Monitoring of the ketosis in the dairy cows in periparturient period with laboratory and stable methods

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Abstract: Ketosis is a serious metabolic disease in high-producing dairy cows. It causes not only other health problems but also economic losses. The aims of the study were to monitor the ketosis (especially according to blood β-hydroxybutyrate concentration) and to draw a comparison between the hand-held device measuring with the laboratory methods. There were included 16 Holstein dairy cows in the trial. The animals were divided into two groups according to the Body Condition Score (BCS) – lower BCS group (BCS ≤ 3.5; n = 8) and higher BCS group (BCS ≥ 3.75; n = 8). Cows were observed once a week in almost 3 months. The first blood samples were collected by coccygeal venipuncture 2–3 weeks before the parturition (concentrations of triglycerides – TAG, non-esterified fatty acids – NEFA, β-hydroxybutyrate – BHB, total bilirubin – BILI, and activity of aspartate aminotransferase – AST and gamma-glutamyl transferase – GGT were determined). Another blood metabolic profile test was done at the end of the experiment that means 2 months after calving. In addition, the values of BHB and glucose (GLU) were measured by the FreeStyle Optium Neo hand-held device every week after calving till the end of the trial. According to the trial results, the higher concentrations of BHB were observed in the lower BCS group in the 2nd and 3rd week after parturition. Also, the low levels of glucose were observed in the lower BCS animals. The high correlation between laboratory and hand-held device measuring was calculated (r = 0.94, P < 0.01). The levels of most biochemical parameters were increased after parturition in both lower and higher BCS group. The incidence of ketosis was similar in both groups of cows.

Key Words: ketosis, dairy cows, β-hydroxybutyrate (BHB), blood parameters, hand-held device

INTRODUCTION

Ketosis is the metabolic disease caused by the lack of energy and following development of the negative energy balance. The prevalence of metabolic disease is typical in high-producing dairy cows during the periparturient period (Vanholder et al. 2015). Incidence of ketosis impacts cow´s health as well as financial implications on the farm. The study from 2017 calculated economical loss caused by the ketosis for $77 in primiparous cows and $181 for multiparous cows (Liang et al. 2017). The transition period is the most demanding time for the organism because it must adapt to the high metabolic demands of lactation (Drackley 1999). When the energy intake is lower than the energy expenditure, the organism gets into the negative energy balance. That causes an increased uncontrolled rate of body fat mobilization. The TAGs are disintegrated to glycerol and NEFA, which one enter to β-oxidation. Acetyl-CoA is the final product of β-oxidation and is metabolized in the Krebs cycle. Nevertheless, in case of lack of oxaloacetate, the ketogenesis goes instead. That is the reason why ketone bodies as BHB or acetoacetate can be identified in milk, blood and urine samples (Hofírek 2009, Murray et al. 2002). The negative energy balance (NEB) is characterized by increased levels of NEFA and BHB. Some degree of NEB is expected in dairy cows in postpartum period (Ospina et al. 2010). According to the extensive research done in the UK, the prevalence of subclinical ketosis in the first 20 days of lactation was 28.5%, 17.3% and 11.75% with the thresholds of 1.0, 1.2, 1.4 mmol/l respectively (Macrae et al. 2019). Development of subclinical ketosis influences the immune system and the higher incidence of milk fever, retained placenta, metritis, mastitis, pneumonia, and digestive disorders were recorded (Piñeiro et al. 2019).

MATERIAL AND METHODS

Animals

The trial took place on the private dairy farm in South Moravia in the Czech Republic. The experimental animals (n = 16) were Holstein dairy cows. The animals were kept on free-stall housing with no bedding. None of the animals showed any signs of the disease. Cows were fed with TMR (total mixture ration) fulfilling the different energy requirements according to the lactation and reproduction phase. The animals were divided into two groups according to their BCS, that is the lower BCS group (BCS ≤ 3.5; n = 8) and the higher BCS group (BCS ≥ 3.75; n = 8). The one animal of each group died or was culled because of the disease after calving, so at the end of the trial there was only 14 samples of the second sampling instead of 16 at first.

Clinical biochemistry

The blood samples were collected 2–3 weeks before the parturition and 2 months after. The blood was sampled to the non-heparinized tubes from the coccygeal vein. These samples at the beginning and at the end of the trial were centrifuged at 3000 rotation per minute for ten minutes the next day. That is the reason why the level of glucose was not defined with the laboratory analyser. The biochemical parameters evaluating the energy metabolism and liver function such as triglycerides (TAG), non-esterified fatty acids (NEFA), BHB, total bilirubin (BIL), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) were determined in the laboratory. Moreover, the glucose and BHB were measured from the drop of the blood sampled from the coccygeal vein. The values were measured immediately after the sampling in the stable with the FreeStyle Optium Neo hand-held device once a week after calving.

Statistical analysis

Data has been processed by Microsoft Excel (USA) and Statistica version 12.0 (CZ). One-way analysis (ANOVA) was used for data evaluation. To ensure evidential differences Scheffe's test was applied and $p < 0.05$ was regarded as a statistically significant difference. The relationship between the set parameters was tested by correlation analysis. For the relationship of methods of measuring, the correlation coefficient (r) was calculated.

RESULTS AND DISCUSSION

The results of the average values of blood BHB and glucose are shown in Table 1. One part of the study focused on the comparison of hand-held devide and laboratory methods, therefore, Table 2 shows the results of BHB of two different measurements. Figure 1 shows the average values of the parameters characterizing the energy metabolism and liver function.

The increased values of BHB and NEFA are the markers of lipomobilisation and developing of the ketosis. On the other hand, the reduction in glucose concentration is observed. The threshold for BHB in lactating cows is 1.0 mmol/l (Doubek 2007, Macrae et al. 2019). The reference range in dry cows is the concentration of BHB above 0.8 mmol/l, including. Another source states the presence of subclinical ketosis in BHB concentration above 1.2 mmol/l, and clinical ketosis with the blood values above 2.6 mmol/l (Djokovic et al. 2019). In Table 1 there are shown the average values of blood BHB and glucose measured by the hand-held device FreeStyle Optium Neo. The BHB average values of any group, neither lower BCS nor higher BCS, were not above the reference range. Increased results were measured in the lower BCS group at the 2nd and 3rd week after the parturition, where the BHB concentrations were marginal. According to the information written above, increase of NEFA and BHB in the early postpartum period is predictable. The study comparing the blood parameters in different lactation period group confirms this thesis (Djokovic et al. 2019). Based on the results of this trial the hypothesis that the cows with BCS ≥ 4 are supposed to suffer from the ketosis was not confirmed. Moreover, the average blood BHB concentration was higher in the lower BCS group (BCS ≤ 3.5).

The glycaemia in ruminants is relatively constant. The reference range for glucose from 2.5 to 4.1 mmol/l was used (Doubek 2007). The other publication uses threshold glucose ≤ 3.0 mmol/l (Macrae et al. 2019). If the same reference range is used, the lower BCS group will be evaluated as being in hypoglycaemia for the whole time of the trial. According to another study, by progress in lactation 27% cows had had glucose concentration less than 2.5 mmol/l (Mohebbi-Fani et al. 2019). The statistically significant difference was established between the lower BCS and the higher BCS group in the 2nd, 6th and 7th week after calving (1.87 ± 0.42 mmol/l and 2.59 ± 0.46 mmol/l, 2.66 ± 0.39 mmol/l and 3.36 ± 0.46 mmol/l, 2.67 ± 0.29 mmol/l and 3.41 ± 0.33 mmol/l respectively) as can be seen in Table 1. In all cases, the lower glucose concentrations were established in the lower BCS group.

Table 1 The values of BHB (β-hydroxybutyrate) and GLU (glucose) in periparturient period in dairy cows (L—lower BCS group, H—higher BCS group) every week after parturition (W1—W8). Blood sample before parturition (I) and two months after (II).

	BHB [mmol/l]			GLU [mmol/l]	
	L	H		L	H
I	0.53 ± 0.09	0.46 ± 0.16	I	2.33 ± 0.21	2.35 ± 0.17
W1	0.81 ± 0.28	0.59 ± 0.13	W1	2.37 ± 0.44	2.68 ± 0.65
W2	1.00 ± 0.42	0.59 ± 0.31	W2	1.87 ± 0.42 ^{A}	2.59 ± 0.46 ^{B}
W3	1.02 ± 0.76	0.40 ± 0.10	W3	2.73 ± 0.45	3.05 ± 0.18
W4	0.62 ± 0.20	0.66 ± 0.37	W4	2.83 ± 0.37	3.06 ± 0.33
W5	0.66 ± 0.31	0.66 ± 0.38	W5	2.87 ± 0.31	3.01 ± 0.41
W6	0.86 ± 0.30	0.61 ± 0.26	W6	2.66 ± 0.39 ^{A}	3.36 ± 0.46 ^{B}
W7	0.79 ± 0.19	0.66 ± 0.26	W7	2.67 ± 0.29 ^{a}	3.41 ± 0.33 ^{b}
II (W8)	0.91 ± 0.51	0.64 ± 0.32	II (W8)	2.76 ± 0.62	3.24 ± 0.23

Legend: $^{a, b}$—different letter in column means statistically significant difference between groups P < 0.05

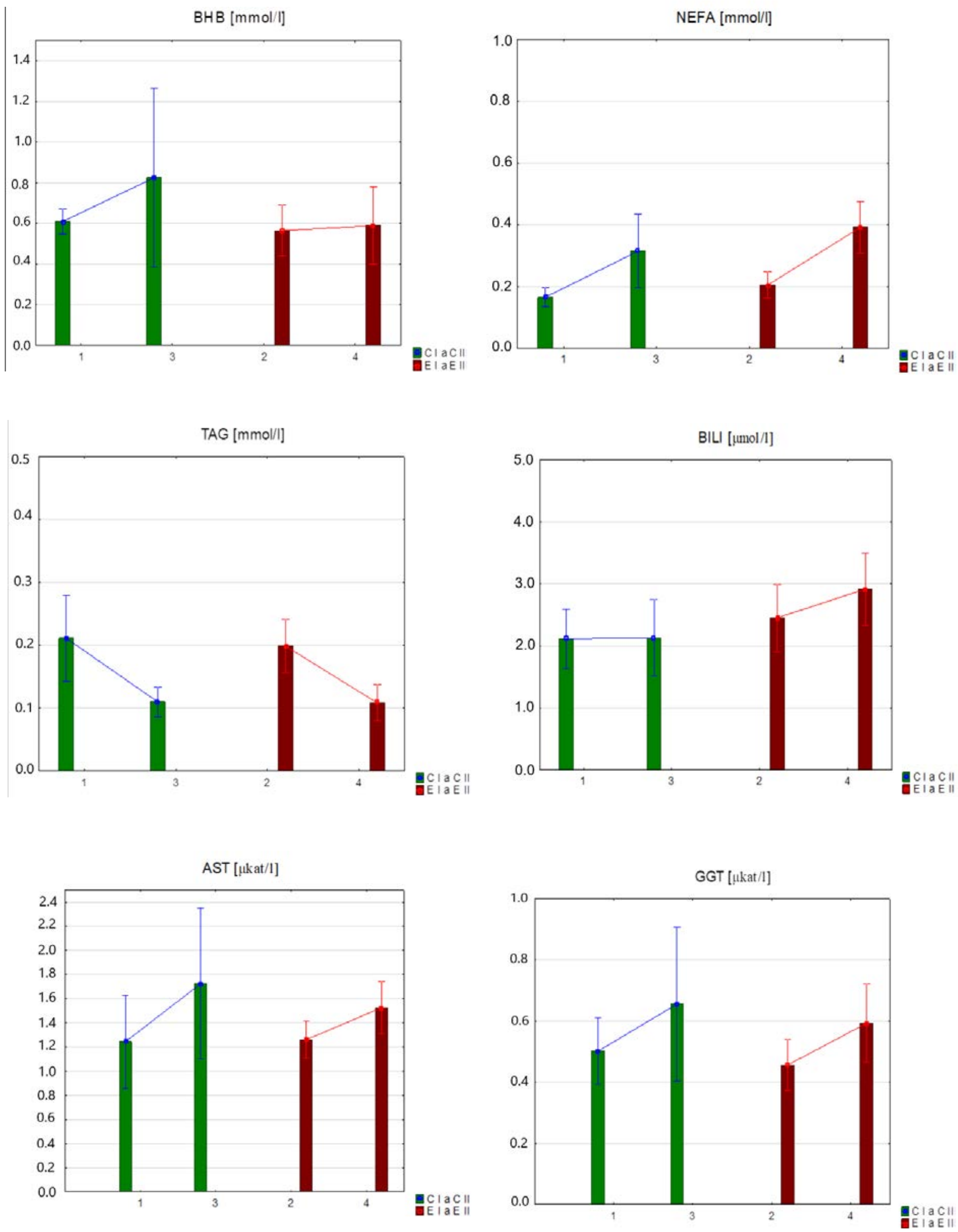
^{A,B}—different letter in column means statistically significant difference between groups P < 0.01

Table 2 compares two different methods of measuring the concentration of the BHB, immediately after the sampling directly in the stable with the hand-held device and the laboratory establishing from the serum centrifuged the next day. The highly significant correlation r = 0.94 was proved between the hand-held device and laboratory tests. In comparison to another study with the FreeStyle Precision device, where the correlation coefficient was 83%. The sensitivity and the specificity depend on the level of BHB and the device that is used. Based on a serum BHB laboratory concentration of 1.4 mmol/l the sensitivity is 100% and the specificity 92% for the FreeStyle Precision devise. When the BHB concentration of 1.2 mmol/l was used, the sensitivity was the same for this device, but the specificity was lower (76%) (Kanz et al. 2015).

The serum parameters like BHB, NEFA, TAG, BILI, AST, and GGT were determined. Using BHB, NEFA, and TAG can be assessed the level of the energy metabolism, the increased concentrations of BILI, AST, and GGT show the liver damage. The average serum BHB concentrations before parturition were not raised in any group (0.61 ± 0.07 mmol/l, 0.56 ± 0.15 mmol/l). The values sampled 2 months after parturition were not beyond the range, either. Those were higher but there was no statistically significant difference established (0.82 ± 0.48 mmol/l, 0.59 ± 0.20 mmol/l). On the other hand, the statistically significant difference was proved between the prepartum and postpartum samplings in the TAG concentration (C I: 0.21 ± 0.08 mmol/l, E I: 0.20 ± 0.05 mmol/l, C II: 0.11 ± 0.03 mmol/l, E II: 0.11 ± 0.03 mmol/l). But no average value was over the threshold (0.35 mmol/l). In non-esterified fatty acids, where the over reference range is the value up to 0.7 mmol/l, none of the average samples gets over it. As the significant difference was observed between the 1st and the 2nd sampling in the lower BCS and also in the higher BCS group. That can be seen in Figure 1. There is a strong increase in the NEFA level that conversely replicates the TAG curve. There was improved the negative correlation between TAG and NEFA. There are some studies that have shown that elevated NEFA is more objective parameter of negative downstream health and production outcomes than BHB. The incidence of subclinical ketosis according to the increased BHB values (over 1.0 mmol/l) was 28.5%. But 40.3% of animals had NEFA values equal or over 0.7 mmol/l (Macrae et al. 2019).

The concentration of the total bilirubin and levels of the liver enzyme are the parameters of the liver function. The thresholds of the liver enzymes (AST and GGT) are ≤ 1.5 μkat/l and ≤ 0.5 μkat/l, respectively (Doubek 2007). As can be seen in Figure 1, there are the increased values

Figure 1 The average blood values of some biochemical parameters of the metabolic profile in lower BCS (C) and higher BCS (E) group before (I) and 2 months (II) after calving



Legend: 1 (C I), 2 (E I), 3 (C II), 4 (E II)

for both of the groups (lower BCS and higher BCS) after the calving, but no significant difference was proved. There was extended the strong positive correlation between the AST and GGT values. The bilirubin concentration was in the reference range for the whole trial period. There can be seen the higher values in the higher BCS group, but no significant difference was proved between the groups.

Table 2 The comparation of the BHB (β-hydroxybutyrate) values measured by the hand-held device in the stable (device) and defined in the laboratory (lab) in the dairy cows in periparturient period

	BHB [mmol/l]		%		BHB [mmol/l]		%
	lab	device			lab	device	
L I	0.88	0.8	91	H I	0.62	0.5	80
	0.45	0.4	89		0.70	0.6	86
	0.58	0.4	69		0.56	0.5	89
	0.61	0.5	82		0.60	0.5	83
	0.62	0.6	97		0.73	0.7	96
	0.45	0.3	67		0.60	0.4	67
	0.40	0.3	75		0.52	0.5	96
	0.52	0.4	77		0.54	0.6	111
x ± sd	0.56 ± 0.14	0.46 ± 0.16	81 ± 10	x ± sd	0.61 ± 0.07	0.54 ± 0.09	89 ± 12
L II	0.82	1.3	159	H II	0.91	0.9	99
	0.45	0.5	111		0.46	0.4	87
	0.49	0.6	122		0.64	0.8	125
	0.44	0.4	91		1.85	2.1	114
	0.45	0.3	67		0.78	0.9	115
	0.94	0.9	96		0.53	0.6	113
	0.53	0.5	94		0.60	0.7	117
x ± sd	0.59 ± 0.19	0.64 ± 0.32	106 ± 27	x ± sd	0.82 ± 0.44	0.91 ± 0.51	110 ± 12
correlation					**0.94**		

Legend: x – mean, sd – standard deviation, L – lower BCS group, H – higher BCS group; I – blood sample 2–3 week before parturition and II – 2 months after parturition, % – BHB (device)/BHB (lab) × 100

CONCLUSION

The results of the study confirm the higher metabolic and liver burden in the postpartum period. Nevertheless, no statistically significant difference was proved between the lower BCS and the higher BCS group. The measurement of the BHB with the hand-held device was established as sufficient methods for ketosis prevention.

ACKNOWLEDGEMENTS

The research was financially supported by the IGA FA MENDELU (AF-IGA2019-IP079) and the authors would like to express the thanks for that.

REFERENCES

Djokovic, R. et al. 2019. Estimation Metabolic Status in High Yielding Dairy Cows During Transition Period and Full Lactation. Acta Scientiae Veterinariae [Online], 47: 1667. Available at: https://www.researchgate.net/publication/334466518_Estimation_of_Metabolic_Status_in_High_Yielding_Dairy_Cows_During_Transition_Period_and_Full_Lactation. [2019-08-24].

Doubek, J. 2007. Interpretace základních biochemických a hematologických nálezů u zvířat. 1st ed., Brno: Noviko.

Drackley, J.K. 1999. Biology of Dairy Cows During the Transition Period: the Final Frontier? Journal of Dairy Science [Online], 82(11): 2259–2273. Available at: https://www.mendeley.com/catalogue/biology-dairy-cows-during-transition-period-final-frontier/. [2019-08-19].

Hofírek, B. 2009. Poruchy metabolismu. In Nemoci skotu. Brno: Noviko, pp. 668—673.

Kanz, P. et al. 2015. Suitability of Capillary Blood Obtained by a Minimally Invasive Lancet Technique to Detect Subclinical Ketosis in Dairy Cows by Using 3 Different Electronic Hand-held Devices. Journal of Dairy Science [Online], 98(9): 6108–6118. Available at: https://www.sciencedirect.com/science/article/pii/S0022030215004403. [2019-08-29].

Liang, D. et al. 2017. Estimating US Dairy Clinical Disease Costs with a Stochastic Simulation Model. Journal of Dairy Science [Online], 100(2): 1472–1486. Available at: https://www.sciencedirect.com/science/article/pii/S0022030216308992. [2019-08-24].

Macrae, A.I. et al. 2019. Prevalence of Excessive Negative Energy Balance in Commercial United Kingdom Dairy Herds. The Veterinary Journal [Online], 248: 51–57. Available at: https://www.sciencedirect.com/science/article/pii/S1090023319300401. [2019-08-22].

Mohebbi-Fani, M. et al. 2019. A Field Study on Glucose, Non-esterified Fatty Acids, Beta-hydroxybutyrate and Thyroid Hormones in Dairy Cows During the Breeding Period in Fars Province, Iran. Iranian Journal of Veterinary Research [Online], 20(1): 55–59. Available at: https://www.researchgate.net/publication/332538708_A_field_study_on_glucose_non-esterified_fatty_acids_beta-_hydroxybutyrate_and_thyroid_hormones_in_dairy_cows_during_the_breeding_period_in_Fars_province_Iran. [2019-08-24].

Murray, R.K. et al. 2002. Harperova Biochemie. 4th ed., Jinočany: H & H.

Ospina, P.A. et al. 2010. Evaluation of Nonesterified Fatty Acids and β-hydroxybutyrate in Transition Dairy Cattle in the Northeastern United States: Critical Thresholds for Prediction of Clinical Diseases. Journal of Dairy Science [Online], 93(2): 546–554. Available at: https://www.sciencedirect.com/science/article/pii/S0022030210714971. [2019-08-23].

Piñeiro, J.M. et al. 2019. Associations of Pre- and Postpartum Lying Time with Metabolic, Inflammation, and Health Status of Lactating Dairy Cows. Journal of Dairy Science [Online], 102(4): 3348–3361. Available at: https://www.sciencedirect.com/science/article/pii/S0022030219301638. [2019-08-23].

Vanholder, T. et al. 2015. Risk Factors for Subclinical and Clinical Ketosis and Association with Production Parameters in Dairy Cows in the Netherlands. Journal of Dairy Science [Online], 98(2): 880–888. Available at: https://www.researchgate.net/publication/269711773_Risk_factors_for_subclinical_and_clinical_ketosis_and_association_with_production_parameters_in_dairy_cows_in_the_Netherlands. [2019-08-19].