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Comparison of milk protein composition and rennet coagulation properties in native Swedish dairy cow breeds and high-yielding Swedish Red cows

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ABSTRACT

Recent studies have reported a very high frequency of noncoagulating milk in Swedish Red cows. The underlying factors are not fully understood. In this study, we explored rennet-induced coagulation properties and relative protein profiles in milk from native Swedish Mountain and Swedish Red Polled cows and compared them with a subset of noncoagulating (NC) and well-coagulating (WC) milk samples from modern Swedish Red cows. The native breeds displayed a very low prevalence of NC milk and superior milk coagulation properties compared with Swedish Red cows. The predominant variants in both native breeds were α_{S1}-casein (α_{S1}-CN) B, β-CN A^2 and β-lactoglobulin (β-LG) B. For κ-CN, the B variant was predominant in the Swedish Mountain cows, whereas the A variant was the most frequent in the Swedish Red Polled. The native breeds displayed similar protein composition, but varied in content of α_{S1}-CN with 9 phosphorylated serines (9P) form. Within the Swedish Mountain cows, we observed a strong inverse correlation between the relative concentration of κ-CN and micelle size and a positive correlation between ionic calcium and gel firmness. For comparison, we investigated a subset of 29 NC and 28 WC milk samples, representing the extremes with regard to coagulation properties based on an initial screening of 395 Swedish Red cows. In Swedish Red, NC milk properties were found to be related to higher frequencies of β-CN A^2, κ-CN E and A variants, as well as β-LG B, and the predominant composite genotype of β- and κ-CN in the NC group was A^2A^2/AA. Generally, the A^2A^2/AA composite genotype was related to lower relative concentrations of κ-CN isoforms and higher relative concentrations of α_{S1}-, α_{S2}-, and β-CN. Compared with the group of WC milk samples, NC milk contained a higher fraction of α_{S2}-CN and α-lactalbumin (α-LA) but a lower fraction of α_{S1}-CN 9P. In conclusion, milk from native Swedish breeds has good characteristics for cheese milk, which could be exploited in niche dairy products. In milk from Swedish Mountain cows, levels of ionic calcium seemed to be more important for rennet-induced gel firmness than variation in the relative protein profile. In Swedish Red, lower protein content as well as higher fraction of α_{S2}-CN and lower fraction of α_{S1}-CN 9P were related to NC milk. Further, a decrease in the frequency of the composite β-κ-CN genotype A^2A^2/AA through selective breeding could have a positive effect on milk coagulation properties.
Key words: genetic polymorphism, mass spectrometry, liquid chromatography, indigenous cattle

INTRODUCTION

There is an increasing recognition of the importance of conserving local native dairy breeds, as they might possess unique genetic variation that can affect the milk composition and quality, potentially resulting in distinct milk characteristics that could be exploited in niche dairy products. Further, local breeds embed an awareness of our cultural heritage and often reflect a contrast to modern intensive production systems. In Sweden, Swedish Red Polled and Swedish Mountain cattle are recognized as native breeds. Because of their low effective population sizes, these breeds have, to a large extent, been diluted through crossbreeding with imported high-yielding breeds; the number of living descendants from these breeds is small; 1,000 Swedish Mountain and 450 Swedish Red Polled (Tapio et al., 2006).

Lien et al. (1999) examined 17 native and 5 modern Nordic dairy breeds for common milk protein polymorphisms. Based on these markers, Nordic native breeds could be divided into northern and southern groups, which was later confirmed by Kantanen et al. (2000). Molecular characterizations of 35 northern European breeds suggested 3 distinct phylogenetic groups: Black-and-White type, Baltic Red, and Nordic cattle. Swedish Mountain cattle and Swedish Red Polled belonged to

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the Nordic cattle group; however, sub-structure within the group indicated that Swedish Mountain cattle were more closely related to a group comprising native Finnish breeds, Icelandic cattle, Sided Tränder and Nordland cattle, Fjällnära cattle, and Bohus Poll, than to Swedish Red Polled. Likewise, a grouping of Norwegian Red, Finnish Ayrshire, and Swedish Red documented the common history of these breeds (Tapio et al., 2006). A more detailed cluster analysis conducted by Li and Kantanen (2010) identified 6 genetic clusters and confirmed the grouping of Swedish Mountain cattle and Swedish Red, whereas Swedish Red Polled had a more admixed ancestry but still exhibited the strongest classification to the group also containing Swedish Mountain cattle.

Proteins in bovine milk can be classified into caseins (α_{S1}-, α_{S2}-, β-, and κ-CN) and whey proteins (α-LA and β-LG). Examination of genetic variation of the major milk proteins is of interest due to the documented correlation with compositional and technological traits (Wedholm et al., 2006; Hallén et al., 2008; Heck et al., 2009; Poulsen et al., 2013). Protein variant polymorphism previously reported in Swedish cattle breeds documented variation in the number of variants identified as well as their frequencies (Lien et al., 1999). For *CSN1S1*, encoding α_{S1}-CN, Swedish Mountain cattle had a high frequency of variant C (31%) compared with Swedish Red Polled cattle (10%), whereas frequencies of these variants were not reported in Swedish Red. For *CSN2*, encoding β-CN, variants A^1, A^2, and B were found in similar frequencies in the 2 native breeds compared with Swedish Red, in which only variants A^1 and A^2 were identified. In *CSN3*, encoding κ-CN, variant B was found at a very high frequency in Swedish Mountain cattle (71%) compared with the 2 other breeds. In contrast, *CSN3* variant E had a relatively high frequency in Swedish Red Polled (20%) and in Swedish Red (12%). For β-LG, variants A and B were found in all breeds, but with a predominance of β-LG B (63–84%). For Swedish Red, a larger genotyping study (Poulsen et al., 2013) confirmed the reported frequencies by Lien et al. (1999).

Variation in cheese milk quality and high frequencies of noncoagulating (**NC**) milk samples have been reported in several northern European cattle populations (Ikonen et al., 1999; Tyrisevä et al., 2004; Wedholm et al., 2006; Hallén et al., 2007). In Swedish Red, among the 400 milk samples analyzed, milk from 16% of the cows did not coagulate (Poulsen et al., 2013) and recently a comparable high frequency was documented in native Red Danish 1970 cows (Poulsen et al., 2017a). The underlying cause of NC milk is not fully understood, but the phenomenon is partly under genetic influence, with moderate heritability in Swedish Red cows (Gustavsson et al., 2014a). A major QTL explaining 9 to 15% of the phenotypic variation in different coagulation traits in Swedish Red was identified around the casein gene cluster on chromosome 6 (Gregersen et al., 2015), but genes playing a role in *o*-glycosylation of κ-CN have also been suggested as candidate genes affecting milk coagulation properties (Tyrisevä et al., 2008; Gregersen et al., 2015). Variation in *CSN3* have been related to higher relative and absolute content of κ-CN as well as shorter rennet coagulation time and improved curd firmness (Bonfatti et al., 2010a,b). Furthermore, Jensen et al. (2012) found that good coagulation ability was associated with a higher degree of glycosylated κ-CN. In addition, variability in the phosphorylated fraction of α_{S1}- and α_{S2}-CN may play a role (Frederiksen et al., 2011). Gustavsson et al. (2014b) related NC milk to lower ionic and total calcium contents, whereas casein micelle size was more important for variation in gelation properties, when NC milk samples were excluded.

The aim of the present study was to apply liquid chromatography combined with electrospray ionization-mass spectrometry (**LC/ESI-MS**) to detect genetic variation and relative protein composition in milk from Swedish Red and 2 native Swedish breeds, Swedish Mountain and Swedish Red Polled. Variability in protein composition was associated with rennet-induced milk coagulation properties as well as total and ionic calcium contents and casein micelle size. Further, a subset consisting of NC and well-coagulating (**WC**) milk samples from Swedish Red was compared in relation to variant polymorphism of the major milk proteins and variation of posttranslational modifications.

MATERIALS AND METHODS

Milk Sampling

Morning milk samples from Swedish Red (n = 58) were collected as described by Gustavsson et al. (2014a). Morning milk samples from Swedish Mountain cattle (n = 23) and Swedish Red Polled (n = 8) were collected from southern Sweden (Mjölby and Vellinge, respectively). On the day of sampling, the collected milk samples were aliquoted, skimmed (centrifuged for 30 min, 2,000 × *g* at 4°C), and refrigerated at 4°C for up to 6 h before being subjected to rheological analyses or frozen for later analyses (−20°C). Individual fresh milk samples were analyzed for SCC using flow cytometry (Fossomatic 5000, Foss Analytical, Hillerød, Denmark) and overall milk composition (contents of total protein, fat and lactose) using infrared spectroscopy (MilkoScan FT2, Foss Analytical) at a certified