



High Frequencies of the α_{S1} -Casein Zero Variant in Milk from Swedish Dairy Goats

Monika Johansson^{1,3}, Madeleine Högberg², Anders Andrén¹

¹ Department of Food Science, Uppsala BioCenter, Swedish University of Agricultural Sciences, Sweden, SE-750 07 Uppsala

² Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden

³ Corresponding author: Department of Food Science, Uppsala BioCenter, Swedish University of Agricultural Sciences, SE-750 07, Uppsala, Sweden, tel: +4618672041, fax: +4618672995, monika.johansson@slu.se

Acknowledgements

We thank leg. veterinary Ylva Persson, who kindly helped us with collecting the milk. We also thank the goat farmers from Sweden, where the milk sampling was achieved.

Summary

A high concentration of caseins in milk is a prerequisite for a high cheese yield. Over 70 percent of Norwegian Landrace goats have been shown to have a mutation that reduces or removes the ability to produce α_{S1} -casein (α_{S1} -CN), with a subsequent lower cheese yield as a consequence. Swedish Landrace goats

are closely related to Norwegian goats, so it is very likely that Swedish goats carry the gene for zero-synthesis of α_{S1} -CN. The objective of this study was to survey the Swedish goat population in order to estimate the frequency of Swedish goats producing low amounts of α_{S1} -CN. By use of capillary zone electrophoresis, milk samples from 283 goats from ten different

geographical regions of Sweden were analysed. Sixty-five percent of goats were found to produce low or no concentrations of α_{S1} -CN. Only 12 percent of the dairy goats showed a high expression of this protein.

Key Words: Swedish Goats, α_{S1} -Casein, Capillary Zone Electrophoresis

Introduction

Milk with a higher proportion of α_{S1} -CN and κ -CN has improved cheese-making properties due to greater total protein, fat and calcium contents (Pierre et al., 1998; Clark and Sherbon, 2000). Goats generally have a lower proportion of α_{S1} -CN in their milk than cows and sheep, but the proportions of different caseins vary among breeds (Clark and Sherbon, 2000; Moatsou et al., 2004). Norwegian Landrace goats belong to a population in which some dairy goats through a mutation lost the ability to produce α_{S1} -CN (Hayes et al., 2006). The frequency of this “zero” variant has been measured in several studies to be more than 70 percent, which significantly reduces the milk casein and fat content and thereby the cheese yield (Dagnachew et al., 2011; Devold et al., 2011). The reason for the high frequency of the zero variant of the α_{S1} -CN in Norway is believed to be associated with brown cheese production (whey cheese), which historically has been the main Norwegian goat milk product. When manufacturing this type of cheese, the dry matter content in milk is of less importance because the whey is the main component and not the curd.

As many as 18 different variants of α_{S1} -CN have been recognized in goat breeds (Caroli et al., 2007). Differences existing among the genetic variants A, B, C, D, E, F, G and O have been described (Grosclaude et al., 1994). The ability to produce α_{S1} -CN is controlled mainly by the alleles number 1, 3 and 6, of which the A, B and C variants produce 3.6 g/l, E allele 1.6 g/l per, the F-variant produces 0.6 g/l of α_{S1} -CN. The O variant produces no α_{S1} -CN (Martin et al., 1999). Depending on their ability to produce α_{S1} -CN, goats have been classified into strong-, medium-, weak-, or zero-goats (Feligini et al., 2003). The strong goats produce the most suitable milk for production of cheese with good rennet coagulation properties and high cheese yield. In most goat breeds of Southern Europe, the frequency of weak and zero variants is very low. Exceptions are the Spanish Canaria and the Italian Garganica breeds, which have gene frequencies of 20 percent and 23 percent, respectively,

for the zero variant (Albenzio et al., 2009; Caroli et al., 2007).

Thus, the ability of goats to produce milk with high contents of α_{S1} -CN has a huge economic impact for dairy goat farms producing goat cheeses. Swedish Landrace goats are closely related to the Norwegian Landrace, and bucks from Norway have been used in the breeding of Swedish goats, so it is very likely that Swedish goats carry the gene for zero-synthesis of α_{S1} -CN. The objective of this study was to provide a random survey of the Swedish goat population with the aim to estimate the frequency of Swedish goats producing low levels of α_{S1} -CN.

Materials and Methods

Milk samples were collected from 283 goats from 28 farms in ten different geographical regions of Sweden. A sample of 5 ml was taken from each goat during milking, and the samples were stored at -20°C until analysis of the protein profile by capillary zone electrophoresis (CZE). Before analyses, the samples were defatted at $3000 \times g$ at 4°C for 10 min. Electro-migration of the goat milk proteins was carried out with a CZE (G-

1600AX, Agilent Technologies Co., SE-164 94, Kista, Sweden), controlled by Chemstation software (version A 10.02). Separations were performed using unfused silica standard capillaries ($50 \mu\text{m}$ inner diameter, 40 cm active length (Chrom Tech, SE-195 30, Märsta, Sweden) according to the methods described by Åkerstedt et al. (2012) with the following minor modifications: 2.33 mL of sample buffer was added to 1 mL of defatted goat milk, and an extended running time of 55 min was used. The goat milk proteins were identified by comparison with reference samples (kindly provided by Dr. Andrea Criscione, University of Catania, Italy) and confirmed by previously published electropherograms (Feligini et al., 2005; García-Ruiz et al., 2000). The calculation of relative concentrations of the individual proteins was based on the peak area and expressed as a percentage of the total areas recorded for all peaks in the electropherogram. The percentages of α_{S1} -CN compared to the other caseins were calculated and classified as strong (15 percent to 25 percent), medium (7 percent to 14.9 percent) and low (0 percent to 6.9 percent) to get a grouping on the expression of α_{S1} -CN.

Figure 1: Representative electropherograms of goat milk protein profile.

1A. Protein profile with a high expression of α_{S1} -CN.

1B. Protein profile with a low expression of α_{S1} -CN. The individual proteins are indicated. α -LA, α -lactalbumin; β -LG, β -lactoglobulin; α_{S1} -CN, α_{S1} -casein; α_{S2} -CN, α_{S2} -casein; κ -CN, κ -casein; β -CN, β -casein.

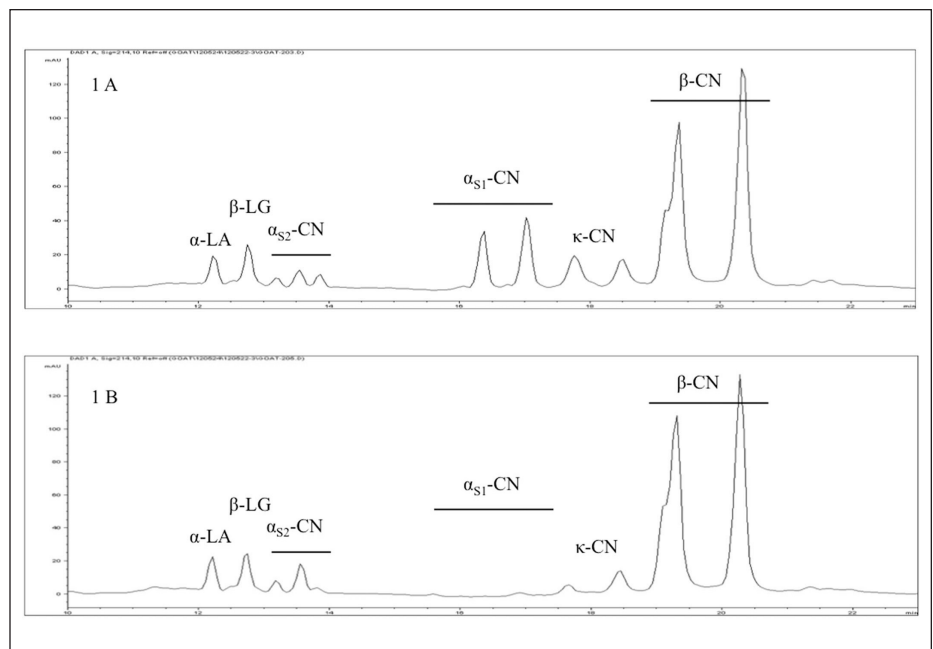


Table 1. Overview of goats from Swedish counties expressing strong, medium and low amounts of α_{S1} -CN.

County	Classes of expressed α_{S1} -CN			
	Herds (id)	Strong (n)	Medium (n)	Low (n)
Uppland	1	4	5	5
	2	0	0	10
	3	5	7	0
	4	1	0	9
Västergötland	1	1	2	4
	2	1	1	8
	3	1	3	6
Västmanland	1	2	1	7
Dalsland	1	2	2	6
Gävleborg	1	0	0	9
	2	2	2	6
	3	0	2	12
	4	1	1	8
Jämtland	1	1	7	2
	2	3	5	1
	3	4	8	2
	4	0	1	3
	5	0	0	10
Värmland	6	3	0	7
	1	0	0	7
	2	2	8	0
Dalarna	3	0	5	8
	1	0	0	10
Ångermanland	2	0	1	10
	1	0	0	10
Härjedalen	2	1	1	7
	1	1	1	8
Σ	2	0	0	10
	28	35	63	185
Frequency (%)		12	22	65

Results and Discussion

Examples of protein profiles of milk from individual goats with a high and low expression of α_{S1} -CN based on capillary zone electrophoresis (CZE) electropherograms are presented in Figures 1A and 1B, respectively. The amount of α_{S1} -CN, as well as the number of peaks of α_{S1} -CN, varied considerably among goats. There was considerable variation in the amount of the other milk proteins, although not as much variation as for the α_{S1} -CN. For example, the peaks for α_{S2} -CN and κ -CN in Figure 1A and 1B varied in area depending on differences in phosphorylation (Heck et al., 2008) and glycosylation (Jensen et al., 2012) of the caseins, respectively.

Table 1 shows that there was a very

high frequency of Swedish dairy goats with low expression of α_{S1} -CN. Of 283 goat milk samples, 185 (65 percent) had an expression of α_{S1} -CN below 7 percent. Only 35 goats (12 percent) produced high concentrations of α_{S1} -CN, and 63 (22 percent) were considered medium producers of α_{S1} -CN.

Due to slight variations between assays, the samples located around the breakpoints of 7 percent and 15 percent α_{S1} -CN could be incorrectly classified. However, our results were very close to the results of the Norwegian studies (Dagnachew et al., 2011; Devold et al., 2011), which showed that 70 percent of the Norwegian goat population is characterized by low expression of α_{S1} -CN. However, there is need for a deeper characterization of the Swedish goat popula-

tion, including genotyping, in order to identify the frequency of E, F and O alleles. These are the most common alleles associated with low- or non-production of α_{S1} -CN in non-Scandinavian populations.

It is known that the gross composition of goat milk is similar to cow and sheep. However, there are differences in fat, solids-non-fat, proteins, caseins and whey proteins and total ash (Jandal, 1996). The unfavorable mutations of the α_{S1} -CN gene result in very negative economic consequences for goat-cheese production, and it is very important to start breeding the Scandinavian dairy goats for the A-variant (allele 6) of α_{S1} -CN.

The economic value of increasing the frequency of the A-variant in Swedish dairy goats could be estimated as follows: If you have goats homozygous for the D-variant (allele 1; no α_{S1} -CN produced) and through breeding replace these individuals with goats homozygous for the A-variant, each A-variant allele will raise the α_{S1} -CN-production by 3.6 grams per liter, for a total increase of 7.2 grams per liter (0.72 percent). If 10 liters of milk with the casein concentration 2.1 percent from DD-goats gives 1 kg of cheese of a given variety and moisture, the yield of the same type of cheese will increase to more than 1.3 kg when using milk from AA-goats, because the cheese yield is proportional to the amount of casein. A change from goats carrying the DD to the AA genotype, thus gives a theoretical increase of the cheese yield of at least 30 percent; and a likely higher fat concentration in the milk of AA-goats is not taken into account.

Conclusions

The frequency of Swedish goats producing low amounts of α_{S1} -CN was high. The low expression of α_{S1} -CN results in negative economic consequences for goat-milk cheese production. Therefore, it is important to start breeding Scandinavian dairy goats to increase the frequency of the A-variant of α_{S1} -CN.

Literature Cited

- Åkerstedt, M., E. Wredle, V. Lam, and M. Johansson. 2012. Protein degradation in bovine milk caused by *Streptococcus agalactiae*. *J. Dairy Res.* 79:297-303.
- Albenzio, M., A. Santillo, F. D'Angelo, and A. Sevi. 2009. Focusing on casein gene cluster and protein profile in garganica goat milk. *J. Dairy Res.* 76:83-89.
- Caroli, A., F. Chiatti, S. Chessa, D. Rignanese, E.M. Ibeagha-Awemu, and G. Erhardt. 2007. Characterization of the casein gene complex in West African goats and description of a new α_{S1} -casein polymorphism. *J. Dairy Sci.* 90:2989-2996.
- Clark, S., and J.W. Sherbon. 2000. Alpha(s1)-casein, milk composition and coagulation properties of goat milk. *Small Rumin. Res.* 38:123-134.
- Dagnachew, B.S., G. Thaller, S. Lien, and T. Ådnøy, 2011. Casein SNP in Norwegian goats: additive and dominance effects on milk composition and quality. *Genetics Sel. Evol.* 43:31-42.
- Devold, T.G., R. Nordbø, T. Langsrud, C. Svenning, M.J. Brovold, E.S. Sørensen, B. Christensen, T. Ådnøy, and G.E. Vegarud. 2011. Extreme frequencies of the α_{S1} -casein "null" variant in milk from Norwegian dairy goats - implications for milk composition, micellar size and renneting properties. *Dairy Sci. Technol.* 91:39-51.
- Feligini, M., S. Frati, S. Vlaco, P. Parma, and G. Enne. 2003. Polymorphism of α_{S1} -casein in goat milk: Identification of A, B, E and F variants by biochemical and genetic analysis. *Italian J. Anim. Sci.* 2:103-105.
- Feligini, M., S. Frati, V.C. Curik, A. Brambilla, P. Parma, I. Curik, G.F. Greppi, and G. Enne. 2005. Caprine α_{S1} -casein polymorphism: Characterisation of A, B, E and F variants by means of various biochemical and molecular techniques. *Food Technol. Biotech.* 43:123-132.
- García-Ruiz, A., R. López-Fandiño, L. Lozada, J. Fontecha, M.J. Fraga, and M. Juárez. 2000. Distribution of nitrogen in goats' milk and use of capillary electrophoresis to determine casein fractions. *J. Dairy Res.* 67:113-117.
- Grosclaude, F., G. Ricordeau, P. Martin, F. Remeuf, and L. Vassal. 1994. Du gène au fromage: Lepolymorphisme de la caséine α_{S1} caprine, ses effets, son évolution (In France). *INRA Prod. Animal.* 7:3-19.
- Hayes, B., N. Hagesæther, T. Ådnøy, G. Pellerud, P.R. Berg, and S. Lien. 2006. Effects on production traits of haplotypes among casein genes in Norwegian goats and evidence for a site of preferential recombination. *Genetics* 174:455-464.
- Heck, J.M.L., C. Olieman, A. Schennink, H.J.F. van Valenberg, M.H.P.W. Visker, R.C.R. Meuldijk, and A.C.M. van Hooijdonk. 2008. Estimation of variation in concentration, phosphorylation and genetic polymorphism of milk proteins using capillary zone electrophoresis. *Int. Dairy J.* 18:548-555.
- Jandal, J. M. 1996. Comparative aspects of goat and sheep milk. *Small Ruminant Res.* 2:177-185.
- Jensen, H.B, J.W. Holland, N.A. Poulsen, and L.B. Larsen. 2012. Milk protein genetic variants and isoforms identified in bovine milk representing extremes in coagulating properties. *J. Dairy Sci.* 95:2891-2903.
- Martin, P., M. Ollivier-Bousquet, and F. Grosclaude. 1999. Genetic polymorphism of caseins: a tool to investigate casein micelle organization. *Int. Dairy J.* 9:163-171.
- Moatsou, G., M. Samolada, P. Panagiotou, and E. Anifantakis. 2004. Casein fraction of bulk milks from different caprine breeds. *Food Chem.* 87:75-81.
- Pierre, A., J.L. Le Qéré, M.H. Famelart, A. Riaublanc, and F. Rousseau. 1998. Composition, yield, texture and aroma compounds of goat cheeses as related to the A and O variants of α_{S1} -casein in milk. *Lait* 78:291-301.