Metabolic adaptation in first week after calving and early prediction of ketosis type I and II in dairy cows

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SUMMARY

The aim of this study is to determine differences in metabolic adaptation in first week after calving between healthy and cows that develop ketosis type I (diagnosed 3-6 weeks after calving) or II (diagnosed 2-3 weeks after calving) after calving. Experiment included 50 healthy cows, 50 ketosis I type cows and 50 ketosis II cows. Animals were selected retrospectively form 435 Holstein cows that were constantly monitored. Monitoring is regular procedures during all season. Blood samples were taken in first week after calving. Concentration of ketone body was measured every two day by test stripes from the end of 1st until the end of 6th week. Any color change in test strip (5 mg/dL, trace, or higher) was indicator of ketosis in cows. Cows were clinically evaluated to determine any clinical symptoms (reduced appetite, rumen atony, behavioral changes). In cows with ketosis type I were noted higher concentrations of nonesterified fatty acid (NEFA) and lower concentrations of glucose and insulin compared to control group. Higher concentrations of beta hydroxybutyrate (BHB), tumor necorsis factor alpha (TNF-α) and total bilirubin were noted in cows with ketosis type II compared to control group. Value of revised quantitative insulin sensitivity check index (RQUICKI) was lower, and aspartate aminotransferase (AST) was higher in blood of ketosis II cows compared to ketosis I cows. Prediction of ketosis type I is significant by logistic regression model which include insulin, NEFA and glucose as independent predictor (area under ROC curve, AUC=0.78, $p<0.05$). Possibility of ketosis I development increases with NEFA increase and decrease of glucose and insulin concentrations. Prediction of ketosis type II development is significant by logistic regression model which include BHB, TNF-α and total bilirubin where increase of these parameters indicates higher possibility of ketosis II development (AUC=0.87, $p<0.01$). Differentiation of ketosis type I and II is significant by logistic regression model which include value of RQUICKI and AST (AUC=0.74, $p<0.05$), so with increasing of AST and decreasing of RQUICKI in first week of lactation increase risk for type II ketosis. Percentage of variation in the metabolic parameters that is predictable from the BHB in first week after calving was significantly higher in ketotic (10.9-18.5%) then in healthy cows (2.5-9.1%). Cows in ketosis type I and II show different metabolic adaptations in first week after calving. These differences allow prediction of development a exact type of ketosis. Metabolic adaptation in function of ketogenesis was developed in first week after calving, early before manifestation of ketosis.

KEY WORDS

Cows, ketosis, inflammation, insulin resistance, liver function.

INTRODUCTION

Ketosis is significant metabolic disorder of cows that is developed as a consequence of negative energetic balance and it is presented with higher concentrations of ketone bodies in blood, milk and urine. Subclinical ketosis may be diagnosed when blood serum BHB concentrations are above 1.2 mmol/L, and clinical ketosis with blood BHB level above 2.6 mmol/L as gold standard test[1].

Basal metabolic adaptations in cows that develop ketosis are: higher concentrations of BHB, higher concentrations of NEFA, lower concentrations of glucose, higher concentrations of liver enzymes and bilirubin, disturbances in macro and micro elements, greater values of inflammatory markers, oxidative stress and insulin resistance. It can be followed with abomasal dislocation, mastitis, metritis, laminitis and behavioral changes[2-4].

On pathohysiologic aspect, ketosis can be divided into ketosis type I and II[5-7]. Type I is developed 3-6 weeks after calving while type II develops 1-3 weeks after calving and it is tightly related to fatty liver. Ketosis I develops as a consequence of underfeeding and increase in milk production, while ketosis II is developed as a consequence of homeorhetic processes in early lactation. Use of some biochemical markers from blood and milk can be predicted development of ketosis in cows. Milk parameters may be also used for objective ketosis diagnosis[8-10]. Chuang et al.[11] have been determined differences in values of some laboratory parameters in blood between ketosis I and II measured from moment of calving until fourth week of lactation.

Early prediction of ketosis in the first week after calving may be possible. Classification of cows according to lipolysis, ketogenesis or activity of anabolic hormones in first week after calving shows metabolic differences between the classes for seve-

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ral consecutive weeks after calving. Kessel et al.[12] noted that retrospectively classified cows with high BHB concentration (over 1 mmol/L) have higher concentrations of BHB, NEFA, AST, GGT and lower concentrations of IGF-I in first 14 weeks after calving. Cincović et al.[13] founded that intensive lipolysis and ketogenesis in first week before and after calving cause lower concentrations of glucose, total proteins, cholesterol and urea and higher concentrations of bilirubin and AST compared to cows in control group. Cows loaded with metabolic stress (high catabolic and low anabolic blood indicators) showed significant difference in metabolic adaptation in relation to control group: higher values of STH, BHB, higher values of bilirubin, AST, ALT, GGT, AP and MDA and lower levels of glucose, total proteins, albumin and body condition[14].
The aim of this study is to determine differences in metabolic adaptations in first week after calving in ketotic cows type I and II and to determine possibility of prediction of ketosis development and type of ketosis with metabolic parameters in first week after calving. The aim is, also, to determine if there is any correlation of metabolic parameters and BHB and to determine differences in function of type of ketosis in cows in first week after calving.

MATERIALS AND METHODS

Animals and model: 150 animals were included in experiment: 50 healthy, 50 cows in type I ketosis and 50 cows in type II ketosis. Animals were selected retrospectively form 435 Holstein cows that were constantly monitored. Monitoring is regular procedures during all season. Cows were from second to fourth parity. During the early lactation the daily ration consisted of 4 kg alfalfa hay, 15 kg maize silage (30%DM), 8 kg alfalfa haylage, 4 kg ear maize silage (68%DM), 2 kg dry sugar beet pulp, 2 kg extruded soybean meal, and 4.5 kg concentrate (18%CP), NEL 153 MJ. Energy balance was evaluated according to body weight, offered meal and average milk production using NRC [2001] standards[15]. Blood samples were taken in first week after calving. From the end of the 1st to the end of the 6th week concentration of ketone body in urine was measured every two day by test stripes (Ketostix, Bayer, GER). Any color change in test strip (5 mg/dL, trace, or higher) was indicator of ketosis in cows. Cows were clinically evaluated to determine any clinical symptoms (reduced appetite, rumen atony, behavioral changes). Healthy cows did not show clinical symptoms of ketosis and urinary ketone bodies were not diagnosed in those cows. In cows with clinical symptoms and moderate or large color change on Ketostix was determined blood BHB concentration. Concentration of BHB >1.2 mmol/L in blood was cut-off value for final diagnosis of ketosis[1]. Based on the time when ketosis occurred, cows were classified into ketosis type I (diagnosed 3-6 weeks after calving), ketosis type II cows (diagnosed 2-3 weeks after calving) and healthy cows.
Blood sample analyses: In first week after calving concentrations of BHB, NEFA, glucose, total proteins, albumins, bilirubin, AST, GGT, cholesterol, triglycerides were determined by biochemical colorimetric kits (Biosystem, SP ans Randox, UK). Concentrations of observed parameters were determined by the Chemray analyzer (Rayto, PRC). Insulin concentration was determined by ELISA method (Cusabio, PRC) just like TNF-α concentration (Cloud-Clone Corp., USA). Determined values were read on reader RT-2100 C (Rayto, PRC). Insulin resistance indices were calculated by standard methodology[16]: RQUICKI = 1/[log (conc.t0 glucose mg/dL) + log (conc.t0 insulin mmol/L)+log (conc.t0 NEFA mmol/L)].
Statistics: Differences in concentrations of metabolic parameters and indexes of insulin resistance between groups were determined by ANOVA analysis and post hock LSD-test. Possibility of ketosis diagnosis was determined by multiple logistic regressions in which model was included metabolic parameters that were showed significant differences in LSD-test. Odds ratio (OR) and area under ROC curve (AUC) were determined for every model. Model that questions possibility of differential diagnosis of ketosis type I and II was made with metabolic parameters that are different in ketosis I and II by multiple logistic regressions. Early changes in metabolism in function of ketogenesis were determined by coefficient of correlation and determination (percentage of variation) in linear model between BHB and other metabolic parameters concentrations in first week after calving. Using the Fisher r-to-z transformation, will be calculate a value of z that can be applied to assess the significance of the difference between two correlation coefficients (healthy vs. ketosis type I; healthy vs. ketosis type II and ketosis type I vs. ketosis type II).

RESULTS

In first week after calving differences in metabolic adaptations in cows with different type of ketosis were found (Table 1). Higher concentrations of BHB, TNF-α and total bilirubin were founded in cows that developed ketosis type II in comparison to control group. Higher concentrations of NEFA and lower concentrations of glucose and insulin were noted in cows that developed ketosis type I compared to control group during first week after calving. Determination of ketosis type I or II by metabolic parameters can be done by lower RQUICKI index and higher AST levels in blood of cows that develop ketosis type II.
Prediction of ketosis type I is significant by logistic regression model which include insulin, NEFA and glucose as independent predictor (area under ROC curve, AUC=0.78, $p<0.05$). Possibility of ketosis I development increases with NEFA increase and decrease of glucose and insulin concentrations. Prediction of ketosis type II development is significant by logistic regression model which include BHB, TNF-α and total bilirubin where increase of these parameters indicates higher possibility of ketosis II development (AUC=0.87, $p<0.01$). Differentiation of ketosis type I and II is significant by logistic regression model which include value of RQUICKI and AST (AUC=0.74, $p<0.05$), so with increasing of AST and decreasing of RQUICKI in first week of lactation increase risk for type II ketosis. Results are presented in Table 2, Figure 1 and 2.
Linear correlation analysis between BHB and metabolic parameters was analysed in first week after calving. Percentage of variation in the metabolic parameters that is predictable from the BHB in first week after calving was significantly higher in ketotic (10.9-18.5%) then in healthy cows (2.5-9.1%). We found statistically significant difference in coefficient correlation between healthy and ketotic cows. Those results indicated that metabolic pathways are changed in function of ketogenesis in first week after calving and before ketosis manifestation.

DISCUSSION

Ketosis development in cows and its early prediction is researched with different models. All models showed that great significance in ketosis prediction have values of NEFA, AST, glucose and other parameters which is in compliance with our results. Asl et al.[8] founded that cut-off point for NEFA concentrations of 0.26 mmol/L can be used during early lactation for diagnosis of subclinical ketosis in first 8 weeks after calving. Sun et al.[9] founded that thresholds were more than 0.76 mmol/L for NEFA, more than 104 U/L for AST, less than 140 U/L for cholinesterase and more than 3.3 µmol/L

Table 1 - Blood parameters in healthy cows and cows with ketosis type I and II.

Parameter	Healthy control	Ketosis I	Ketosis II	Significance
BHB (mmol/L)	0.52±0.19^{a}	0.71±0.16^{b}	0.89±0.14^{b}	*
NEFA (mmol/L)	0.49±0.18^{a}	0.71±0.2^{b}	0.54±0.19^{ab}	**
Glucose (mmol/L)	2.51±0.35^{a}	2.13±0.36^{b}	2.4±0.32^{a}	**
T. bilirubin (µmol/L)	5.6±2.1^{a}	6.2±2.9^{a}	8.9±2.4^{b}	*
AST (IU/L)	94.5±20.5^{ab}	89±19.1^{a}	119.3±21.2^{b}	**
GGT (IU/L)	33.5±2.5^{a}	32.4±1.7^{a}	34.3±3.2^{a}	NS
Albumin (g/L)	33.6±2.1^{a}	32.4±1.7^{a}	31.2±2.6^{a}	NS
T. Protein (g/L)	62±4.4^{a}	59±3.9^{a}	58±3.8^{a}	NS
Cholesterol (mmol/L)	3.01±0.29^{a}	2.92±0.18^{a}	2.86±0.24^{a}	NS
Triglycerides (mmol/L)	0.14±0.014^{a}	0.14±0.017^{a}	0.13±0.016^{a}	NS
TNF-α (ng/mL)	0.78±0.18^{a}	0.83±0.19^{ab}	1.01±0.17^{b}	**
Insulin (µIU/L)	4.5±1.4^{a}	3.1±1.5^{b}	4.3±1.6^{ab}	*
RQUICKI	0.51±0.015^{ab}	0.52±0.016^{a}	0.50±0.014^{b}	**

NS: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$. Means followed by different letters differ significantly at $p < 0.05$.

Table 2 - Combination of biomarkers in evaluation of ketosis I and II development.

	Estimated	OR	% of explained variance	Model significance
Ketosis type I from Healthy control				
Insulin	-0.2	1.1-1.6	76%	**
NEFA	0.14	1.5-2.8		
Glucose	-0.78	0.9-1.8		
Ketosis type II from Healthy control				
BHB	0.21	1.8-3.1	82%	***
TNF-α	0.52	1.9-2.8		
T. bilirubin	1.3	1.5-2.6		
Ketosis type I from Ketosis type II				
RQUICKI	-0.092	1.05-1.7	71%	*
AST	8.9	1.2-1.8		

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

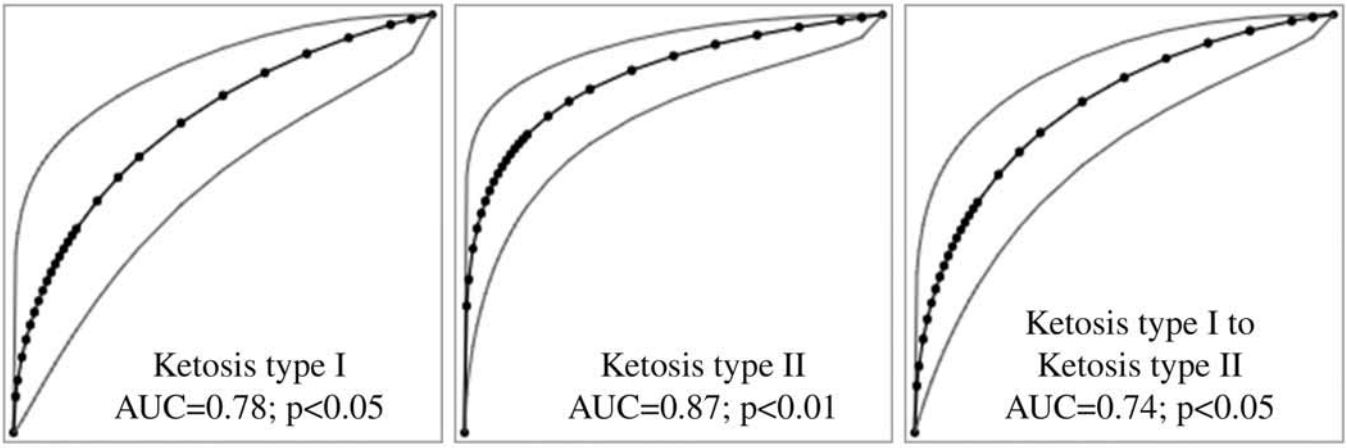

Figure 1 - Prediction of ketosis I and II development and prediction of ketosis.

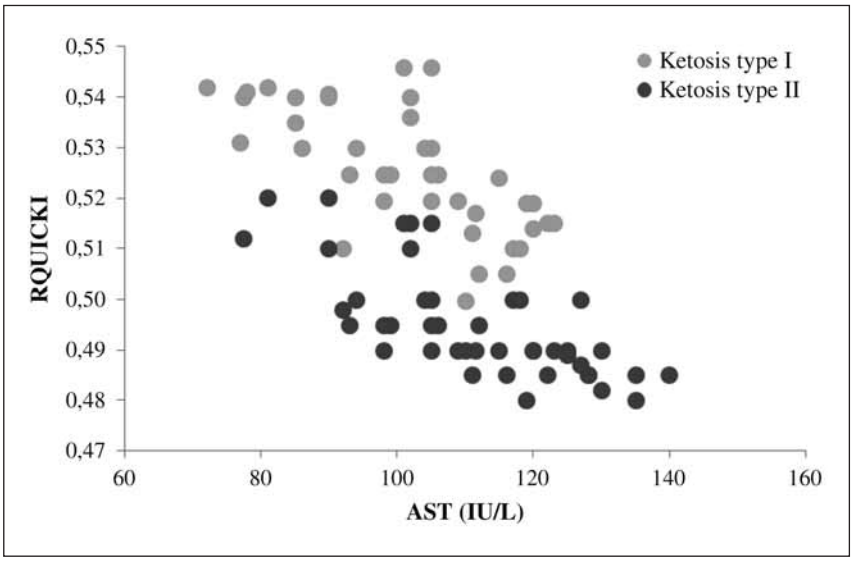

Figure 2 - RQUICKI and AST in cows in ketosis I and II.

for total bilirubin. Cao et al.[17] confirmed significance of glucose, AST and NEFA levels in evaluation of healthy and ketotic cows (GLU >3.04 mmol/L, AST <100 U/L, and NEFA <0.82 mmol/L). In listed experiments cut-off values are calculated on the moment of ketosis diagnosis no matter the type of ketosis. Unlike these studies, our experiment examined possibility of ketosis prediction based on values of blood parameters in first week after calving. Comparison of AUC value in our and mentioned studies showed that our model (that considers values of parameters in first week after calving) with similar efficiency determines ketotic cows just like model that includes single blood parameters (measured in the moment of ketosis diagnosis). Parity effect on metabolic parameters was not significant in our research. The lack of influence of parity is in compliance with the results found by Fiore et al.[18].

Cows in ketosis type I have higher concentrations of BHB and NEFA and lower concentration of insulin and glucose compared to control group. Ketosis type I is developed 3-6 weeks after calving as a consequence of reduced food intake. Experiment of Bjerre-Harpøth et al.[19] showed significant increase of NEFA and reduction of insulin and glucose in cows that had food restriction in early lactation. Similar metabolic changes were found in our trial. More recent results show that decreased dry matter intake in the prepartum period is a significant predictor of ketosis in cows, with the additional finding that cows in ketosis had lower dry matter intake during postpartal period[20]. Our results are consistent with characteristics of ketosis I that are described in classification of the authors cited[6-8].

Cows in ketosis type II have higher concentration of BHB, total bilirubin and TNF-α compared to healthy cows and/or ketosis type I type group. Oetzel[7] showed that fatty liver is the main characteristic of ketosis II type. Mostafavi et al.[21] noted that concentrations of BHB, NEFA, AST and NEFA/cholesterol relation are very specific in evaluation of fat liver development in cows. The increase in AST and NEFA concentrations during the postpartum period is considered a suitable indicator of ketosis associated to hepatic steatosis or liver damage[22,23].

Recent studies showed that cows in ketosis have higher concentrations of TNF-α[24]. Aplication of ketoprofen as non-steroid anti-inflammatory drug in cows during early lactation decrease sign of liver inflammation and AST activity in blood[25]. Concentration of TNF-α was higher in monocyte cell culture, during cultivation with BHB[26]. High expression of TNF-α in the liver is upregulated by interleukin-8 (IL-8) and IL-1β secreted from placenta, which is liver-related metabolic disorder mechanism[27]. Excessive inflammation in periparturient period is in basis of ketosis in dairy cows[28].

Comparison of ketosis type showed that cows that developed ketosis type II had greater activity of AST and lover value of RQUICKI index compared to ketosis type I group of cows. Higher degree of insulin resistance (reflected in lower RQUICKI index) with higher AST level indicated development of ketosis II type. Chuang et al.[11] founded higher concentrations of BHB, NEFA, glucose, bilirubin, AST and ALT in ketosis II type compared to ketosis type I. Xu et al.[29] noted lower RQUICKI index in ketotic cows compared to control group. Djoković et al.[3] showed that insulin resistance indexes that are measured in basal conditions significantly affect dynamical changes in concentrations of insulin, glucose and NEFA in ketotic cows.

Possibility of early prediction of ketosis development and type of ketosis in cows by metabolic markers in first week after calving was proved in this research. In model of logistic regression given markers showed higher risk for ketosis development and question of correlation of these markers with

Table 3 - Coefficient of determination of significant ketosis predictors with BHB in first week after calving.

Type 2	Healthy control	Ketosis type I	Ketosis type II	Ketosis type I: Healthy control	Ketosis type II: Healthy control	Ketosis type I: Ketosis type II
TNF-α	2.5%	15.2%	11.3%	**	*	NS
T. bilirubin	6.4%	14.5%	12.6%	**	**	NS
Insulin	8.4%	14.6%	16.4%	**	**	NS
NEFA	9.1%	15.4%	18.5%	*	**	NS
Glucose	2.4%	13.1%	15.7%	**	**	NS
RQUICKI	6.3%	11.3%	13.8%	**	**	NS
AST	5.2%	10.9%	11.7%	**	**	NS

NS: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$.

BHB had appeared. Healthy cows have lower degree of commonly explained variation between BHB and parameters in healthy then in ketotic cows. That was founded in this research and indicates early adaptation of metabolism in ketosis function in cows. Results of Djoković et al.[30] showed that in early lactation correlation of BHB with insulin and other indicators of functional status of liver is not in function of energetic balance in cows that indicates importance of BHB in metabolic adaptations of cows in early lactation.

CONCLUSIONS

Cows in ketosis type I and II show different metabolic adaptations in first week after calving. These differences allow prediction of development a exact type of ketosis. Ketosis type I is determined by values of glucose, insulin and NEFA (lipolysis and insulin secretion). Ketosis type II is determined by values of BHB, TNF-α and bilirubin (ketogenesis, inflammation and functional status of liver). Differentiation of ketosis type I and II is possible by RQUICKI and AST (insulin resistance and hepatocytes damage). Proportion of the variation of metabolic parameters that is predictable from the BHB was higher in ketotic than in healthy cows. Metabolic adaptation in function of ketogenesis was developed in first week after calving, early before manifestation of ketosis.

ACKNOWLEDGEMENT

This research is supported by projects “Influence and clinical evaluation of blood serum tumor necrosis factor alpha (TNF-α) in inflammatory response of ruminants and dogs” - Provincial secretariat of high education and science, Vojvodina, Serbia.

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