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Effects of breed and casein genetic variants on protein profile in milk from Swedish Red, Danish Holstein, and Danish Jersey cows

F. Gustavsson,*[1] A. J. Buitenhuis,† M. Johansson,‡ H. P. Bertelsen,† M. Glantz,* N. A. Poulsen,§ H. Lindmark Månsson,*# H. Stålhammar,‖ L. B. Larsen,§ C. Bendixen,† M. Paulsson,* and A. Andrén‡
*Department of Food Technology, Engineering and Nutrition, Lund University, PO Box 124, SE-221 00 Lund, Sweden
†Department of Molecular Biology and Genetics, Aarhus University, Blichers Allé 20, PO Box 50, DK-8830 Tjele, Denmark
‡Department of Food Science, BioCenter, Swedish University of Agricultural Sciences, PO Box 7051, SE-750 07 Uppsala, Sweden
§Department of Food Science, Aarhus University, Blichers Allé 20, PO Box 50, DK-8830 Tjele, Denmark
#LRF Dairy Sweden, Ideon Science Park, SE-223 70 Lund, Sweden
‖VikingGenetics, PO Box 64, SE-532 21 Skara, Sweden

ABSTRACT

In selecting cows for higher milk yields and milk quality, it is important to understand how these traits are affected by the bovine genome. The major milk proteins exhibit genetic polymorphism and these genetic variants can serve as markers for milk composition, milk production traits, and technological properties of milk. The aim of this study was to investigate the relationships between casein (CN) genetic variants and detailed protein composition in Swedish and Danish dairy milk. Milk and DNA samples were collected from approximately 400 individual cows each of 3 Scandinavian dairy breeds: Swedish Red (SR), Danish Holstein (DH), and Danish Jersey (DJ). The protein profile with relative concentrations of α-lactalbumin, β-lactoglobulin, and α_{S1}-, α_{S2}-, κ-, and β-CN was determined for each milk sample using capillary zone electrophoresis. The genetic variants of the α_{S1}- (*CSN1S1*), β- (*CSN2*), and κ-CN (*CSN3*) genes for each cow were determined using TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA). Univariate statistical models were used to evaluate the effects of composite genetic variants, α_{S1}-β-κ-CN, on the protein profile. The 3 studied Scandinavian breeds differed from each other regarding CN genotypes, with DH and SR having similar genotype frequencies, whereas the genotype frequencies in DJ differed from the other 2 breeds. The similarities in genotype frequencies of SR and DH and differences compared with DJ were also seen in milk production traits, gross milk composition, and protein profile. Frequencies of the most common composite α_{S1}-β-κ-CN genotype BB/A^2A^2/AA were 30% in DH and 15% in SR, and cows that had this genotype gave milk with lower relative concentrations of κ- and β-CN and higher relative concentrations of α_S-CN, than the majority of the other composite genotypes in SR and DH. The effect of composite genotypes on relative concentrations of the milk proteins was not as pronounced in DJ. The present work suggests that a higher frequency of BB/A^1A^2/AB, together with a decrease in BB/A^2A^2/AA, could have positive effects on DH and SR milk regarding, for example, the processing of cheese.
Key words: milk protein composition, casein, genetic polymorphism, composite genotype

INTRODUCTION

Milk composition affects both the processing of milk and its nutritional value. It is well known that variation in milk composition is influenced by environmental factors such as feeding, the physiological condition of the cow, and the genetic makeup (Walstra et al., 2006). Differences also exist in milk composition between breeds and individual cows within a breed, which are partly due to genetic variation (e.g., McLean et al., 1984; Wedholm et al., 2006; Poulsen et al., 2012). The detailed protein composition is important for the processability and functionality of different dairy products, such as yogurt, milk powder, and cheese (Walstra et al., 2006). As detailed milk protein composition is not included in the Swedish and Danish national milk recording systems (Swedish Dairy Association, 2011; RYK, 2012), knowledge of how the detailed protein composition varies between and within the Scandinavian breeds is limited. The milk proteins are subdivided into caseins and whey proteins. The caseins α_{S1}-, α_{S2}-, β-, and κ-CN, represent approximately 80% of total milk protein, whereas the other 20% consists of whey proteins, mainly β-LG and α-LA (Farrell et al., 2004). For decades, it has been known that the major milk proteins are present in different genetic variants in milk. Extensive research has shown that these genetic variants can serve as markers for both milk yield and composition and, consequently,

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[1]Corresponding author: Frida.Gustavsson@food.lth.se

milk processability (e.g., Ikonen et al., 1997; Hallén et al., 2007; Bonfatti et al., 2010a). Some of these studies have shown that genetic protein variants affect absolute concentrations (McLean et al., 1984; Ikonen et al., 1997; Hallén et al., 2008) and relative concentrations (Bobe et al., 1999; Heck et al., 2009a; Bonfatti et al., 2010b) of the individual milk proteins, especially κ-CN genetic variant B, which has been shown to have a positive effect on κ-CN concentration (Bobe et al., 1999; Hallén et al., 2008; Heck et al., 2009a).

As no large-scale study has been performed on detailed protein composition in the most common dairy cow breeds in Sweden and Denmark, milk was collected from about 400 each of Swedish Red (**SR**; 41% of the Swedish cattle population), Danish Holstein (**DH**; 71% of the Danish cattle population), and Danish Jersey (**DJ**; 13% of the Danish cattle population) breeds. The aim of this study was to investigate the relationship between casein genetic variants and detailed protein composition in Swedish and Danish milk. Both the knowledge of how detailed milk composition varies between breeds and how it varies between composite α_{S1}-β-κ-CN genotypes within a breed could help to direct and improve the quality of milk used for further processing. This could be done using organized breeding programs or by using milk from different breeds for the production of different dairy products.

MATERIALS AND METHODS

Milk Samples

Morning milk samples and tissue/blood samples were collected from the following cows: 415 DH (20 dairy herds, collected between October and December 2009), 406 DJ (22 dairy herds, between February and April 2010), and 392 SR (20 dairy herds, between April and May 2010 and September 2010 and April 2011). The cows were selected to be as unrelated as possible using recorded ancestry and, in most cases, only 1 daughter was chosen per sire and farm. The majority of the cows were in lactations 1 to 3 and in DIM 70 to 245. In SR, 1% of the animals were in lactation 4 and 5 and 25% of the animals were milked before lactation d 70 or after lactation d 245, respectively. Milk yield was recorded at time of sampling and the daily milk yield was estimated from milk yield at each milking and how many times the cows were milked per day and farm. Fresh milk samples were analyzed for contents of total protein, total casein, and fat using the infrared technique (MilkoScan FT2; Foss Analytical A/S, Hillerød, Denmark). This method has previously been validated for casein measurements (Sørensen et al., 2003). Somatic cell counts were measured by flow cytometry. The DJ and DH samples were measured using a Fossomatic 5000 somatic cell counter (Foss Analytical A/S) at a Danish certified dairy analysis laboratory (Eurofins Steins Laboratorium A/S, Holstebro, Denmark). The SR samples were measured using a CombiFoss 5000 analyzer (Foss Analytical A/S) at a certified Swedish dairy analysis laboratory (Eurofins Steins Laboratorium AB, Jönköping, Sweden). All samples had SCC below 500,000 cells/mL.

Sample and Capillary Zone Electrophoresis Buffer Preparation

At the day of sampling, the collected milk samples were defatted by centrifugation (SR samples at 2,000 × *g* and DH and DJ samples at 2,643 × *g*) for 30 min at 4°C, with subsequent removal of the fat layer. Skim milk samples were frozen and stored at −20°C until the day of analysis. The samples and capillary electrophoresis buffers were prepared according to Åkerstedt et al. (2012). In short, the sample solution was prepared by mixing 300 μL of milk with 700 μL of sample buffer and, after mixing, the sample solution was left at room temperature for 1 h. In the sample buffer, M-D,L-dithiothreitol (DTT; Sigma, Stockholm, Sweden) was used as reducing agent. The sample solutions were filtered through a 0.45-μm nylon membrane filter before analyses by capillary zone electrophoresis (**CZE**).

CZE Analysis

Milk analyses were carried out with CZE according to Åkerstedt et al. (2012). The CZE equipment (G-1600AX; Agilent Technologies Sweden AB, Kista, Sweden) was controlled by ChemStation software (version A 10.02; Agilent Technologies Sweden AB). In summary, milk protein separations were performed using an unfused silica standard capillary with a 50-μm i.d., 40-cm active length (ChromTech AB, Märsta, Sweden), and separations were carried out at 45°C at a constant voltage of 25 kV. Sample solutions were injected at the anode by pressure injection at 5 kPa for 7 s.

Identification of Peaks and Quantification of the Individual Milk Proteins

Milk protein standards of α_S-CN, β-CN, κ-CN, α-LA, and β-LG (Sigma) were used for peak identification. Furthermore, the peak identifications were confirmed by a comparison with previously published electropherograms (Otte et al., 1997; Miralles et al., 2003; Heck et al., 2008). Multiple peaks found around the main peak of α_{S2}-CN were interpreted as α_{S2}-CN isoforms according to Heck et al. (2008). The relative concentration of α_{S1}-CN was divided into α_{S1}-CN 8P, α_{S1}-CN