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Relation between α_{S1}-casein, genotype, and quality traits of milk from Swedish dairy goats

Monika Johansson,[1]* Åse Lundh,[1] and Anna Maria Johansson[2]
[1]Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala 750 07, Sweden
[2]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala 750 07, Sweden

ABSTRACT

Locally produced food is becoming popular among Swedish consumers. One product that has increased in popularity is artisan-manufactured goat cheese, and although the dairy goat industry in Sweden is small-scale, production is gradually increasing. In goats, the *CSN1S1* gene regulates expression of the protein α_{S1}-casein (α_{S1}-CN), which has been found to be important for cheese yield. Over the years, breeding animals have been imported to Sweden from Norway. Historically, a high frequency of the Norwegian goat population carried a polymorphism at the *CSN1S1* gene. This polymorphism, called the Norwegian null allele (D), leads to zero or significantly reduced expression of α_{S1}-CN. Using milk samples from 75 goats, this study investigated associations between expression of α_{S1}-CN and genotype at the *CSN1S1* gene on milk quality traits from Swedish Landrace goats. Milk samples were grouped according to relative level of α_{S1}-CN (low: 0–6.9% of total protein; medium-high: 7–25% of total protein) and genotype (DD, DG, DA/AG/AA). While the D allele leads to extremely low expression of α_{S1}-CN, the G allele is low expressing and the A allele is highly expressing for this protein. Principal component analysis was used to explore the total variation in milk quality traits. To evaluate the effect of different allele groups on milk quality attributes, 1-way ANOVA and Tukey pairwise comparison tests were used. The majority (72%) of all goat milk samples investigated showed relative α_{S1}-CN content of 0% to 6.82% of total protein. The frequency of individuals homozygous for the Norwegian null allele (DD) was 59% in the population of sampled goats, and only 15% carried at least one A allele. A low relative concentration of α_{S1}-CN was associated with lower total protein, higher pH, and higher relative concentration of β-casein and levels of free fatty acids. Milk from goats homozygous for the null allele (DD) showed a similar pattern as milk with low relative concentration of α_{S1}-CN, but total protein was only numerically lower, and somatic cell count and α_{S2}-CN were higher than for the other genotypes. The associations between levels of α_{S1}-CN and the investigated genotype at the *CSN1S1* gene indicate a need for a national breeding program for Swedish dairy goats.

Key words: Swedish Landrace goat, goat milk quality traits, α_{S1}-CN polymorphism, null allele, levels of α_{S1}-CN in milk

INTRODUCTION

In Scandinavia, Norway is the leading country in dairy goat farming, whereas in Sweden local goat milk production is small-scale. However, it is now increasing, with the number of goats in Sweden nearly tripling in recent years, to approximately 20,000 animals (Jordbruksverket, 2019). The interest in locally produced food (e.g., goat's cheese) is increasing among Swedish consumers (Bosona and Gebresenbet, 2018) due to concerns about the diversity, purity, and authenticity of food and a desire to support rural areas (Ditlevsen et al., 2020). The most common dairy goat breed in Sweden is the Swedish Landrace, which is closely related to the Norwegian Landrace. This breed is primarily used for small-scale local milk and cheese production, since it is high yielding, producing between 1,000 and 1,400 L of milk per year (Mason, 1996). Historically, with high frequency (>70%) the Norwegian goat population contained a deletion in exon 12 of the *CSN1S1* gene where α_{S1}-CN is encoded. This deletion, also known as the Norwegian null allele (allele D), leads to zero or heavily reduced expression of α_{S1}-CN compared with heterozygote or noncarrier goats (Devold et al., 2011). For α_{S1}-CN in general, 20 genetic variants have been reported in goat breeds (Caroli et al., 2007; Park et al., 2007; Mestawet et al., 2013). These variants are usually divided into 4 classes—strong, medium, weak, or null—depending on their protein expression. Alleles A, B1, B2, B3, B4, C, H, L, and M are highly expressing (~3.5 g/L), alleles E and I are medium expressing

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*Corresponding author: monika.johansson@slu.se

(~1.1 g/L), and the F and G variants are low expressing (~0.45 g/L). The null variants 01, 02, and N only express traces of α_{S1}-CN (Martin et al., 1999; Marletta et al., 2007). The frequency of these alleles varies between breeds, and genotypes with high expression are known to exist in the French (Pierre et al., 1998), Spanish (Jordana et al., 1996), and Italian (Sacchi et al., 2005) goat populations, whereas the null variant is predominant in Norwegian dairy goats (Dagnachew et al., 2011; Devold et al., 2011). In a study on the Swedish dairy goat population, extremely low levels of α_{S1}-CN (0%–6.9% of total protein) were detected in 65% of 283 goats investigated (Johansson et al., 2014).

In addition to low levels of α_{S1}-CN in milk, the defective null allele has been associated with significantly lower content of total protein, total casein, and TS compared with milk with high levels of α_{S1}-CN (Ambrosoli et al., 1988; Pierre et al., 1998; Clark and Sherbon, 2000; Devold et al., 2011; Johansson et al., 2015). Milk with a high content of α_{S1}-CN has been found to have beneficial cheese-making properties, resulting in high cheese yield (Skeie, 2014; Johansson et al., 2015). Goat milk with low levels of α_{S1}-CN may result in a less firm gel, from which caseins are easily lost with the whey if not properly bound to the casein gel network (Skeie, 2014). Bonfatti et al. (2011) attributed the beneficial coagulation properties of high α_{S1}-CN milk to the superior self-association properties of this casein. A higher fat content has been reported for milk with high levels of α_{S1}-CN (Pierre et al., 1998; Clark and Sherbon, 2000), and Dagnachew et al. (2011) reported elevated levels of free fatty acids (**FFA**) and a rancid flavor associated with the Norwegian null allele. Cebo et al. (2012) found that the physicochemical characteristics of the milk fat globule membrane were affected by the genotype of the α_{S1}-CN gene. Other studies have found a negative association between α_{S1}-CN and pH (Ambrosoli et al., 1988; Pierre et al., 1998; Johansson et al., 2015). For all these reasons, the polymorphism in the *CSN1S1* gene is perhaps one of the most studied in the field of goat milk proteins (Park et al., 2007).

The objective of the present study was to explore the variation in the detailed composition of Swedish dairy goat milk and investigate how milk quality traits are affected by the content of α_{S1}-CN and the genotype at the *CSN1S1* gene.

MATERIALS AND METHODS

Experimental Design and Milk Sampling

Because milk samples were collected in association with the farms' routine daily milking procedure, this study did not require ethical approval with an animal experiment ethics committee. Milk samples were collected from 75 goats on 5 farms in 5 different geographical regions of Sweden during February and March 2020. The goats were randomly selected animals of the Swedish Landrace breed, and no specific factors [e.g., lactation stage, lactation number, type of production (extensive or intensive), average milk production, or diet] were considered. The number of goats from each farm was as follows: farm 1: 26 samples; farm 2: 37 samples; farm 3: 3 samples; farm 4: 5 samples; and farm 5: 4 samples. A sample of 50 mL was taken from each goat during evening milking. The milk samples were stored at −20°C and transported frozen to the Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala, for analysis of milk quality traits. Upon arrival, the milk was thawed overnight at 4°C. After equilibration of the thawed milk samples for 60 min at room temperature, the pH was measured using a pH meter (Seven Compact S210, Mettler-Toledo). In 2 cases, the sample was insufficient to measure the milk pH. Gross composition, FFA content, and SCC were analyzed in whole milk samples. For investigation of milk protein profile, plasmin and plasminogen activity, and total proteolysis, the samples were defatted by centrifugation at 2,093 × *g* at 4°C for 10 min (Himac CT 15RE; Hitachi Koki Co. Ltd.). After centrifugation, the fat layer on top of each sample was removed with a cotton stick. If not analyzed on the same day as defatting, samples were stored at −20°C until analysis.

Milk Gross Composition and Somatic Cell Count

Milk samples from each individual goat were analyzed for concentrations of total fat, protein, lactose, TS, SFA, UFA, MUFA, PUFA, myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1 *cis*-9), using a Fourier transform infrared spectrometer (FOSS Electric A/S). The SCC was analyzed by a fluorescence-based cell counting technique, using flow cytometry (Fossomatic Foss FT 120).

Free Fatty Acids

Concentrations of FFA in goat milk samples were determined by an extraction-titration method as described by Vidanarachchi et al. (2015). In brief, milk fat was extracted using diethyl ether and hexane (80:20, vol/vol) with methyl orange as indicator. Samples were acidified using H_2SO_4 until the color of the solution turned pink (pH 2–3). After centrifugation, the supernatant was transferred to a new tube, and the FFA concentration was determined by titration of the supernatant

with KOH in ethanol, using α-naphtholphthalein and phenolphthalein as indicators. Titration was stopped when the color of the solution turned a lilac/blue that persisted for a few seconds.

Casein and Whey Protein Composition

Protein separation was performed with a 7100 capillary electrophoresis system (Agilent Technologies Co.) using unfused silica standard capillary as described by Johansson et al. (2013) with the following minor changes: D,L-dithiothreitol was added to the sample buffer on the day of sample preparation to disrupt disulfide bridges in the milk proteins. Milk (300 μL) was mixed with 700 μL of sample buffer, incubated at room temperature for 1 h, and defatted after centrifugation for 10 min at 2,093 × *g* at 4°C (Himac CT 15RE; Hitachi Koki Co. Ltd.). An extended running time of 55 min was used. The capillary was washed for 10 min with 1 *M* NaOH after every 5 separations. Relative concentrations of the individual proteins were calculated based on peak area and expressed as a percentage of the total integrated area in the electropherogram. In one case, α-LA could not be detected.

Plasmin and Plasminogen-Derived Activities

Determination of plasmin and plasminogen-derived activities was performed according to de Vries et al. (2016). Plasmin and plasminogen were dissociated from casein micelles by incubation of milk with ε-amino-n-caproic acid, followed by ultracentrifugation (Optima MAX-XP, Beckman Coulter Inc.) at 4°C for 1 h at 100,000 × *g*. Plasmin activity in the resulting milk serum was measured using a chromogenic substrate, pyro-Glu-Phe-Lys-p-nitroanilide hydroxychloride (Aniara). Plasminogen was activated with urokinase to measure the sum of plasmin and plasminogen-derived activities. Absorbance was recorded every 3 min for 120 min at 37°C in a multimode microplate reader (POLARstar Omega, BMG LABTECH), and activity was expressed as change in absorbance per time unit (ΔA405/Δt). Plasminogen-derived activity was calculated by subtracting plasmin activity from total activity.

Determination of Total Proteolysis by Fluorescamine Assay

Total proteolysis was measured using the method of Wiking et al. (2002), as modified by Johansson et al. (2017). The method is based on the reaction of primary amino groups of trichloroacetic acid–soluble peptides and free amino acids with fluorescamine. The milk samples were mixed with an equal volume of 24% trichloroacetic acid and kept on ice for 30 min before centrifugation at 16,000 × *g* for 20 min at 4°C. Supernatant (20 μL) was mixed with freshly made sodium tetraborate pH 8, fluorescamine was added, and the mixture was loaded in a 96-microwell plate. Fluorescence (excitation wavelength 390 nm, emission wavelength 480 nm) was measured after 23 min in a fluorescence reader (POLARstar Omega, BMG LABTECH). The extent of proteolysis was expressed as leucine equivalence (eq. m*M*) based on a standard curve with 5 different concentrations (1, 0.75, 0.5, 0.3, and 0.05 m*M*) of 0.1 *M* L-leucine dissolved in 1 m*M* HCl. Each milk sample was analyzed in triplicate.

DNA Extraction

Collection of DNA for genotyping of the goats was performed using nasal swabs. The sponge of the swab was rubbed in the animal's nostril for a couple of seconds and then placed in the collection tube. Samples were stored and mailed to the Swedish University of Agricultural Sciences at ambient temperature. Before analyses, the samples were incubated in water at 50°C for 1 h. A solution (PG-L2P) provided with the nasal swabs was used to extract DNA in accordance with the manufacturer's instructions (DNA Genotek Inc.).

DNA Amplification, Sequencing, and Sequence Analysis

Polymerase chain reactions were run using the BigDye Direct Cycle Sequencing Kit (Applied Biosystems) and the primer pair 4 from Mestawet et al. (2013). The forward primer was GAGCTTCAACAAAAGTCTTTCCA and started at intron 11 at gene *CSN1S1*; the reverse primer was TGACTTCATAGTTCAAATGCACA and ended at intron 13 on the same gene. The primers had M13 tails. The DNA was diluted to 4 ng/μL in a total volume of 50 μL. For the first PCR, 0.8 μ*M* of each primer was mixed, and 1.5 μL of the primer mix, together with 1.0 μL of DNA, 5.0 μL of BigDye Direct PCR Master Mix, and 2.5 μL of deionized water, was used for each reaction. The conditions for the first PCR were as follows: an initial denaturation step of 96°C for 5 min, followed by 35 amplification cycles at 94°C for 30 s, 62°C for 45 s, and 68°C for 45 s, with a final extension step at 72°C for 2 min. The PCR for cycle sequencing was performed with 8 μL of the first PCR reaction and a mix of 2.0 μL of BigDye Direct Sequencing Master Mix and 1.0 μL of BigDye Direct M13 forward or reverse primer. The cycle sequencing was then run as follows: 37°C for 15 min, 80°C for 2 min,

96°C for 1 min, followed by 25 cycles at 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Sequencing was done by capillary electrophoresis (3500xL Genetic Analyzer, Applied Biosystems). The sequences were analyzed using the software 4Peaks, and the genotypes at the site of the deletion of one base pair in exon 12 described in Hayes et al. (2006) were determined. All 3 alleles at this site observed by Hayes et al. (2006) in Norwegian dairy goats were present in our data and were denoted D, A, and G in this study.

Statistical Analyses

For all statistical analyses, the goat population was categorized in 2 ways: (1) in groups, according to the relative content of α_{S1}-CN in the milk (i.e., in the same way as in our previous study, allowing comparison of results; Johansson et al., 2014); and (2) according to genotype at the *CSN1S1* gene (Devold et al., 2011). The frequency of goats expressing high levels of α_{S1}-CN was low, and to obtain reliable results in terms of number of samples, medium- and high-expressing goats were combined into one group. The relative concentration of α_{S1}-CN in milk from this group was within the range of 7.02% to 25.29%. The goat population showed 5 different genotypes at the *CSN1S1* gene, but because just a few goats carried an A allele, all genotypes containing at least one A allele were combined into one group (DA/AG/AA). The DD and DG genotypes were grouped separately. The groups of alleles were arranged according to Devold et al. (2011), classifying the D allele as a null allele in terms of α_{S1}-CN expression. Similarly, the G allele was classified as a weak allele, while A was classified as a strong allele according to Martin et al. (1999).

Minitab 19.2020.20 (Minitab Inc.) and Simca 17.0 (Sartorius Stedim Data Analytics AB) software was used for the statistical analyses, with a confidence interval of 95%. The SCC values were transformed to $\log_{10}$-values due to nonnormal distribution. The variation in milk quality traits was evaluated by 1-way ANOVA and Tukey pairwise comparison. In these analyses, the level of α_{S1}-CN (low, medium-high) and genotype (DD, DG, DA/AG/AA) at the *CSN1S1* gene were used as fixed factors, and farm was used as a random factor in 2 separate ANOVA. Milk quality traits were used as responses. The model took the following form:

$$Y_{ijk} = \mu + \alpha_i + b_j + e_{ijk},$$

where Y_{ijk} is the response (dependent variable, milk quality attributes) for observation k in farm j (1–5), with α_{S1} group i (low or medium-high) or genotype i (DD, DG, DA/AG/AA); μ is the general mean; α_i is the effect of α_{S1} group or genotype i; b_j is the random effect of farm j; and e_{ijk} is a random residual.

A boxplot of the levels of α_{S1}-CN for the different genotypes of exon 12 in the *CSN1S1* gene was made using the command boxplot in R (R Core Team, 2021).

Principal component analysis (**PCA**; Wold et al., 1987) was used to explore the total variation in milk quality traits. All milk quality traits were unit-variance-scaled, autotransformed, and displayed in the PCA loading plot. Milk from goats with low and medium-high levels of α_{S1}-CN and milk from goats with the different genotypes at the *CSN1S1* gene were visualized in the PCA score plots by using different colors.

RESULTS

Milk Level of α_{S1}-Casein and Genotype at the CSN1S1 Gene in the Investigated Goat Population

On categorizing goat milk samples according to their relative concentration of α_{S1}-CN (low: 0%–6.9%; medium-high: 7%–25% of total protein), it was found that 72% of the goats produced milk with a low α_{S1}-CN level, while 28% produced milk with a medium-high level (Table 1).

Table 2 shows the number of animals with each *CSN1S1* genotype and the percentage of the total (75 animals). The majority of the goats, 59%, were homozygous for the D allele, 27% carried the DG genotype, and only 15% carried at least one A allele (DA/AG/AA).

Table 1. Distribution of goat milk samples based on their relative concentration of α_{S1}-casein[1]

Group of goats	Range of the relative concentration of α_{S1}-casein (%) in milk[2]	Number (n) of animals[3]	Percentage of all investigated animals[3]
Low	0.00–6.82	54	72
Medium-high	7.02–25.29	21	28

[1]Milk samples were categorized in groups of low (0%–6.9%) and medium-high (7%–25%) concentration of α_{S1}-casein out of total protein in the milk, to facilitate comparison with findings in our recent study (Johansson et al., 2014).

[2]α_{S1}-casein as percentage of total protein.

[3]Number and percentage of individuals in each group of the investigated goat population (n = 75).

Table 2. Distribution of the *CSN1S1* genotype in the Swedish Landrace goat population investigated (n = 75)[1]

Genotype distribution	DD	DG	DA	AG	AA
Number (n)	44	20	8	2	1
Percentage (%)	59	26	10	2	1

[1]Number of individuals (n) with each genotype and percentage (%) of each genotype in the total population.

The genotypes containing an A allele clearly show higher relative concentration of α_{S1}-CN than goats with genotype DD or DG. The mean level of α_{S1}-CN is slightly higher for goats with genotype DG than for goats with genotype DD (Figure 1).

Total Variation in Goat Milk Quality Traits

A PCA model, explaining 38% and 13% of the variation in the first and second component, respectively, was used to describe the overall variation in the investigated milk quality traits (Figure 2). According to the loading plot (Figure 2A), the first component was strongly negatively associated with milk fat traits, clustered close to each other in the upper left quadrant of the plot. In contrast, FFA were positively associated with the axis in the right upper quadrant. The second component explained the variability to a lesser extent, mostly discriminating protein levels. It was strongly negatively associated with the relative concentration of α_{S1}-CN and positively associated with β-CN and also with pH.

The score plot in Figure 2B illustrates the total variation in milk quality traits for the investigated goat milk samples, with colors representing samples with low and medium-high relative concentrations of α_{S1}-CN. The concentration of α_{S1}-CN was consequently negatively associated with the second component, strongly discriminating milk samples from goats in the low versus the medium-high groups. In the second score plot (Figure 2C), colors instead illustrate genotypes at the *CSN1S1* gene. The A allele was, with one exception, negatively associated with the second component. The exception was a sample with the genotype DA, categorized in the group DA/AG/AA, which was located in the upper left quadrant of the plot. This milk sample deviated from the others in terms of higher levels of SFA, C14:0, and C18:0. Milk samples from goats homozygous for the weak D allele or with the D allele in combination with the G allele were typically more scattered in the score plot, although the majority were positively associated with the second component and located in the upper quadrants.

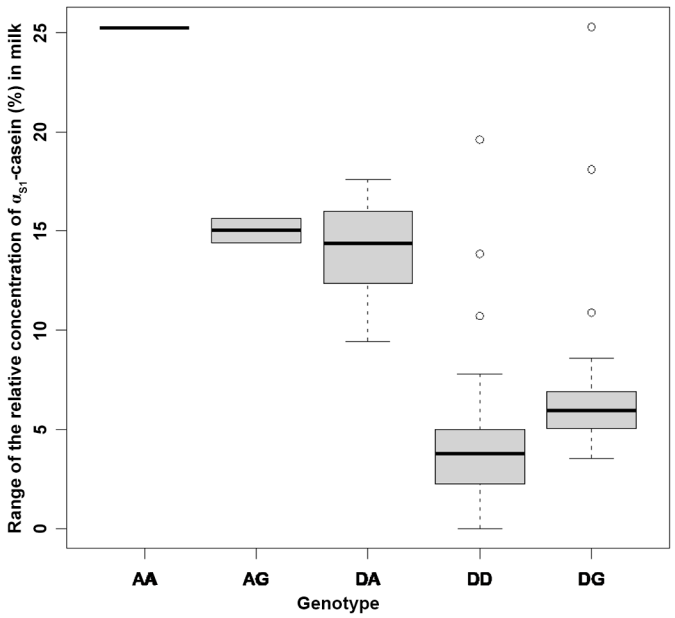

Figure 1. Boxplot showing the distribution of relative concentrations of α_{S1}-CN in milk for the different genotypes of exon 12 in the *CSN1S1* gene. The horizontal line in each box is the median value. The lower and upper extents of the boxes indicate the 25th and 75th percentiles of the distribution, respectively. The whiskers indicate the maximum and minimum values. Dots indicate values considers as outliers.

Quality Traits in Milk from Swedish Landrace Goats in Relation to α_{S1}-Casein Content

Table 3 shows average values for the quality traits investigated in milk from Swedish Landrace goats and for the groups with low and medium-high levels of α_{S1}-CN. The relative concentration of α_{S1}-CN in the low group was on average 3.93%, compared with 14.15% in milk from goats in the medium-high group ($P < 0.001$). A large variation in many of the other variables analyzed illustrates the influence of feed, farm management, and the individual animal. Despite this variation, results from 1-way ANOVA indicated significant differences between the 2 groups for several quality traits. Total protein and κ-CN were 17% and 10% lower, respectively ($P = 0.042$, $P < 0.001$), in milk from the low group than in milk from the medium-high group. Moreover, α_{S2}-CN and β-CN were both 14% higher ($P = 0.017$, $P < 0.001$) in milk from the low-α_{S1}-CN group, whereas the FFA content was 62% lower ($P = 0.004$) in the group with a medium-high level of α_{S1}-CN. Additionally, milk pH was higher in the low-α_{S1}-CN group compared with the medium-high group ($P = 0.009$). No other parameters investigated differed significantly between the 2 groups.

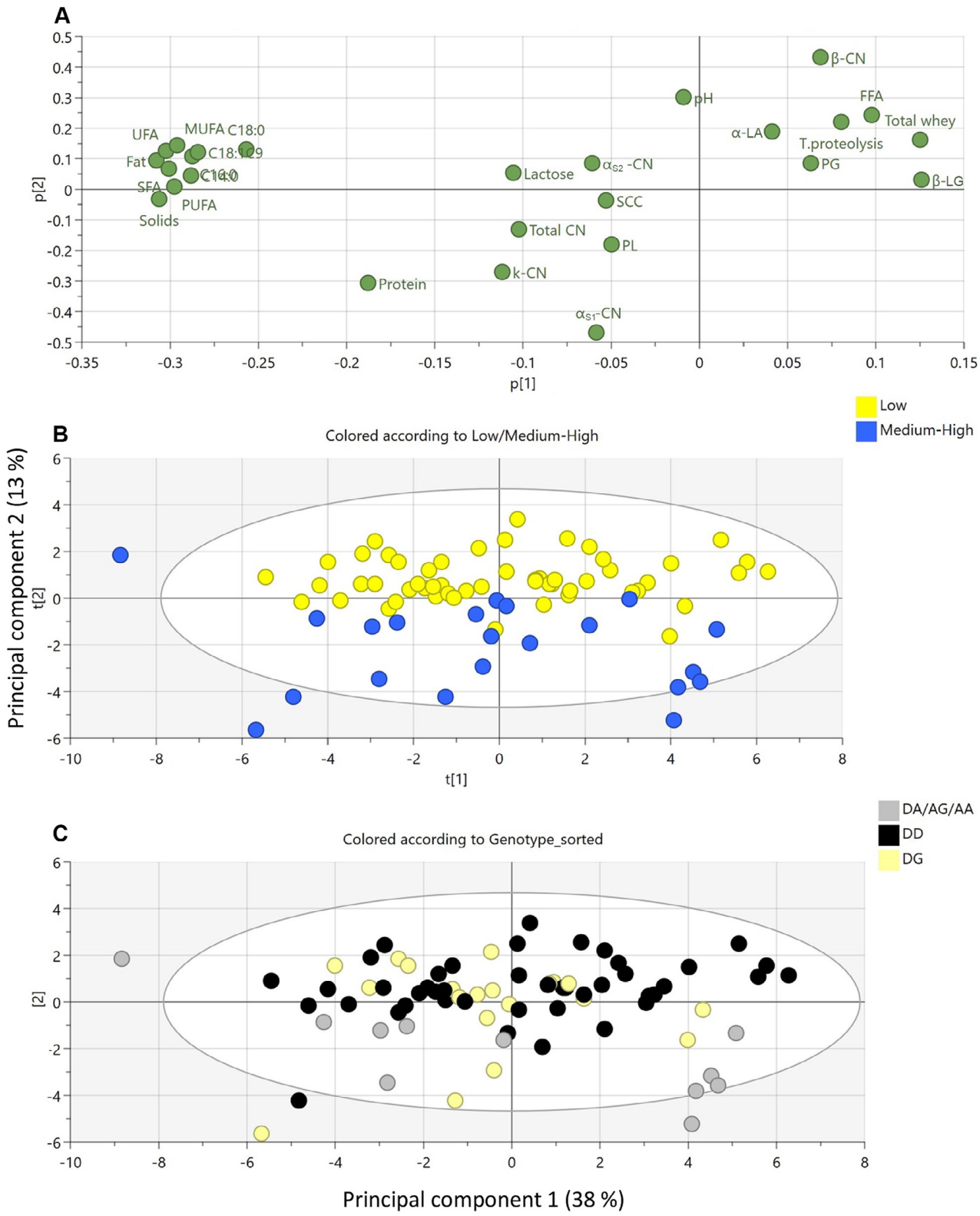

Figure 2. Principal component analysis plots illustrating the total variation in milk quality traits associated with Swedish Landrace goats (n = 75). The loading plot (A) illustrates associations between the investigated traits, with variables grouped together being related. The greater the distance to the origin, the greater the contribution of the variable to the total variation. In the 2 score plots, different colors indicate milk from goats with (B) low and medium-high relative concentrations of α_{S1}-CN and (C) milk from goats with different genotypes at the *CSN1S1* gene. T.proteolysis = total proteolysis; FFA = free fatty acids; PL = plasmin; PG = plasminogen.

Table 3. Quality traits of goat milk samples (n = 75) based on their relative concentrations of α_{S1}-CN[1]

Milk quality trait	Mean	Min.	Max.	Low (n = 54)	Medium-high (n = 21)	*P*-value
Total protein (g/100 g)	2.45 ± 0.44	1.68	4.23	2.32 ± 0.31	2.80 ± 0.55	<0.001
Total fat (g/100 g)	2.85 ± 1.01	0.89	5.88	2.89 ± 0.90	2.73 ± 1.27	0.545
SFA (g/100 g)	1.93 ± 0.69	0.61	4.06	1.94 ± 1.61	1.90 ± 0.86	0.829
UFA (g/100 g)	0.84 ± 0.28	0.28	1.50	0.86 ± 0.26	0.78 ± 0.34	0.253
MUFA (g/100 g)	0.53 ± 0.23	0.04	1.11	0.55 ± 0.21	0.47 ± 0.28	0.174
PUFA (g/100 g)	0.13 ± 0.06	0.02	0.04	0.13 ± 0.05	0.13 ± 0.07	0.937
C14:0 (g/100 g)	0.44 ± 0.15	0.18	0.92	0.43 ± 0.13	0.44 ± 0.19	0.813
C16:0 (g/100 g)	0.78 ± 0.27	0.25	1.50	0.79 ± 0.24	0.73 ± 0.34	0.388
C18:0 (g/100 g)	0.27 ± 0.10	0.07	0.60	0.28 ± 0.09	0.25 ± 0.12	0.299
C19:1 *cis*-9 (g/100 g)	0.37 ± 0.18	0.01	0.79	0.38 ± 0.17	0.33 ± 0.20	0.236
Free fatty acids (m*M*/100 g of fat)	0.94 ± 0.56	0.13	3.69	1.05 ± 0.58	0.64 ± 0.36	0.004
SCC (× 10^3 cells/mL)	408 ± 362	29	1,429	432 ± 352	347 ± 389	0.090
pH[2]	6.58 ± 0.11	6.32	6.78	6.60 ± 0.11	6.53 ± 0.10	0.009
Lactose (%)	4.39 ± 0.22	3.98	5.02	4.37 ± 0.20	4.43 ± 0.26	0.348
TS (%)	10.53 ± 1.40	7.55	14.46	10.41 ± 1.21	10.84 ± 1.79	0.225
Protein fractions as % of total protein						
Total casein	89.89 ± 2.13	82.21	93.31	89.70 ± 2.16	90.36 ± 2.04	0.229
α_{S1}-CN	6.79 ± 5.54	0.00	25.29	3.93 ± 1.72	14.15 ± 5.16	<0.001
α_{S2}-CN	5.33 ± 1.33	1.85	8.53	5.55 ± 1.11	4.75 ± 1.68	0.017
β-CN	67.09 ± 6.51	41.01	75.89	69.89 ± 3.25	59.91 ± 7.33	<0.001
κ-CN	10.68 ± 2.32	5.61	16.46	10.34 ± 2.14	11.55 ± 2.59	0.042
Whey protein (% of total protein)	7.33 ± 1.73	2.04	12.19	7.76 ± 1.89	6.85 ± 1.90	0.065
α-LA[3]	2.61 ± 0.71	1.17	4.26	2.83 ± 1.59	2.52 ± 0.71	0.400
β-LG	4.76 ± 1.45	0.24	9.21	4.93 ± 1.26	4.32 ± 1.83	0.103
Proteolytic activity						
Plasmin (U/mL)	7.19 ± 4.51	1.94	21.75	6.83 ± 3.63	8.12 ± 6.25	0.268
Plasminogen (U/mL)	18.69 ± 3.92	10.64	28.73	18.98 ± 3.52	17.93 ± 4.83	0.298
Total proteolysis (eq. m*M* leucine)	43.73 ± 13.90	13.88	70.91	45.56 ± 13.96	39.00 ± 12.89	0.066

[1]Mean values ± SD. Min. = minimum; Max. = maximum. To facilitate comparison with findings in our recent study (Johansson et al., 2014), milk samples were categorized into 2 groups: low (0–6.9%) and medium-high relative concentrations (7–25%) of α_{S1}-CN. Differences between the 2 groups were evaluated by 1-way ANOVA and were considered significant at $P < 0.05$.

[2]Number of observations = 73.

[3]Number of observations = 74.

Table 4. Comparison of quality traits of milk samples from goats (n = 75) categorized according to genotype at the *CSN1S1* gene[1]

Milk quality trait	Genotype[2]		
	DD (n = 44)	DG (n = 20)	DA/AG/AA (n = 11)
Total protein (g/100 g)	2.38 ± 0.42	2.52 ± 0.53	2.61 ± 0.30
Total fat (g/100 g)	2.81 ± 0.90	3.03 ± 0.83	2.64 ± 1.65
SFA (g/100 g)	1.88 ± 0.60	2.05 ± 0.59	1.88 ± 1.11
UFA (g/100 g)	0.85 ± 0.26	0.89 ± 0.21	0.74 ± 0.45
MUFA (g/100 g)	0.54 ± 0.21	0.57 ± 0.17	0.43 ± 0.36
PUFA (g/100 g)	0.13 ± 0.06	0.14 ± 0.05	0.13 ± 0.09
C14:0 (g/100 g)	0.42 ± 0.12	0.46 ± 0.14	0.46 ± 0.24
C16:0 (g/100 g)	0.78 ± 0.24	0.82 ± 0.25	0.64 ± 0.41
C18:0 (g/100 g)	0.27 ± 0.09	0.29 ± 0.09	0.24 ± 0.16
19:1 *cis*-9 (g/100 g)	0.37 ± 0.17	0.41 ± 0.13	0.29 ± 0.26
Free fatty acids (m*M*/100 g fat)	1.04 ± 0.48[a]	0.93 ± 0.70[ab]	0.52 ± 0.42[b]
SCC ($\times 10^3$ cells/mL)	467 ± 361[a]	394 ± 403[ab]	191 ± 186[b]
pH[3]	6.61 ± 0.09[a]	6.56 ± 0.13[ab]	6.5 ± 0.10[b]
Lactose (%)	4.41 ± 0.22	4.33 ± 0.21	4.40 ± 0.23
TS (%)	10.42 ± 1.29	10.74 ± 1.22	10.60 ± 2.09
Protein fractions in % of total protein			
Total casein	89.59 ± 2.45	90.31 ± 1.66	90.29 ± 1.27
α_{S1}-CN	4.32 ± 3.47[a]	7.57 ± 5.23[b]	15.26 ± 4.01[c]
α_{S2}-CN	5.22 ± 1.50	5.73 ± 1.50	5.03 ± 1.09
β-CN	69.70 ± 4.65[a]	65.60 ± 7.16[b]	59.35 ± 4.65[c]
κ-CN	10.36 ± 2.57	11.40 ± 2.52	10.65 ± 2.57
Total whey protein (% of total protein)	7.68 ± 2.22	7.56 ± 1.41	6.71 ± 1.34
α-LA	2.82 ± 1.74	2.63 ± 0.73	2.66 ± 0.67
β-LG	4.80 ± 1.56	4.93 ± 1.19	4.05 ± 1.32
Proteolytic activity			
Plasmin (U/mL)	8.02 ± 4.43	5.68 ± 4.40	6.61 ± 4.62
Plasminogen (U/mL)	19.14 ± 3.68	19.03 ± 3.92	16.26 ± 3.81
Total proteolysis (eq. m*M* leucine)	44.71 ± 14.13	42.47 ± 14.88	42.08 ± 11.83

[a–c]Values within rows with different letters are significantly different ($P < 0.05$).

[1]Because only a few goats carried an A allele, all genotypes containing at least one A allele were combined into one group (DA/AG/AA). Mean values ± SD. Differences between the groups were evaluated by Tukey pairwise comparison and were considered significant at $P < 0.05$.

[2]n = number of goats with each genotype.

[3]For the DD genotype, pH was measured in 42 goat milk samples (n = 42).

Differences in Milk Quality Traits Between the CSN1S1 Genotypes

Table 4 shows differences in milk quality traits between the *CSN1S1* genotypes DD, DG, and DA/AG/AA. Comparing the DD group to DG and DA/AG/AA, the relative concentration of α_{S1}-CN was 43% and 72% lower, respectively, in milk from the DD group ($P < 0.001$), while the relative concentration of α_{S1}-CN in milk from the DG group was 50% lower than in milk from the DA/AG/AA group ($P < 0.001$). The allele groups also showed significant differences in SCC, FFA, pH, and relative concentration of β-CN. The SCC and FFA values were 59% and 50% lower, respectively, in milk from DA/AG/AA goats than in milk from DD goats ($P = 0.006$, $P = 0.022$), while the relative concentration of β-CN in milk from DD goats was 6% and 16% higher, respectively, than in milk from DG and DA/AG/AA goats ($P < 0.001$). Additionally, pH was lower in milk from DA/AA/AG compared with DD goats ($P = 0.007$). No other milk quality traits investigated differed between the groups of genotypes.

DISCUSSION

Expression of α_{S1}-CN within the Swedish goat population has been studied previously (Johansson et al., 2014, 2015). However, to our knowledge the present study was the first to examine associations between α_{S1}-CN levels in milk, goat genotype at the *CSN1S1* gene, and quality traits of milk from Swedish Landrace goats. In this study, the analysis included a wide range of variables, leading to a better understanding of problems associated with low expression of α_{S1}-CN in the Swedish dairy goat population.

The distribution of goats producing low (72%) and medium-high (28%) levels of α_{S1}-CN in this study was similar to the distribution previously reported by Johansson et al. (2014), suggesting that the mutation at the *CSN1S1* gene in Swedish goats is not under control. This was confirmed by the distribution of genotypes at the *CSN1S1* gene, with 59% of the goats investigated being homozygous for the Norwegian null allele D (Table 2). This incidence is lower than in a Norwegian survey by Devold et al. (2011), which genotyped 70% of

the studied goat population as DD. Moreover, only 15% of the goat population in our study carried at least one A allele (i.e., the strong allele). Milk from goats with the A allele has been associated with higher content of total protein, fat, and calcium and with properties favoring cheese production (e.g., shorter coagulation time, higher cheese yield, and superior product quality; Clark and Sherbon, 2000). On investigating different milk quality traits in Norwegian goat milk in relation to the class of genetic variants of α_{S1}-CN, Devold et al. (2011) found that the average protein content in milk from goats with the strong variant was significantly higher (2.98 g/100 g) than in milk from goats with the weak and null variants (2.94 and 2.87 g/100 g, respectively).

For many years, the frequency was high in the Norwegian goat population of a defective allele variant with a deletion of a single nucleotide in exon 12, a defect so far only reported in Norwegian dairy goats (Devold et al., 2011). Homozygote goats with this allele expressed low levels or no α_{S1}-CN compared with heterozygote goats. In 2003, the frequency of Norwegian bucks carrying this defective allele was 80%, but after introduction of a national breeding goal to reduce the occurrence of the defective allele, its frequency in the Norwegian goat population was reduced to 16% by 2012 (Ådnøy, 2014).

Despite only a few animals having at least one A allele in this study, that allele had a clear effect, with carriers having higher levels of α_{S1}-CN than goats with only D or G alleles (Figure 1). This is in agreement with the results from Norwegian goat investigations (Dagnachew et al., 2011). Unfortunately, none of the goats included in this study had the GG genotype, and only one had the AA genotype, which makes conclusions about the effect of all genotypes impossible. Since the A and G alleles are less common than the D allele, homozygotes of G and A will be rare in the population.

There was wide variation in the goat milk quality traits investigated, as illustrated by the PCA (Figure 2). Several milk quality traits were associated with level of α_{S1}-CN, while the PCA results also suggested an association between genotype at the *CSN1S1* gene and milk quality, with allele groups separated along principal component (**PC**) 2. With some exceptions, values for milk from goats with medium-high relative concentration of α_{S1}-CN in their milk and for milk from goats with a genotype including at least one A allele were negatively associated with PC2 and located in the lower quadrants of the score plot. Although PC2 explained only a smaller proportion of the variation in the data, the distribution could be attributed to differences in milk composition between the groups, as visualized in the loading plot (Figure 2A). In our study and in studies by Ambrosoli et al. (1988) and Clark and Sherbon (2000), the total protein content was significantly lower in milk from goats with low levels of α_{S1}-CN (Table 3). This was also indicated in the PCA plots, where lower contents of total protein and lower relative concentrations of κ-CN were associated with the group of goats with a low relative concentration of α_{S1}-CN (Figure 2). Although the PCA suggested an association between genotypes with the A allele and higher total protein content, differences between the groups of genotypes were not significant. This lack of significance is possibly due to the limited number of individuals in the sample (n = 11 goats), and numerical values for total protein were highest in the group with at least one A allele. Goats with low levels of α_{S1}-CN in their milk showed higher relative concentrations of α_{S2}-CN and β-CN. Likewise, higher relative concentrations of β-CN were observed in milk associated with the DD genotype. Similar associations were observed by Balia et al. (2013), who found that Sarda goats with lower production of α_{S1}-CN showed partial compensation in their production of β-CN, α_{S2}-CN, and κ-CN. This suggests that when expression of one casein gene is downregulated, the others can be upregulated to compensate (Bovenhuis et al., 1992; Leroux et al., 2003). In our study, however, the relative concentration of κ-CN, known as a technologically important protein in milk coagulation and significant for cheese yield, was lower in milk from goats with lower production of α_{S1}-CN.

Comparing levels of total protein, fat, lactose, and TS in milk from Swedish Landrace goats to those reported for other breeds showed that levels were generally lower in the Swedish goats. The total protein content in milk from the Swedish goat population investigated was on average 2.45 g/100 g, compared with 3.87 and 2.53 g/100 g for the Nubian and Alpine breeds, respectively (Soryal et al., 2005). The genotype distribution of goats of the Alpine type is characterized by higher frequencies of intermediate and weak alleles, while in the Mediterranean breeds, strong alleles prevail (Balia et al., 2013)

The SCC in milk is used as a measure of udder health status and as an indicator of milk quality, with goat milk having naturally higher SCC values than cow milk (Albenzio et al., 2015). Mean SCC in milk from the goat population investigated in this study was 408 × 10^3 cells/mL (Table 4), which is well below the 700 × 10^3 cells/mL threshold indicating impaired udder health status in goats (Albenzio et al., 2015). The SCC was significantly higher in milk from goats homozygous for the null allele than in milk from goats with at least one A allele. Balia et al. (2013) also investigated the association between SCC and genotype but found no association. Although the difference was not significant ($P = 0.09$), SCC was lower in milk with medium-high levels of α_{S1}-CN than in milk with low levels of α_{S1}-CN.

A negative association between level of α_{S1}-CN and pH, as observed in this study, has previously been reported in studies by Ambrosoli et al. (1988), Pierre et al. (1998), and Johansson et al. (2015). Consequently, pH was significantly lower in the group with a medium-high level of α_{S1}-CN compared with a low level of α_{S1}-CN (Table 3). Likewise, when comparing the genotypes with at least one A allele to the group homozygous for the null allele (DD), milk from goats with the strong A allele had significantly lower pH than milk from goats with the DD genotype (Table 4). The lower pH in milk with higher levels of α_{S1}-CN could benefit coagulation rate and curd firmness, which is well documented for cow milk (Ambrosoli et al., 1988) as well as for goat milk (Johansson et al., 2015). Pierre et al. (1998) suggested that the lower pH of milk from goats with the A allele is associated with the difference in total nonfat solids in the milk.

Elevated levels of FFA in raw milk, as a result of spontaneous or induced lipolysis, are responsible for rancidity (Deeth, 2006), a quality defect that is generally unacceptable to the consumer. In our study, the content of FFA was significantly higher in milk from the group with low α_{S1}-CN than in milk from the group with medium-high levels (Figure 2 and Table 3). Similarly, milk from goats with the null genotype DD had significantly higher levels of FFA (Table 4), an association reported previously by Pierre et al. (1998). Ådnøy et al. (2014) found that milk from goats with the specific deletion of the *CSN1S1* on exon 12 was associated with a markedly stronger milk taste. Interestingly, Cebo et al. (2012) found that the genetic polymorphism at the α_{S1}-CN gene affected both the structure and the composition of milk fat globules. Those authors suggested that the higher content of FFA in milk with a low level of α_{S1}-CN may be explained by increased lipolytic enzyme-fat interactions due to a larger specific surface area of fat globules in milk from goats with the null allele.

CONCLUSIONS

Results of this study indicated a strong association between milk levels of α_{S1}-CN and allele combination at the *CSN1S1* gene in Swedish Landrace goats. Although the D allele, leading to extremely low expression of α_{S1}-CN, was found to be the most common allele, one A allele alone was sufficient to significantly increase expression of α_{S1}-CN. However, the low frequency of the A allele or other strong alleles in the Swedish goat population is troublesome, affecting not only expression of α_{S1}-CN but also total protein content of the milk. In Sweden, as in many other countries, goat milk is mainly used for different types of cheeses, and despite small-scale and local production, Swedish goat cheese has a significant number of consumers. Following on successful work in Norway, a national breeding program to reduce the unwanted D allele is therefore of great value to increase the profitability of Swedish goat cheese producers.

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REFERENCES

Ådnøy, T. 2014. The dairy goat industry in Norway: Challenges in a historical perspective. Small Rumin. Res. 122:4–9. https://doi.org/10.1016/j.smallrumres.2014.07.011.

Albenzio, M., A. Santillo, A. L. Kelly, M. Caroprese, R. Marino, and A. Sevi. 2015. Activities of indigenous proteolytic enzymes in caprine milk of different somatic cell counts. J. Dairy Sci. 98:7587–7594. https://doi.org/10.3168/jds.2015-9762.

Ambrosoli, R., L. Di Stasio, and P. Mazzocco. 1988. Content of α_{S1}-casein and coagulation properties in goat milk. J. Dairy Sci. 71:24–28. https://doi.org/10.3168/jds.S0022-0302(88)79520-X.

Balia, F., M. Pazzola, M. L. Dettori, M. C. Mura, S. Luridiana, V. Carcangiu, G. Piras, and G. M. Vacca. 2013. Effect of *CSN1S1* gene polymorphism and stage of lactation on milk yield and composition of extensively reared goats. J. Dairy Res. 80:129–137. https://doi.org/10.1017/S0022029912000702.

Bonfatti, V., A. Cecchinato, G. Di Martino, M. De Marchi, L. Gallo, and P. Carnier. 2011. Effect of κ-casein B relative content in bulk milk κ-casein on Montasio, Asiago, and Caciotta cheese yield using milk of similar protein composition. J. Dairy Sci. 94:602–613. https://doi.org/10.3168/jds.2010-3368.

Bosona, T., and G. Gebresenbet. 2018. Swedish consumers' perception of food quality and sustainability in relation to organic food production. Foods 7:54. https://doi.org/10.3390/foods7040054.

Bovenhuis, H., J. A. Van Arendonk, and S. Korver. 1992. Associations between milk protein polymorphisms and milk production traits. J. Dairy Sci. 75:2549–2559. https://doi.org/10.3168/jds.S0022-0302(92)78017-5.

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. https://doi.org/10.3168/jds.2006-674.

Cebo, C., C. Lopez, C. Henry, C. Beauvallet, O. Ménard, C. Bevilacqua, F. Bouvier, H. Caillat, and P. Martin. 2012. Goat α_{s1}-casein genotype affects milk fat globule physicochemical properties and the composition of the milk fat globule membrane. J. Dairy Sci. 95:6215–6229. https://doi.org/10.3168/jds.2011-5233.

Clark, S., and J. Sherbon. 2000. Alpha$_{s1}$-casein, milk composition and coagulation properties of goat milk. Small Rumin. Res. 38:123–134. https://doi.org/10.1016/S0921-4488(00)00154-1.

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. Genet. Sel. Evol. 43:31. https://doi.org/10.1186/1297-9686-43-31.

de Vries, R., M. Brandt, Å. Lundh, K. Holtenius, K. Hettinga, and M. Johansson. 2016. Short communication: Influence of shortening the dry period of Swedish dairy cows on plasmin activity in milk. J. Dairy Sci. 99:9300–9306. https://doi.org/10.3168/jds.2016-11502.

Deeth, H. C. 2006. Lipoprotein lipase and lipolysis in milk. Int. Dairy J. 16:555–562. https://doi.org/10.1016/j.idairyj.2005.08.011.

Devold, T. G., R. Nordbø, T. Langsrud, C. Svenning, M. Jansen 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. https://doi.org/10.1051/dst/2010033.

Ditlevsen, K., S. Denver, T. Christensen, and J. Lassen. 2020. A taste for locally produced food—Values, opinions and sociodemographic differences among "organic" and "conventional" consumers. Appetite 147:104544. https://doi.org/10.1016/j.appet.2019.104544.

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. https://doi.org/10.1534/genetics.106.058966.

Johansson, M., M. Akerstedt, S. Li, G. Zamaratskaia, and Å. S. Lundh. 2013. Casein breakdown in bovine milk by a field strain of *Staphylococcus aureus*. J. Food Prot. 76:1638–1642. https://doi.org/10.4315/0362-028X.JFP-13-112.

Johansson, M., M. Högberg, and A. Andrén. 2014. High frequencies of the αS1-casein zero variant in milk from Swedish dairy goats. Sheep Goat Res. J. 29:1–4.

Johansson, M., M. Högberg, and A. Andrén. 2015. Relation between α_{S1}-casein content and coagulation properties of milk from Swedish dairy goats. Open Food Sci. J. 9:1–4. https://doi.org/10.2174/1874256401509010001.

Johansson, M., Å. Lundh, R. de Vries, and K. S. Sjaunja. 2017. Composition and enzymatic activity in bulk milk from dairy farms with conventional or robotic milking systems. J. Dairy Res. 84:154–158. https://doi.org/10.1017/S0022029917000140.

Jordana, J., M. Amills, E. Diaz, C. Angulo, J. M. Serradilla, and A. Sanchez. 1996. Gene frequencies of caprine α_{s1}-casein polymorphism in Spanish goat breeds. Small Rumin. Res. 20:215–221. https://doi.org/10.1016/0921-4488(95)00813-6.

Jordbruksverket. 2019. Gethållning 2018. Rapportserie 2019:01. Accessed Mar. 29, 2023. https://jordbruksverket.se/download/18.29196bdf172db848a9e1384f/1592839852350/201901.pdf.

Leroux, C., F. Le Provost, E. Petit, L. Bernard, Y. Chilliard, and P. Martin. 2003. Real-time RT-PCR and cDNA macroarray to study the impact of the genetic polymorphism at the *α_{s1}-casein* locus on the expression of genes in the goat mammary gland during lactation. Reprod. Nutr. Dev. 43:459–469. https://doi.org/10.1051/rnd:2003032.

Marletta, D., A. Criscione, S. Bordonaro, A. M. Guastella, and G. d'Urso. 2007. Casein polymorphism in goat's milk. Lait 87:491–504. https://doi.org/10.1051/lait:2007034.

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. https://doi.org/10.1016/S0958-6946(99)00055-2.

Mason, I. L. A. 1996. World Dictionary of Livestock Breeds, Types and Varieties. 4th ed. CAB International.

Mestawet, T. A., A. Girma, T. Ådnøy, T. G. Devold, and G. E. Vegarud. 2013. Newly identified mutations at the *CSN1S1* gene in Ethiopian goats affect casein content and coagulation properties of their milk. J. Dairy Sci. 96:4857–4869. https://doi.org/10.3168/jds.2012-6467.

Park, Y. W., M. Juárez, M. Ramos, and G. F. W. Haenlein. 2007. Physico-chemical characteristics of goat and sheep milk. Small Rumin. Res. 68:88–113. https://doi.org/10.1016/j.smallrumres.2006.09.013.

Pierre, A., J.-L. Le Quéré, A. Riaublanc, Y. Le Graët, D. Demaizieres, and F. Michel. 1998. Composition and physico-chemical characteristics of goat milks containing the A or O α_{sl} casein variants. Lait 78:191–202. https://doi.org/10.1051/lait:1998222.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.R-project.org.

Sacchi, P., S. Chessa, E. Budelli, P. Bolla, G. Ceriotti, D. Soglia, R. Rasero, E. Cauvin, and A. Caroli. 2005. Casein haplotype structure in five Italian goat breeds. J. Dairy Sci. 88:1561–1568. https://doi.org/10.3168/jds.S0022-0302(05)72825-3.

Skeie, S. B. 2014. Quality aspects of goat milk for cheese production in Norway: A review. Small Rumin. Res. 122:10–17. https://doi.org/10.1016/j.smallrumres.2014.07.012.

Soryal, K., F. A. Beyene, S. Zeng, B. Bah, and K. Tesfai. 2005. Effect of goat breed and milk composition on yield, sensory quality, fatty acid concentration of soft cheese during lactation. Small Rumin. Res. 58:275–281. https://doi.org/10.1016/j.smallrumres.2004.11.003.

Vidanarachchi, J. K., S. Li, Å. S. Lundh, and M. Johansson. 2015. Short communication: Lipolytic activity on milk fat by *Staphylococcus aureus* and *Streptococcus agalactiae* strains commonly isolated in Swedish dairy herds. J. Dairy Sci. 98:8560–8564. https://doi.org/10.3168/jds.2015-9559.

Wiking, L., M. B. Frost, L. B. Larsen, and J. H. Nielsen. 2002. Effects of storage conditions on lipolysis, proteolysis and sensory attributes in high quality raw milk. Milk Wissenschaft 57:190–194.

Wold, S., K. Esbensen, and P. Geladi. 1987. Principal component analysis. Chemom. Intell. Lab. Syst. 2:37–52. https://doi.org/10.1016/0169-7439(87)80084-9.