis of high-yielding dairy cows is amongst the most significant health issues in global dairy farming leading to economic losses incurred from reduced milk production, low insemination rate, treatment costs etc. (Gordon *et al.,* 2013). The etiological basis of ketosis is related to the fact that during the pregnancy the metabolism of cows is adapted to the developing embryo, while during early lactation the mammary gland is of higher metabolic priority because most of the nutrients are redirected to it, including glucose – a main and essential element for lactogenesis (Aeberhard *et al.,* 2001; Sordillo & Raphael, 2013). Both processes predispose to emerging energy deficiency and ketosis development. The occurrence of negative energy balance is further due to the physiological phenomenon of reduced dry matter intake (by over 30%) in the period immediately before and after cal-

ving (Roche *et al.,* 2013; Samiei *et al.,* 2015).

Historically, ketosis has been classified as primary and secondary, depending on food intake and the presence of a secondary disease such as retained placenta, metritis, mastitis and other disorders (Rajala & Gröhn, 1998; Herdt, 2000). From a clinical point of view, it can be classified as subclinical (SCK) or clinical (CK), depending on the amount of ketone bodies in the organism and the presence of clinical symptoms (Sakha *et al.,* 2007; Gordon *et al.,* 2013). Ketosis is characterised by glycogen depletion and reduced gluconeogenesis activity in the liver, hypoglycaemia, ketonaemia, ketonuria, ketolactia, acetone-smelling breath and development of hepatic lipidosis (Allen & Piantoni, 2013). Grummer (1993) and Drackley (1999) report changes in the parameters reflecting the liver functions (carbohydrate, protein, fat, pigment, etc.) in dairy cows with ketosis caused by liver dystrophy.

The aim of this study was to investigate high-yielding cows in different physiological conditions (pregnant, recently calved and lactating) with diagnosed SCK and CK to detect changes in some blood liver parameters (glucose, total protein, albumin and total bilirubin).

MATERIALS AND METHODS

The present study was performed on 157 Holstein cows (1st to 4th lactation) with annual milk yield of 9000–11000 L and average body weight 450–550 kg. Cows were fed rations in concordance with their physiological condition and norms for dietary roughage and concentrate contents for each physiological condition.

The cows were divided into 3 groups according to their physiological condition: I group – pregnant cows (from day 15 to day 0 pre-calving); II group – recently calved (from day 0 to 15 postpartum) and III group – lactating (from day 30 to 45 postpartum). Blood concentrations of BHBA were assayed in all target animals and on the basis of results, they were classifies as healthy (control, BHBA <1.2 mmol/L), affected with SCK (BHBA from 1.2 to 2.6 mmol/L) and CK (BHBA >2.6 mmol/L). The first group included 21 pregnant cows: 9 healthy and 12 (57%) – with SCK. Blood BHBA concentrations indicative for CK were not established in this group. The second group comprised 90 recently calved cows: 55 healthy, 27 (30%) – with SCK and 8 (8.88%) – with CK. The third group (47 lactating cows) included 24 healthy (control) cows; 15 animals (32%) affected with SCK and 8 (17%) – with CK.

Blood samples were collected through puncture of the coccygeal vein using sterile 21G needles and vacutainers (Biomed, Bulgaria) with heparin (5 mL) and without anticoagulant (6 mL). Samples were obtained in the morning before feeding and were stored and transported at 4 °C. Analysis was performed within 24 hours after sampling. Blood BHBA and glucose concentrations were determined *in situ* using a portable Xpress-I system (Nova Biomedical, UK). The following indices were determined: total protein (g/L), albumin (g/L) and total bilirubin (μmol/L) using commercial colorimetric test kits (Biolabo Diagnostics, France) on an automated biochemical analyser Mindray BS-120 (China) and Integra 400 plus Roche (Hoffmann – La Roche Ltd., Switzerland).

Statistical analysis

Results were presented as mean ± standard deviation (SD). Statistical analysis (ANOVA) was done with Statistica 6.0

StatSoft, Inc. (USA, 1993). The level of statistical significance was P<0.05.

RESULTS

Blood BHBA levels in control cows from the three groups were within the reference range (Fig. 1). In cows with SCK they were statistically significantly increased as vs controls (Fig. 1). The analysis of changes in this blood parameter in cows with CK from groups II and III, showed that β-hydroxybutyrate concentrations were statistically significantly higher (P<0.001) than respective control and subclinical ketosis groups (Fig. 1). In cows from group I (pregnant) concentrations higher than 2.6 mmol/L were not exhibited, e.g. clinical ketosis was not present.

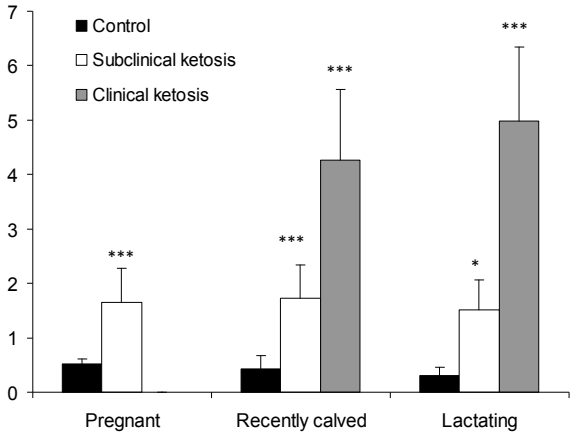

Fig. 1. Changes in blood β-hydroxybutyrate (BHBA) levels in cows with subclinical and clinical ketosis (mean ± SD); *P<0.05; **P<0.01; ***P<0.001 vs control cows.

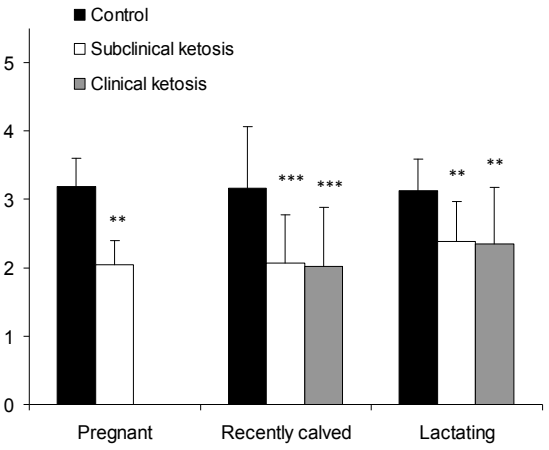

Fig. 2. Changes in blood glucose levels in cows with subclinical and clinical ketosis (mean ± SD); *P<0.05; **P<0.01; ***P<0.001 vs control cows.

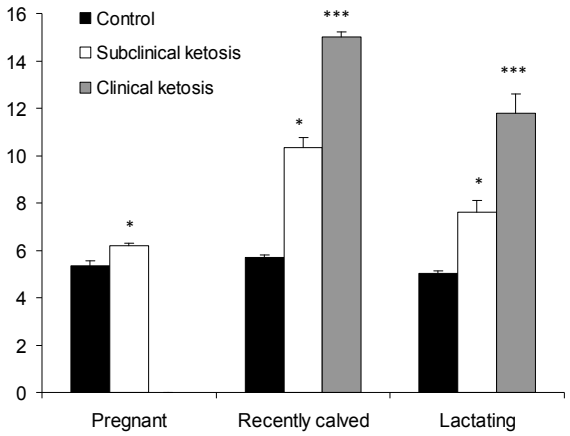

Fig. 3. Changes in blood total bilirubin levels in cows with subclinical and clinical ketosis (mean ± SD); *P<0.05; **P<0.01; ***P<0.001 vs control cows.

Table 1. Changes in blood total protein and albumin levels in cows from groups I, II and III with SCK and CK (mean ± SD)

		Total protein (g/L)	Albumin (g/L)
Group I	Control	75.22±1.2	35.10±0.3
(15–0 days pre-partum)	Subclinical ketosis	63.40±1.6	32.88±0.2*
Group II	Control	73.85±0.4	35.07±0.4
(0–15 days postpartum)	Subclinical ketosis	63.60±0.5	23.36±0.3**
	Clinical ketosis	53.78±0.8**	22.75±0.3**
Group III	Control	76.82±1.0	36.10±0.2
(30–45 days postpartum)	Subclinical ketosis	57.36±0.6*	25.56±0.3**
	Clinical ketosis	46.28±0.7***	22.51±0.5***

*P<0.05; **P<0.01; ***P<0.001 vs control cows

The blood glucose levels in control cows were within the respective physiological ranges (Fig. 2). In cows from groups with SCK and CK, blood glucose values were significantly lower (P<0.01) than controls (Fig. 2).

The blood total bilirubin in cows from control groups (pregnant, recently calved and lactating) were within the reference interval. Blood chemistry analysis in cows with SCK and CK showed statistically significant bilirubinaemia (P<0.001) vs control groups (Fig. 3).

The analysis of blood in the three control groups for detection of changes in total protein and albumin quantities demonstrated levels close to the physiological norms (Table 1). The blood total protein and albumin in cows with SCK and CK decreased with different levels of statistical significance compared to control groups.

DISCUSSION

The negative energy balance occurring in the transition period plays an important role in the occurrence of metabolic diseases in high-yielding cows such as ketosis, hepatic lipidosis, abomasal displacement, endometritis, leading to significant economic losses (Rajala & Gröhn, 1998). Therefore, early diagnosis of ketosis in dairy cows through clinical and blood laboratory tests is economically justifiable for farmers (LeBlanc, 2010; Garro *et al.,* 2014). In general for the diagnosis of ketosis, samples are obtained for determination of ketone bodies in blood, urine and milk, as well as glucose, total protein, albumin, bilirubin and others. Blood BHBA levels are accepted as the primary diagnostic test for SCK and CK in dairy cows (Oetzel, 2007; Garro *et al.,* 2014). Some researchers (Seifi *et al.,* 2011; McArt *et al.,* 2013) accept blood BHBA concentrations up to 1.2 mmol/L as normal; those from 1.2 to 2.6 mmol/L – indicative for SCK and those over 2.6 mmol/L corresponding to CK. Significant differences in BHBA values in the blood of the three groups of cows studied by us are particularly important for the early diagnosis of SCK throughout the pregnancy, parturition (calving) and lactation (up to 45 days) and taking measures to prevent the disease, confirmed by other authors (Duffield, 2004). Glucose is an important energy source for many organs and systems in the body of the dairy animal. Some vital cells (erythrocytes, brain and kidney cells) rely on glucose as the only energy substrate (Aschenbach *et al.,* 2011). Changes in blood glucose levels in our comparative studies of dairy cows with SCK and CK showed unidirectional changes (hypoglycaemia), as also confirmed by other authors (İssi *et al.,* 2016). The metabolism of glucose in the ruminants is regulated by various hormones, depending on the degree of uptake and utilisation of food, as well as the available glucose precursors (Sordillo & Raphael, 2013). The amount of glucose precursors varies depending on lactation, food intake, fat mobilisation, and energy balance. Volatile fatty acids (especially propionate) produced in the rumen are the main precursors for gluconeogenesis in the liver (50–60%) (Lean *et al.,* 2013; Sordillo & Raphael, 2013). Another important source of glucose is glycogen. Stored in the liver or skeletal muscles, it directly and indirectly maintains glucose levels in the body (Butler, 2000; Kuhla *et al.,* 2011). Blood glucose concentrations in cows with SCK and CK statistically significantly decresead ($P<0.001$), as a result of depletion of glycogen from the liver (West, 1990; Drackley *et al.,* 2001; Marutsova & Marutsov, 2016).

The changes in the development of SCK and CK in the cows of the three groups established by us and by other authors (Bertoni *et al.,* 2010; Djoković *et al.,* 2013) showed that the pigmentary function of the liver is disturbed, as seen from increased ($P<0.001$) total bilirubin concentrations, particularly obvious in recently calved and lactating cows with SCK and CK. The impaired pigmentary function of the liver (bilirubinaemia) is due to the dystrophic changes, which on one hand, mechanically obstruct bilirubin secretion in the bile and, on the other, disturb permeability of bile and blood capillaries in the damaged hepatocytes (karyopyknosis and karyolysis, lipidosis, etc.). The integrity of this barrier decreases and bilirubin passes into the bloodstream. Bilirubinaemia is proportional to the accelerated metabolic processes in the liver induced by stress at calving as well as to the lower rate of synthe-

sis of enzymes responsible for its elimination.

The protein metabolism in the liver of cows with SCK and CK is also impaired, with statistically significantly lower serum total protein and albumin values (hypoproteinaemia) ($P<0.001$). Levels of total protein may be influenced to a large extent by the condition of the liver, as the plasma proteins are synthesised there. Albumin, synthesised in the liver, plays a central role in the regulation of colloid osmotic pressure. It retains water and is involved in the transport of bilirubin, fatty acids, hormones and vitamins. Albumin values also decrease as a result of urinary excretion found in our studies (proteinuria) (Marutsova, 2016) when the glomerular basement membrane permeability is increased from dystrophic changes in the kidneys. The decrease in total protein in the early lactation can also be explained by redirecting albumin and globulins from the blood to the udder. The hypoproteinaemia in cows with ketosis is due to the dystrophic processes and fatty infiltration of the liver and kidneys (Djoković *et al.,* 2013; Abba *et al.,* 2015).

In conclusion, pregnant cows from group I were affected by SCK but not by CK (BHBA<2.6 mmol/L). During the postpartum period and intensive lactation, cows could suffer from both forms of ketosis. We recommend mandatory blood screening of 10% of cows in dairy farms to determine BHBA and glucose values and appropriate monitoring to evaluate and prevent SCK and CK. Hypoglycaemia, bilirubinaemia and hypoproteinaemia in cows with SCK and CK are parameters of impaired liver function.

REFERENCES

Abba, Y., F. F. J. Abdullah, E. L. T. Chung, M. A. Sadiq, K. Mohammed, A. Y. Osman, N. R. Rahmat, I. A. Razak, M. A. M. Lila, A. W. Haron & A. A. Saharee, 2015. Biochemical and pathological findings of pregnancy toxemia in Saanen doe: A case report. *Journal of Advanced Veterinary and Animal Research*, **2**, 236–239.

Aeberhard, K. I., R. M. Bruckmaier & J. W. Blum, 2001. Metabolic, enzymatic and endocrine status in high-yielding dairy cows - Part 2. *Journal of Veterinary Medicine A, Physiology, Pathology, Clinical medicine,* **48**, 111–127.

Allen, M. S. & P. Piantoni, 2013. Metabolic control of feed intake. Impli-cations for metabolic disease of fresh cows. *The Veterinary Clinics of North America. Food Animal Pracitice*, **29**, 279–297.

Aschenbach, J. R., G. B. Penner, F. Stumpff & G. Gäbel, 2011. Ruminant nutrition symposium: role of fermentation acid absorption in the regulation of ruminal pH. *Journal of Animal Science*, **89**, 1092–1107.

Bertoni, G., R., Lombardelli, F. Piccioli-Cappelli & E. Trevisi, 2010. Main endocrine-metabolic differences between 1st and 2nd lactation of the dairy cows around calving. *Journal of Animal Science*, **1**, 116.

Butler, W. R., 2000. Nutritional interactions with reproductive performance in dairy cattle. *Animal Reproduction Science*, **2**, 449–457.

Djoković, R., V. Kurćubić, Z. Ilić, M. Cincović, M. Petrović, N. Fratrić & B. Jašović, 2013. Evaluation of metabolic status in Simmental dairy cows during late pregnancy and early lactation. *Veterinarski Arhiv*, **6**, 593–602.

Drackley, J. K., 1999. Biology of dairy cows during the transition period: The final frontier? *Journal of Dairy Science*, **82**, 2259–2273.

Drackley, J. K., T. R. Overton & N. G. Douglas, 2001. Adaptations of glucose and long

chain fatty acid metabolism in liver of dairy cows during the periparturient period. *Journal of Dairy Science*, **84**, 100–112.

Duffield, T. F., 2004. Monitoring strategies for metabolic disease in transition dairy *cows. IVIS, 23rd WBC Congress*, Québec, Canada.

Garro, C. J., L. Mian & M. C. Roldan, 2014. Subclinical ketosis in dairy cows: prevalence and risk factors in grazing production system. *Journal of Animal Physiology and Animal Nutrition (Berlin),* **98***,* 838–844.

Gordon, J. L., S. J. LeBlanc & T. F. Diffield, 2013. Ketosis treatment in lactating dairy cattle. *The Veterinary Clinics of North America. Food Animal Pracitice*, **29**, 433–445.

Grummer, R. R., 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. *Journal of Dairy Science*, **76**, 12.

Herdt, T. H., 2000. Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. *The Veterinary Clinics of North America. Food Animal Pracitice*, **16**, 215–230.

İssi, M., Y. Gül & O. Başbug, 2016. Evaluation of renal and hepatic function in cattle with subclinical and clinical ketosis. *Turkish Journal of Veterinary Animal Science*, **40**, 47–52.

Kuhla, B., G. Nurnberg, D. Albrecht, S. Görs, H. M. Hammon & C. C. Metges, 2011. Involvement of skeletal muscle protein, glycogen, and fat metabolism in the adaptation on early lactation of dairy cows. *Journal of Proteome Research*, **10**, 4252–62.

Lean, I. J., R.Van Saun & P. J. DeGaris, 2013. Energy and protein nutrition management of transition dairy cows. *The Veterinary Clinics of North America. Food Animal Pracitice*, **29**, 337–366.

LeBlanc, S., 2010. Monitoring metabolic health of dairy cattle in the transition period. *Journal of Reproduction Development*, **56**, 29–35.

Marutsova, V., 2016. Comparative studies on the ketosis in ruminants. PhD thesis. Department of Internal medicine, Faculty of Veterinary medicine, Trakia University, Bulgaria.

Marutsova, V. & P. Marutsov, 2016. Body condition scoring and changes in some blood biochemical parameters in cows with subclinical and clinical ketosis. *International Journal of Advanced Research*, **4**, 405–412.

McArt, J. A., D. V. Nydam, G. R. Oetzel, T. R. Overtond & P. A. Ospina, 2013. Elevated non-esterified fatty acids and β-hydroxybutyrate and their association with transition dairy cow performance. *The Veterinary Journal*, **198**, 3, 560–570.

Oetzel, G. R., 2007. Herd-level ketosis - diagnosis and risk factors. Preconf. Sem. 7C: Dairy herd problem investigation strategies: Transition cow troubl. *American Association of Bovine Practitioners, 40th Annual Conference*, Sept. 19, 2007 – Vancouver, BC, Canada.

Rajala, P. J. & Y. T. Gröhn, 1998. Disease occurrence and risk factor analysis in Finnish Ayrshire cows. *Acta Veterinaria Scandinavica.*, **39**, 1–13.

Roche, J. R., A. W. Bell, T. R. Overton & J. J. Loor, 2013. Invited review: Nutritional management of the transition cow in the 21st century – a paradigm shift in thinking. *Animal Production Science*, **53**, 1000–1023.

Sakha, M., M. Ameri, H. Sahrifi & I. Taheri, 2007. Bovine subclinical ketosis in dairy herds in Iran. *Veterinary Research Communication*, **31**, 637–679.

Samiei, A., J. B. Liang, G. R. Ghorbani, H. Hirooka, S. Ansari-Mahyari, H. Sadri & V. Tufarelli, 2015. Relationship between dietary energy level, silage butyric acid and body condition score with subclinical ketosis incidence in dairy cows. *Advances in Animal and Veterinary Sciences*, **3**, 354–361.

Seifi, H. A., S. J. LeBlanc, K. E. Leslie & T. F. Duffield, 2011. Metabolic predicttors of post-partum disease and culling risk in dairy cattle. *The Veterinary Journal*, **188**, 216–220.

Sordillo, L. M. & W. Raphael, 2013. Significance of metabolic stress, lipid mobilization and inflammation on transition cow disorders. *The Veterinary Clinics of North America. Food Animal Practice*, **29**, 267–278.

West, H. J., 1990. Effect on liver function of acetonemia and the fat cow syndrome in cattle. *Research of Veterinary Science*, **48**, 221–227.

Paper received 05.07.2017; accepted for publication 30.11.2017

Correspondence:

V. J. Marutsova, PhD, Assistant Professor
Department of Internal Diseases,
Faculty of Veterinary Medicine,
Trakia University,
6000 Stara Zagora, Bulgaria;
mobile:+0359883253737;
e-mail:vaniamarutsova@abv.bg