# Genetic variants and posttranslational modifications of milk proteins in relation to non-coagulating milk

*K. Nilsson[1], L. Buhelt Johansen[2], S. I. Duchemin[3], M. Stenholdt Hansen[2], H. Stålhammar[4], W. F. Fikse[5], D. J. de Koning[3], H. Lindmark-Månsson[1], M. Paulsson[1] and M. Glantz[1]*

[1] *Department of Food Technology, Engineering and Nutrition, Lund University, Lund, SE*
[2] *Arla Foods amba, Aarhus, DK*
[3] *Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, SE*
[4] *Viking Genetics, Skara, SE*
[5] *Växa Sverige, Uppsala, SE*

The ability of milk to coagulate after addition of rennet is a crucial step in cheese production. We have previously shown that Swedish Red Dairy Cattle (RDC) has a high prevalence of individuals, 18%, that produce non-coagulating (NC) milk. This has a negative effect on cheese yield, and thus it is of high interest for dairies to eliminate NC milk. Previous studies have tried to link NC milk to different genetic variants of milk proteins and other studies have linked posttranslational modifications (PTM) of milk proteins to coagulation ability of milk. One type of PTM is glycosylation of κ-casein, but the results from earlier studies are contradictory, since it has been found that glycosylation of κ-casein could both increase or decrease the rennet-coagulation ability of milk. The aim of this study was to determine the protein profile in milk from Swedish RDC, including PTMs of the caseins, in order to explore links between the milk proteins and NC milk. Additionally, the results from this study can be used to investigate the overall protein profile of Swedish RDC cows and compare with other breeds.

Milk samples from 619 individual Swedish RDC cows were analysed using liquid chromatography-high resolution mass spectrometry and the obtained masses were compared to Uniprot database to determine genetic variants of both casein and whey proteins. PTMs were determined by using masses, where phosphorylations of the genetic variants of casein, as well as glycosylations of κ-casein, were obtained. Both the number of glycosylations as well as the glycosylation variant (A-E) were determined. The genetic variants and PTMs were combined with coagulation parameters measured in the milk samples, in order to distinguish the protein profile of NC milk from Swedish RDC. In addition, composition and physical properties of the milk samples were used in the study to fully explore the pattern for NC milk.

Genetic variants of milk proteins can be determined both genetically and phenotypically, and by measuring the variants in milk samples, the results will show which variants are expressed at protein level in the milk. Thus far, we have found that κ-casein variant E, which previously has been shown to have a negative impact on rennet-induced coagulation, has decreased in the Swedish RDC breed. In our data set, we have also found at least six different phosphorylation isoforms of $\alpha_{S2}$-casein, which is very uncommon. Further analyses should reveal additional insights into the relations between detailed milk protein profile and NC milk within Swedish RDC. By gaining more knowledge of genetic variants and PTMs of milk proteins affecting NC milk, this can be used in the breeding strategy of Swedish RDC in order to reduce the frequency of NC milk, and also to get a better understanding of the mechanisms behind the inability of milk to coagulate.