J. Dairy Sci. 96:1–9
http://dx.doi.org/10.3168/jds.2013-6808

Alterations in milk metabolome and coagulation ability during the lactation of dairy cows

H. Harzia,*†‡[1] A. Ilves,*†‡ M. Ots,*† M. Henno,* I. Jõudu,*† T. Kaart,*† K. Ling,* O. Kärt,* K. Kilk,‡ and U. Soomets‡
*Institute of Veterinary Medicine and Animal Science, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu 51014, Estonia
†Bio-Competence Centre of Healthy Dairy Products, Kreutzwaldi 1, 51014 Tartu, Estonia
‡Department of Biochemistry, Medical Faculty, University of Tartu, the Centre of Excellence for Translational Medicine, 50411 Tartu, Estonia

ABSTRACT

Milk composition has been known to change during lactation. To help understand the changes in metabolic profile throughout the whole lactation, liquid chromatography mass-spectrometry was used to analyze 306 milk samples from 82 primi- and multiparous dairy cows. Changes in metabolic profile common to all cows throughout lactation were ascertained based on principal component and general linear model analysis. Sets of specific markers; for instance, 225, 397, and 641–642 m/z (positive mode), and 186, 241, and 601–604 (negative mode), with at least a 1.5-fold higher intensity during the first 60 d compared with the last 60 d of lactation were observed. The metabolome was affected by parity and milking time. Markers, identified as peptides differentiating parity, were observed. A significant increase for citrate was observed in evening milk. Milk coagulation traits were strongly animal specific. The curd firmness values were influenced by milking time. Sets of markers were associated with curd firmness in positive (197 m/z) and negative (612, 737, 835, 836, 902, 1000, 1038, and 1079 m/z) ion mode.
Key words: lactation curve, milk metabolite, coagulation

INTRODUCTION

Metabolomics has been introduced recently to animal and dairy science. Being routinely collected, milk is a suitable substance for monitoring analyses. A better understanding of the milk metabolome would advance its use in evaluating the state of the animals and milk technological properties. Milk composition and production are affected by inherent and external factors, directly or indirectly. Differences in milk composition may be caused by nutritional (Malossini et al., 1996) or nonnutritional factors such as stage of lactation (Jõudu et al., 2008; Stoop et al., 2009). Using nuclear magnetic resonance (Klein et al., 2010, 2012) and GC-MS (Klein et al., 2010) for targeted analyses, a change in the metabolic composition of milk throughout the lactation has been observed. In a recent study by Ilves et al. (2012), using a mass spectrometric approach (liquid chromatography-tandem MS; **LC-MS/MS**), changes in the milk metabolome in early lactation were observed, specifically decreases in phosphorylated saccharides, citrate, and lactose concentrations. Melzer et al. (2013) also showed changes in the metabolome up to d 120 of lactation; metabolites that correlated with milk traits were detected. However, one limitation of the study was the lack of multiple samples from the same cow over the lactation (Melzer et al., 2013). Hence, the aim of this study was to provide greater understanding of the changes in the metabolic profile throughout the whole lactation, involving several samples per cow and using untargeted global metabolomics with LC-MS/MS.

Coagulation, an important trait of milk technological quality, is influenced by lactation stage and other factors (Grandison et al., 1984; Auldist et al., 2002; Cassandro et al., 2008). Previously, Harzia et al. (2012) identified the difference in the metabolome of noncoagulating and coagulating milk, and differences in metabolic profiles of milk with different coagulation abilities have been reported by Sundekilde et al. (2011). The metabolites related to coagulation properties include citrate, choline, carnitine, lactose, and other oligosaccharides such as N-acetyllactosamine (NAcLac; Sundekilde et al., 2011; Harzia et al., 2012). Nevertheless, the change in milk metabolome throughout lactation and relationships with coagulation ability need to be investigated further; therefore, a second objective of this study was to identify correlations between metabolome and coagulation ability.

Received March 14, 2013.
Accepted June 19, 2013.
[1]Corresponding author: hedi.harzia@emu.ee

MATERIALS AND METHODS

Animal Management and Feeding

Animal use and care were in accordance with the Estonian Animal Protection Act. Cows in a loose housing system on the Estonian University of Life Sciences' experimental farm (Eerika Farm LLC, Märja, Estonia) were milked twice a day and fed TMR ad libitum year round. Three rations were used: ration 1 consisted of (DM) 40% silage, 58% concentrates, and 2% minerals, 11.3 MJ of ME, 160 g/kg CP, and 103 g/kg MP; ration 2 consisted (DM) 48% silage, 50% concentrates, and 2% minerals, 10.7 MJ of ME, 159 g/kg CP, and 97 g/kg MP; and ration 3 consisted of (DM) 73% silage, 25% concentrates, and 2% minerals, 9.7 MJ of ME, 143 g/kg CP, and 85 g/kg MP. The rations comprised grass (75%) and clover (25%) silage; barley, wheat, and maize meal; heat-treated rapeseed cake, limestone, sodium chloride, and a vitamin-mineral mix for lactating cows. After calving, the cows were fed with ration 2 up to 14 DIM, after which ration 1 was offered up to 6.5 mo of lactation or if milk production was still >30 kg/d. Thereafter, the cows were again fed ration 2 and, at 1 mo before drying off, ration 3 was applied.

Sample Collection and Analysis

Milk samples (40 mL; n = 306, 3.73 replicates per cow, 6, ..., 307 DIM) from 82 primi- and multiparous (n = 156 and 150, respectively) Estonian Holstein (n = 70), Estonian Red (n = 7), and Estonian Native (n = 5) dairy cows were collected from February 2011 to February 2012 once a month with in-line milk meters within the framework of regular animal recording.

Milk samples were analyzed for fat, protein, and SCC content at the laboratory of Estonian Animal Recording Centre. Curd firmness ($\mathbf{E_{30}}$, mm) and coagulation time (**RCT**, min) were analyzed with an Optigraph as described by Kübarsepp et al. (2005), and milk pH was recorded (pH meter, SevenMulti; Mettler Toledo GmbH, Greifensee, Switzerland). Milk samples were prepared and MS analyses were performed on an LC-MS/MS (3200 Q TRAP; AB Sciex Instruments, Framingham, MA) as described by Harzia et al. (2012).

Statistical Analysis

Mass spectral data were preprocessed by binning data to atomic mass unit resolution. The principal component analyses (**PCA**) were performed for m/z in positive and negative ion mode to discover the potential patterns in MS data for differently ionized metabolites. To study the alterations of milk metabolome during the lactation, the identified principal components (**PC**) were modeled following the general linear mixed (GLM) model:

$$y_{ijklm} = \mu + B_i + P_j + D_k + M_l + b_1 \times LDIM_{ijklm} + b_2 \times LDIM^2_{ijklm} + b_3 \times LDIM^3_{ijklm} + C_m + e_{ijklm},$$

where y_{ijklm} is the dependent variable, μ is the model intercept, B_i is the breed effect ($i = 1, 2, 3$), P_j is the parity effect ($j = 1, 2$; primi- and multiparous), D_k is the diet effect ($k = 1, 2, 3$), M_l is the milking time effect ($l = 1, 2$; morning and evening), $b_1 \times LDIM_{ijklm} + b_2 \times LDIM^2_{ijklm} + b_3 \times LDIM^3_{ijklm}$ is the third-order Lagrange polynomial of DIM, C_m is the random cow effect ($m = 1, \ldots, 82$), and e_{ijklm} is the model error. The same model was applied to study alterations in milk coagulation and production and composition traits during the lactation. To examine the relationships between different milk traits and milk metabolome during 3 different lactation stages—the beginning (the first 60 d), middle (mo 3 to 8), and the end of lactation (the last 60 d)—Spearman rank correlation analysis was performed considering also the binary dummy variables of parity and milking time.

Statistical significance between m/z intensities in the spectra of early and late lactation milk, morning and evening milkings, and the first and second to third parities was determined by Student's t-tests. Results were displayed on a volcano plot as the distribution of signal intensities relative to the mean at respective m/z values, which were ranked based on calculated statistical differences. Statistical analyses were performed with SAS software (version 9.1; SAS Institute Inc., Cary, NC) and with R 2.8.1 /BioConductor algorithms (R Development Core Team, 2009).

RESULTS AND DISCUSSION

Lactational Curves of Milk Characteristics

As the biological needs of the calf changes with age, milk composition alters as lactation progresses (Walstra, 1999). The GLM analyses of production, composition, and quality traits during lactation are presented in Figure 1; no abnormal dynamics were observed. Changes in milk yield and fat and protein contents were similar to those of Mucha and Strandberg (2011) and Stoop et al. (2009). Fat content changed contrarily to milk yield, declining during the first 100 d (from 4.49 to 3.85%) and then increasing to d 259 (4.29%). Milk yield reached its peak at the end of mo 2 (30.5 kg/d) and then declined to d 251 (24.16 kg/d). Protein percentage declined over