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

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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/A²A²/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_{S1} -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/A¹A²/AB, together with a decrease in BB/A²A²/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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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 $2,000 \times g$ and DH and DJ samples at $2,643 \times g$) 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 μ L of milk with 700 μ L 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- μ m 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- μ m 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

9P, and total α_{S1} -CN. Total α_{S1} -CN is the sum of relative concentrations of α_{S1} -CN 8P and α_{S1} -CN 9P and also the peak migrating just before the 8P peak, all according to Heck et al. (2008). As κ -CN is present in different phosphorylated and glycosylated forms, it separates into multiple peaks, with the monophosphorylated form in the highest concentration (Otte et al., 1997; Miralles et al., 2001). The different genetic variants of β -CN can be distinguished using CZE, where β -CN B separates first, followed by A¹, A²/I (which cannot be distinguished from each other because of similar migration times), and A³ (Recio et al., 1997). Depending on β -CN genotype, some κ -CN isoforms will be visible, whereas other will comigrate with β -CN. In the electropherograms where a κ -CN peak was visible because of the absence of a genetic protein variant of β -CN, the relative concentration was determined for that peak. The average of these relative concentrations was then subtracted from and added to the relative concentrations of β -CN and κ -CN, respectively, in electropherograms where the κ -CN peak was hidden. The monophosphorylated form of κ -CN was assigned κ -CN 1P and the estimated relative concentration of total κ -CN was assigned adjusted total κ -CN. The estimated relative concentration of β -CN was assigned adjusted total β -CN. Hence, β -CN was the relative concentration of β -CN and the comigrated κ -CN, all according to Heck et al. (2008). As this was an assumption, both the adjusted and unadjusted values were included in the analyses. The relative concentrations of the individual milk proteins were estimated the same way as by Heck et al. (2008). Equation [1] was used to estimate the relative concentration of an individual protein:

$$C_x = \frac{A_x/t_x}{\sum_{i=1}^n (A_i/t_i)} \times 100\%, \quad [1]$$

where C_x is the relative concentration, A_x is the specific area of interest in the electropherogram, t_x is the migration time in minutes of protein x , i is the index of summation, and n is the total number of peaks that together form 100% of the area.

Some of the electropherograms showed inadequate separation of whey proteins and, therefore, several protein profiles were excluded from the analysis. The inadequate separation was thought to be due to insufficient fat removal, as traces of milk fat can alter the migration behavior of the proteins in CZE (C. Olieman, NIZO Food Research BV, Ede, the Netherlands, personal communication). After disregarding samples with inadequate separation of whey proteins, 381 DH, 221 DJ, and 325 SR (protein profile sample set) were included in the protein profile analysis.

Genotyping of Genetic Variants

Blood samples (SR) and ear tissue samples (DH and DJ) were used to extract DNA according to Poulsen et al. (2013). The extracted genomic DNA from all animals was genotyped for α_{S1} - (*CSN1S1*), β - (*CSN2*), and κ -CN (*CSN3*) genetic variants using TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA), using the primer sequence according to Poulsen et al. (2013). The assays distinguished between *CSN1S1* variants A, B, C, D, and F; *CSN2* variants A¹, A², A³, B, and I; and *CSN3* variants A, B, and E, which are reported to be the most common casein variants (Farrell et al., 2004). Both the present study and the study by Poulsen et al. (2013) is part of the Swedish-Danish milk genomics initiative where the same samples have been used in several studies with different analyses. However, the present study differs from Poulsen et al. (2013) regarding the number of cows included in the analyses.

Statistical Analysis

One-way ANOVA was used to determine differences in milk composition and milk production traits between breeds using the full sample set (Minitab version 14; Minitab Ltd., Coventry, UK). The protein profile was compared between breeds in the protein profile subset.

The effect of each of the composite α_{S1} - β - κ -CN genotypes on relative concentrations of the different milk proteins was estimated within each breed using a linear mixed model approach in DMU software (Madsen and Jensen, 2007).

For DH, the model was as follows:

$$Y_{ijk} = \mu + \text{herd}_i + \text{parity}_j + \text{DIM} + \text{compngen}_2 + \text{compngen}_3 + \text{compngen}_4 + \text{compngen}_5 + \text{compngen}_6 + \text{compngen}_7 + A_k + e_{ijk}. \quad [2]$$

For DJ, the model was as follows:

$$Y_{ijk} = \mu + \text{herd}_i + \text{parity}_j + \text{DIM} + \text{compngen}_2 + \text{compngen}_3 + \text{compngen}_4 + \text{compngen}_5 + \text{compngen}_6 + \text{compngen}_7 + \text{compngen}_8 + \text{compngen}_9 + A_k + e_{ijk}. \quad [3]$$

For SR, the model was as follows:

$$Y_{ijk} = \mu + \text{herd}_i + \text{parity}_j + \text{DIM} + \text{compngen}_2 + \text{compngen}_3 + \text{compngen}_4 + \text{compngen}_5 + \text{compngen}_6 + \text{compngen}_7 + \text{compngen}_8 + \text{compngen}_9 + A_k + e_{ijk}. \quad [4]$$

The results of models [2] to [4] were compared with model [5]:

$$Y_{ijk} = \mu + \text{herd}_i + \text{parity}_j + \text{DIM} + A_k + e_{ijk} \quad [5]$$

for each breed separately, where Y_{ijk} is the phenotype of the k th individual in herd i and parity j ; μ is the overall mean of the trait; herd is a fixed effect ($i = 1, 2, \dots, 22$); parity is a fixed effect ($j = 1, 2, 3$, or 4), DIM is a covariate of DIM (d 129 to 229 in DH, d 130 to 252 in DJ, and d 18 to 427 in SR), **compgen** is a vector of 0 and 1 fitted as a covariate, indicating the composite α_{S1} - β - κ -CN genotype of an animal; A_k is the random additive genetic effect of animal k ; and e_{ijk} is the random residual effect. In Equations [2] and [3], **compgen**₁ is the composite genotype BB/A²A²/AA, which is the most common one in DH and the third most common in SR. In Equation [4], **compgen**₁ is the most common DJ composite genotype, BC/A²A²/AB; **compgen**₂₋₉ are the other composite genotypes present at a higher frequency than 2.5%, both in the full sample set and in the protein profile sample set, in each breed separately. When disregarding animals with composite genotypes present at lower frequencies than 2.5%, the models estimating the effects of composite genotypes on protein profile included 309 DH, 309 SR, and 199 DJ (protein profile subset). Relationships between animals were accounted for and the pedigrees were traced back for 5 generations for each breed separately. The DH pedigree consisted of 4,811 animals, the DJ pedigree consisted of 4,351 animals, and the SR pedigree consisted of 4,636 animals. The genetic variance explained by the composite genotypes was calculated as

$$\sum \left[\text{fr} \times (1 - \text{fr}) \times (\text{effect of the composite genotype})^2 / (\sigma_G^2 + \sigma_e^2) \times 100 \right], \quad [6]$$

where fr is the frequency of the composite genotype, σ_G^2 is the genetic variance, and σ_e^2 is the residual variance.

RESULTS

Effect of Breed on Milk Composition, Milk Production Traits, and Protein Profile

It was evident that breed affected both milk composition and milk production traits as shown in Table 1. A difference existed in mean SCC between the breeds, with SR giving milk with a lower mean SCC ($P < 0.05$) than DH and DJ. The DJ cows gave milk with lower milk, protein, and casein yields ($P < 0.05$) and with higher fat, protein, and casein contents than SR and DH, whereas no difference existed in fat yield. Milk from SR cows had higher protein contents than

milk from DH cows, whereas no differences existed in fat and casein contents, or in milk, fat, protein, and casein yields. Observations on breed differences in milk composition and milk production traits agree well with data from the national cow databases in Sweden and Denmark (Swedish Dairy Association, 2011; RYK, 2012). Further, the results agree with published studies on individual cows (Wedholm et al., 2006; Frederiksen et al., 2011b). Differences in protein profile could be noticed between milk from the 3 breeds (Table 2). The highest relative concentrations of α -LA, β -LG, α_{S1} -CN 9P, β -CN, and adjusted total β -CN were found in DH, whereas the highest relative concentrations of α_{S2} -CN, α_{S1} -CN 8P, total α_{S1} -CN, and κ -CN 1P were found in DJ. Milk from DJ and SR had higher relative concentrations of adjusted total κ -CN than milk from DH.

Genotype Frequencies

The presence and frequencies of different casein alleles and genotypes were quite similar between DH and SR, whereas the DJ breed was quite different from the other 2 breeds (Table 3). Regarding the κ -CN genotypes, the DJ cows were distinguished from SR and DH regarding the high occurrence of the B variant and the absence of the E variant. In SR and DH, the κ -CN A variant was the most common, whereas κ -CN E was more common in SR than in DH. Five β -CN variants were represented in these 3 breeds (i.e., A¹, A², A³, B, and I). The β -CN A² variant was the most common variant in all 3 breeds, whereas β -CN A¹ was more common in SR than in DH and DJ. Variant β -CN B was more common in DJ than in the other 2 breeds and β -CN I was present in low frequencies in DH and DJ. Regarding α_{S1} -CN, almost all of the DH and SR cows were homozygous for the B variant and only a few animals carried the C variant. In DJ, both α_{S1} -CN B and C variants were present at high frequencies.

As the aim of the present study was to investigate the effect of composite genetic variants on the protein profile, the frequencies of composite α_{S1} - β - κ -CN genotypes was presented for the protein profile sample set, which included 381 DH, 221 DJ, and 325 SR (Table 4). The most common composite casein genotype in DH was BB/A²A²/AA, which was present at a frequency of 30%. This composite genotype was also quite common in SR at a frequency of 13%, whereas the most common composite genotype in SR was BB/A¹A²/AE (18%). Genotype BB/A¹A²/AA was the second most common composite genotype in both DH and SR, with a frequency of 15 and 17%, respectively. The most common composite genotype in DJ was BC/A²A²/AB (23%), followed by BC/BA²/BB (19%) and CC/A²A²/

Table 1. Descriptive statistics for lactation data, milk production traits, and milk composition in Danish Holstein (DH), Swedish Red (SR), and Danish Jersey (DJ) cows using the full sample set (415 DH, 392 SR, and 406 DJ)

Trait ¹	Breed								
	DH			SR			DJ		
	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)
DIM (d)	179.0 ^a	21.3	11.9	187.0 ^b	76.8	41.1	186.1 ^{ab}	22.8	12.2
Parity	1.7	0.8	45.1	1.7	0.8	43.5	1.7	0.8	45.3
SCC ($\times 1,000/\text{mL}$)	110.3 ^a	109.8	99.5	54.1 ^b	64.0	118.3	113.1 ^a	108.6	96.0
Fat (g/100 g)	4.0 ^a	0.8	19.6	4.1 ^a	0.9	21.0	6.0 ^b	0.9	14.4
Protein (g/100 g)	3.4 ^a	0.3	7.4	3.7 ^b	0.4	9.6	4.3 ^c	0.3	7.4
Casein (g/100 g)	2.7 ^a	0.1	4.5	2.7 ^a	0.1	3.4	3.0 ^b	0.2	4.8
MY (kg/d)	30.7 ^a	8.1	26.5	30.2 ^a	9.9	33.0	20.5 ^b	5.2	25.1
FY (kg/d)	1.2	0.4	30.1	1.2	0.4	35.2	1.2	0.3	23.7
PY (kg/d)	1.1 ^a	0.3	25.4	1.1 ^a	0.3	29.5	0.9 ^b	0.2	23.6
CY (kg/d)	0.8 ^a	0.2	25.9	0.8 ^a	0.3	31.7	0.6 ^b	0.2	24.2

^{a-c}Means within a row with different superscript letters differ significantly ($P < 0.05$).

¹MY = milk yield; FY = fat yield; PY = protein yield; CY = casein yield.

BB (16%), whereas the frequency of BB/A²A²/AA was much lower in DJ than in SR and DH (4%).

Effect of Composite Genotypes

The effects of composite α_{S1} - κ - β -CN genotypes on milk production traits, gross milk composition, and protein profile were investigated. No effect could be seen on milk production traits in any of the breeds or on gross milk composition in SR (fat, protein, and casein contents; data not shown). Only a few composite genotypes had an effect on gross composition in DH and DJ (data not shown). However, an effect of α_{S1} - κ - β -CN genotypes was detected on the protein profile in all 3 breeds.

Whey Proteins. No significant effects were detected of composite α_{S1} - κ - β -CN genotypes on relative

concentrations of α -LA in either of the breeds (data not shown). Regarding relative concentrations of β -LG in SR and DH, the composite genetic variant BB/A¹A²/AB gave milk with a significantly higher relative concentration of β -LG ($P = 0.02$ – 0.09) compared with BB/A²A²/AA (Table 5). In SR, this was also true for BB/A¹A¹/EE ($P = 0.04$). In DJ, milk from cows with the composite genetic variant BB/BB/BB had higher relative concentrations of β -LG than milk from cows with BC/A²A²/AB ($P = 0.08$). The variance explained by the composite genotypes on relative concentrations of β -LG was 4.3, 6.1, and 2.6% in DH, SR, and DJ, respectively (Supplementary Table S1, <http://dx.doi.org/10.3168/jds.2013-7312>).

α_{S1} - and α_{S2} -CN. As presented in Table 6, all composite α_{S1} - β - κ -CN genotypes in SR resulted in milk with lower relative concentrations of α_{S1} -CN 9P

Table 2. Descriptive statistics for the relative concentration of the individual milk proteins grouped by breed [Danish Holstein (DH), Swedish Red (SR), and Danish Jersey (DJ) cows], using the protein profile sample set (381 DH, 325 SR, and 221 DJ)

Protein (%, wt/wt ¹)	Breed								
	DH			SR			DJ		
	Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)
α -LA	2.56 ^a	0.69	27.08	2.32 ^b	0.76	32.79	2.14 ^c	0.86	39.89
β -LG	10.12 ^a	2.17	21.47	8.80 ^b	1.84	20.89	8.76 ^b	2.71	30.91
α_{S1} -CN 8P	21.29 ^a	1.70	7.99	21.65 ^b	1.47	6.78	23.60 ^c	1.88	7.98
α_{S1} -CN 9P	6.53 ^a	1.39	21.24	6.43 ^{ab}	1.10	17.12	6.21 ^b	1.14	18.38
α_{S1} -CN	29.06 ^a	2.03	6.97	29.33 ^a	1.56	5.32	31.51 ^b	2.55	8.10
α_{S2} -CN	6.56 ^a	1.21	18.49	6.82 ^b	1.25	18.27	7.32 ^c	1.10	15.01
κ -CN 1P	5.05 ^a	0.98	19.30	5.36 ^b	1.05	19.55	6.39 ^c	0.91	14.21
Adj. ² κ -CN	10.13 ^a	1.31	12.92	11.69 ^b	1.15	9.86	11.68 ^b	1.09	9.37
β -CN	38.54 ^a	2.70	7.00	36.99 ^b	2.77	7.49	36.41 ^c	2.94	8.08
Adj. β -CN	35.82 ^a	2.39	6.68	33.72 ^b	2.55	7.56	34.40 ^c	2.86	8.30

^{a-c}Means within a row with different superscript letters differ significantly ($P < 0.05$).

¹wt/wt = wt/wt of total protein.

²Adj. = adjusted average, which is adjusted for overlapping κ - and β -CN peaks.

Table 3. Genotype and allele frequencies of α_{S1} -, β -, and κ -CN genotypes in Danish Holstein (DH), Swedish Red (SR), and Danish Jersey (DJ) cows in the full sample set (415 DH, 392 SR, and 406 DJ)

Protein	Genotype	DH	SR	DJ	Allele	DH	SR	DJ
α_{S1} -CN	BB	0.990	0.992	0.319	B	0.995	0.996	0.572
	BC	0.010	0.008	0.505	C	0.005	0.004	0.428
	CC	0	0	0.176				
β -CN	A ¹ A ¹	0.067	0.218	0.007	A ¹	0.266	0.482	0.081
	A ¹ A ²	0.332	0.519	0.093	A ²	0.614	0.509	0.634
	A ² A ²	0.377	0.246	0.424	A ³	0.005	0	0
	BA ¹	0.022	0.010	0.044	B	0.046	0.009	0.218
	BA ²	0.060	0.008	0.245	I	0.070	0	0.067
	BI	0.010	0	0.035				
	BB	0	0	0.056				
	II	0.007	0	0.002				
	A ¹ I	0.038	0	0.012				
	A ² I	0.077	0	0.083				
	A ¹ A ³	0.005	0	0				
	A ² A ³	0.005	0	0				
κ -CN	AA	0.481	0.373	0.037	A	0.697	0.620	0.206
	AB	0.346	0.195	0.338	B	0.239	0.160	0.794
	AE	0.087	0.298	0	E	0.064	0.219	0
	BB	0.050	0.023	0.625				
	BE	0.031	0.080	0				
	EE	0.005	0.030	0				

($P = 0.005$ – 0.03) and total α_{S1} -CN ($P = 0.002$ – 0.09) than milk from cows with composite genotype BB/A²A²/AA. Further, all but 2 composite genotypes gave milk with lower relative concentrations of α_{S2} -CN ($P = 0.001$ – 0.07) than milk from cows with composite genotype BB/A²A²/AA. In DH, milk from cows with composite genotype BB/A²I/AB had higher relative concentrations of α_{S2} -CN ($P = 0.02$) and milk from cows with the other composite genotypes had lower relative concentrations of α_{S2} -CN than milk from cows with BB/A²A²/AA (Table 6). Further, milk from DH cows with composite genotype BB/A²I/AB gave milk with significantly lower relative concentrations of α_{S1} -CN 8P, α_{S1} -CN 9P and total α_{S1} -CN. In DJ, cows with the composite genotype BB/BB/BB gave milk with significantly lower relative concentrations of α_{S1} -CN 8P and total α_{S1} -CN than the most common composite genotype in DJ, BC/A²A²/AB (Table 7). Further, cows with the composite genotype CC/A²A²/BB gave milk with higher relative concentrations of α_{S1} -CN 9P and total α_{S1} -CN and lower relative concentrations of α_{S2} -CN than cows with BC/A²A²/BB. The variance explained by the composite genotypes on α_{S1} -CN was 13.6, 31.9, and 16.9% and on α_{S2} -CN, the variance was 16.7, 35.2, and 15.8% in DH, SR, and DJ, respectively (Supplementary Table S1, <http://dx.doi.org/10.3168/jds.2013-7312>).

κ -CN. In SR, milk from cows with all composite α_{S1} - β - κ -CN genotypes had higher relative concentrations of κ -CN 1P ($P = 0.001$ – 0.01) and adjusted total κ -CN ($P = 0.001$ – 0.09) than milk from cows with BB/A²A²/AA (Table 8). In DH, milk from cows with composite

genotypes BB/A¹A²/AA, BB/A¹A²/AB, and BB/BA²/AB had higher relative concentrations of κ -CN 1P ($P < 0.001$) and adjusted total κ -CN ($P = 0.01$ – 0.06) than milk from cows with BB/A²A²/AA (Table 8). In

Table 4. Frequencies of the most common composite α_{S1} - β - κ -CN genotypes in Danish Holstein (DH), Danish Jersey (DJ), and Swedish Red (SR) cows using the protein profile sample set (381 DH, 325 SR, and 221 DJ)

Composite casein genotype	Breed		
	DH	SR	DJ
BB/A ¹ A ¹ /AA	0.019	0.067 ¹	
BB/A ¹ A ¹ /AE	0.011	0.119 ¹	
BB/A ¹ A ¹ /EE	0.005	0.032 ¹	
BB/A ¹ A ² /AA	0.146 ¹	0.170 ¹	
BB/A ¹ A ² /AB	0.090 ¹	0.077 ¹	0.028 ²
BB/A ¹ A ² /AE	0.080 ¹	0.176 ¹	
BB/A ¹ A ² /BE	0.008	0.064 ¹	
BB/A ² A ² /AA	0.297 ¹	0.131 ¹	0.041 ¹
BB/A ² A ² /AB	0.080 ¹	0.109 ¹	
BB/A ² I/AB	0.069 ¹		0.018
BB/BA ¹ /BB			0.041 ¹
BB/BA ² /AB			0.060 ¹
BB/BB/BB	0.050 ¹		0.055 ¹
BC/A ¹ A ² /BB			0.060 ¹
BC/A ² A ² /AB		0.006	0.229 ¹
BC/A ² I/BB			0.064 ¹
BC/BA ² /BB			0.193 ¹
CC/A ² A ² /BB			0.156 ¹
Other ³	0.146	0.048	0.055

¹Composite casein genotypes included in the statistical model (frequency >2.5%, both in the full sample set and the protein profile sample set).

²Composite casein genotype not present at a frequency higher than 2.5% in the full sample set.

³Other refers to cows with composite genotypes with a frequency below 2.5% in all breeds.

Table 5. Estimated differences $\pm$ SE and trait general means $\pm$ SD in relative concentrations of β -LG, comparing all composite α_{S1} - β - κ -CN genotypes with a frequency higher than 2.5% to the composite genotype BB/A²A²/AA in Danish Holstein (DH) and Swedish Red (SR) cows and to BC/A²A²/AB in Danish Jersey (DJ) cows, respectively¹

Composite casein genotype	β -LG (% wt/wt ²)		Composite casein genotype	β -LG (% wt/wt)
	DH	SR		
BB/A ² A ² /AA	0	0	BC/A ² A ² /AB	0
BB/A ¹ A ¹ /AA		-0.13 $\pm$ 0.50	BB/A ² A ² /AA	-0.03 $\pm$ 1.08
BB/A ¹ A ¹ /AE		0.33 $\pm$ 0.43	BC/BA ² /BB	-0.16 $\pm$ 0.61
BB/A ¹ A ¹ /EE		1.40 $\pm$ 0.64*	CC/A ² A ² /BB	0.34 $\pm$ 0.63
BB/A ¹ A ² /AA	0.34 $\pm$ 0.37	0.20 $\pm$ 0.38	BB/BB/BB	1.65 $\pm$ 0.92†
BB/A ¹ A ² /AB	1.10 $\pm$ 0.45*	0.83 $\pm$ 0.48†	BB/BA ² /AB	0.66 $\pm$ 0.91
BB/A ¹ A ² /AE	0.65 $\pm$ 0.46	0.28 $\pm$ 0.38	BC/A ² I/BB	-0.10 $\pm$ 0.87
BB/A ¹ A ² /BE		0.80 $\pm$ 0.52	BC/A ¹ A ² /BB	-0.18 $\pm$ 0.89
BB/A ² A ² /AB	0.34 $\pm$ 0.48	0.17 $\pm$ 0.42	BB/BA ¹ /BB	-0.40 $\pm$ 1.09
BB/A ² I/AB	-0.11 $\pm$ 0.50			
BB/BA ² /AB	0.51 $\pm$ 0.56			
Trait mean	10.12 $\pm$ 2.17	8.80 $\pm$ 1.84	Trait mean	8.76 $\pm$ 2.71

¹Cows from the protein profile subset were used (309 DH, 309 SR, and 199 DJ).

²wt/wt = wt/wt of total protein.

† $P < 0.1$; * $P < 0.05$.

DJ, milk from cows with the composite genotype BC/A¹A²/BB had significantly higher relative concentration of κ -CN 1P ($P = 0.001$) and higher adjusted total κ -CN ($P = 0.08$) than milk from cows with BC/A²A²/BB (Table 7). The variance explained by the composite genotypes on κ -CN 1P was 32.9, 87.0, and 10.2%, and on adjusted total κ -CN, the variance was 7.9, 55.8, and 5.8% in DH, SR, and DJ, respectively (Supplementary Table S1, <http://dx.doi.org/10.3168/jds.2013-7312>).

β -CN. In DH, milk from cows with composite α_{S1} - β - κ -CN genotypes BB/A¹A²/AA, BB/A¹A²/AE, and BB/BA²/AB had higher relative concentrations of total β -CN and adjusted total β -CN ($P = 0.001$ – 0.08) than milk from cows with BB/A²A²/AA (Table 8). Further, milk from cows with BB/A¹A²/AB had significantly higher relative concentrations of total β -CN ($P < 0.001$) than milk from cows with BB/A²A²/AA. In SR, milk from cows with all but BB/A¹A¹/EE, BB/A²A²/AB, and BB/A¹A¹/AE had significantly higher relative concentrations of β -CN ($P = 0.001$ – 0.05) than milk from cows with BB/A²A²/AA (Table 8). Regarding the relative concentration of adjusted total β -CN, milk from cows with BB/A¹A²/AB, BB/A¹A²/BE, BB/A¹A¹/AA, and BB/A¹A¹/AE had significantly higher relative concentrations ($P = 0.02$ – 0.09) than milk from cows with BB/A²A²/AA. In DJ, milk from cows with BC/BA²/BB, BC/A¹A²/BB, and BB/BA¹/BB had higher relative concentrations of β -CN and adjusted total β -CN ($P = 0.001$ – 0.09) than milk from cows with BC/A²A²/BB (Table 7). The variance explained by the composite genotypes on β -CN was 53.8, 66.3, and 20.8%, and on adjusted total β -CN, the variance was 7.8, 12.3, and 15.3% in DH, SR, and DJ, respectively (Supplementary Table S1, <http://dx.doi.org/10.3168/jds.2013-7312>).

DISCUSSION

Effect of Breed on Protein Profile

The results from this study show a significant effect of breed on relative concentrations of all individual proteins. Studies reporting breed differences in protein profile are scarce; however, a few studies have reported breed effects on absolute concentrations of the individual milk proteins (e.g., McLean et al., 1984; Auldist et al., 2004; Wedholm et al., 2006). It could be misleading to compare the present work with those studies, as relative concentrations might not increase or decrease in the same direction as absolute concentrations (Hallén et al., 2008). However, those studies confirm that breed differences are to be expected. As DH samples were collected in the autumn, the DJ samples in the winter, and the SR samples in both winter and autumn, season could be a confounding factor when estimating breed effects. However, Heck et al. (2009b) and Lindmark-Månsson et al. (2003) argued that most of the seasonal variation depends on the diet change during the grazing season and the samples collected in the current study was collected before and after the grazing season.

Interestingly, the present study showed that DH milk had the lowest relative concentrations of κ -CN 1P and the highest relative concentrations of β -CN of the 3 breeds. This was also true when correcting for hidden κ -CN peaks, adjusted total κ -CN, and adjusted total β -CN. However, looking at the differences between DJ and SR, DJ milk had higher relative concentrations of κ -CN 1P than SR milk but the relative concentrations of adjusted total κ -CN did not differ. This suggests that SR milk contains higher relative concentrations of glycosylated κ -CN than DJ milk. This is important, as variations in

Table 6. Estimated differences $\pm$ SE and trait general means $\pm$ SD in relative concentrations of α_{S2} - and α_{S1} -CN, comparing all composite α_{S1} - β -CN genotypes with a frequency higher than 2.5% to the composite genotype BB/A²A²/AA in Danish Holstein (DH) and Swedish Red (SR) cows, respectively¹

Composite casein genotype	α_{S1} -CN 8P (% wt/wt ²)		α_{S1} -CN 9P (% wt/wt)		α_{S1} -CN (% wt/wt)		α_{S2} -CN (% wt/wt)	
	DH	SR	DH	SR	DH	SR	DH	SR
BB/A ² A ² /AA	0	0	0	0	0	0	0	0
BB/A ¹ A ¹ /AA		-0.38 $\pm$ 0.40		-0.71 $\pm$ 0.27*		-1.03 $\pm$ 0.43*		-0.68 $\pm$ 0.34†
BB/A ¹ A ¹ /AE		-0.02 $\pm$ 0.34		-0.58 $\pm$ 0.24*		-0.65 $\pm$ 0.37†		-1.11 $\pm$ 0.29***
BB/A ¹ A ¹ /EE		-0.04 $\pm$ 0.52		-0.79 $\pm$ 30.35*		-1.00 $\pm$ 0.56†		-0.90 $\pm$ 0.43*
BB/A ¹ A ² /AA	-0.28 $\pm$ 0.28	-0.40 $\pm$ 0.31	-0.14 $\pm$ 0.23	-0.59 $\pm$ 0.21**	-0.48 $\pm$ 0.33	-1.10 $\pm$ 0.33**	-0.34 $\pm$ 0.19†	-0.17 $\pm$ 0.26
BB/A ¹ A ² /AB	-0.69 $\pm$ 0.34*	-0.66 $\pm$ 0.39†	-0.43 $\pm$ 0.27	-0.62 $\pm$ 0.26*	-1.21 $\pm$ 0.40**	-1.38 $\pm$ 0.42**	-0.62 $\pm$ 0.23*	-1.17 $\pm$ 0.32**
BB/A ¹ A ² /AE	-0.40 $\pm$ 0.34	0.08 $\pm$ 0.31	0.13 $\pm$ 0.28	-0.63 $\pm$ 0.21**	-0.48 $\pm$ 0.41	-0.57 $\pm$ 0.33†	-0.49 $\pm$ 0.23*	-0.08 $\pm$ 0.26
BB/A ¹ A ² /BE		-0.47 $\pm$ 0.41		-0.71 $\pm$ 0.29*		-1.17 $\pm$ 0.44*		-1.63 $\pm$ 0.35***
BB/A ² A ² /AB	-0.11 $\pm$ 0.36	-0.63 $\pm$ 0.34†	-0.33 $\pm$ 0.29	-0.51 $\pm$ 0.23*	-0.40 $\pm$ 0.42	-1.12 $\pm$ 0.37**	-0.69 $\pm$ 0.24**	-0.52 $\pm$ 0.28†
BB/A ² I/AB	-0.80 $\pm$ 0.37*		-1.07 $\pm$ 0.30**		-1.82 $\pm$ 0.44***		0.64 $\pm$ 0.25*	
BB/BA ² /AB	-0.49 $\pm$ 0.41		-0.25 $\pm$ 0.34		-0.86 $\pm$ 0.50†		-0.91 $\pm$ 0.28**	
Trait mean	21.29 $\pm$ 1.70	21.65 $\pm$ 1.47	6.53 $\pm$ 1.39	6.43 $\pm$ 1.10	29.06 $\pm$ 2.03	29.33 $\pm$ 1.56	6.56 $\pm$ 1.21	6.82 $\pm$ 1.25

¹Cows from the protein profile subset were used (309 DH and 309 SR).²wt/wt = wt/wt of total protein.† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.**Table 7.** Estimated differences $\pm$ SE and trait general means $\pm$ SD in relative concentrations of α_{S2} -, α_{S1} -, κ -, and β -CN, comparing all composite α_{S1} - β -CN genotypes with a frequency higher than 2.5% to the composite genotype BC/A²A²/AB in Danish Jersey (DJ) cows¹

Composite casein genotype	Concentration (% wt/wt ²)							
	α_{S1} -CN 8P	α_{S1} -CN 9P	α_{S1} -CN	α_{S2} -CN	κ -CN 1P	Adj. ³ κ -CN	β -CN	Adj. β -CN
BC/A ² A ² /AB	0	0	0	0	0	0	0	0
BB/A ² A ² /AA	0.64 $\pm$ 0.67	0.24 $\pm$ 0.42	0.53 $\pm$ 0.89	-0.63 $\pm$ 0.39	-0.12 $\pm$ 0.34	-0.43 $\pm$ 0.41	1.66 $\pm$ 1.04	1.72 $\pm$ 1.03†
BC/BA ² /BB	-0.27 $\pm$ 0.38	0.26 $\pm$ 0.23	-0.15 $\pm$ 0.50	-0.52 $\pm$ 0.22*	0.35 $\pm$ 0.19†	0.12 $\pm$ 0.24	1.65 $\pm$ 0.59**	0.98 $\pm$ 0.58†
CC/A ² A ² /BB	0.62 $\pm$ 0.39	0.78 $\pm$ 0.24***	1.28 $\pm$ 0.52*	-0.68 $\pm$ 0.23**	-0.10 $\pm$ 0.20	-0.47 $\pm$ 0.24†	-0.62 $\pm$ 0.60	-0.56 $\pm$ 0.60
BB/BB/BB	-2.27 $\pm$ 0.57***	-0.26 $\pm$ 0.35	-2.95 $\pm$ 0.76***	-0.23 $\pm$ 0.34	0.21 $\pm$ 0.30	-0.01 $\pm$ 0.36	0.03 $\pm$ 0.89	1.17 $\pm$ 0.88
BB/BA ² /AB	-0.72 $\pm$ 0.56	0.26 $\pm$ 0.35	-0.77 $\pm$ 0.75	-0.45 $\pm$ 0.33	-0.03 $\pm$ 0.29	-0.28 $\pm$ 0.35	1.35 $\pm$ 0.88	0.72 $\pm$ 0.87
BC/A ² I/BB	-0.89 $\pm$ 0.54	-0.05 $\pm$ 0.34	-0.90 $\pm$ 0.72	0.44 $\pm$ 0.32	0.14 $\pm$ 0.27	0.08 $\pm$ 0.33	0.04 $\pm$ 0.84	0.11 $\pm$ 0.83
BC/A ¹ A ² /BB	-0.17 $\pm$ 0.55	0.02 $\pm$ 0.34	-0.39 $\pm$ 0.73	-0.10 $\pm$ 0.32	0.96 $\pm$ 0.27***	0.57 $\pm$ 0.33†	2.96 $\pm$ 0.84***	2.26 $\pm$ 0.83*
BB/BA ¹ /BB	-0.82 $\pm$ 0.67	-0.03 $\pm$ 0.42	-1.35 $\pm$ 0.90	-0.48 $\pm$ 0.40	-0.03 $\pm$ 0.35	0.37 $\pm$ 0.42	2.42 $\pm$ 1.05*	2.81 $\pm$ 1.04*
Trait mean	23.60 $\pm$ 1.88	6.21 $\pm$ 1.14	31.51 $\pm$ 2.55	7.32 $\pm$ 1.10	6.39 $\pm$ 0.91	11.68 $\pm$ 1.09	36.41 $\pm$ 2.94	34.40 $\pm$ 2.86

¹Cows from the protein profile subset were used (199 DJ).²wt/wt = wt/wt of total protein.³Adj. = adjusted average, which is adjusted for overlapping κ - and β -CN peaks.† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 8. Estimated differences $\pm$ SE and trait general means $\pm$ SD in relative concentrations of κ - and β -CN, comparing all composite α_{S1} - β - κ -CN genotypes with a frequency higher than 2.5% to the composite genotype BB/A²A²/AA in Danish Holstein (DH) and Swedish Red (SR) cows, respectively¹

Composite casein genotype	κ -CN 1P (% wt/wt ²)			Adj. ³ κ -CN (% wt/wt)			β -CN (% wt/wt)			Adj. β -CN (% wt/wt)		
	DH	SR	0	DH	SR	0	DH	SR	0	DH	SR	0
BB/A ² A ² /AA	0			0			0			0		
BB/A ¹ A ¹ /AA		1.49 $\pm$ 0.26***			1.34 $\pm$ 0.30***			1.31 $\pm$ 0.64†			1.56 $\pm$ 0.64*	
BB/A ¹ A ¹ /AE		1.06 $\pm$ 0.22***			1.15 $\pm$ 0.26***			0.51 $\pm$ 0.55			0.92 $\pm$ 0.54†	
BB/A ¹ A ¹ /EE		0.91 $\pm$ 0.33**			1.37 $\pm$ 0.39**			-0.92 $\pm$ 0.83			-0.48 $\pm$ 0.83	
BB/A ¹ A ² /AA	0.67 $\pm$ 0.15***	0.80 $\pm$ 0.20***		0.48 $\pm$ 0.21*	0.89 $\pm$ 0.23***		2.48 $\pm$ 0.37***	2.17 $\pm$ 0.49***		0.67 $\pm$ 0.36†	0.23 $\pm$ 0.49	
BB/A ¹ A ² /AB	1.02 $\pm$ 0.18***	1.50 $\pm$ 0.25***		0.76 $\pm$ 0.26**	1.06 $\pm$ 0.29**		2.06 $\pm$ 0.46***	2.99 $\pm$ 0.62***		0.30 $\pm$ 0.45	1.17 $\pm$ 0.61†	
BB/A ¹ A ² /AE	0.13 $\pm$ 0.18	0.70 $\pm$ 0.20**		-0.09 $\pm$ 0.26	0.40 $\pm$ 0.23†		3.22 $\pm$ 0.46***	2.62 $\pm$ 0.49***		1.36 $\pm$ 0.45**	0.78 $\pm$ 0.49	
BB/A ¹ A ² /BE		0.74 $\pm$ 0.27**			0.81 $\pm$ 0.31*			3.40 $\pm$ 0.65***			1.48 $\pm$ 0.65*	
BB/A ² A ² /AB	0.32 $\pm$ 0.19†	0.89 $\pm$ 0.21***		0.32 $\pm$ 0.27	0.53 $\pm$ 0.25*		-0.02 $\pm$ 0.49	0.18 $\pm$ 0.54		-0.04 $\pm$ 0.48	0.24 $\pm$ 0.54	
BB/A ² A ² /AB	0.13 $\pm$ 0.20			0.04 $\pm$ 0.28			0.67 $\pm$ 0.51			0.68 $\pm$ 0.50		
BB/BA ² /AB	1.05 $\pm$ 0.23***			0.61 $\pm$ 0.32†			2.39 $\pm$ 0.56***			1.21 $\pm$ 0.55*		
Trait mean	5.05 $\pm$ 0.98	5.36 $\pm$ 1.05		10.13 $\pm$ 1.31	11.69 $\pm$ 1.15		36.99 $\pm$ 2.77	36.41 $\pm$ 2.94		35.82 $\pm$ 2.39	33.72 $\pm$ 2.55	

¹Cows from the protein profile subset were used (309 DH and 309 SR).

²wt/wt = wt/wt of total protein.

³Adj. = adjusted average, which is adjusted for overlapping κ - and β -CN peaks.
† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

glycosylation of κ -CN affects CN micelle stability and, in turn, milk processability (Holland, 2008).

Genotype Frequencies

The allele and genotype frequencies in the present study have earlier been published and discussed by Poulsen et al. (2013). Regarding frequencies of composite α_{S1} - β - κ -CN genotypes, differences can be observed in all 3 breeds compared with earlier studies (Bech and Kristiansen, 1990; Lundén et al., 1997). It is evident that in the last 10 to 20 yr, a decrease in cows with BB/A¹A¹/AA (from ~20 to ~2% in our study) and BB/A¹A²/AA (from ~40 to ~15% in our study) has occurred in both DH (Bech and Kristiansen, 1990) and SR (Lundén et al., 1997). Furthermore, a drastic increase of cows with BB/A²A²/AA has occurred in in DH (from 9 to 30% in our study; Bech and Kristiansen, 1990) and BB/A¹A²/AE in SR (from 0% to 18% in our study; Lundén et al., 1997). In DJ, the number of cows with the composite α_{S1} - β - κ -CN genotype BB/BA²/AB has decreased (from 20 to 6% in our study) and the number of cows with CC/A²A²/BB have increased (from <7 to 16% in our study) compared with the number observed by Bech and Kristiansen (1990). The results presented here confirm earlier studies, that casein variants affect both milk composition and milk yields (Bovenhuis et al., 1992; Ikonen et al., 1999; Heck et al., 2009a). Current breeding goals may have induced a change in the genotype frequencies of the studied breeds. In turn, this may have large effects on the protein profile and technological properties of different dairy products.

Effect of Composite Genotypes

The concentration and conformation of β -LG affects properties of many different milk products and is especially important in the production of fermented products and milk powders (Anema, 2008; Lucey, 2008). In the present study, only a few composite α_{S1} - β - κ -CN genotypes affected relative concentrations of β -LG, which indicates that these composite genotypes would not be suitable markers for altering β -LG content in DH, SR, and DJ breeds. Visker et al. (2011) previously showed that Holstein-Friesian cows homozygous for β - κ -CN haplotype A²/A gave milk with higher relative concentrations of β -LG compared with cows with alternate genotypes. However, in the present study, the composite genotypes that affected relative concentrations of β -LG were associated with higher relative concentrations of β -LG than BB/A²A²/AA. It is known that genetic variants of β -LG can affect relative concentrations of β -LG (Heck et al., 2009a) and as these genetic variants are not accounted for in the models

used in the present study or in the study by Visker et al. (2011), this could explain the inconsistencies. To be able to breed cows with altered whey protein content, it is probable that genetic variants of β -LG must be considered.

Caseins are important for many different dairy products and especially in cheese where they are the building blocks in the cheese gel network (Walstra et al., 2006). Most of the composite genotypes in SR and many of the composite genotypes in DH were associated with low relative concentrations of α_{S1} - and α_{S2} -CN and high relative concentrations of κ - and β -CN compared with the common composite genotype BB/A²A²/AA. In addition to BB/A²A²/AA, 4 of the other composite genotypes had frequencies above 2.5% in both DH and SR and it might be expected that the effects of these composite genotypes on protein profile would be the same in both breeds. Both SR and DH cows with the composite genotype BB/A¹A²/AB gave milk with lower relative concentrations of α_{S1} - and α_{S2} -CN as well as higher relative concentrations of κ -CN 1P, adjusted total κ -CN and β -CN than SR and DH cows with BB/A²A²/AA. However, regarding the other composite genotypes, the effects were not as consequent across SR and DH. An explanation for this could be that many more composite genetic variants were present at low frequencies in DH than in SR. This means that 20% of the DH cows were not accounted for in this comparison, whereas only 5% were not accounted for in SR.

To our knowledge, the only study investigating the effect of composite α_{S1} - β - κ -CN genotypes on relative concentrations of milk proteins is that of Ehrmann et al. (1997). However, Ehrmann et al. (1997) did not account for relationships between animals, in contrast to the present study, and their results could be biased, as 8 different breeds were investigated simultaneously even though all composite genotypes were not present in all breeds. Therefore, it is not appropriate to compare the results from the present study with Ehrmann et al. (1997). However, studies exist investigating the effect of composite β - κ -CN haplotypes on relative concentrations of the milk proteins. Visker et al. (2011) found that the relative concentration of α_{S1} -CN was significantly higher and that the relative concentrations of κ -CN 1P and β -CN were lower in milk from Dutch Holstein-Friesian cows homozygous for β - κ -CN haplotype A²/A than in milk from cows not carrying this haplotype. Further, Bonfatti et al. (2010b) found that the β - κ -CN haplotype A²/A was associated with lower relative concentrations (per total casein) of α_{S1} - and α_{S2} -CN and higher relative concentrations of κ -CN in Italian Simmental cows. The effect of β - κ -CN haplotype A²/A on β -CN was not as obvious. The effects of composite α_{S1} - β - κ -CN BB/A²A²/AA on relative

concentrations of the caseins in the present study are, therefore, corroborated both by what has been seen in Dutch Holstein-Friesian (Visker et al., 2011) and in Italian Simmental (Bonfatti et al., 2010b) cows.

The recently discovered β -CN I variant in European cattle breeds (Jann et al., 2002) is present both in DH and DJ. Interestingly, DH cows with the composite genotype BB/A²I/AB and DJ cows with BC/A²I/BB gave milk with higher relative concentrations of α_{S2} -CN than cows with BB/A²A²/AA. This agrees with Visker et al. (2011), who found that β - κ -CN haplotype I/B was the only haplotype associated with higher relative concentrations of α_{S2} -CN. Visker et al. (2011) also found that casein haplotype I/B was associated with lower relative concentrations of α_{S1} -CN and higher relative concentrations of κ -CN 1P, which agrees with the present study regarding relative concentrations of α_{S1} -CN. Both Jensen et al. (2012) and Jöndu et al. (2008) suggest that higher relative concentrations of α_{S2} -CN are associated with poor coagulation in rennet gels, which indicates that an increase in the β -CN I variant in DH and DJ could have a negative effect on milk coagulation properties of the milk from these breeds.

In DJ, the effects of composite α_{S1} - β - κ -CN genotypes on relative concentrations of the milk proteins are not as evident as in the other 2 breeds. Notably, CC/A²A²/BB is the only composite genotype in the 3 breeds that is associated with high levels of α_{S1} -CN compared with the most common composite genotype. Furthermore, composite genotype BC/A¹A²/BB was found to be favorable for high relative concentrations of κ -CN 1P and adjusted total κ -CN as well as high relative concentrations of β -CN compared with the most common DJ composite genotype BC/A²A²/AB. As CC/A²A²/BB is the third most common composite genotype in DJ (15.6%; Table 4) this composite genotype could be one of the reasons for the higher relative concentration of α_{S1} -CN in DJ compared with SR and DH. Furthermore, BC/A¹A²/BB is present in only 6% of DJ and, therefore, it can be used as a marker for increased relative concentrations of κ - and β -CN in DJ, which could be favorable for, as an example, rennet-induced and acid-induced gelation (Lucey, 2008).

Possible Effects on Milk Processability

Lindmark-Månsson et al. (2003) found a significant decrease in casein content and a subsequent increase in whey proteins in Swedish dairy milk from 1970 to 1996, whereas no change in total protein concentration was observed. The authors suggested that this indicated a change in the distribution of different milk proteins and that this change could partly be due to selection strategies in dairy cows. As the present study has shown

a significant effect of composite genotypes on detailed protein composition, changes in frequencies could affect the functionality of dairy milk. Compared with earlier studies (Bech and Kristiansen, 1990; Lundén et al., 1997), the frequencies of the different composite genotypes have changed drastically. When looking at the results from the present study, it is possible that the increase in DH and SR cows with composite genotype BB/A²A²/AA has resulted in milk with higher relative concentrations of α_{S1} - and α_{S2} -CN and lower relative concentrations of κ - and β -CN. This indicates that if the relationships between genetics and milk composition are not known and not properly accounted for in breeding programs, then a risk exists for milk composition to become less suitable for processing of various dairy products.

The lower relative concentrations of κ - and β -CN and higher relative concentrations of α_S -CN, which is associated with α_{S1} - β - κ -CN genotype BB/A²A²/AA, could be the underlying reason for the poor rennet-induced gelation properties observed for BB/A²A²/AA cows in Poulsen et al. (2013). Likewise, this and other studies have shown that milk from Jersey cows have superior coagulation properties compared with Holstein-Friesian cows (Auld et al., 2004; Frederiksen et al., 2011b; Poulsen et al., 2013). Jensen et al. (2012) found that within the DJ and DH breeds, significantly higher protein and casein contents existed as well as higher relative concentrations of κ -CN (in both DH and DJ) and lower relative concentrations of α_{S2} -CN (only in DJ) in well-coagulating milk than poorly coagulating milk. The relative concentrations of β -CN and α_{S1} -CN did not differ between these groups. Frederiksen et al. (2011a) and Jensen et al. (2012) found that a higher proportion of the more phosphorylated forms of α_{S1} - and α_{S2} -CN were associated with non- and poorly coagulating milk in DH. As the DJ breed gives milk with a higher relative concentration of α_{S1} -CN 8P than DH together with the differences in protein profiles as well as in protein and casein contents, this could explain the superior coagulation properties in DJ.

The present work suggest that a higher frequency of BB/A¹A²/AB, which is present in 8% of SR and 9% of DH, together with a decrease in BB/A²A²/AA could have positive effects on DH and SR milk, as the relative concentrations of α_S -CN would decrease and the relative concentrations of κ - and β -CN would increase in both breeds. Also, BB/A¹A²/AB has previously been shown to have a positive effect on rennet-induced gelation properties compared with BB/A²A²/AA in both SR and DH using the same sample set (Poulsen et al., 2013). However, further research is needed to more thoroughly investigate how detailed milk protein composition affects processing of different dairy products.

CONCLUSIONS

Significant differences exist in milk composition, protein profile, and frequencies of casein genotypes in 3 Scandinavian breeds (DH, SR, and DJ). Danish Holsteins and SR are quite similar regarding casein genotype frequencies, whereas the differences in casein genotype frequencies in DJ compared with the other 2 breeds are more pronounced. The same trend can be seen regarding the differences in milk composition and protein profile. Danish Holstein and SR cows with the common composite genotype BB/A²A²/AA, with frequencies of 30 and 15%, resulted in milk with lower relative concentrations of κ - and β -CN and higher relative concentrations of α_S -CN than most of the other composite genotypes. The differences in relative protein concentration were not as pronounced between milk from cows with the most common composite genotype in DJ (BC/A²A²/AB) and milk from cows with the other composite genotypes present at a frequency higher than 2.5%. The present work suggest that a higher frequency of BB/A¹A²/AB, which is present in 8% of SR and 9% of DH, together with a decrease in BB/A²A²/AA frequency could have positive effects on DH and SR milk regarding, for example, cheese processability. Furthermore, this study shows that the effect of composite casein genotypes on protein profile should be considered in the national breeding programs to prevent changes in the detailed protein composition that could have negative effects on, for example, cheese production.

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