

ORIGINAL ARTICLE

## Comparison of milk protein composition in a Swedish and a Danish dairy herd using reversed phase HPLC

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### Abstract

The milk composition in a Swedish herd, consisting of Swedish Red and White cows (SRB) and Swedish Holstein cows (SLB), and in a Danish herd, consisting of Danish Holstein-Friesian cows (SDM), was evaluated. Concentrations of the major milk proteins ( $\kappa$ -casein,  $\alpha_{S1}$ -casein,  $\beta$ -casein,  $\beta$ -lactoglobulin A,  $\beta$ -lactoglobulin B and  $\alpha$ -lactalbumin) of 134 individual milk samples were determined by reversed phase (RP) HPLC. Other parameters determined included milk fat, urea, lactose, calcium, lactoferrin, somatic cell count and protein degradation (determined as level of free amino-terminals). Analysis of variance was used to compare concentrations of analysed milk variables between SRB and SLB or between SLB and SDM. Concentration of total protein, total casein,  $\beta$ -casein and  $\kappa$ -casein were significantly higher in SRB milk compared with SLB milk. Concentration of  $\alpha$ -lactalbumin and calcium were significantly higher in SDM milk than in SLB milk. The concentration of urea was higher in SLB than in SDM milk and is suggested to reflect differences in feeding regimes between the investigated Swedish and Danish herd.

**Keywords:** Calcium, casein, dairy cows, free amino terminals, urea, whey protein.

### Introduction

Milk proteins are crucial for the technological quality of bovine milk. However, the concentration of total protein and casein in Swedish bulk milk shows geographical variation which partly can be explained by differences in feeding practices and feed quality in different regions (Lindmark-Månsson et al., 2003). This is likely to occur in other countries and is important to study because it can influence the quality and yield of dairy products, e.g., cheese.

In Scandinavia, the dominant milk producing countries are Denmark and Sweden. It would be of interest to evaluate differences in milk protein composition between herds in Sweden and Denmark, in order to obtain a better understanding of any differences in technological properties of milk observed in these countries.

The two most common dairy breeds in Sweden are the Swedish Red and White (SRB, related to Ayrshire) and the Swedish Holstein (SLB), whereas the most common breed in Denmark is the Danish Holstein-Friesian (SDM). Several studies have shown that protein and milk fat concentrations are higher in milk from Ayrshire cows compared to Holstein (Cerbulis & Farrell, 1975; Custer, 1979; UK, Milk Marketing Board of England and Wales, 1984; Tyriseva et al., 2004). However, little attention has been paid to evaluate the protein composition in milk from SRB and SLB cows, whereas the protein profile in milk from SDM cows has been studied earlier (Ostersen et al., 1997). All major milk proteins have been simultaneously separated and quantified using reverse-phase high performance liquid chromatography (RP-HPLC) (Visser et al.,

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1991; Bobe et al., 1998; Bordin et al., 2001) or capillary zone electrophoresis (de Jong et al., 1993).

The protein composition in milk is highly dependent on genetic polymorphism of milk proteins. It is well documented that the  $\kappa$ -casein ( $\kappa$ -CN) concentration is higher in milk containing the B allele of  $\kappa$ -CN (McLean et al., 1984; van den Berg et al., 1992; Bobe et al., 1999). It has also been demonstrated that concentration of  $\beta$ -lactoglobulin ( $\beta$ -LG) is higher in milk with the AA genotype than with the AB or BB genotype (McLean et al., 1984; Ng-Kwai-Hang et al., 1987; Graml et al., 1989).

The individual milk components are important for various milk characteristics and suitability for further processing. One of the minor milk proteins, lactoferrin, is of particular significance due to its antimicrobial activity (Bullen, 1978; Smith & Schanbacher, 1977). This property might be desirable in consumption milk, but could be detrimental in cheese or other fermented products because it is negatively correlated with growth of starter cultures (Lindmark-Månsson et al., 2000). Another important factor influencing several processing properties of milk is proteolysis, which is related to the somatic cell count (SCC; Andrews, 1983). Proteolysis in milk may reduce curd strength (Okigbo et al., 1985) and increase rennet coagulation time (Grufferty & Fox, 1988). A non-protein component, which also is important for the technological quality of milk, is calcium. It is important in the milk clotting process, because it neutralizes negative charges of para-casein micelles as well as building salt linkages between them, i.e., calcium phosphate linkages (Walstra et al., 1999).

The main objective of this investigation was to evaluate variations in milk protein composition between SRB and SLB cows within one Swedish herd, and between herds of SLB and SDM cows in Sweden and Denmark.

## Materials and methods

### *Milk collection and experimental design*

Evening whole milk samples (the total milk volume) from 16 SRB, 15 SLB and 14 SDM cows were collected in September 2003. Milk from SRB and SLB cows was collected at the experimental dairy herd at Jälla (Swedish University of Agricultural Sciences) and milk from SDM was collected at the Research Centre Foulum (Danish Institute of Agricultural Sciences). A second sampling from 22 SRB, 8 SLB and 15 SDM took place in January 2004 and the last sampling from another 20 SRB, 9 SLB and 15 SDM was carried out in May 2004. A total of 134 individual cows were thus included in the study and

no cow was sampled twice. Feeding and management were carried out according to standard practices at the two experimental herds and the cows were stalled indoors on the three sampling occasions. All cows included in the study were healthy and milked twice a day. Milk yield in kg was recorded at the specific milking occasion. The cows were grouped into four classes according to stage of lactation: early (week 6 to 15; 10 SRB, 9 SLB and 9 SDM), mid (week 16 to 30; 36 SRB, 15 SLB and 18 SDM), late (week 31 to 45; 11 SRB, 7 SLB and 11 SDM), and very late (week 46 or later; 1 SRB, 1 SLB and 6 SDM). The cows were also grouped into four classes according to lactation number: first lactation (23 SRB, 10 SLB and 17 SDM), second lactation (19 SRB, 10 SLB and 13 SDM), third lactation (10 SRB, 8 SLB and 4 SDM) and fourth or more lactation (7 SRB, 3 SLB and 10 SDM). A comparison was carried out between SRB and SLB, to evaluate differences between breeds within the Swedish herd. A second comparison between SLB and SDM was accomplished to evaluate differences between the Swedish and the Danish herd. All milk samples were cold stored (4°C) for two days, then preheated to 40°C and defatted. The skimmed milk was heat treated in a pilot plate heating equipment (72°C for 15 s), as described previously by Allmere et al. (1998).

### *Determination of milk protein composition by RP-HPLC*

**Materials.** Urea (purity >99.5%) and DL-dithiothreitol (DTT >99%) and purified bovine  $\kappa$ -CN (>90%)  $\alpha$ -casein ( $\alpha$ -CN; >70%),  $\beta$ -casein ( $\beta$ -CN; >90%),  $\beta$ -LG A (>90%),  $\beta$ -LG B (>90%) and  $\alpha$ -lactalbumin ( $\alpha$ -LA; >85%) were purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile (HPLC grade) was purchased from Fisher (Loughborough, UK) and Spectranal® Trifluoroacetic acid (>99.8%) from Sigma-Aldrich (Seelze, Germany). Water was purified using a Milli-Q® system (Millipore Corp, Bedford, USA). The water was also filtered through a 0.45  $\mu$ m Sartorius regenerated cellulose filter (Sartorius AG, Göttingen, Germany) prior to use. Buffer solutions were sonicated 15 min in a Branson 3210 ultrasonic bath (Branson Ultrasonic Corp., Danbury, USA).

**Analysis.** Protein composition in individual skimmed and heat-treated milk samples was determined by RP-HPLC. The chromatographic system D-7000 from Merck-Hitachi (Darmstadt, Germany) consisted of an HPLC pump model L-7100, an auto injector model L-7200 and a UV detector model L-7400 equipped with the chromatographic system

software D-7000 HPLC System Manager. The RP-HPLC method used was modified according to Bordin et al. (2001). Milk samples (100  $\mu$ l) were diluted in reducing buffer (400  $\mu$ l) containing 8 M urea and 20 mM DTT in ultrapure water and left for 1 h at room temperature for a complete reduction of milk proteins. The samples were further diluted 1:3 in buffer A (10% acetonitrile in ultrapure water and 0.1% TFA) and centrifuge filtered (Ultrafree-CL, PVDF membrane, 0.45  $\mu$ m, Millipore, Bedford, UK). The separations were performed on a reversed phase analytical column C<sub>4</sub> (BioBasic-4, Thermo, Runcorn, UK) 150  $\times$  3 mm, 300-Å pore diameter and 5  $\mu$ m particle size. A volume of 20  $\mu$ l was injected into the column. Elution started with a linear gradient from 26 to 28% buffer B (10% ultrapure water in acetonitrile and 0.1% TFA) for 7 min followed by a linear gradient to 36% B for 10 min, an isocratic elution for 13 min, a linear gradient to 43% B for 20 min and a final isocratic elution for 15 min. Separations were performed at room temperature with the flow rate 0.300 ml min<sup>-1</sup>.

*Protein identification and quantification.* Proteins were identified according to retention times of peaks from the purified milk proteins. Purified milk protein (3 mg) was dissolved in 1 ml reducing buffer and then prepared in the same way as the milk samples. Standard curves were obtained by measuring peak areas with various known amounts of injected purified milk proteins and were adjusted for impurities of purified milk proteins. The standard curves were used to calculate the concentration of protein represented by peak areas obtained from milk samples. The concentration of total protein analysed was calculated as the sum of the concentrations of  $\alpha$ <sub>S1</sub>-CN,  $\beta$ -CN,  $\kappa$ -CN,  $\beta$ -LG A,  $\beta$ -LG B and  $\alpha$ -LA. Total casein concentration analysed was calculated as the sum of the concentrations of  $\alpha$ <sub>S1</sub>-CN,  $\beta$ -CN and  $\kappa$ -CN, and total whey protein concentration as the sum of the concentrations of  $\beta$ -LG A,  $\beta$ -LG B and  $\alpha$ -LA.

#### *Determination of other milk variables*

Samples of fresh milk were analysed for concentrations of milk fat, lactose, urea and pH with a Milkoscan FT 6000 (A/S N. Foss electric, Denmark) and for SCC with a Fossomatic 5000 (A/S N. Foss electric, Denmark) at Steins Laboratory (Holstebro, Denmark). The SCC was logarithmically transformed to obtain normally distributed data. Calcium concentration, both dissolved and colloidal calcium, was determined using atom

absorption spectrophotometry after drying to ash at 525°C for 6 h followed by solution in acid and dilution in lanthanchloride solution. The content of lactoferrin in heat-treated whey samples was analysed using a commercial enzyme-linked immunosorbent assay (ELISA), Bovine Lactoferrin ELISA Quantification Kit (Bethyl Laboratories, Inc, Montgomery, TX, USA). The procedure was carried out according to the manufacturer's instructions. Protein degradation was determined as the level of free amino terminals using the fluorescamine method modified for milk samples (Larsen et al., 2004). Free amino terminals were determined in individual heat-treated skimmed milk samples and expressed as equivalents of leucine (mM) according to a standard curve.

#### *Genotyping for $\kappa$ - and $\beta$ -casein genetic variants*

Blood samples were collected from SRB and SLB and the DNA was genotyped for genetic variants of  $\kappa$ - and  $\beta$ -CN using Pyrosequencing<sup>TM</sup> (Biotage AB, Uppsala, Sweden), as described by Hallén et al. (Department of Food Science, University of Agricultural Sciences, Uppsala, Sweden, personal communication)

#### *Statistical analysis*

Statistical analysis of variance (ANOVA) was performed using the GLM (General Linear Model) procedure in SAS (Ver. 9.1. SAS Institute Inc., Cary, NC, USA). Two models (numbers 1 and 2) were applied for studying the effect of breed or herd on concentrations (in g per 100 g) of total protein analysed, total casein analysed,  $\kappa$ -CN,  $\alpha$ <sub>S1</sub>-CN,  $\beta$ -CN,  $\beta$ -LG A,  $\beta$ -LG B,  $\alpha$ -LA, milk fat, lactose and calcium and casein number, concentration of lactoferrin ( $\mu$ g ml<sup>-1</sup>), free amino terminals (mM leucine), urea (mM), and SCC (log ml<sup>-1</sup>). Model 1 contained the fixed effect of SRB ( $n = 58$ ) or SLB ( $n = 32$ ) whereas model 2 contained the fixed effect of SLB ( $n = 32$ ) or SDM ( $n = 44$ ). The fixed effect of sampling occasion (September, January and May), stage of lactation (early, mid, late or very late) and lactation number (1, 2, 3 or 4) were included in both models. Additionally, one model (model 3) was applied to evaluate the effect of breed on the  $\beta$ -CN concentration when genetic polymorphism of  $\beta$ -CN was included. Model 3 contained the fixed effect of breed (SRB or SLB),  $\beta$ -CN genetic variant (A1A1, A1A2, A2A2 and A2B), sampling occasion, stage of lactation and lactation number. Two-way interactions were included when significant. Significant differences

between least squares means (LSM) were evaluated using the option pdiff.

## Results

### *Determination of milk protein composition by RP-HPLC*

Concentrations of major milk proteins, except  $\alpha_{S2}$ -CN, were determined in all milk samples ( $n = 134$ ) by RP-HPLC. Figure 1 shows chromatograms of a milk sample and the purified milk proteins  $\kappa$ -CN,  $\alpha_S$ -CN,  $\beta$ -CN,  $\beta$ -LG B,  $\beta$ -LG A and  $\alpha$ -LA. The purified  $\beta$ -lg B resulted in two peaks and  $\kappa$ -casein and  $\beta$ -casein resulted in three major peaks each.

### *Variations in milk composition between SRB and SLB*

Table I shows LSM and standard errors (SE) of analysed milk proteins from cows of SRB and SLB. Concentrations of total protein analysed, total casein analysed,  $\beta$ -CN and  $\kappa$ -CN were significantly higher in SRB milk ( $p < 0.01$ ,  $p < 0.05$ ,  $p < 0.05$  and  $p < 0.05$ , respectively). The same variables were also influenced by sampling occasion ( $p < 0.001$ ,  $p < 0.001$ ,  $p < 0.01$  and  $p < 0.01$ , respectively). In addition, concentrations of total protein analysed, total casein analysed and  $\beta$ -CN were influenced by lactation number ( $p < 0.01$ ,  $p < 0.05$  and  $p < 0.05$ , respectively). Lactoferrin concentration did not differ among the breeds within the investigated herd. LSM and SE of other analysed milk variables

from cows of SRB and SLB are presented in Table II. Concentration of milk fat was significantly higher in SRB milk ( $p < 0.05$ ) whereas concentrations of lactose, level of free amino terminals and pH were higher in SLB milk ( $p < 0.001$ ,  $p < 0.05$  and  $p < 0.001$ , respectively). Milk fat concentration was also influenced by sampling occasion ( $p < 0.001$ ).

### *Variations in milk composition between SLB and SDM*

Only the concentration of  $\alpha$ -LA differed between SLB and SDM with a significantly higher concentration in SDM milk ( $p < 0.05$ ; results not shown). Table III shows LSM and SE of other analysed milk variables from cows of SLB and SDM. The urea concentration was significantly higher in SLB milk than in SDM milk ( $p < 0.001$ ) and was also influenced by sampling occasion ( $p < 0.01$ ). Concentration of calcium was significantly higher in milk from SDM ( $p < 0.01$ ) whereas protein degradation determined as free amino terminals was significantly lower ( $p < 0.001$ ) compared with SLB milk. Calcium concentration was also influenced by stage of lactation and lactation number ( $p < 0.01$  and  $p < 0.05$ , respectively).

### *Frequencies of $\kappa$ -CN and $\beta$ -CN genotypes within investigated SRB and SLB cows*

Table IVa shows frequencies of  $\kappa$ - and  $\beta$ -CN genotypes within the Swedish herd. The AB and AE genotypes of  $\kappa$ -CN were more frequent within

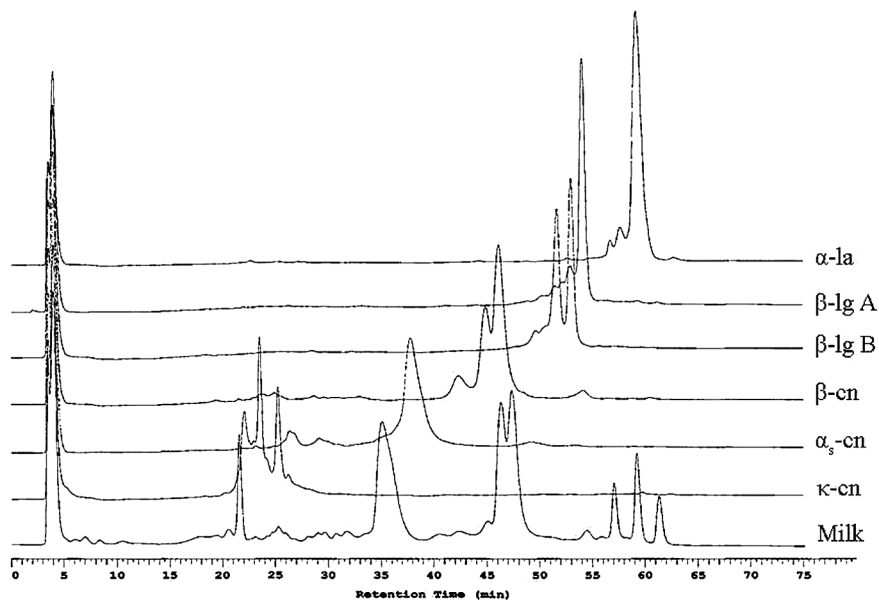


Figure 1. Examples of chromatograms obtained from milk and from purified milk proteins. Elution started with a linear gradient from 26 to 28% buffer B (10% ultrapure water in acetonitrile and 0.1% TFA) in 7 min followed by a linear gradient to 36% B in 10 min, an isocratic elution for 13 min, a linear gradient to 43% B for 20 min and a final isocratic elution for 15 min. The purified milk proteins were eluted in the following order:  $\kappa$ -casein ( $\kappa$ -CN),  $\alpha_S$ -casein ( $\alpha_S$ -CN),  $\beta$ -casein ( $\beta$ -CN),  $\beta$ -lactoglobulin B ( $\beta$ -lgB),  $\beta$ -lactoglobulin A ( $\beta$ -lgA) and  $\alpha$ -lactalbumin ( $\alpha$ -la).

Table I. Concentrations of analysed milk proteins from cows of Swedish Red and White breed (SRB) and Swedish Holstein Breed (SLB).

| Breed                                                  | SRB               | SLB               |                    | Influence of | Influence of       | Influence of |
|--------------------------------------------------------|-------------------|-------------------|--------------------|--------------|--------------------|--------------|
| Least squares means $\pm$ Standard error               | LSM $\pm$ SE      | LSM $\pm$ SE      | Influence of       | stage of     | lactation          | sampling     |
| Number of individuals ( <i>n</i> )                     | ( <i>n</i> = 58)  | ( <i>n</i> = 32)  | breed              | lactation    | number             | occasion     |
| Total protein <sup>1)</sup> (g 100 g <sup>-1</sup> )   | 3.39 $\pm$ 0.06   | 3.14 $\pm$ 0.07   | **                 | n.s.         | **                 | ***          |
| Total casein <sup>2)</sup> (g 100 g <sup>-1</sup> )    | 2.76 $\pm$ 0.05   | 2.57 $\pm$ 0.07   | *                  | n.s.         | *                  | ***          |
| Casein number <sup>3)</sup>                            | 0.81 $\pm$ 0.01   | 0.81 $\pm$ 0.01   | n.s.               | n.s.         | n.s.               | *            |
| $\alpha_{S1}$ -casein (g 100 g <sup>-1</sup> )         | 1.06 $\pm$ 0.02   | 1.04 $\pm$ 0.03   | n.s.               | n.s.         | n.s.               | n.s.         |
| $\beta$ -casein <sup>4)</sup> (g 100 g <sup>-1</sup> ) | 1.26 $\pm$ 0.03   | 1.16 $\pm$ 0.04   | *                  | n.s.         | *                  | **           |
| $\kappa$ -casein (g 100 g <sup>-1</sup> )              | 0.46 $\pm$ 0.01   | 0.41 $\pm$ 0.02   | *                  | n.s.         | n.s.               | **           |
| $\beta$ -lactoglobulin A (g 100 g <sup>-1</sup> )      | 0.32 $\pm$ 0.03   | 0.25 $\pm$ 0.04   | n.s.               | n.s.         | n.s.               | n.s.         |
| $\beta$ -lactoglobulin B (g 100 g <sup>-1</sup> )      | 0.23 $\pm$ 0.02   | 0.25 $\pm$ 0.02   | n.s.               | n.s.         | n.s.               | n.s.         |
| Total $\beta$ -lactoglobulin (g 100 g <sup>-1</sup> )  | 0.55 $\pm$ 0.02   | 0.50 $\pm$ 0.02   | n.s.               | n.s.         | n.s.               | n.s.         |
| $\alpha$ -lactalbumin (g 100 g <sup>-1</sup> )         | 0.087 $\pm$ 0.002 | 0.083 $\pm$ 0.003 | n.s. <sup>5)</sup> | n.s.         | n.s. <sup>5)</sup> | n.s.         |
| Lactoferrin (mg ml <sup>-1</sup> )                     | 0.12 $\pm$ 0.01   | 0.12 $\pm$ 0.02   | n.s.               | **           | *                  | n.s.         |

\*\*\*:  $p < 0.001$ ; \*\*:  $p < 0.01$ ; \*:  $p < 0.05$ ; n.s.: not significant ( $p > 0.05$ ).

<sup>1)</sup>The sum of the concentrations of  $\alpha_{S1}$ -casein,  $\beta$ -casein, and  $\kappa$ -casein,  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin determined by HPLC.

<sup>2)</sup>The sum of the concentrations of  $\alpha_{S1}$ -casein,  $\beta$ -casein, and  $\kappa$ -casein determined by HPLC.

<sup>3)</sup>The casein to protein ratio determined from HPLC-analysis.

<sup>4)</sup>The influence of breed when  $\beta$ -casein genetic polymorphism was included in the statistical analysis is presented in Table IVb.

<sup>5)</sup>Not significant due to a significant interaction between breed and lactation number ( $p < 0.01$ ).

SRB than within SLB. The frequency of BB or BE was low or absent within both breeds in the investigated herd. The  $\beta$ -CN genotype A1A1 was more frequent within SRB than within SLB. The A2B genotype was less frequent within both breeds (Table IVa) and it was not represented within all sampling occasions or lactation numbers (results not shown). This means that there was a confounding between  $\beta$ -CN genotype and sampling occasion as well as between  $\beta$ -CN genotype and lactation number (results not shown). However, the  $\beta$ -CN concentration was higher in SRB than in SLB milk, even though the effect of  $\beta$ -CN genotype was included in the statistical analysis (Table IVb).

## Discussion

The main finding of this investigation was that concentrations of  $\beta$ -CN and  $\kappa$ -CN were higher in

SRB than in SLB milk (Table I). Previous results (Wedholm et al., 2006), showed that the  $\kappa$ -CN concentration was higher in milk from cows carrying the  $\kappa$ -CN AB genotype than the AA genotype. The higher frequency of  $\kappa$ -CN AB within SRB than within SLB (Table IVa) may therefore explain the higher  $\kappa$ -CN concentration in the SRB milk analysed. The effect of different  $\beta$ -CN genotypes on  $\beta$ -CN concentration could, however, not be determined because of the confounding between  $\beta$ -CN genotype and sampling occasion as well as between  $\beta$ -CN genotype and lactation number (results not shown). McLean et al. (1984) reported a significantly higher concentration of  $\beta$ -CN in milk from cows carrying the  $\beta$ -CN A2B genotype compared with the A1A1, A1A2 or A2A2 genotype. However, in the present study the frequency of the A2B genotype was lower in SRB than in SLB (Table IVa). Most probably it is a combined effect of  $\beta$ -CN

Table II. Concentrations of analysed milk variables from cows of Swedish Red and White breed (SRB) and Swedish Holstein Breed (SLB).

| Breed                                      | SRB               | SLB               |                    | Influence of | Influence of | Influence of       |
|--------------------------------------------|-------------------|-------------------|--------------------|--------------|--------------|--------------------|
| Least squares means $\pm$ Standard error   | LSM $\pm$ SE      | LSM $\pm$ SE      | Influence of       | stage of     | lactation    | sampling           |
| Number of individuals ( <i>n</i> )         | ( <i>n</i> = 58)  | ( <i>n</i> = 32)  | of breed           | lactation    | number       | occasion           |
| Milk yield (kg) <sup>1)</sup>              | 10.82 $\pm$ 0.62  | 12.70 $\pm$ 0.70  | n.s. <sup>2)</sup> | ***          | n.s.         | n.s. <sup>2)</sup> |
| Fat (g 100 g <sup>-1</sup> )               | 5.22 $\pm$ 0.12   | 4.77 $\pm$ 0.15   | *                  | n.s.         | n.s.         | ***                |
| Lactose (g 100 g <sup>-1</sup> )           | 4.63 $\pm$ 0.03   | 4.84 $\pm$ 0.03   | ***                | n.s.         | n.s.         | n.s.               |
| Urea (mM)                                  | 5.24 $\pm$ 0.29   | 5.47 $\pm$ 0.21   | n.s.               | n.s.         | n.s.         | n.s.               |
| Calcium (g 100 g <sup>-1</sup> )           | 0.114 $\pm$ 0.002 | 0.115 $\pm$ 0.002 | n.s.               | n.s.         | n.s.         | n.s.               |
| Free amino terminals (mM Leucine)          | 1.01 $\pm$ 0.03   | 1.12 $\pm$ 0.04   | *                  | n.s.         | n.s.         | n.s.               |
| Somatic cell count (log ml <sup>-1</sup> ) | 5.07 $\pm$ 0.05   | 5.04 $\pm$ 0.07   | n.s.               | n.s.         | n.s.         | ***                |
| pH                                         | 6.67 $\pm$ 0.01   | 6.73 $\pm$ 0.01   | ***                | n.s.         | n.s.         | n.s.               |

\*\*\*:  $p < 0.001$ ; \*\*:  $p < 0.01$ ; \*:  $p < 0.05$ ; n.s.: not significant ( $p > 0.05$ ).

<sup>1)</sup>Evening milk yield from the specific sampling occasion.

<sup>2)</sup>Not significant due to significant interaction between breed and sampling occasion ( $p > 0.01$ ).

Table III. Concentrations of analysed milk variables from cows of Swedish Holstein Breed (SLB) and Danish Holstein Breed (SDM).

| Breed                                      | SLB               | SDM               |                    | Influence of | Influence of | Influence of       |
|--------------------------------------------|-------------------|-------------------|--------------------|--------------|--------------|--------------------|
| Least squares means $\pm$ Standard error   | LSM $\pm$ SE      | LSM $\pm$ SE      | Influence of       | stage of     | lactation    | sampling           |
| Number of individuals ( <i>n</i> )         | ( <i>n</i> = 32)  | ( <i>n</i> = 44)  | breed              | lactation    | number       | occasion           |
| Milk yield (kg) <sup>1)</sup>              | 13.02 $\pm$ 0.66  | 19.10 $\pm$ 0.50  | n.s. <sup>2)</sup> | ***          | n.s.         | n.s. <sup>2)</sup> |
| Fat (g 100 g <sup>-1</sup> )               | 4.75 $\pm$ 0.16   | 4.64 $\pm$ 0.14   | n.s.               | n.s.         | n.s.         | **                 |
| Lactose (g 100 g <sup>-1</sup> )           | 4.83 $\pm$ 0.03   | 4.93 $\pm$ 0.03   | n.s.               | n.s.         | n.s.         | n.s.               |
| Urea (mM)                                  | 5.56 $\pm$ 0.20   | 3.62 $\pm$ 0.18   | ***                | n.s.         | n.s.         | **                 |
| Calcium (g 100 g <sup>-1</sup> )           | 0.117 $\pm$ 0.003 | 0.126 $\pm$ 0.002 | **                 | **           | *            | n.s.               |
| Free amino terminals (mM Leucine)          | 1.12 $\pm$ 0.04   | 0.92 $\pm$ 0.04   | ***                | n.s.         | n.s.         | n.s.               |
| Somatic cell count (log ml <sup>-1</sup> ) | 5.02 $\pm$ 0.05   | 4.88 $\pm$ 0.05   | n.s.               | n.s.         | n.s.         | ***                |
| pH                                         | 6.73 $\pm$ 0.01   | 6.71 $\pm$ 0.01   | n.s.               | n.s.         | n.s.         | n.s.               |

\*\*\*:  $p < 0.001$ ; \*\*:  $p < 0.01$ ; \*:  $p < 0.05$ ; n.s.: not significant ( $p > 0.05$ ).

<sup>1)</sup>Evening milk yield from the specific sampling occasion.

<sup>2)</sup>Not significant due to significant interaction between breed and sampling occasion ( $p < 0.001$ ).

and  $\kappa$ -CN genotype that influences the  $\beta$ -CN concentration because the  $\beta$ -CN and  $\kappa$ -CN loci are linked closely on the same chromosome (Grosclaude et al., 1973).

The higher level of free amino terminals in SLB milk (Table II) compared with SRB milk could be related to the lower concentration of  $\beta$ -CN in the SLB milk examined. Protein degradation in milk is mainly due to plasmin activity which hydrolyses  $\beta$ -CN into  $\gamma$ -CN and proteose peptones (Fox, 1992). The higher level of free amino terminals in SLB than in SDM milk (Table III) can be related to a higher SCC in the SLB milk samples (though not significant) and is thought to reflect increased proteolysis as previously reported by Andrews (1983).

That SLB milk had a higher urea concentration than SDM milk could be related to differences in the protein to carbohydrate balance (Gustafsson & Palmquist, 1993) fed to the Swedish and the Danish experimental herds. It has been shown that milk urea has a negative influence on the milk clotting properties (Martin et al., 1997). The higher concentration of calcium in SDM than in SLB milk is interesting, both from a technological and a nutritional point of view. It would be relevant to further evaluate the reason for this variation, because there are few reports examining how to influence the calcium concentration in milk (Banks et al., 1984).

This report also describes a method, modified according to Bordin et al. (2001), to determine the protein composition in skimmed milk resulting in separation of  $\kappa$ -CN,  $\alpha_{S1}$ -CN,  $\beta$ -CN,  $\beta$ -LG variant A and B and  $\alpha$ -LA in a single step. Unfortunately, the  $\alpha_{S2}$ -CN concentration could not be determined owing to difficulties in distinguishing between the  $\alpha_{S2}$ -CN peak and other minor peaks with similar retention time. The minor peak that appeared just after 25 min ( $\alpha_S$ -CN chromatogram, Figure 1) most probably corresponds to  $\alpha_{S2}$ -CN, but the corresponding peak was difficult to assign unambiguously in the milk samples (milk chromatogram, Figure 1). The purified  $\beta$ -LG B unexpectedly resulted in a double peak (Figure 1). This may be explained by the presence of different oligomers of  $\beta$ -LG B, in spite of the denaturing and reducing sample buffer used. However, the exact nature of the two peaks is not known and both peak areas were included in the calculation of  $\beta$ -LG B concentration in milk samples. The purified  $\kappa$ -CN and  $\beta$ -CN resulted in three major peaks each (Figure 1). These correspond to different genetic variants and glycosylation or phosphorylation forms of  $\kappa$ -CN and  $\beta$ -CN (Ng-Kwai-Hang, 2002) and were all included in the calculation of the concentrations of these proteins in milk samples.

Ostersen et al. (1997) evaluated the protein composition in SDM at different stages of lactation.

Table IVa. Frequencies of  $\kappa$ - and  $\beta$ -casein genotypes within the Swedish Red and White (SRB) and Swedish Holstein (SLB) cows investigated.

|                                             | $\kappa$ -casein genotype (frequency in percent) |    |    |    |    | $\beta$ -casein genotype (frequency in percent) |      |      |     |
|---------------------------------------------|--------------------------------------------------|----|----|----|----|-------------------------------------------------|------|------|-----|
|                                             | AA                                               | AB | AE | BB | BE | A1A1                                            | A1A2 | A2A2 | A2B |
| Swedish Red and White cows ( <i>n</i> = 58) | 43                                               | 31 | 22 | 2  | 2  | 17                                              | 52   | 28   | 3   |
| Swedish Holstein cows ( <i>n</i> = 32)      | 75                                               | 13 | 9  | 3  | 0  | 10                                              | 50   | 34   | 6   |

*n*: number of individuals.

Table IVb. Influence of breed on concentration of  $\beta$ -casein ( $\text{g } 100 \text{ g}^{-1}$ ; Least squares means and standard errors) in milk samples from Swedish Red and White (SRB) and Swedish Holstein (SLB) cows when  $\beta$ -casein genotype was included in the statistical model.

|                                                    | Influence of breed |                   | Influence of $\beta$ -casein genotype <sup>1)</sup> , lactation number or sampling occasion | Influence of stage of lactation |
|----------------------------------------------------|--------------------|-------------------|---------------------------------------------------------------------------------------------|---------------------------------|
|                                                    | SRB ( $n=59$ )     | SLB ( $n=31$ )    |                                                                                             |                                 |
| $\beta$ -casein ( $\text{g } 100 \text{ g}^{-1}$ ) | $1.34 \pm 0.04^a$  | $1.25 \pm 0.06^b$ | $p < 0.05$                                                                                  | n.s.                            |

$n$ : number of individuals.

n.s.: not significant ( $p > 0.05$ ).

<sup>1)</sup>The cows were genotyped A1A1, A1A2, A2A2 or A2B. Frequency within SRB and SLB are presented in Table IVa.

<sup>a-b</sup>Means with different superscripts differ ( $p < 0.05$ ).

In medium lactation (24 weeks after parturition) they found the following ratio of individual caseins in relation to total casein, 48:38:10 of  $\alpha_S$ -CN,  $\beta$ -CN and  $\kappa$ -CN, respectively. The corresponding distribution calculated from our data, on average for SDM, was 38:46:16 for  $\alpha_{S1}$ -CN,  $\beta$ -CN and  $\kappa$ -CN, respectively (results not shown). However, we would expect a more similar distribution to that reported from Ostensen et al. (1997) if  $\alpha_{S2}$ -CN had also been included.

From the results of this study we could conclude that the higher concentration of casein analysed in SRB milk was related to higher concentration of  $\beta$ -CN and  $\kappa$ -CN. The higher  $\kappa$ -CN concentration is most probably due to the effect of  $\kappa$ -CN genetic polymorphism whereas the higher  $\beta$ -CN concentration could partly be related to the lower degree of protein degradation in SRB milk. The effect of variations in milk protein composition on milk clotting properties and cheese yield has also been evaluated (Wedholm et al., 2006).

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