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Short communication: Genetic variation of riboflavin content in bovine milk

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ABSTRACT

Riboflavin (vitamin B_2) is an essential water-soluble vitamin; elderly people and adolescents in particular can have poor riboflavin status. In Western diets, milk and dairy products are primary sources of riboflavin, but little is known about the natural variation within and among bovine breeds, and how genetic and environmental factors can affect the riboflavin content in milk. As a part of the Danish-Swedish Milk Genomics Initiative, the aim of the study was to quantify milk riboflavin content using reverse-phase HPLC in 2 major Danish dairy breeds. The results showed substantial interbreed differences in milk riboflavin content. Milk from Danish Jersey cows contained significantly higher levels of riboflavin (1.93 mg/L of milk) than milk from Danish Holstein cows (1.40 mg/L of milk). Furthermore, genetic analyses revealed high heritabilities in both breeds (0.52 for Danish Holstein and 0.31 for Danish Jersey). A genomic association study found 35 significant single nucleotide polymorphisms (false discovery rate < 0.10) to be associated with riboflavin content in milk in Jersey cows (all on BTA14 and BTA17), and 511 significant single nucleotide polymorphisms in Holstein cows spread over 25 different autosomes with BTA13 and BTA14 having the most promising quantitative trait loci. The best candidate gene found within the identified quantitative trait loci was *SLC52A3*, a riboflavin transporter gene, which was among the significant markers on BTA13 in Holstein cows.
Key words: genomic heritability, quantitative trait loci, vitamin B_2

Short Communication

Milk, as an important source of nutrients, is recommended as part of an everyday, balanced diet. Milk contains saturated and unsaturated fatty acids, proteins, carbohydrates, minerals, and vitamins, which in their natural or hydrolyzed form potentially promote positive health effects. Consumption of milk and dairy products can thereby exert protective effects on human health (Haug et al., 2007; Givens, 2010). Undoubtedly bovine milk is one of the best sources for several vitamins and minerals in human nutrition, including riboflavin (vitamin B_2) and cobalamin (vitamin B_{12}; Rooke et al., 2010). Riboflavin belongs to the essential water-soluble vitamins in milk, and according to Haug et al. (2007), the content in bovine milk is around 1.83 mg/L. Riboflavin is important due to its key role in numerous metabolic pathways and redox reactions through the biologically active coenzymes, flavin adenine dinucleotide, and flavin mononucleotide (Powers, 2003). For elderly people and adolescents in particular, low intake of riboflavin-containing foods can result in riboflavin deficiencies, and in Western diets milk and dairy products account for 51% of the intake in preschool children (Powers, 2003).

Despite the acknowledged value of milk and dairy products as riboflavin sources (Sunaric et al., 2012), very few studies have documented the drivers for riboflavin variation in milk within and across bovine breeds. The primary origin of riboflavin and other B vitamins is biosynthesis in the rumen (Schwab et al., 2006), and documented effects of feed and breed (Shingfield et al., 2005; Poulsen et al., 2015) are related to the rumen environment and the microbial processes responsible for the riboflavin synthesis or transport and secretion of riboflavin into milk.

The aim of this study was to determine the content of riboflavin in milk from Danish Holstein (**DH**) and Danish Jersey (**DJ**) cows and to explore to what extent this variation can be explained by genotype. Furthermore, the aim was to identify QTL for milk riboflavin content using the BovineHD BeadChip. To our knowledge, this is the first study to screen riboflavin variation in milk from a large number of animals and to provide heritability estimation and QTL identification for milk riboflavin content.

This study was conducted as part of the Danish-Swedish Milk Genomics Initiative. Ear tissue and morning milk samples were collected from 456 DH (20 dairy herds, October–December 2009) and 436 DJ (22 dairy

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herds, February–April 2010) as described by Poulsen et al. (2012). Parity and days in milk at sampling were registered for all cows. Milk yield at morning milking was recorded, and representative milk samples of at least 0.5 L were placed on ice and transported to the laboratory. Immediately after arrival to the laboratory, milk samples were skimmed (centrifuged for 30 min, 2,643 × *g* at 4°C), aliquoted, and stored at –20°C until analysis of riboflavin.

The day before analysis, samples were moved to 4°C and riboflavin content in skim milk samples was determined by reverse-phase HPLC essentially as described in Poulsen et al. (2015). The HPLC system consisted of a Zorbax SB-C8 column (4.6 × 150 nm, 5 μm) equipped with a fluorescence detector (Agilent FLD 1200, Agilent Technologies, Waldbronn, Germany). All analytical procedures were conducted under subdued lighting conditions using glassware wrapped in aluminum foil. In total, riboflavin content was determined in milk from 395 DJ and 428 DH cows.

Genomic DNA was extracted from ear tissue, which was used for genotyping 347 DH and 290 DJ cows with the bovine HD beadchip (Van Tassell et al., 2008). These cows were daughters of 200 DH and 133 DJ sires. The genotyping was performed with the Illumina Infinium II Multisample assay device (Illumina Inc., San Diego, CA). The SNP chips were scanned using iScan and analyzed by Beadstudio, version 3.1 (Illumina Inc.). The quality parameters, used for the selection of SNP in the genome-wide association studies, were minimum call rates of 80% for individuals and 95% for loci. Marker loci with minor allele frequencies below 1% were excluded. The quality of the markers was assessed using the GenCall data analysis software of Illumina. Individuals with average GenCall scores below 0.65 were excluded (Teo et al., 2007). The *Bos taurus* genome assembly (*Btau_4.0*; Liu et al., 2009) was used to base the physical positions of the markers.

Breed difference in milk riboflavin content was estimated by one-way ANOVA:

$$Y_{ik} = \mu + \text{breed}_i + e_{ik}, \quad [1]$$

where Y_{ik} is the phenotype of individual k in breed i, μ is the overall mean of the trait, and breed is a fixed effect (i = DJ, DH).

Variance components were estimated within breed following a linear model:

$$Y_{ijk} = \mu + \text{parity}_i + \text{DIM} + \text{herd}_j + \text{animal}_k + e_{ijk}, \quad [2]$$

where Y_{ijk} is the phenotype of individual k in parity i and herd j, μ is the fixed mean effect, parity is a fixed effect (i = 1, 2, 3 DH, i = 1, 2, 3 DJ), DIM is a covariate of days in milk (d 129 to 229 in DH, d 130 to 252 in DJ), herd is a random effect, and animal is the random additive genetic effect based on **G** of animal k (Yang et al., 2010).Univariate analyses were performed to estimate the heritability, which was defined as

$$h^2 = \sigma_a^2 \Big/ \left(\sigma_a^2 + \sigma_{\text{herd}}^2 + \sigma_e^2\right), \quad [3]$$

where σ_a^2 was the genetic variation, σ_{herd}^2 was the herd variation, and σ_e^2 was the residual variation obtained from model 2.The proportion of the total variance caused by the herd was calculated as

$$h_{\text{herd}} = \sigma_{\text{herd}}^2 \Big/ \left(\sigma_a^2 + \sigma_{\text{herd}}^2 + \sigma_e^2\right). \quad [4]$$

A genomic relationship matrix was calculated for each breed separately as described by the first method presented in VanRaden (2008). A genomic relationship matrix was calculated as follows: Let **M** be a matrix with dimensions of the number of individuals (n) by the number of loci (m) that specifies which marker alleles each individual inherited. The elements of **M** were set to −1, 0, 1 for the homozygote, heterozygote, and the other homozygote, respectively. The diagonals of **M′M** show the number of homozygous loci for each individual, and off diagonals measure the number of alleles shared by relatives. Let the frequency of the second allele at locus i be p_i, and let **P** contain the allele frequencies, such that column i of **P** equals $2(p_i - 0.5)$. Subtraction of **P** from **M** gives **Z**, which is needed to set the expected mean value to 0. The genomic relationship matrix **G** was then calculated as $\mathbf{ZZ'}/[2\Sigma p_i(1 - p_i)]$ (VanRaden, 2008).

Association Mapping. The analysis was performed using the following linear model for each breed separately:

$$Y_{ijk} = \mu + \text{herd}_i + \text{parity}_j + \text{DIM} + b \times \text{SNP} + \text{animal}_k + e_{ijk}, \quad [5]$$

where Y_{ijk} is the phenotype of individual k in herd i and parity j, μ is the fixed mean effect, herd is a fixed effect (i = 1, 2, …, 20 DH; i = 1, 2,…, 21 DJ), parity is a fixed effect (j = 1, 2, 3 DH, j = 1, 2, 3 DJ), DIM is a covariate of days in milk (d 129 to 229 in DH, d 130 to 252 in DJ), b is the allele substitution effect, which is a count in individual k of one of the 2 alleles (with arbitrary labeling), and animal is the random additive genetic effect based on **G** of animal k (Yang et al., 2010). A Wald test with a null hypothesis of H_0: b =