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Milk metabolites and their genetic variability

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ABSTRACT

The composition of milk is crucial to evaluate milk performance and quality measures. Milk components partly contribute to breeding scores, and they can be assessed to judge metabolic and energy status of the cow as well as to serve as predictive markers for diseases. In addition to the milk composition measures (e.g., fat, protein, lactose) traditionally recorded during milk performance test via infrared spectroscopy, novel techniques, such as gas chromatography-mass spectrometry, allow for a further analysis of milk into its metabolic components. Gas chromatography-mass spectrometry is suitable for measuring several hundred metabolites with high throughput, and thus it is applicable to study sources of genetic and nongenetic variation of milk metabolites in dairy cows. Heritability and mode of inheritance of metabolite measurements were studied in a linear mixed model approach including expected (pedigree) and realized (genomic) relationship between animals. The genetic variability of 190 milk metabolite intensities was analyzed from 1,295 cows held on 18 farms in Mecklenburg-Western Pomerania, Germany. Besides extensive pedigree information, genotypic data comprising 37,180 single nucleotide polymorphism markers were available. Goodness of fit and significance of genetic variance components based on likelihood ratio tests were investigated with a full model, including marker- and pedigree-based genetic effects. Broad-sense heritability varied from zero to 0.699, with a median of 0.125. Significant additive genetic variance was observed for highly heritable metabolites, but dominance variance was not significantly present. As some metabolites are particularly favorable for human nutrition, for instance, future research should address the identification of locus-specific genetic effects and investigate metabolites as the molecular basis of traditional milk performance test traits.

Key words: metabolome, genomic relationship, single nucleotide polymorphism, heritability

INTRODUCTION

In dairy cattle, a multitude of milk components are recorded during milk performance tests. Besides monitoring performance traits (e.g., fat or protein content), it is especially important to control udder health by means of indicator traits, such as cell count. Other milk composition traits, for instance, FA levels or acetone, are also involved in indicating management status of the cow. Most of these traits can be measured with infrared spectroscopy. Infrared spectroscopy has been extended to a more detailed analysis of milk components; for example, protein content has been further resolved into its components, such as κ-CN and β-LG (Rutten et al., 2011). The same technique is used to measure the FA composition of milk (Soyeurt et al., 2006). Although infrared spectroscopy is suitable for population-wide animal recording, novel techniques allow for a further analysis of milk (Töpel, 2004). Coupling GC-MS breaks milk down into its metabolic intermediates. Besides GC-MS, other technical processes (e.g., nuclear magnetic resonance spectroscopy; Nicholson et al., 1999) are available, which differ from GC-MS in terms of quantity, reproducibility, and sensitivity in matter determination. Klein et al. (2010) have applied GC-MS to milk samples and obtained a few metabolites for further investigation; in plants, however, GC-MS has been shown to be suitable for identification and relative quantification of several hundred metabolites in high throughput (40 min per sample; Lisec et al., 2006). Thus, GC-MS is applicable to study sources of genetic and nongenetic variation of milk metabolites in dairy cows. The milk metabolome is a snapshot of the metabolic state of a cow; thus, metabolites may primarily help to explore metabolic (production) diseases, such as ketosis, milk fever, rumen acidosis, or fatty liver syndrome (Littledike et al., 1981; Goff and Horst, 1997), and to infer the risk of a disease. For instance, 3-hydroxybutanoic acid is typically used as a biomarker for ketosis (Geishauser et al., 2000), and a related study

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found a direct relationship between ketosis and the ratio of nuclear magnetic resonance spectroscopy-derived milk components from glycerophosphocholine to phosphocholine (Klein et al., 2012). Furthermore, some milk components may be of particular interest; for example, bovine milk oligosaccharides (**BMO**) resemble human milk oligosaccharides in structure and have a similar protective role on infants' intestines and immune system (Zivkovic and Barile, 2011) and may be a target for functional food production. Thus, milk metabolites may not only be used as biomarkers, they can also generally be seen as novel milk traits from which other production or fitness traits may be deduced and whose genetic background should be elucidated. Then, it is necessary to quantify the extent to which the observed variability of metabolic profiles is due to either genetic variation or environmental or temporal variation.

The primary interest of this study was to estimate genetic parameters and explore mode of inheritance of milk metabolites which were obtained from milk samples collected during performance testing. Toward these objectives, genetic variability of metabolite measurements was investigated in a linear mixed model approach including expected (pedigree) and realized (genomic) relationship between animals for a large sample of dairy cows. The significance of genetic variance components was tested and the ability of milk metabolites to predict genetic values for nonphenotyped animals was studied to verify their meaning for breeding purposes. The heritable effect on chemically related metabolites as well as functionally related metabolites is discussed with respect to selected groups and pathways.

MATERIALS AND METHODS

Milk Samples and Metabolite Data

This field study was designed to gather samples under the same conditions as performance testing. Milk samples were collected from 1,344 Holstein cows held on 18 dairy farms in Mecklenburg-Western Pomerania (in the northeast of Germany) from May to November 2009. The cows were sampled between d 21 and 120 of their first lactation. Only first-lactation cows were selected to avoid variation due to parity and effects of selection due to culling of cows with low milk yield. As this project cooperated with commercial dairy farms only, a logistical challenge existed to separate samples of preselected cows that lay in the required lactation period. For this purpose, the LKV (Association for Quality Inspection, Güstrow, Germany) had access to a farm-specific animal list published and updated weekly on a website. The quality inspector drew 2 samples from the aliquot of the cow's daily milk yield—one according to the usual procedure for milk performance tests, and the other drawn for use in the experiment. Preservatives (natrium acid) were added to the milk samples. The additional samples were transported in separate boxes to the LKV, and analyzed via infrared spectroscopy (Foss, Hillerød, Denmark). Afterward, the remaining milk was aliquoted into 2-mL tubes (Eppendorf, Germany) and frozen using liquid nitrogen until sample collection was finished (39 collection dates). Samples were deep-frozen within a few hours after being collected on a regular test day. All samples (one tube per cow) were sent to the laboratory at the Max Planck Institute for Molecular Plant Physiology (MPIMP; Potsdam, Germany) to measure the metabolite profiles. Twelve animals were later removed from the data set due to an invalid milk measurement (outside the desired lactation interval or because of decreased pH value).

Profiles for the hydrophilic fraction of metabolites were obtained from GC-MS according to Lisec et al. (2006) with minor adjustments [no ball milling, 1.1-mL total extraction volume; 100 μL of milk sample + 1 mL of MeOH:$CHCl_3$:H_2O; 10:90 N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) derivatization]. Sample data were unbalanced in terms of farm, sample date, and half-sib families. For example, a sire had, on average, 6.6 daughters, ranging from 1 to 106. Thus, for GC-MS application, a specific randomized design based on a Latin square was developed, which was as balanced as possible with respect to the factors mentioned above (Melzer et al., 2010). Because of laboratory restrictions, the final design was slightly modified. The laboratory delivered molecule spectra, measured in 47 batches, in which molecule retention time (GC step), the mass:charge ratios, and the corresponding relative intensities of molecule fragments (MS step) were recorded for each sample. These spectra were further processed with the R package TargetSearch version 1.10 (Cuadros-Inostroza et al., 2009; R Development Core Team, 2011). The retention time of each molecule was converted into a retention index based on the retention time standards of FA methyl esters added to the sample in the GC step. Both retention index and molecule spectrum were used to annotate each molecule obtained from the MS step. Molecule spectra from narrow time windows (0.5 s), which showed highly correlated intensity values (correlation >0.95) over all samples, were combined to build a metabolite spectrum. Median values of these mass spectra were then compared with reference spectra in a database (Golm Metabolome Database, **GMD**; http://gmd.mpimp-golm.mpg.de/search.aspx). The assignment of a metabolite spectrum to the reference was accepted in case that the similarity score ($\in[0; 1{,}000]$) between them was >500; otherwise, the metabolite was labeled

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