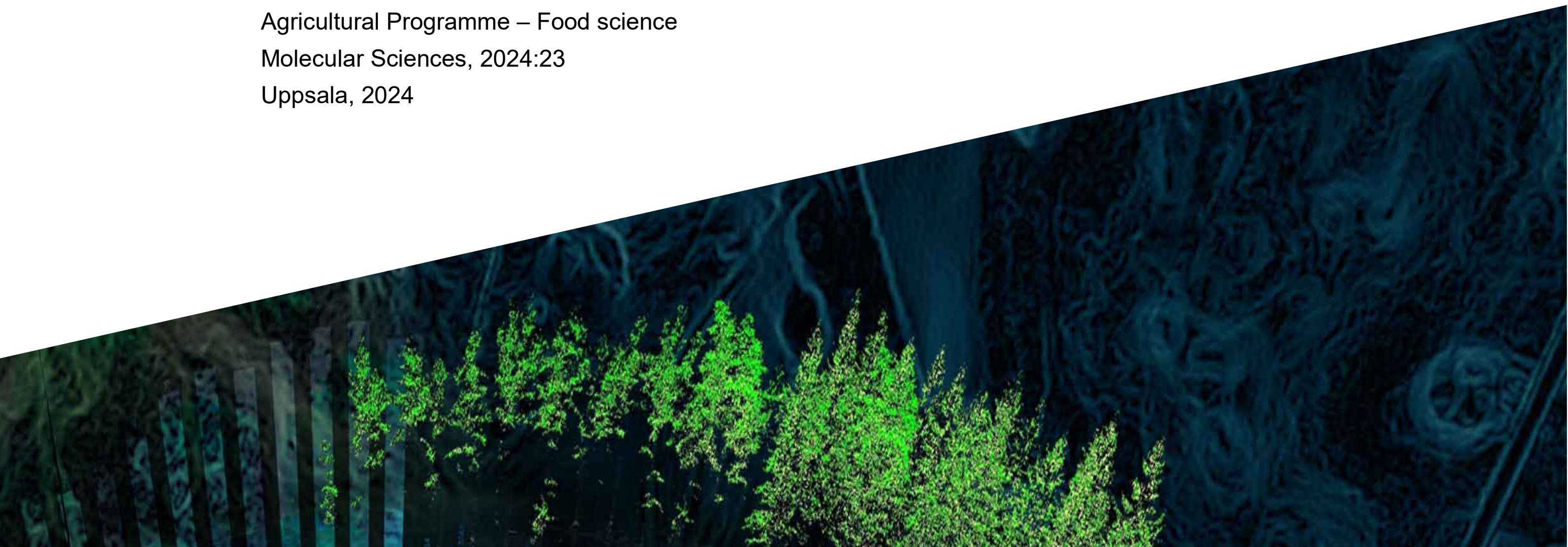
Relation between curd yield and αs_1-casein in milk from Swedish dairy goats

Sambandet mellan utbyte av ostmassa och αs1-kasein i mjölk från svenska getter

Ida Åkerberg

Degree project/Independent project • 30 credits
Swedish University of Agricultural Sciences, SLU
Department of Molecular Sciences
Agricultural Programme – Food science
Molecular Sciences, 2024:23
Uppsala, 2024



Relation between curd yield and αs_1-casein in milk from Swedish dairy goats

Sambandet mellan utbyte av ostmassa och αs_1-kasein i mjölk från svenska getter

Ida Åkerberg

Supervisor: **Monika Johansson, SLU, Department of Molecular Sciences**

Examiner: Åse Lundh, SLU, Department of Molecular Sciences

Credits:	30 credits
Level:	A2E
Course title:	Master thesis in Food science
Course code:	EX0877
Programme/education:	Agricultural Programme – Food science
Course coordinating dept:	Department of Molecular Sciences
Place of publication:	Uppsala
Year of publication:	2024
Title of series:	Molecular Sciences
Part number:	2024:23

Keywords: αs_1-casein, Swedish Landrace goats, goat milk, cheese yield

Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Sciences
Department of Molecular Sciences

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for **YES**, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for **NO**, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file. If you are more than one author, the checked box will be applied to all authors. You will find a link to SLU's publishing agreement here:

- https://libanswers.slu.se/en/faq/228318.

☒ YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

☐ NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.

Abstract

The demand for locally produced food, such as artisan-made goat cheese, is growing among Swedish consumers. In Sweden, the most commonly used goat breed for dairy production is the Swedish Landrace goat which is closely related to the Norwegian Landrace goat. The Norwegian Landrace goat has been found to have extremely low levels of αs_1-casein (αs_1-CN) due to a mutation in the *CSN1S1* gene which results in a gene variant called D. The expression of αs_1-CN in goat milk might influence the amounts of total protein, fat and calcium in the milk, affecting the milk quality in connection to cheesemaking.

The aim of this study was to investigate the relation between αs_1-CN, *CSN1S1* genotype and curd yield in milk from Swedish Landrace goats. Milk samples were collected from 143 Swedish Landrace goats from 3 farms. A mini-manufacturing method for assessing curd yield from the individual milk samples was used. The milk samples were also analysed for gross composition, milk protein profile, and somatic cell count. Milk samples were grouped according to their relative concentration of αs_1-CN (low: 0–6.9% of total protein, medium-high: 7–25% of total protein) and according to their genotype at the *CSN1S1* gene (AA, AG, DA, DD, DG/GG). Obtained data were evaluated by 1-way ANOVA and Tukey pairwise comparison test. The results showed that 48% of the samples had a low relative concentration of αs_1-CN (0–6.9% of total protein), while 52% of the samples contained a medium-high relative concentration of this casein (7–25% of total protein). The frequency of goats carrying the DD genotype was 19% in the sampled population and 24% carried the AA genotype classified as "strong", leading to high expression of αs_1-CN. The relative concentration of αs_1-CN in milk from goats with the DD genotype was 69%, 55% and 54% lower, respectively, then in milk from goats carrying the AA, AG and DA genotypes ($p < 0.001$). For goats with the DG/GG genotype, the relative concentration of αs_1-CN in the milk was 57%, 37% and 35% lower, respectively, then in milk from the AA, AG and DA goats ($p < 0.001$). No significant association between the relative concentration of αs_1-CN and curd yield was found in this study. Curd yield was also not significantly different between genotypes, and goats carrying the DD genotype had the highest curd yield numerically. Milk samples with medium-high relative content of αs_1-CN had 8% higher total protein ($p = 0.006$) and 13 % higher fat content ($p = 0.007$) than samples of the group with low relative content of αs_1-CN. Milk from goats with low relative content of αs_1-CN had 5% higher total casein content ($p < 0.001$), 13% higher β-CN content ($p = 0.001$) and 21% higher κ-CN content ($p = 0.001$). Significant differences in total solids and fat content were found between the AA, DA and DG/GG genotypes ($p = 0.002$, $p = 0.003$). Pearson's correlation indicated a trend for a negative association between the curd yield and the relative concentration of αs_1-CN, but the correlation was not significant ($p = 0.543$). However, curd yield was positively related to total solids ($p < 0.001$), total fat ($p = 0.018$), total protein ($p < 0.001$), casein ($p < 0.001$), and casein ratio ($p = 0.007$). In conclusion, the total protein, fat and casein content were found to be more associated with the curd yield than αs_1-CN. These results can be useful for further improvement of the breeding program of Swedish dairy goats but also for better understanding of parameters important for cheesemaking.

Keywords: αs_1-casein, Swedish Landrace goats, goat milk, curd yield

Sammanfattning

Efterfrågan på närproducerad mat, som hantverksmässigt tillverkad getost, växer bland svenska konsumenter. I Sverige utgör Svensk lantras den viktigaste getrasen för mejeriproduktion, en ras som är nära besläktad med den norska lantrasgeten. Den norska lantrasgeten har visat sig ha extremt låga halter αs_1-kasein (αs_1-CN) i sin mjölk. Detta förklaras av en oönskad mutation i *CSN1S1* genen vilket har gett upphov till genvarianten D. Uttrycket av αs_1-CN påverkar mängden totalprotein, fett och kalcium i mjölken, vilket i sin tur påverkar mjölkkvalité i samband med osttillverkning. Syftet med denna studie var att undersöka sambandet mellan αs_1-CN, *CSN1S1* genotyp och utbyte av ostmassa i mjölk från svenska lantrasgetter. Mjölkprover samlades in från 143 getter av svensk lantras från 3 svenska gårdar. En laborativ metod för produktion av ostmassa användes för att bedöma ostutbytet från individuella mjölkprover. Utöver detta analyserades också mjölksammansättning, mjölkens proteinprofil och celltal. Mjölkproverna grupperades utifrån deras relativa koncentration av αs_1-CN (låg: 0–6,9% av totalprotein och medel/hög: 7-25% av totalprotein), och utifrån genotyp (AA, AG, DA, DD, DG/GG). Data utvärderades med hjälp av envägs variansanalys (ANOVA) och parvis jämförelse utfördes med Tukeys test. Resultaten visade att 48 % av mjölkproverna innehöll låg relativ koncentration αs_1-CN (0–6,9% av totalprotein), medan 52 % av proverna innehöll medel/höga relativ koncentration (7–25% av totalprotein). DD genotypen återfanns hos 19% av getterna och 24% bar AA genotypen i *CSN1S1*-genen, en stark variant som leder till ett högt uttryck av αs_1-CN. Den relativa koncentration av αs_1-CN i mjölk från getter med DD genotypen var 69 %, 55 % respektive 54 % lägre än i mjölk från getter med genotyperna AA, AG och DA ($p < 0,001$). För getter med DG/GG genotyperna var den relativa koncentrationen av αs_1-CN i mjölken 57 %, 37 % respektive 35 % lägre än i mjölk från getter med AA, AG och DA genotyper ($p < 0,001$). Det fanns inget signifikant samband mellan utbytet av ostmassa och den relativa koncentrationen av αs_1-CN i mjölken. Utbytet av ostmassa skilde sig inte signifikant mellan de olika genotyperna, men numeriskt så gav mjölk från getter som bar på DD genotypen högst mängd ostmassa. Mjölk från getter med medel/hög relativ koncentration av αs_1-CN hade 8% högre mängd totalprotein ($p = 0,006$) och 13% högre fetthalt ($p = 0,007$) än mjölkprover inom den låga gruppen. Mjölk från getter med låg relativ koncentration av αs_1-CN hade 5% högre mängd totalkasein ($p < 0.001$), 13 % högre mängd β-CN ($p = 0,001$) och 21 % högre mängd κ-CN ($p = 0,001$). Signifikanta skillnader i mängd torrsubstans och fetthalt fanns mellan genotyperna AA, DA och DG/GG ($p = 0,002$, $p = 0,003$). Pearsons korrelation påvisade ett negativt samband mellan mängden ostmassa och nivåer av αs_1-CN, men skillnader var enbart numeriska ($p = 0,543$). En positiv korrelation fanns dock mellan utbyte av ostmassa och halten total torrsubstans ($p < 0,001$), fetthalt ($p = 0,018$), totalprotein ($p < 0,001$), kasein ($p < 0,001$) och kasein-kvoten ($p = 0,007$). Sammanfattningsvis så visade det sig att totala mängden torrsubstans samt totalprotein, fett och kasein var mer kopplat till utbytet av ostmassa än halten αs_1-CN. Dessa resultat kan vara användbara för att få ett förbättrat avelsprogram och kunskap om osttillverkningsprocesser kopplad till den svenska getmjölken.

Nyckelord: αs_1-kasein, svensk lantrasget, getmjölk, utbyte av ostmassa

Table of contents

List of tables

List of figures

Abbreviations

CE	Capillary electrophoresis
CN	Casein
GMP	Glycomacropeptide
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
SCC	Somatic cell count
SFA	Saturated fatty acids
TWP	Total whey protein
UFA	Unsaturated fatty acids

1. Introduction

Goat milk has a long history in human nutrition and has played an important role providing high quality food (Boyazoglu et al. 2005). It is believed that goats were one of the first species to be domesticated approximately 10 000 years ago (Nomura et al. 2013). Goats are high productivity animals. Considering their small size and feed intake, the milk yield is often exceptional. They are also great at adapting to different environmental conditions, have a low value feed acceptance and are easy to keep (Monteiro et al. 2018).

The dairy goat industry is rapidly expanding over the world, with a continuous increase in the number of dairy goats. There are approximately one billion goats globally and the number of goats has more than doubled during the last four decades (Utaaker et al. 2021). About 200 millions of these were used as dairy goats, producing roughly 15 million tons of goat milk yearly (ALKaisy et al. 2023). Most of the goats (>90%) are found in developing countries, mainly in Asia followed by Africa (Utaaker et al. 2021). It is likely that more people consume goat milk than milk from any other animals (Silanikove et al. 2010). The demand for goat cheese is increasing among consumers. Europe stands for approximately 35% of the global goat cheese production (Morales et al. 2019). In countries such as France and Italy, cheese production is a significant industry, and both countries are known for producing high quality goat cheeses (Silanikove et al. 2010).

Locally produced food, such as artisan-made goat cheese, is becoming more and more popular among Swedish consumers (Bosona & Gebresenbet 2018). In earlier times, goat breeding had an important role in the traditional Swedish agriculture. It was largely an activity for the rural population and served as an important livelihood source to resource-poor farmers (Rytkönen et al. 2013). Today, goat farming is rather small scaled but increasingly growing in the Swedish agriculture. However, there is no official registration for goats in Sweden and the last census of the Swedish goat population was carried out in 2018. At that time, the total number of goats was estimated at 20 000 (Jordbruksverket, 2019a), which is low in comparison to the Swedish population of sheep and cattle which at that time were approximately 587 000 and 1.4 million, respectively, (Jordbruksverket, 2019a; Jordbruksverket, 2019b). The number of registered goat farmers was approximately

746 in 2018 and the amount of goat milk and goat milk cheese produced was approximately 1374 and 126 tons, respectively (Jordbruksverket 2019a).

There are four Swedish goat breeds of which the Swedish Landrace goat is the most common dairy goat breed used for milk and cheese production. This breed is high yielding and has an average annual yield of 700 litres of milk per goat. However, individual goats producing up to 2000 litres per year have been recorded (Svenska Getavelsförbundet 2021). Studying goat farming in other Nordic countries, Norway has the largest established dairy goat industry. Here, goat milk has traditionally been used and is still largely used to produce brown "whey cheese" (Skeie 2014; Ådnøy 2014). Studies on the Norwegian Landrace goat population have previously shown that this breed has a high prevalence of polymorphism at the *CSN1S1* gene, affecting αs_1-casein (αs_1-CN) levels in the milk. A "null" variant of the gene, leading to zero or very low expression of αs_1-CN, was found to be predominant (>70%) in the breed (Devold et al. 2010; Dagnachew et al. 2011). It has been revealed that polymorphism in the *CSN1S1* locus effects the composition and technological properties of milk which is believed to affect the development of dairy goat products (Dagnachew et al. 2011). The Swedish Landrace goat and the Norwegian Landrace goat are closely related to each other since cross-breeding between these two has been going on over time. During the 80's and 90's, the Norwegian goat was frequently used to avoid inbreeding in the Swedish population (Svenska Getavelsförbundet 2021). Similar to the situation for the Norwegian goats, previous studies conducted on Swedish goats have reported low relative concentrations of αs_1-CN in the milk (Johansson et al. 2014, 2015, 2023), which is thought to negatively affect cheesemaking properties. The Swedish Board of Agriculture has not really invested in any official control of the goat farming in Sweden. Instead, the Swedish Goat Breeding Association is the organization responsible for the goats, however, on a voluntary basis. This organization is currently working with developing strategies for breeding against the mutation of the *CSN1S1* gene in order to benefit the producers of dairy products based on goat milk (Svenska Getavelsförbundet 2021).

1.1 Aim

The aim of this study was to investigate if the expression of αs_1-CN in the milk from Swedish dairy goats is associated to the composition and properties, specifically the curd yield, of the milk. The hypothesis was that the concentration of αs_1-CN in the milk influences the curd yield.

2. Background

2.1 Composition of goat milk

Information about the composition of goat milk is essential for successful development of dairy goat products. The average composition of goat milk compared to cow milk is shown in Table 1. However, there are great variations in the composition of milk, both among the different goat breeds and due to factors, such as lactation stage, environmental conditions, feeding, health status and genetics (Park et al. 2007). Generally, it can be said that goat milk composition is similar to that of bovine milk. They both contain approximately 3-3.5 g protein/100g, with casein constituting 80% and whey protein 20% of total proteins (Prosser 2021). The caseins are divided into four subtypes: αs1-casein (αs_1-CN), αs2-casein (αs_2-CN), β-casein (β-CN) and κ-casein (κ-CN) where the genes encoding the caseins are *CSN1S1*, *CSN1S2*, *CSN2* and *CSN3*, respectively (Rahmatalla et al. 2022). The main whey proteins in milk are α-lactalbumin (α-La) and β-lactoglobulin (β-Lg) (Martin et al. 2002). αs_1-CN is one of the major proteins in bovine milk, constituting up to 40% of the total caseins (Farrell et al. 2004). In goat milk, the αs_1-CN concentration varies between 0-25% (Rahmatalla et al. 2022). The amount of κ-CN and αs_2-CN are similar for both goat and cow milk; however, goat milk contains more of β-CN than cow milk (Clark & Sherbon 2000; Martin et al., 2002).

Caseins organize themselves with calcium phosphate in the form of a structure called micelles. The casein micelles have a key role in preventing precipitation of caseins and, along with fat globules, whey proteins and minerals, make sure that milk is stable as an emulsion (Runthala et al. 2023). Casein micelles in goat milk in general contain more calcium and inorganic phosphorus and are larger in size than bovine casein micelles (Park et al. 2007; Roy et al. 2021). The size of the casein micelles in goat milk is related to the content of αs_1-CN in the milk. Smaller mean sizes are associated with high αs_1-CN levels in the milk while larger mean sizes are associated with low αs_1-CN levels (Pierre et al. 1999; Panthi et al. 2017).

The fat contents of goat and cow milk are relatively similar, but the fatty acid profiles differs to some extent (Prosser 2021). Goat milk contains more short-, medium- and branched-chained fatty acids, which is what gives the goat milk its distinct flavour (Prosser 2021; Yurchenko et al. 2018). Goat milk fat has higher digestibility compared to cow milk fat, due to the smaller mean milk fat globule size and the higher concentration of short- and medium chained fatty acids. The quantity and composition of fat in goat milk influences the texture, aroma and flavour of dairy goat products and is highly affected by feed, stage of lactation and breed (Yurchenko et al. 2018; Currò et al. 2019).

Table 1. Average composition of goat- and cow milk

Component	Goat	Cow	Reference
Total solids	13.60	11.40	Ceballos et al. 2009
Total protein (g/100 ml)	3.30	3.40	Prosser 2021
CN (% of total protein)	83.00	83.00	Prosser 2021
TWP (% of total protein)	17.00	17.00	Prosser 2021
Lactose (g/100 ml)	4.10	4.50	Prosser 2021; Ceballos et al. 2009
Total fat (g/100 ml)	3.50	4.00	Prosser 2021; Walstra et al. 2006
SFA (% of total FA)	70.42	71.24	Ceballos et al. 2009
MUFA (% of total FA)	25.67	25.56	Ceballos et al. 2009
PUFA (% of total FA)	4.08	3.20	Ceballos et al. 2009

CN = casein; FA = fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; TWP = total whey protein.

2.2 Genetic polymorphism of *CSN1S1* in goats

The protein coding gene *CSN1S1* is responsible for encoding of αs_1-CN, which is one of the four casein proteins in goat milk. αs_1-CN has an important role in the transportation of calcium phosphate and the transport of other caseins from the endoplasmic reticulum. It plays a part in the casein transportation in the secretory pathway and in the biosynthesis of casein micelles (Rahmatalla et al. 2022). *CSN1S1* is polymorphic, meaning that the gene carries more than one allele on its locus, which leads to several types of existing variants. These different variants have been reported to affect milk quality traits such as milk composition, milk yield as well as coagulation properties (Tumino et al. 2023). In total 18 different allelic variations of the gene have been identified, and they are divided into four groups depending on their expression of αs_1-CN. “Strong” variants (A, B_1, B_2, B_3, B_4, B’, C, H, L and M) are linked to high expression of αs_1-CN, “medium” variants (E and I) and “weak” variants (F and G) are associated with medium and low expressions, respectively. The last class comprises the “null” variants (01, 02, N) meaning that the milk only has small traces of αs_1-CN or that αs_1-CN is totally absent in animals which are homozygote for these alleles (Dagnachew et al. 2011; Devold et al. 2010).

Recent studies reported that a large part of the Norwegian goat population carried a polymorphism at the *CSN1S1* gene, i.e. the Norwegian null allele (D). This allele leads to zero or extremely low expression of αs_1-CN (Devold et al. 2011; Skeie et al. 2014; Johansson et al. 2023). Variants expressing high levels of αs_1-CN have been found in the Italian (Dettori et al. 2023), Spanish (Jordana et al. 1996) and in the French (Carillier-Jacquin et al. 2016) goat populations. Studies by Johansson et al (2014, 2015, 2023) investigated the expression of αs_1-CN within the Swedish goat population. Results showed that a large proportion of the tested goat population expressed low relative levels of αs_1-CN (0%–6.9% of the total protein). The estimated percentage of goats that produced low levels of αs_1-CN were 65%, 44% and 72% respectively over the years of investigations. Several studies have been performed to investigate how the different genetic variants affect the composition of goat milk. Variants leading to high expression of αs_1-CN have been positively correlated with a higher amount of total protein, casein, fat, and calcium in milk leading to higher milk quality, specifically in connection to cheesemaking (Clark & Sherbon 2000; Ambrosoli et al. 1988; Devold et al. 2010). It has also been shown that higher αs_1-CN levels lead to lower pH in the milk (Ambrosoli et al. 1988; Johansson et al. 2015).

2.3 Processing properties in goat cheese production

Milk gelation is crucial for the structural development of dairy products such as cheese (Lucey 2002). The casein micelle is naturally covered by κ-casein (κ-CN) which stabilizes the micelle due to both electrostatic and steric repulsion. There are various ways of destabilizing the micelle to cause the gelation of milk. The most used method for cheesemaking is enzymatic hydrolysis of κ-CN by rennet (Li et al. 2023). One of the key enzymes in rennet is chymosin, which cleaves the κ-CN chain at the Phe105-Met106 bond, which results in κ-CN being split into two parts: an insoluble peptide (para κ-CN) and water-soluble glycomacropeptide (GMP). This results in the destabilization of casein micelles which will start to aggregate and form a gel. Finally, the gel is cut to promote syneresis i.e., expulsion of the whey, and a fresh curd is retained (Pazzola 2019).

High quality milk, e.g., milk with a high total solids content, is associated with improved cheesemaking (Ådnøy 2014). Higher concentration of αs_1-CN in the milk has been positively correlated with higher amount of total casein, fat, and calcium (Clark & Sherbon 2000; Ambrosoli et al. 1988; Devold et al. 2010). Casein and fat retain moisture in the curd which contributes to higher cheese yield (Clark &

Sherbon 2000; Pazzola et al. 2019). Moreover, a high content of αs_1-CN has been correlated with higher total protein content and lower pH of the milk which results in firmer gels (Ambrosoli et al. 1988; Devold et al. 2011; Johansson et al. 2015). The level of αs_1-CN has also been found to affect the coagulation properties of milk. Ambrosoli et al. (1988) and Clark & Sherbon (2000) showed that the coagulation time was longer in case of milks with higher content of αs_1-CN. On the other hand, Devold et al. (2011) and Johansson et al. (2015) found the coagulation time to be longer for milks with low level of αs_1-CN. Moreover, the size of the casein micelle in milk is affected by the level of αs_1-CN, with high levels of αs_1-CN resulting in smaller micelles (Pierre et al. 1999; Panthi et al. 2017). Milk with smaller casein micelles coagulated faster and formed firmer curds compared to milk with larger micelles (Devold et al. 2011). The smaller micelles contain more κ-CN, which may result in increased bridging between proteins and calcium during coagulation resulting in increased gel firmness (Panthi et al. 2017). The αs_1-CN content has also been reported to affect sensory quality attributes of cheese. High αs_1-CN level in milk resulted in cheese with improved structure and taste with less typical goat flavour than cheese produced from milk with low αs_1-CN (Rahmatalla et al. 2022). Cheese made from milk with low levels of αs_1-CN were found to have more of a rancid flavour, likely due to more free fatty acids being formed by lipolysis of milk fats (Skeie et al. 2014).

Cheese yield is one of the most important indicators of the efficiency of cheesemaking. A firm curd results in higher retention of milk solids which increase the cheese yield (Clark & Sherbon 2000). Goat milk with low levels of αs_1-CN may be inferior in forming firm gels which results in decreased cheese yield, since casein is lost in the whey as it is not properly bound to the casein network (Skeie 2014). Pirisi et al. (1994) and Frattini et al. (2014) observed that milk with high levels of αs_1-CN from Saanen and Alpine goat breeds led to higher cheese yield compared to milk with low levels of αs_1-CN. Cheese yield is the final economic target for many dairy goat farmers. Therefore, it is important for goat farmers and the cheese industry to understand the effect of milk composition, in relation to αs_1-CN, on the cheesemaking ability.

3. Materials and method

3.1 Animals and milk collection

Goat milk samples were collected from three different farms in the counties of Västerbotten and Ångermanland, Sweden. Out of in total 143 milk samples from individual goats, 69 came from farm 1, 34 from farm 2 and 40 from farm 3. All the collected milk samples were from goats of the Swedish Landrace breed. At two farms, the milk samples were collected during the period November – December 2022, and at one farm they were collected in July 2023. Milk samples were transported to SLU and were stored at -20℃ until use. Factors such as age, lactation stage, number of lactations, diet and health status were not taken into account in this study. This study was conducted in the research facilities at the Department of Molecular Sciences, Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden.

3.2 Production of goat milk curd

3.2.1 Rennet preparation

For each cheesemaking occasion, bovine rennet consisting of 75% chymosin and 25% pepsin and with a concentration of 180 International Milk Clotting Units (IMCU) (Scandirenn Kemikalia AB, Skurup, Sweden), was diluted in water to a final concentration of 18 IMCU.

3.2.2 Analysis of milk pH

Milk pH was measured on the day of the curd making occasion with a pH meter (SevenCompact pH meter S210, Mettler-Toledo, LCC., Columbus, OH, USA). The pH was measured after the frozen milk had been warmed in a water bath at 32°C for 30 min and allowed to cool down to reach a temperature of 22-26°C.

3.2.3 Production of goat milk curd

Frozen whole goat milk samples were incubated in a water bath at 32-35°C for 30 min to thaw the samples and to allow the milk fat to melt. 10 g of milk was then weighed in pre-weighted 15 ml falcon tubes in four technical replicates. The milk samples were incubated in the water bath for a second time at 32°C for 30 min before 100 µl rennet was added, resulting in a final concentration of 0.18 IMCU/ml in the milk samples. The tubes were turned upside down three times to allow even distribution of rennet in the milk. The milk samples were then kept in the water bath at 32°C for 30 min for coagulation to occur. The resulting curd was cut using a cross-shaped tool, which before usage, had been pre-warmed in the water bath at 32°C for 30 min. The curds were cut one time with the cross-shape tool which was wiped off between each sample to avoid whey transfer between the samples. The curds were incubated again in the water bath at 32°C for 30 min to allow syneresis to occur. To separate the whey and the curd, the tubes were centrifuged at 1650 RPM (Sorvall Super T21, Sorvall Products, L.P., Newtown, CT, USA) at 22°C for 20 min. The whey was poured off and collected in a new 50 ml falcon tube and weighed. The remaining curd was weighed and the weight from the falcon tube was subtracted to calculate the curd yield. Mean curd yield for 10 g milk (g/10g milk) was calculated from the four technical replicates of each sample. By multiplying the mean values with 10, the mean curd yield for 100g of milk (g/100g milk) was determined.

3.3 Analysis of milk protein profile

To determine the protein profile of the milk samples, milk from each individual goat and control samples were analysed by capillary electrophoresis (CE; 7100 CE system, Agilent Technologies Co., Santa Clara, CA, USA) controlled by Chemstation software version A 10.02. (Agilent Technologies Co., Santa Clara, CA, USA).

3.3.1 Buffer preparation

For the CE analysis, urea stock solution, run buffer and sample buffer were prepared. The chemicals were obtained from Sigma-Aldrich (Sigma-Aldrich Co., St. Louis, MO, USA) if not stated differently. An urea stock solution (0.30 L, 6 M) was prepared by mixing 108.10 g urea (Mw 60.06), 0.15 g hydroxypropyl methyl cellulose (MHEC) (0.05%) and 5.40 g ion exchange resin (AG 501-X8 Resin, Bio-Rad Laboratories Inc., CA, USA). The compounds were mixed with water to reach a total volume of 0.30 L, and the solution was mixed and left at room temperature overnight before being filtered through a 0.45 µm membrane (Agilent Captiva

Econofilter, Agilent Technologies Co., Santa Clara, CA, USA). Run buffer was prepared by mixing 0.29 g trisodium citrate dehydrate (Mw 294.10) and 1.996 g citric acid monohydrate (Mw 210.14) with 6 M urea stock to reach a total volume of 50 ml. Sample buffer was prepared by mixing 4.046 g hydroxymethyl-aminomethane (TRISS; Mw 121.14), 4.988 g ethylenediaminetetraacetic acid (EDTA; CAS no: 6381-92-6, VWR International, Radnor, PA, USA) (Mw 372.24) and 1.758 g 4-Morpholinepropanesulfonic acid (MOPS; Mw 209.26), and diluting with 6 M urea stock to reach a total volume of 200 ml. Both run buffer and sample buffer were aliquoted and stored at -20°C until use. On the day of analysis, both buffers were thawed at room temperature and filtered through a 0.45 µm membrane before use.

3.3.2 Sample preparation

Frozen milk samples (2 ml in Eppendorf tubes) were thawed overnight in the refrigerator or were prewarmed in a water bath at 32°C for 15 min. The samples were de-fatted by centrifugation (Himac CT 15RE, Hitachi Koki Co., Ltd, Tokyo, Japan) at 10 000 RPM at 4℃ for 10 minutes, and the fat layer on the surface of the milk was removed by using a cotton stick. The samples were vortexed to allow even distribution. From each sample, 200µl of milk were pipetted to a new Eppendorf tube and mixed with 400µl of sample buffer, to which 0.079g D,L-dithiothreitol (DTT) had been freshly added for each 10 ml of sample buffer. Samples were vortexed and incubated at room temperature for one hour. The samples were then defatted a second time by centrifugation for 10 min at 10 000 RPM and 4℃, and the fat was removed with a cotton stick. After that the samples were filtered through a 0.45 µm membrane into new Eppendorf tubes. The filtrated samples (30µl) were transferred to conic vials to be run in the capillary electrophoresis system. Control samples were prepared according to the same procedure from pasteurized, non-homogenized cow milk.

3.3.3 Capillary electrophoresis analysis

Separation of the proteins was performed as described by Johansson et al. (2013), using an unfused silica capillary. From the obtained electropherogram, the relative concentration of the different proteins in the samples was calculated based on obtained peak areas and expressed as percentage of the total integrated area. Two milk samples could for unknown reasons not be analysed by CE.

3.4 Analysis of milk and whey gross composition and somatic cell count

Milk samples from each individual goat as well as the whey obtained after milk coagulation, were analysed for gross composition at the milk testing laboratory at Department of Animal Nutrition and Management, SLU, Uppsala, Sweden. Fourier Transform Infrared Spectroscopy; FTIR; (Foss Electric A/S, Hilleröd, Denmark) was conducted to measure concentrations of total solids, fat, protein, and lactose, density, the content of saturated fatty acids (SFA), unsaturated fatty acids (UFA), mono unsaturated fatty acids (MUFA), and poly unsaturated fatty acids (PUFA), as well as the individual fatty acids myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1 cis-9. Somatic cell count (SCC) was analysed by electronic fluorescence-based cell counting (Fossomatic Foss FT 120, Foss electric A/S, Hilleröd, Denmark). Four milk samples could not be analysed for gross composition, and 45 milk samples failed in the evaluation of SCC and whey because of technical problems in the laboratory. The percentage of casein in the milk was estimated by subtracting the amount of protein in the whey fraction from the total amount of protein in the milk. The casein ratio was calculated as the amount of casein in the milk divided by the total amount of protein in the sample.

3.5 DNA extraction and determination of genotype at the *CSN1S1* gene

Prior to this thesis project was initiated, the individual goats had been sampled, and DNA extracted and sequenced to determine genotype at the Department of Animal breeding and Genetics, SLU. Corresponding data from genotyping of the *CSN1S1* gene was obtained for 55% of the total goat population (n = 78). For this, nasal swabs and reagents included in the kit were used in accordance with the manufacturer's instructions (DNA Genotek Inc., Ottawa, ON, Canada). The procedure for the external analyses is not described in detail in the thesis.

3.6 Statistical analyses

Data for gross composition, SCC and *CSN1S1* genotype were acquired as raw data and statistically analysed with data generated in this study. For the statistical analyses, the goat population was categorized into two groups according to the relative concentration of αs_1-CN (low: 0%–6.9% and medium-high: 7%–25% of total protein) which is in accordance with the studies by Johansson et al. (2015, 2023).

In short, in this study the low expressing group showed a relative concentration of αs_1-CN within the range 1.09% - 6.99%. The number of goats expressing high concentration of αs_1-CN was low (n = 2), whereby goats expressing medium and high concentrations were combined into one group. In this study, the relative concentration of αs_1-CN in milk from the medium and high concentrations group was within the range of 7.02% to 16.87%. Likewise, there was only one goat which was homozygote for the G allele, and therefore the GG and DG genotypes were combined whereas the AA, AG, DA and DD genotypes were evaluated separately.

Statistical analyses were performed using Minitab software (Minitab, LLC., State College, PA, USA) with a confidence interval of 95%. One-way ANOVA and Tukey pairwise comparison were used to evaluate the variation in milk quality traits among all samples and between the groups. Pearson's correlation was used for correlation analysis of traits related to αs_1-CN concentration and curd yield. Two boxplots were made, one to illustrate the distribution of the relative concentration of αs_1-CN and the curd yield, respectively, and one to illustrate the distribution of relative concentration of αs_1-CN in milk and curd yield for the different genotypes of the *CSN1S1* gene.

4. Results

4.1 Relative concentrations of αs_1-casein in the investigated goat population

Out of all tested goat milk samples (n = 137) 48% of the samples had a low relative concentration of αs_1-CN, while 52% had a medium-high content (Table 2).

Table 2. Categorisation of goat milk samples (n = 137) based on their relative concentration of αs_1-casein (αs_1-CN)

Expression rate	Range of the relative concentration of αs_1-CN (%)	Number of animals	Percentage of total animals
Low[1]	1.09-6.99	66	48
Medium-high[2]	7.02-16.87	71	52

Milk samples were categorized into two groups: [1] low (0–6.9%) and [2] medium-high (7–25%) relative concentrations of αs_1-casein out of the total protein in the milk.

Curd yield within the group low (0-6.9%) and medium-high (7-25%) relative concentration of αs_1-CN, respectively, was determined (Figure 1a). The median curd yield for the low group was 25.8 g/100g which was slightly higher than for the medium-high group which had a median value of 23.1 g/100g. However, the range of the curd yield was higher for the medium-high αs_1-CN group (6.15-55.33 g/100g) than for the low group (11.4–38.15 g/100g). The relative concentration of αs_1-CN ranged between 1.09%-6.99% for the low group and 7.02%-16.87% for the medium-high group (Figure 1b).

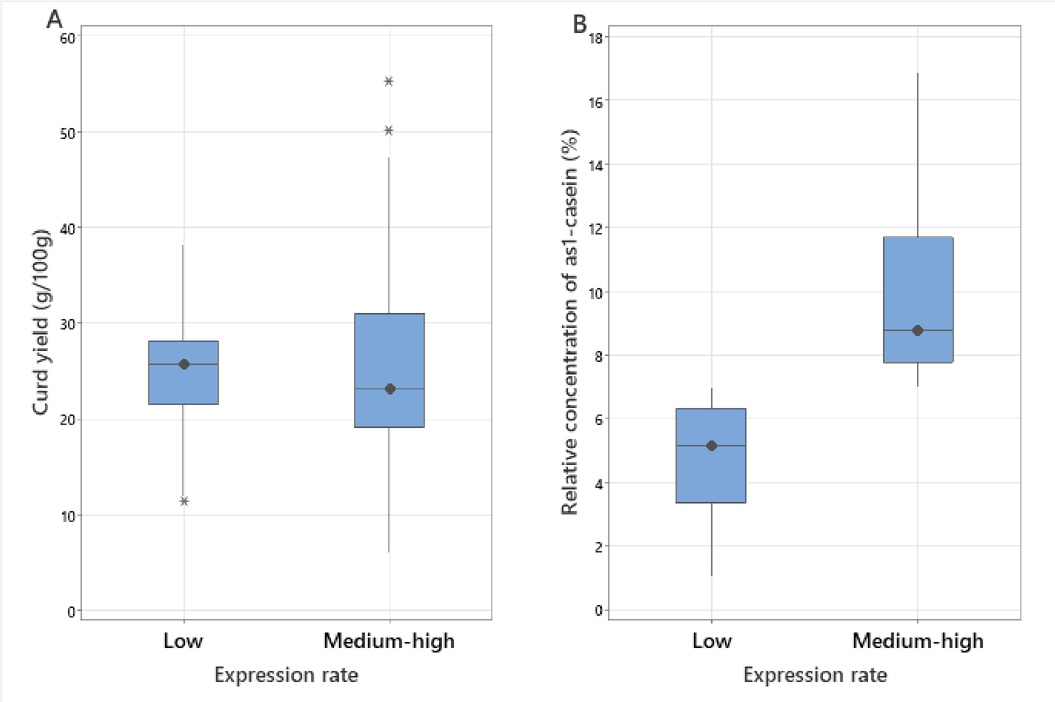


Figure 1. Boxplot showing the range of curd yield (A) and relative concentration of αs_1-casein (B) for the two groups: low (0–6.9%) and medium-high (7–25%). The middle line represents the median and the lower and upper extents of the boxes indicate the 25th and 75th percentiles of the distribution, respectively. The whiskers show the minimum and maximum values. Asterisks (*) indicates values identified as outliers.

4.2 Relation between *CSN1S1* genotype and expression of αs_1-casein

The number and percentage of each genotype out of the total number of goats that were genotyped for the *CSN1S1* gene (n = 78) were determined (Table 3). Of the whole studied goat population (n = 137), 59 goats (43%) were not genotyped and of the tested animals, six different variations were discovered in the following study: AA (24%), AG (9%), DA (31%), DD (19%), DG (15%) and GG (1%). Of all goats, 65% carried at least one D allele (DA/DD/DG) and 64% carried at least one A allele (AA/AG/DA).

The distribution of the different *CSN1S1* genotypes in the groups low (0-6.9%) and medium-high (7-25%) relative concentration of αs_1-CN, were determined (Table 4). Among goats carrying at least one A allele (AA/AG/DA), six (12%) were in the low group while 44 (88%) were in the medium-high group. Of goats with at least one D allele (DA/DD/DG), 30 (59%) were in the low group and 21 (41%) were in the medium-high group. Only one goat was homozygote for the G allele, and it was in the low group.

Table 3. Distribution of the *CSN1S1* genotype in the investigated Swedish Landrace goats (n = 78)[1]

	Genotype					
	AA	AG	DA	DD	DG	GG
Number (n)	19	7	24	15	12	1
Percentage (%)	24	9	31	19	15	1

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

Table 4. Relationship between genotype at the *CSN1S1* gene and relative concentration of αs_1-casein (αs_1-CN) (n = 78)[1]

	Genotype					
Expression rate	AA	AG	DA	DD	DG	GG
Low (n)	0	1	5	14	11	1
Medium-high (n)	19	6	19	1	1	0

[1]Number of individuals (n) from each genotype categorized into the two groups: low (0–6.9%) and medium-high (7–25%) relative concentrations of αs_1-casein.

The range of curd yield and relative concentration of αs_1-CN, respectively, was determined for the different genotypes (Figure 2). The range of curd yield was 14.50-36.58 g/100g for the AA, 6.15-42.40 g/100g for the AG, and 11.40-35.32 g/100g for the DA genotype, respectively (Figure 2a). For genotypes with only D and G alleles, the range was 13.60-50.15 g/100g for the DD and 12.18-33.23 g/100g for the DG genotype, respectively. The DD and AG genotypes showed the highest median value for curd yield, 28.15 g/100g and 27.63 g/100g, respectively. The lowest median value was shown for the AA (21.57 g/100g) and the DA (22.75 g/100g) genotypes. The DG genotype had a median value of 25.18 g/100g. There was only one goat with the GG allele and the curd yield was 26.98 g/100g for this genotype. Genotypes with at least one A allele showed higher ranges of relative concentrations of αs_1-CN: 7.71-16.87% for AA, 6.91-8.38% for AG and 6.48-10.96% for the DA genotype, compared to goats with genotype DD (1.46-5.05%), DG (2.58-7.12%) and GG (4.15%) (Figure 2b).

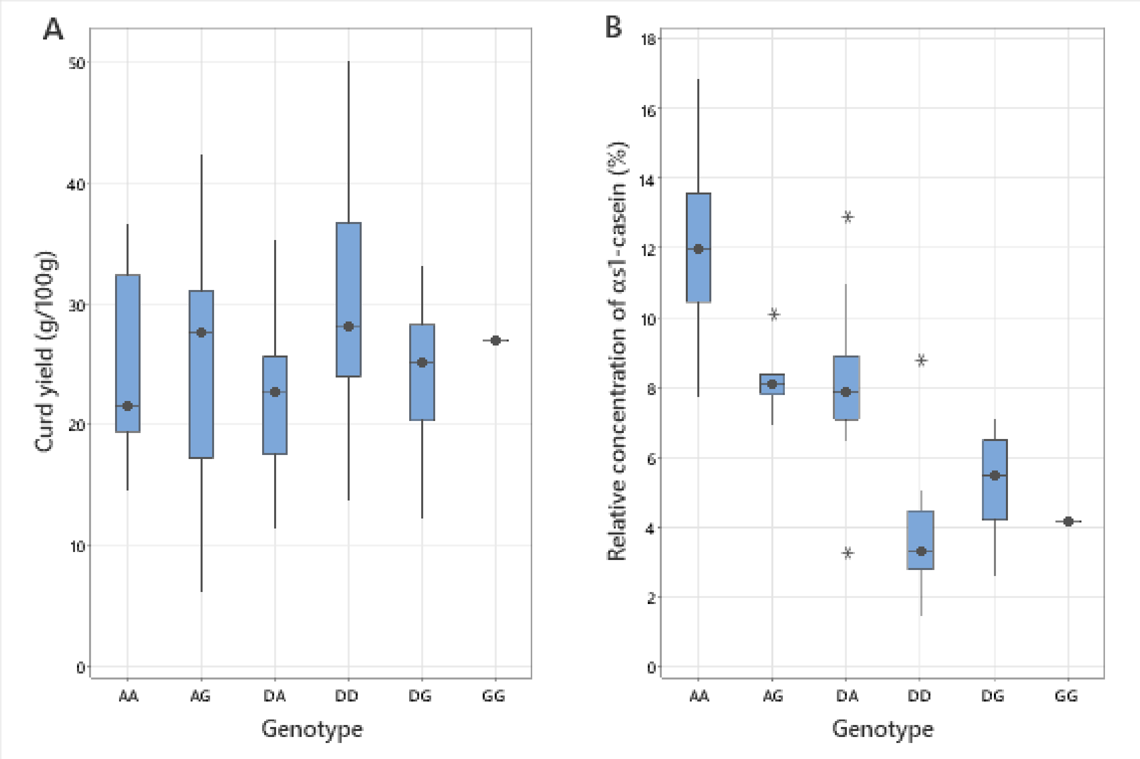


Figure 2. Boxplot showing the range of curd yield (A) and relative concentration of αs_1-casein (B) in milk for the different genotypes (AA, AG, DA, DD, DG and GG) of the CSN1S1 gene. The middle line represents median values, and the lower and upper extents of the boxes indicate the 25th and 75th percentiles of the distribution, respectively. The whiskers show the minimum and maximum values. Asterisks symbols (*) indicate values identified as outliers.

Milk from the AA genotype clearly had the highest median value of αs_1-CN (11.94%) among all genotypes. The median value for the AG genotype was slightly higher (8.14%), compared to the DA genotype (7.85%). The DG genotype showed higher median values of αs_1-CN (5.52%) compared to goats with genotype DD (3.32%) and GG (4.15%).

4.3 Differences in milk protein profile and average curd yield in relation to the relative concentration of αs_1-casein

The relative concentration of αs_1-CN in milk from the low group was on average 4.87% of total proteins, compared to the medium-high group which had an average of 9.62% ($p < 0.001$) (Table 5). Comparing the curd yield between the groups, the medium-high group only had 1% higher curd yield than the low group and the results were non-significant ($p = 0.853$). The low group showed higher contents of both β-CN- and κ-CN content, 13% and 21% respectively ($p = 0.001$, $p = 0.001$).

Overall, the percentage of total casein was 5% higher for the low group than for the medium-high group ($p < 0.001$). Comparing the relative concentration of whey proteins between the groups, the low group contained a 14% higher amount of α-La compared to the medium-high group ($p = 0.044$).
The amounts of protein in the whey fraction after curd separation differed ($p = 0.007$), with 15% more protein in the whey for the medium-high group compared to the low group. No other investigated parameters differed significantly between the two groups.

Table 5. Comparison of relative protein composition and average curd yield of milk samples from goats (n = 137) based on their relative concentration of αs_1-casein (αs_1-CN)[1]

Milk quality traits	Mean	Range	Low	CV low (%)	Medium-high	CV Medium-high (%)
TWP (% of total protein)	9.42 ± 3.47	3.13 - 23.71	9.90 ± 4.29	43.33	8.98 ± 2.42	26.95
α-La (%)	2.29 ± 0.92	0.49 - 6.00	2.45 ± 1.04[a]	42.45	2.14 ± 0.76[b]	35.51
β-Lg (%)	6.39 ± 2.75	0.45 - 17.09	6.67 ± 3.37	50.52	6.14 ± 2.02	32.90
CN (% of total protein)	79.93 ± 4.66	67.05 - 91.59	81.77 ± 4.92[a]	6.02	78.21 ± 3.67[b]	4.69
αs_1-CN (%)	7.33 ± 3.15	1.09 - 16.87	4.87 ± 1.59[b]	32.65	9.62 ± 2.42[a]	25.16
αs_2-CN (%)	7.12 ± 3.11	2.02 - 24.17	6.79 ± 2.08	30.63	7.42 ± 3.82	51.48
β-CN (%)	54.12 ± 6.43	40.83 - 17.09	57.65 ± 5.86[a]	10.16	50.84 ± 5.06[b]	9.95
κ-CN (%)	11.36 ± 3.67	3.56 - 24.28	12.47 ± 4.25[a]	34.08	10.33 ± 2.67[b]	25.85
Casein[2,4] (%)	1.84 ± 0.55	0.30 - 2.88	1.77 ± 0.49	27.68	1.90 ± 0.60	31.58
Casein ratio[3,4] (g/100g)	61.69 ± 11.87	16.76 - 79.29	62.32 ± 11.79	18.92	61.10 ± 11.99	19.62
Protein in whey fraction (%)	1.1 ± 0.34	0.58 - 3.67	1.02 ± 0.23[b]	22.55	1.17 ± 0.40[a]	34.19
Curd yield (g/100g)	25.24 ± 7.79	6.15 - 55.33	25.11 ± 6.30	25.09	25.36 ± 9.00	35.49

α-LA = α-lactalbumin; β-Lg = β-lactoglobulin; CN = casein; CV = coefficient of variation; TWP = total whey protein.
[1]Data are presented as mean values ± SD.
Milk samples were categorized into two groups: low (0–6.9%) and medium-high (7–25%) relative concentrations of αs_1-casein. Differences between groups were evaluated by one-way ANOVA and Tukey test. [a,b] values within rows with different letters are significantly different at $p < 0.05$.
[2]Casein = protein in whey fraction subtracted from total protein in milk.
[3]Casein ratio = amount of casein in milk divided by the total amount of protein in milk.
[4]n = 136

4.4 Differences in milk protein profile and average curd yield in relation to the genotype at the *CSN1S1* gene

Differences in relative protein composition and average curd yield between the *CSN1S1* genotypes AA, AG, DA, DD and DG/GG were determined (Table 6). Comparing the curd yield between the genotypes, the results were not significantly different ($p = 0.178$). However, the result showed significant differences between the genotypes regarding the relative content of caseins. Milk from DD and DG/GG goats had the lowest relative amount of αs_1-CN. Comparing the milk of DD goats to the AA, AG and DA goats, the relative concentration of αs_1-CN in the DD milk was 69%, 55% and 54% lower, respectively ($p < 0.001$). For the DG/GG milk, the relative concentration of αs_1-CN was 57%, 37% and 35% lower than milk from the AA, AG and DA goats ($p < 0.001$). The total casein content was 6% lower in AA milk compared to both the DD and DG/GG milk ($p = 0.001$). The relative concentration of αs_2-CN in the DA milk was 47% higher than AA milk ($p = 0.020$). The relative concentration of β-CN was 11% and 12% higher for milk from the DD and DG/GG goats compared to the AA milk ($p = 0.006$). The relative content of κ-CN was 73%, 37%, 65% and 26% higher in milk from the DD goats compared to AA, AG, DA and DG/GG milk ($p < 0.001$). For DG/GG milk, the relative content of κ-CN was 38% and 31% higher, respectively, than in AA and DA milk ($p < 0.001$). In addition, there were significant differences between the genotypes regarding the relative content of whey proteins. Milk from AA goats had the lowest amount of whey proteins, 28% lower than the DD milk ($p = 0.050$). The relative concentration of α-La was 34% lower for AA milk compared to DG/GG milk ($p = 0.016$). The amount of protein in the whey fraction after curd separation also differed significantly. The AG milk had 57% higher amount of proteins in the whey fraction compared to the DG/GG milk ($p = 0.040$).

Table 6. Comparison of the relative protein composition and the average curd yield of milk samples from goats (n = 78) based on their genotype at the *CSN1S1* gene[1]

	Genotype				
Milk quality traits	AA	AG	DA	DD	DG/GG
TWP (% of total protein)	8.24 ± 2.89[b]	9.08 ± 1.15[ab]	8.73 ± 2.63[ab]	11.49 ± 3.59[a]	9.67 ± 4.63[ab]
α-La (%)	1.72 ± 0.57[b]	1.90 ± 0.44[ab]	1.94 ± 0.68[ab]	2.29 ± 0.88[ab]	2.62 ± 1.07[a]
β-Lg (%)	5.87 ± 2.37	6.38 ± 1.31	6.13 ± 2.44	8.37 ± 2.83	6.29 ± 3.52
CN (% of total protein)	77.43 ± 3.43[b]	77.79 ± 3.68[ab]	78.82 ± 3.78[ab]	82.63 ± 4.73[a]	82.52 ± 5.84[a]
αs_1-CN (%)	11.99 ± 2.30[a]	8.19 ± 0.95[b]	8.01 ± 1.77[b]	3.69 ± 1.74[c]	5.17 ± 1.34[c]
αs_2-CN (%)	4.98 ± 2.56[b]	7.73 ± 2.40[ab]	7.31 ± 3.38[a]	5.78 ± 1.97[ab]	7.27 ± 1.54[ab]
β-CN (%)	50.91 ± 5.60[b]	49.81 ± 4.37[ab]	53.47 ± 5.33[ab]	56.61 ± 6.40[a]	56.94 ± 6.63[a]
κ-CN (%)	9.55 ± 1.84[c]	12.05 ± 3.23[bc]	10.04 ± 1.70[c]	16.56 ± 3.69[a]	13.14 ± 3.91[b]
Casein[2,4] (%)	1.96 ± 0.70	2.16 ± 0.19	1.70 ± 0.71	1.83 ± 0.34	1.81 ± 0.40
Casein ratio[3,4] (g/100g)	62.28 ± 13.64	65.85 ± 4.20	56.93 ± 16.10	59.84 ± 6.58	65.41 ± 6.85
Protein in whey fraction (%)	1.11 ± 0.31[ab]	1.48 ± 0.97[a]	1.15 ± 0.25[ab]	1.22 ± 0.24[ab]	0.94 ± 0.18[b]
Curd yield (g/100g)	24.50 ± 7.44	24.54 ± 11.72	22.29 ± 6.03	28.74 ± 9.36	24.21 ± 6.32

α-LA = α-lactalbumin; β-Lg = β-lactoglobulin; CN = casein; TWP = total whey protein.

[1]Data are presented as mean values ± SD.

Because only one goat carried two G alleles, the DG and GG genotypes were combined into one group (DG/GG). Differences between groups were evaluated by one-way ANOVA and Tukey test. [a,b,c] values within rows with different letters are significantly different at $p < 0.05$.

[2]Casein = protein in whey fraction subtracted from total protein in milk.

[3]Casein ratio = amount of casein in milk divided by the total amount of protein in milk.

[4]n = 77

4.5 Differences in milk gross composition in relation to the relative concentration of αs_1-casein

Significant differences in milk gross composition were observed between the groups low and medium-high levels of αs_1-CN (Table 7). Samples with medium-high relative concentration of αs_1-CN were 6% higher in total solids ($p = 0.001$), 8% higher in total proteins ($p = 0.006$) and 13 % higher in total fat ($p = 0.007$) than samples within the low group. In addition to the differences in fat content, the fat composition differed significantly between the two groups. Milk with a medium-high concentration of αs_1-CN was 12% higher in SFA ($p = 0.013$), 11% higher in UFA ($p = 0.025$), 29% higher in MUFA ($p = 0.001$) as well as 24% higher in C18:1 cis-9 ($p = 0.001$) than samples low in αs_1-CN. Overall, samples in the medium-high group were 6% higher in total solids in the milk ($p = 0.001$). The SCC in milk from the low group was 90% higher than that of the medium-high group ($p = 0.038$). Overall, in the sample set analysed in this study, the SCC varied a lot among samples, which was shown by high coefficient of variation (CV%) for the two groups (Table 6). Also, a large part of the samples could not be analysed for SCC: 21% of samples in the low group and 44% of samples in the medium-high group. Other parameters presented in the table did not significantly differ between the groups.

Table 7. Comparison of gross composition of milk samples from goats (n = 137) based on their relative concentration of as1-casein (αs1-CN)[1]

Milk quality traits	Mean	Range	Low	CV Low (%)	Medium-high	CV Medium-high (%)
Total solids (g/100g)	11.53 ± 1.22	7.55 - 14.87	11.18 ± 1.11[b]	9.93	11.85 ± 1.24[a]	10.46
Total protein (g/100g)	2.92 ± 0.56	1.42 - 4.46	2.79 ± 0.47[b]	16.85	3.04 ± 0.60[a]	19.74
Total fat (g/100g)	3.63 ± 0.94	1.13 - 8.59	3.41 ± 0.83[b]	24.34	3.84 ± 0.99[a]	25.78
SFA (g/100g)	2.52 ± 0.67	1.23 - 6.03	2.37 ± 0.61[b]	25.74	2.66 ± 0.71[a]	26.69
UFA[3] (g/100g)	0.97 ± 0.26	0.33 - 2.10	0.92 ± 0.20[b]	21.74	1.02 ± 0.29[a]	28.43
MUFA[4] (g/100g)	0.64 ± 0.22	0.03 - 1.58	0.57 ± 0.20[b]	35.09	0.70 ± 0.23[a]	32.86
PUFA (g/100g)	0.16 ± 0.07	0.04 - 0.50	0.15 ± 0.07	46.67	0.17 ± 0.07	41.18
C14:0 (g/100g)	0.54 ± 0.17	0.24 - 1.11	0.51 ± 0.18	35.29	0.56 ± 0.17	30.36
C16:0 (g/100g)	1.04 ± 0.29	0.46 - 2.39	0.99 ± 0.27	27.27	1.08 ± 0.30	27.78
C18:0[5] (g/100g)	0.39 ± 0.14	0.01 - 1.20	0.38 ± 0.10	26.32	0.40 ± 0.16	40.00
C18:1 cis-9[6] (g/100g)	0.52 ± 0.17	0.15 - 1.04	0.46 ± 0.13[b]	28.26	0.57 ± 0.18[a]	31.58
Density (g/ml)	1.03 ± 0.002	1.02 - 1.03	1.03 ± 0.00	0.19	1.03 ± 0.00	0.19
Lactose (g/100g)	4.22 ± 0.23	3.47 - 4.76	4.23 ± 0.22	5.20	4.22 ± 0.24	5.69
SCC[7] ($\times 10^3$cells/mL)	262 ± 360	9 - 1779	330 ± 386[a]	116.91	174 ± 305[b]	175.82
pH[2]	6.47 ± 0.55	4.25 - 6.92	6.47 ± 0.55	8.50	6.47 ± 0.55	8.50

SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; C14:0 = myristic acid; C16:0 = palmitic acid; C18:0 = stearic acid; C18:1 cis-9 = oleic acid; SCC = somatic cell count; CV = coefficient of variation.

[1]Data are presented as mean values ± SD.

Milk samples were categorized into two groups: low (0–6.9%) and medium-high (7–25%) relative concentrations of αs1-casein. Differences between groups were evaluated by one-way ANOVA and Tukey test. [a,b] values within rows with different letters are significantly different at $p < 0.05$.

[3]n = 136, [4]n = 132, [5]n = 131, [6]n = 128, [7]n = 92

4.6 Differences in milk gross composition in relation to the genotype at the *CSN1S1* gene

Significant differences in milk gross composition were observed between the different *CSN1S1* genotypes (Table 8). Comparing the AA genotype to the DA and DG/GG genotype, the total solid content was 10% and 15% higher, respectively, for the AA genotype (p = 0.002). The total fat content was 26% and 37% higher, in milk from AA goats compared to milk from DA and DG/GG goats (p = 0.003). Comparing the fat composition, the SFA content of milk from AA goats was 39% higher than milk from goats with the DG/GG genotypes (p = 0.009). The UFA content was 33%, 24% and 30% higher for AA milk compared to DA, DD and DG/GG milk (p = 0.002). The MUFA content was 50% and 40% higher for AA milk compared to DA and DD milk (p = 0.001). Looking at individual fatty acids, the content of C16:0 was 37% higher for milk from AA goats compared to milk from DG/GG goats (p = 0.020). The content of C18:0 was 38% and 52% higher in the milk from AA goats compared to milk from the DA and DD goats (p = 0.020). Milk from AA goats was also 43% higher in C18:1 cis-9 than the milk from the AG, DA and DG/GG goats, and 49% higher than milk from the DD goats (p < 0.001). SCC did not differ significantly between the genotypes (p = 0.055). However, a large part of the milk samples could not be tested for SCC: 58% in AA, 83% in AG, 46% in AD, 33% in DD and 29% in the DG/GG goats. The other parameters: total protein, PUFA, C14:0, density and pH did not significantly differ between the genotypes.

Table 8. Comparison of gross composition of milk samples from goats (n = 78) based on their genotype at the *CSN1S1* gene[1]

	Genotype				
Milk quality traits	AA	AG	DA	DD	DG/GG
Total solids (g/100g)	12.50 ± 1.05[a]	11.78 ± 0.99[ab]	11.39 ± 1.29[b]	11.88 ± 0.98[ab]	10.89 ± 0.95[b]
Total protein (g/100g)	3.07 ± 0.64	3.33 ± 0.21	2.85 ± 0.70	3.04 ± 0.45	2.75 ± 0.43
Total fat (g/100g)	4.47 ± 1.26[a]	3.52 ± 0.84[ab]	3.55 ± 0.85[b]	3.72 ± 0.52[ab]	3.26 ± 0.65[b]
SFA (g/100g)	3.09 ± 0.90[a]	2.46 ± 0.60[ab]	2.52 ± 0.67[ab]	2.63 ± 0.40[ab]	2.23 ± 0.49[b]
UFA (g/100g)	1.17 ± 0.31[a]	0.92 ± 0.24[ab]	0.88 ± 0.22[b]	0.94 ± 0.17[b]	0.90 ± 0.22[b]
MUFA[2] (g/100g)	0.81 ± 0.26[a]	0.58 ± 0.21[ab]	0.54 ± 0.18[b]	0.58 ± 0.14[b]	0.61 ± 0.17[ab]
PUFA (g/100g)	0.17 ± 0.05	0.14 ± 0.04	0.17 ± 0.07	0.15 ± 0.05	0.15 ± 0.07
C14:0 (g/100g)	0.61 ± 0.17	0.50 ± 0.14	0.57 ± 0.20	0.56 ± 0.11	0.47 ± 0.15
C16:0 (g/100g)	1.26 ± 0.36[a]	1.01 ± 0.25[ab]	1.05 ± 0.31[ab]	1.12 ± 0.20[ab]	0.92 ± 0.22[b]
C18:0[3] (g/100g)	0.47 ± 0.23[a]	0.35 ± 0.08[ab]	0.34 ± 0.11[b]	0.31 ± 0.08[b]	0.36 ± 0.13[ab]
C18:1 cis-9[4] (g/100g)	0.67 ± 0.18[a]	0.47 ± 0.17[b]	0.47 ± 0.14[b]	0.45 ± 0.12[b]	0.47 ± 0.15[b]
Density (g/ml)	1.03 ± 0.00	1.03 ± 0.00	1.03 ± 0.00	1.03 ± 0.00	1.03 ± 0.00
Lactose (g/100g)	4.19 ± 0.29	4.16 ± 0.23	4.23 ± 0.25	4.32 ± 0.18	4.16 ± 0.19
SCC[5] ($\times 10^3$cells/mL)	146 ± 105	901 ± 1195	226 ± 276	637 ± 575	362 ± 185
pH	6.58 ± 0.09	6.64 ± 0.12	6.31 ± 0.70	6.64 ± 0.09	6.44 ± 0.63

SFA = saturated fatty acids; UFA = unsaturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; C14:0 = myristic acid; C16:0 = palmitic acid; C18:0 = stearic acid; C18:1 cis-9 = oleic acid; SCC = somatic cell count.

[1]Data are presented as mean values ± SD.

Because only one goat carried two G alleles, the DG and GG genotypes were combined into one group (DG/GG). Differences between groups were evaluated by one-way ANOVA and Tukey test. [a,b] values within rows with different letters are significantly different at $p < 0.05$.

[2]n = 76, [3]n = 77, [4]n = 74, [5]n = 42

4.7 Correlation between milk quality traits related to the relative concentration of αs_1-casein and curd yield

The correlation coefficient of different milk quality traits related to the concentration of αs_1-CN and curd yield were evaluated with Pearsons's correlation (Table 9). The analyses indicated a trend for a negative correlation between the curd yield and the relative concentration of αs_1-CN, but the correlation was not significant ($p = 0.543$). However, the curd yield was significantly positively related to total solids ($p < 0.001$) total fat ($p = 0.018$), total protein ($p < 0.001$), casein ($p < 0.001$), as well as the casein ratio ($p = 0.007$). The concentration of αs_1-CN depicted a significant negative correlation with κ-CN ($p < 0.001$), β-CN ($p < 0.001$) and total CN content ($p < 0.001$). The concentration of αs_1-CN was also significantly positively related with total solids ($p = 0.015$), total fat ($p = 0.040$) and total protein ($p = 0.018$).

Table 9. Pearson's correlations among milk quality traits related to curd yield and concentration of αs_1-casein (αs_1-CN) (n = 137). R-values and p-values for the different correlations are indicated; $p < 0.05$ is considered significant.

Quality trait	R-value	p-value
Curd yield x αs_1-CN	-0.052	0.543
Curd yield x αs_2-CN	0.120	0.161
Curd yield x κ-CN	0.004	0.963
Curd yield x β-CN	-0.126	0.143
Curd yield x Total CN	-0.126	0.144
Curd yield x Total solids	0.288	**<0.001**
Curd yield x Total fat	0.202	**0.018**
Curd yield x Total protein	0.307	**<0.001**
Curd yield x Casein[1,3]	0.331	**<0.001**
Curd yield x Casein ratio[2,3]	0.232	**0.007**
Curd yield x pH	0.036	0.676
αs_1-CN x αs_2-CN	0.120	0.163
αs_1-CN x κ-CN	-0.318	**<0.001**
αs_1-CN x β-CN	-0.621	**<0.001**
αs_1-CN x Total CN	-0.350	**<0.001**
αs_1-CN x Total solids	0.206	**0.015**
αs_1-CN x Total fat	0.175	**0.040**
αs_1-CN x Total protein	0.202	**0.018**
αs_1-CN x Casein[1,3]	0.104	0.226
αs_1-CN x Casein ratio[2,3]	-0.060	0.488
αs_1-CN x pH	-0.005	0.952

CN = casein.

[1]Casein = protein in whey fraction subtracted from total protein in milk.

[2]Casein ratio = amount of casein in milk divided by the total amount of protein in milk.
[3]n = 136

5. Discussion

5.1 Expression of αs_1-casein and genotypes in the *CSN1S1* gene

The aim of this study was to investigate if the expression of αs_1-CN in Swedish dairy goats influences the milk composition and particularly the curd yield. The results indicated that 48% of the samples contained low relative concentrations of αs_1-CN (0-6.9% of total protein), while 52% of the samples contained medium-high levels (7-25% of total protein) (Table 2). It was a lower percentage of goats producing low amounts of αs_1-CN in this study than previously reported by Johansson et al. (2014, 2023). The authors observed an increase from 65% to 75% of investigated goats with low levels αs_1-CN over a time period of 10 years.

Of the goats genotyped for the *CSN1S1* gene in this study, 65% carried at least one D allele (DA/DD/DG) and 64% carried at least one A allele (AA/AG/DA) (Table 3). The number of goats carrying the A allele was considerably higher in this study compared to the study by Johansson et al. (2023), where only 15% of the tested goats carried at least one A allele and most of the goats, 59%, carried the DD genotype. In this study, only 19% of the goats carried the DD genotype while 24% carried the AA genotype. The reduced frequency of goats with a low content of αs_1-CN can be associated with targeted work of the Swedish goat farmers and The Swedish Goat Breeding Association. These parties are now a days jointly working with breeding strategies against the mutation at the *CSN1S1* gene (Svenska Getavelsförbundet 2021). This may be an indication that the work is going in the right direction in the Swedish goat population.

The results of this study also showed that the range of relative concentration of αs_1-CN in the milk samples differed between the *CSN1S1* genotypes (Table 4; Figure 2b). The A allele is classified as a strong allele leading to high expression of αs_1-CN, while G is a weak allele and D is a null allele, leading to low or zero expression of αs_1-CN (Dagnachew et al. 2011; Devold et al. 2010). In this study, genotypes with at least one A allele clearly had higher levels of αs_1-CN than goats with only

D or G alleles. This is in agreement with the results by Johansson et al. (2023) who reported that milk from goats carrying the A alleles had higher relative concentrations of αs_1-CN than DD and DG goats. While only one of the goats had the GG genotype in this study, it is not possible is to draw conclusions about the effect of the genotype αs_1-CN levels in the milk.

5.2 Relation between relative concentration of αs_1-casein and curd yield

No significant association between the relative concentration of αs_1-CN and curd yield was found in this study (Table 5). According to the boxplot (Figure 1a), the median curd yield was slightly higher for the low group (25.8 g/100g) compared to the medium-high group (23.1 g/100g). On the other hand, the numerical value for mean curd yield was slightly lower for the low group (25.11 g/100g) compared to the medium-high group (25.36 g/100g) (Table 5). The high CV for curd yield of both the low- (25.09%) and medium-high group (35.49%) shows that there were large variations in the data sets which may explain the similarity in curd yield. Likewise, there was no difference in average curd yield between the *CSN1S1* genotypes. Comparing numerical values of curd yield for DD and AA genotypes, milk from DD goats had the highest numerical mean (28.74g/100g) and median values (28.15 g/100g). Milk from goats with the AA genotype had mean and median values of 24.50g/100g and 21.57 g/100g, respectively (Table 6, Figure 2a). These results were surprising since previous studies have shown that milk from goats with "strong" alleles have favourable properties in cheese production (Clark & Sherbon 2000; Ambrosoli et al. 1988; Devold et al. 2010). Pirisi et al. (1994) and Frattini et al. (2014) investigated the cheesemaking properties of milk from Saanen and Alpine goat breeds. They observed that milk with high levels of αs_1-CN resulted in higher cheese yield than milk with low levels of αs_1-CN. On the other hand, Caravaca et al. (2011) found no differences in cheese yield between Murciano-Granadina goats carrying strong (BB), intermediate (EE) or weak (EF) genotypes.

Curd yield was significantly positively correlated with total solids, protein, fat, casein and casein ratio, but not with the relative concentration of αs_1-CN, according to the results from Pearsons's correlation (Table 9). Higher content of fat and protein, and casein in particular, has previously been shown to improve cheesemaking properties such as leading to increased curd yield (Pazzola et al. 2019; Devold et al. 2010; Clark & Sherbon 2000; Johansson et al. 2015).

The similarity in the curd yield between the two groups in this study, low and medium-high αs_1-CN, may be explained by the fact that the milk samples in the low group showed higher contents of other caseins apart from αs_1-CN. Goats in the low group had significantly higher relative concentrations of β-CN and κ-CN than the medium-high group (Table 5). Also, milk from goats carrying only D and G alleles had higher relative concentrations of β-CN and κ-CN than goats carrying A alleles (Table 6). The higher κ-CN content of the low group may have resulted in an increase in curd yield for this group, since higher κ-CN content has been associated with firmer gels due to the stronger bridging between proteins and calcium (Panthi et al. 2017). Balia et al. (2013) found that Sarda goats producing lower αs_1-CN levels had higher concentrations of αs_2-CN, β-CN and κ-CN. This may indicate that a reduced αs_1-CN synthesis is compensated by higher synthesis of other caseins (Devold et al. 2010; Balia et al. 2013). This is further indicated by the correlation analysis (Table 9) where the amount of αs_1-CN was found to be negatively related to κ-CN, β-CN and total CN. This suggests the possibility that other caseins are compensating for the low αs_1-CN content which resulted in almost similar curd yield within these two groups. However, as further illustrated in the correlation analysis, curd yield was not significantly correlated with the κ-CN or β-CN content of the milk.

The relative concentration of proteins in the whey fraction significantly differed between the groups and genotypes. The main proteins in the whey fraction are α-La, β-LG, and Glycomacropeptide (GMP) which arise from the cleavage of κ-CN by chymosin in rennet (Pazzola 2019). The group with low relative αs_1-CN concentration had lower amounts of protein in the whey fraction, even if α-La was significantly higher and β-Lg was numerically higher for this group (Table 5). α-La plays an important role in lactose biosynthesis and in turn effects the milk yield (Yang et al. 2020). The increased levels of α-La in the milk from goats in the low group may therefore be a result of higher milk production by these goats. However, as no information was available regarding the milk yield of the individual goats it makes it difficult to draw any conclusions regarding this. Numerically, goats carrying the DG/GG genotypes had the lowest levels of proteins in the whey fraction, but also the highest amounts of α-La in the milk numerically (Table 6). The AG genotype had the highest amount of proteins in the whey fraction numerically, 57% higher compared to the DG/GG genotypes. A low concentration of αs_1-CN in milk has been associated with larger casein micelles being formed. These larger casein micelles contain lower amounts of κ-CN compared to smaller micelles which results in lower levels of GMP being formed and released into the whey (Panthi et al. 2017). Milk from goats with higher expression of αs_1-CN had significantly higher losses of proteins from the curd to the whey, perhaps due to weaker gels formed which may result in caseins being lost in the whey.

However, a high content of αs_1-CN has been associated with increased gel firmness in recent studies, leadings to higher retention of solids in the curd and lower losses in the whey (Clark & Sherbon 2000; Johansson et al. 2015). On the other hand, Caravaca et al. (2011) reported no significant difference in curd firmness when using milk from goats with either strong, intermediate or weak expression of αs_1-CN. The lower concentration of total caseins for the milk in the medium-high group may have led to lower curd firmness which may be an explanation for the increased losses of protein in the whey. Since the curd firmness was not investigated in this study it is hard to draw any conclusion regarding this.

5.3 Relation between αs_1-casein, *CSN1S1* genotype and milk gross composition

Milk samples in the medium-high group had higher contents of total solids, protein and fat compared to the low group (Table 7). The correlation analysis showed that αs_1-CN was significantly positively correlated with the total solids, protein and fat (Table 9). This is in line with previous studies which reported that a higher concentration of αs_1-CN is associated with higher total solids, protein and fat content (Clark & Sherbon 2000; Ambrosoli et al. 1988; Vacca et al. 2014; Devold et al. 2010). Milk from AA goats had the highest levels of total solids and fat numerically (Table 8). Numerical values for total protein were highest for the AG and AA genotypes, and lowest for the DG/GG genotypes. Although not observed in this study, it has been previously shown that the A allele of the *CSN1S1* gene is associated with higher amount of total protein in the milk (Clark & Sherbon 2000; Ambrosoli et al. 1988; Devold et al. 2010). However, some studies have reported similar results as the ones in this study. On investigating different milk quality traits in Swedish goat population in relation to the expression of αs_1-CN and *CSN1S1* genotype, Johansson et al. (2023) found that the total protein content was significantly lower in milk from goats with low levels of αs_1-CN group than in milk from medium-high level goats. However, in that study, total protein content did not significantly differ between the *CSN1S1* genotypes (DD, DG and DA/AG/AA) which is in line with this study. Similar results were reported by Caravaca et al. (2011) who found that the *CSN1S1* genotypes did not significantly affect the total protein content in the milk from Murciano-Granadina goats.

The fat composition also differed between the groups (Table 7), and genotypes (Table 8). Milk with a medium-high relative concentration of αs_1-CN was higher in SFA, UFA, MUFA and C18:1 cis-9 than milk low in αs_1-CN.
Numerically, milk from goats with the AA genotype had the highest levels of SFA, UFA and MUFA as well as C16:0, C18:0 and C18:1C9 among all genotypes.
The effect of different genotype for αs_1-CN synthesis on fatty acid profile in goat milk has been investigated by Bonanno et al. (2013), who reported that the milk fat of goats with higher production of αs_1-CN was richer in SFA which is in line with the findings in this study. However, they also reported that UFA, MUFA and C18:1

cis-9 was lower for goats with higher production of αs_1-CN which was not shown here. Ultimately, they concluded that the different genotypes for αs_1-CN synthesis have a weak effect on the milk fatty acid profile and that feed had the largest influence on fat composition. As the effect of diet was not in the scope of this study, it makes it difficult to draw conclusions about this matter.

SCC differed significantly between the low and medium-high group (Table 7). The total range was 9 000 - 1 779.000 cells/ml. The mean SCC of the low group was approximately 330 000 cells/ml and 170 000 cells/ml for the medium-high group which show that the low group on average had 90% higher SCC. However, 31% of the samples were unable to be tested for SCC due to technical problems in the laboratory. Moreover, the high CV of both the low group (116.91%) and medium-high group (175.82%) shows that there was a large variation between samples within the groups. Estimation of the SCC is widely used for milk quality assessment. The normal level of SCC in goats free from bacterial udder infections, is approximately 300 000 cells/ml which is more than three times as much as cows normally have (Silanikove et al. 2010). In the case of cow health, elevated SCC is highly associated with mastitis, however, it is not always the case for goats (Clark & García 2017). It was shown by Chen et al. (2010) that the composition of goat milk as well as cheese yield were not affected when SCC varied between 214 000 and 1 450 000 cells/ml. Hence it is hard to conclude whether SCC had any effect on curd yield in this study.

5.4 Control samples

Protein profile and curd yield was measured for the control samples (Appendix 1, Table 10). The low CVs for the parameters indicates low variations in the sample group and suggests that the method had high reproducibility.

6. Conclusion

The CE analysis method used in this survey was able to separate caseins and whey proteins which allowed for quantification of αs_1-CN relative to the other proteins in the goat milk samples. It was shown that nearby half of the investigated samples, 48%, contained a low relative concentration of αs_1-CN while 52% of the samples contained medium-high amounts. Six different genotypes of the *CSN1S1* gene were identified in the tested goat population, where 65% carried at least one D allele (DA/DD/DG) and 64% carried at least one A allele (AA/AG/DA). Only one goat (1%) carried the GG genotype. The percentage of goats producing low amounts of αs_1-CN in this study was lower compared to earlier studies investigating the Swedish goat population. This may be an indication that breeding strategies against the mutation at the *CSN1S1* gene is going in the right direction.

Significant differences in fat composition were also discovered between the groups and genotypes. Since fat composition of milk is influenced by factors such as feeding and stage of lactation, which were not included in this study, it was difficult to draw conclusions regarding these results. Moreover, there were significant differences SCC between the groups but not between genotypes. However, large variation within the groups were noticed and large part of the samples were not able to be tested. There were significant differences between groups in protein content of the whey fraction which may be related to the firmness of the gels and losses of protein from the curd. Nevertheless, certain conclusions cannot be drawn since curd firmness was not included in this study. Thus, one suggestion for further studies, is to include additional parameters on curd firmness.

The hypothesis of this study was that the amount of αs_1-CN in the milk will influence the curd yield. The curd yield did not significantly differ between neither groups nor genotypes and thus it was not possible to confirm the hypothesis. It was suggested that the similarity in curd yield between the groups was due to a high variation in αs_1-CN concentration within the groups. Another suggestion was that the higher relative concentrations of β-CN and κ-CN of the low group was compensating for the low αs_1-CN which may contribute to higher curd yield.

Goat milk in the medium-high group had higher content of total solids, protein and fat which was found to significantly correlate with curd yield rather than the amount of αs_1-CN. Numerically, milk from goats with the AA genotype had the highest levels of total solids and fat, and numerical values for total protein were highest for the AG and AA genotypes.

In conclusion, total solids, protein, fat and casein content was found to be more related to the curd yield than the amount of αs_1-CN and might therefore to be a better predictor of cheese yield.

References

ALKaisy, Q. H., Al-Saadi, J. S., Al-Rikabi, A. K. J., Altemimi, A. B., Hesarinejad, M. A. & Abedelmaksoud, T. G. (2023). Exploring the health benefits and functional properties of goat milk proteins. *Food science & nutrition*. 11 (10), 5641–5656. https://doi.org/10.1002/fsn3.3531

Ambrosoli, R., di Stasio, L. & Mazzocco, P. (1988). Content of αs1-Casein and Coagulation Properties in Goat Milk. *Journal of dairy science*. 71 (1), 24–28. https://doi.org/10.3168/jds.S0022-0302(88)79520-X

Balia, F., Pazzola, M., Dettori, M.L., Mura, M.C., Luridiana, S., Carcangiu, V., Piras, G. & Vacca, G.M. (2013). Effect of *CSN1S1* gene polymorphism and stage of lactation on milk yield and composition of extensively reared goats. *Journal of dairy research*. 80 (2), 129–137. https://doi.org/10.1017/S0022029912000702

Bonanno, A., Di Grigoli, A., Montalbano, M., Bellina, V., Mazza, F. & Todaro, M. (2013). Effects of diet on casein and fatty acid profiles of milk from goats differing in genotype for αS1-casein synthesis. *European food research & technology*. 237 (6), 951–963. https://doi.org/10.1007/s00217-013-2069-8

Bosona, T. & Gebresenbet, G. (2018). Swedish consumers' perception of food quality and sustainability in relation to organic food production. *Foods*. 7 (4), 54-. https://doi.org/10.3390/foods7040054

Boyazoglu, J., Hatziminaoglou, I. & Morand-Fehr, P. (2005). The role of the goat in society: Past, present and perspectives for the future. *Small ruminant research*. 60 (1), 13–23. https://doi.org/10.1016/j.smallrumres.2005.06.003

Caravaca, F., Ares, J.L., Carrizosa, J., Urrutia, B., Baena, F., Jordana, J., Badaoui, B., Sànchez, A., Angiolillo, A., Amills, M. & Serradilla, J.M. (2011). Effects of αs1-casein (CSN1S1) and κ-casein (CSN3) genotypes on milk coagulation properties in Murciano-Granadina goats. *Journal of dairy research*. 78 (1), 32–37. https://doi.org/10.1017/S002202991000083X

Carillier-Jacquin, C., Larroque, H. & Robert-Granié, C. (2016). Including α s1 casein gene information in genomic evaluations of French dairy goats. *Genetics selection evolution (Paris)*. 48 (1), 54–54. https://doi.org/10.1186/s12711-016-0233-x

Ceballos, L.S., Morales, E.R., de la Torre Adarve, G., Castro, J.D., Martínez, L.P. & Sampelayo, M.R.S. (2009). Composition of goat and cow milk produced under similar conditions and analyzed by identical methodology. *Journal of food composition and analysis*. 22 (4), 322–329. https://doi.org/10.1016/j.jfca.2008.10.020

Clark, S. & Mora García, M.B. (2017). A 100-Year Review: Advances in goat milk research. *Journal of dairy science*. 100 (12), 10026–10044. https://doi.org/10.3168/jds.2017-13287

Clark, S. & Sherbon, J.W. (2000). Alpha s1-casein, milk composition and coagulation properties of goat milk. *Small ruminant research*. 38 (2), 123–134. https://doi.org/10.1016/S0921-4488(00)00154-1

Currò, S., Manuelian, C.L., De Marchi, M., Claps, S., Rufrano, D. & Neglia, G. (2019). Effects of breed and stage of lactation on milk fatty acid composition of Italian goat breeds. *Animals (Basel)*. 9 (10), 764-. https://doi.org/10.3390/ani9100764

Dagnachew, B.S., Thaller, G., Lien, S. & Ådnøy, T. (2011). Casein SNP in Norwegian goats: additive and dominance effects on milk composition and quality. *Genetics selection evolution (Paris)*. 43 (1), 31–31. https://doi.org/10.1186/1297-9686-43-31

Devold, T.G., Nordbø, R., Langsrud, T., Svenning, C., Jansen Brovold, M., Sørensen, E.S., Christensen, B., Ådnøy, T. & Vegarud, G.E. (2010). Extreme frequencies of the αs1-casein "null"variant in milk from Norwegian dairy goats—Implications for milk composition, micellar size and renneting properties. *Dairy Science and Technology*. 91, 39-51. doi:10.1051/dst/2010033

El-Agamy, E. I. (2007). The challenge of cow milk protein allergy. *Small Ruminant Research*. 68, 64–72. doi: 10.1016/j.smallrumres.2006.09.016.

Farrell, H.M., Jimenez-Flores, R., Bleck, G.T., Brown, E.M., Butler, J.E., Creamer, L.K., Hicks, C.L., Hollar, C.M., Ng-Kwai-Hang, K.F. & Swaisgood, H.E. (2004). Nomenclature of the Proteins of Cows' Milk—Sixth Revision. *Journal of dairy science*. 87 (6), 1641–1674. https://doi.org/10.3168/jds.S0022-0302(04)73319-6

Frattini, S., Nicoloso, L., Coizet, B., Chessa, S., Rapetti, L., Pagnacco, G. & Crepaldi, P. (2014). Short communication: The unusual genetic trend of αS1-casein in Alpine and Saanen breeds. *Journal of dairy science*. 97 (12), 7975–7979. https://doi.org/10.3168/jds.2014-7780

Johansson, M., Högberg, M. & Andrén, A. (2014). High Frequencies of the αS1-Casein Zero Variant in Milk from Swedish Dairy Goats. *Sheep & goat research journal*, 29, 1-

Johansson, M., Högberg, M. & Andrén.A. (2015). Relation between αS1-casein content and coagulation properties of milk from Swedish dairy goats. *Open Food Science Journal*. 9,1–4. https://doi.org/10.2174/1874256401509010001

Johansson, M., Lundh, Å. & Johansson, A.M. (2023). Relation between αS1-casein, genotype, and quality traits of milk from Swedish dairy goats. *Journal of dairy science*. 106 (8), 5582–5592. https://doi.org/10.3168/jds.2022-22857

Johansson, M., Åkerstedt, M., Li, S., Zamaratskaia, G. & Lundh, Å.S. (2013). Casein Breakdown in Bovine Milk by a Field Strain of *Staphylococcus aureus*. *Journal of food protection*. 76 (9), 1638–1642. https://doi.org/10.4315/0362-028X.JFP-13-112

Jordbruksverket (2019a). *Gethållning 2018.* (Statistikrapport 2019:01). https://jordbruksverket.se/download/18.29196bdf172db848a9e1384f/1592839852350/201901.pdf [2023-10-17]

Jordbruksverket (2019b). *Antal nötkreatur i december 2018.* https://jordbruksverket.se/om-jordbruksverket/jordbruksverkets-officiella-statistik/jordbruksverkets-statistikrapporter/statistik/2020-06-22-antal-notkreatur-i-december-2018 [2024-01-12]

Jordana, J., Amills, M., Diaz, E., Angulo, C., Serradilla, J.M. & Sanchez, A. (1996). Gene frequencies of caprine αs1-casein polymorphism in Spanish goat breeds. *Small ruminant research*. 20 (3), 215–221. https://doi.org/10.1016/0921-4488(95)00813-6

Li, S., Delger, M., Dave, A., Singh, H. & Ye, A. (2023).
Acid and rennet gelation properties of sheep, goat, and cow milks: Effects of processing and seasonal variation. *Journal of Dairy Science*. 106 (3). 1611-1625. https://doi.org/10.3168/jds.2022-22561

Lucey, J.A. (2002). Formation and Physical Properties of Milk Protein Gels. *Journal of Dairy Science*. 85 (2), 281-294. https://doi.org/10.3168/jds.S0022-0302(02)74078-2

Martin, P., Szymanowska, M., Zwierzchowski, L. & Leroux, C. (2002). The impact of genetic polymorphisms on the protein composition of ruminant milks. *Reproduction, nutrition, development*, 42 (5), 433–459. https://doi.org/10.1051/rnd:2002036

Monteiro, A., Costa, J.M., & Lima, M.J. (2018). Goat System Production: Advantages and Disadvantages to the Animal, Environment and farmer. In: Kukovics, S. (ed.) *Goat Science*. IntechOpen. 351-366. http://dx.doi.org/10.5772/66562

Morales, F. de A.R., Genis, J.M.C. & Guerrero, Y.M. (2019). Current status, challenges and the way forward for dairy goat production in Europe. *Asian-australasian journal of animal sciences*. 32 (8), 1256–1265. doi: 10.5713/ajas.19.0327

Nomura, K., Yonezawa, T., Mano, S., Kawakami, S., Shedlock, A.M., Hasegawa, M. & Amano, T. (2013). Domestication Process of the Goat Revealed by an Analysis of the Nearly Complete Mitochondrial Protein-Encoding Genes. *PLoS ONE*. 8 (8), 67775. doi: 10.1371/journal.pone.0067775

Panthi, R.R., Jordan, K.N., Kelly, A.L. & Sheehan, J.J. (Diarmuid) (2017). Selection and Treatment of Milk for Cheesemaking. In: McSweeney, P.L.H., Cotter, P.D. & Everett, D.W (eds) *Cheese*. Fourth Edition. Elsevier Ltd, 23–50. https://doi.org/10.1016/B978-0-12-417012-4.00002-8

Park, Y.W., Juárez, M., Ramos, M. & Haenlein, G.F.W. (2007). Physico-chemical characteristics of goat and sheep milk. *Small ruminant research*. 68 (1), 88–113. https://doi.org/10.1016/j.smallrumres.2006.09.013

Pazzola, M., Stocco, G., Dettori, M.L., Bittante, G. & Vacca, G.M. (2019). Effect of goat milk composition on cheesemaking traits and daily cheese production. *Journal of dairy science*. 102 (5), 3947–3955. https://doi.org/10.3168/jds.2018-15397

Pazzola, M. (2019). Coagulation Traits of Sheep and Goat Milk. *Animals (Basel)*. 9 (8), 540-. https://doi.org/10.3390/ani9080540

Pierre, A., Michel, F. & Zahoute, L. (1999). Composition of casein micelles in relation to size in goat milks with A and null αs1-casein variants. *International dairy journal*. 9 (3), 179–182. https://doi.org/10.1016/S0958-6946(99)00057-6

Pirisi, A., Colin, O., Laurent, F., Scher, J. & Parmentier, M. (1994). Comparison of milk composition, cheesemaking properties and textural characteristics of the cheese from two groups of goats with a high or low rate of αS1-casein synthesis. *International dairy journal*. 4 (4), 329–345. https://doi.org/10.1016/0958-6946(94)90030-2

Prosser, C.G. (2021). Compositional and functional characteristics of goat milk and relevance as a base for infant formula. *Journal of food science*. 86 (2), 257–265. https://doi.org/10.1111/1750-3841.15574

Rahmatalla, S.A., Arends, D. & Brockmann, G.A. (2022). Review: Genetic and protein variants of milk caseins in goats. *Frontiers in genetics*. 13, 995349–995349. https://doi.org/10.3389/fgene.2022.995349

Ramos, M. & Juarez, M. (2011). Milk | Sheep Milk. In: Fuquay, J.W. (ed.). *Encyclopedia of Dairy Sciences*. 2nd ed. Academic Press. 494-502. https://doi.org/10.1016/B978-0-12-374407-4.00314-9

Roy, D., Ye, A., Moughan, P.J. & Singh, H. (2021). Structural changes in cow, goat, and sheep skim milk during dynamic in vitro gastric digestion. *Journal of dairy science*. 104 (2), 1394–1411. https://doi.org/10.3168/jds.2020-18779

Runthala, A., Mbye, M., Ayyash, M., Xu, Y. & Kamal-Eldin, A. (2023). Caseins: Versatility of Their Micellar Organization in Relation to the Functional and Nutritional Properties of Milk. *Molecules (Basel, Switzerland)*. 28 (5), 2023-. https://doi.org/10.3390/molecules28052023

Rytkönen, P., Bonow, M., Johansson, M. & Persson, Y. (2013) Goat cheese production in Sweden – a pioneering experience in the re-emergence of local food. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*. 63, 38-46. doi: 10.1080/09064710.2013.798682

Skeie, S.B. (2014). Quality aspects of goat milk for cheese production in Norway: A review. *Small Ruminant Research*. 122 (1–3), 10-17. https://doi.org/10.1016/j.smallrumres.2014.07.012

Silanikove, N., Leitner, G., Merin, U. & Prosser, C.G. (2010). Recent advances in exploiting goat's milk: Quality, safety and production aspects. *Small Ruminant Research.* 89 (2–3), 110-124. https://doi.org/10.1016/j.smallrumres.2009.12.033

Svenska Getavelsförbundet (2021). *Avelsprogram för getter av svensk lantras från år 2020*. [2023-10-21]

Tumino, S., Di Trana, A., Valenti, B., Bordonaro, S., Claps, S., Avondo, M. & Di Gregorio, P. (2023). Polymorphism at the *CSN1S1* Locus and Energy Intake Level Affect Milk Traits and Casein Profiles in Rossa Mediterranea Goats. *Animals (Basel)*. 13 (12), 1982–. https://doi.org/10.3390/ani13121982

Utaaker, K.S., Chaudhary, S., Kifleyohannes, T. & Robertson, L.J. (2021). Global Goat! Is the Expanding Goat Population an Important Reservoir of Cryptosporidium ? *Frontiers in veterinary science*, 8, 648500–648500. https://doi.org/10.3389/fvets.2021.648500

Vacca, G.M., Dettori, M.L., Piras, G., Manca, F., Paschino, P. & Pazzola, M. (2014). Goat casein genotypes are associated with milk production traits in the Sarda breed. *Animal genetics*. 45 (5), 723–731. https://doi.org/10.1111/age.12188

Walstra, P., Wouters, J.T.M. & Geurts, T.J. (2006). *Dairy science and technology*. 2nd ed. Boca Raton, UNITED STATES: Taylor & Francis Group. https://doi.org/10.1201/9781420028010 [2024-01-05]

Yang, F., Zhang, M., Rong, Y., Liu, Z., Yang, S., Zhang, W., Li, J. & Cai, Y. (2020). A novel SNPs in alpha-lactalbumin gene effects on lactation traits in Chinese holstein dairy cows. *Animals (Basel)*. 10 (1), 60-. https://doi.org/10.3390/ani10010060

Yurchenko, S., Sats, A., Tatar, V,. Kaart, T., Mootse, H. & Jõudu. (2018). Fatty acid profile of milk from Saanen and Swedish Landrace goats. *Food chemistry*. 254, 326- 332. https://doi.org/10.1016/j.foodchem.2018.02.041

Ådnøy, T. (2014). The dairy goat industry in Norway: Challenges in a historical perspective. *Small Ruminant Research*. 122 (1–3), 4-9. https://doi.org/10.1016/j.smallrumres.2014.07.011.

Popular science summary

The demand for locally produced food is increasing among Swedish consumers and one of these types of products is locally produced goat cheese. The most used goat breed for milk and cheese production in Sweden is the Swedish Landrace goat which is one of the highest yielding goat breeds in the world. The goat sector is still small in Sweden, but it is steadily increasing as the demand for products from goats is growing.

The composition of milk is crucial for successful production of dairy products such as cheese. Particularly a high protein and fat content of the milk is important as it leads to higher cheese yield. The milk composition is affected by factors such as feeding, health status of the animal, environmental conditions and genetics. In the same way as in cow's milk, casein and whey proteins are the major proteins in goat milk. One of the caseins, i.e., αs_1-casein, has been the topic of several studies over the years, investigating how it affects milk quality and cheese production. It has been reported that high levels of αs_1-casein is related to a higher content of protein, fat and calcium in milk, components which are all very important factors in good cheese quality and high cheese yield. αs_1-casein is encoded by a gene called *CSN1S1* appearing in different variants, so-called genotypes. The different genotypes give rise to different expressions of αs_1-casein which will affect the levels of this protein in the milk. Some of these gene variants cause high expression of αs_1-casein which leads to high levels of this protein in the milk, while other variants give rise to medium, low or even zero levels.

The Swedish Landrace goat is closely related to the Norwegian Landrace goat since many Norwegian goats have been imported to Sweden. It has been found that a large percentage of Norwegian goats have a mutation in the αs_1-casein producing gene that cause extremely low or even zero levels of this protein. Since Swedish and Norwegian goats are closely related, this defect has also been observed in the Swedish goat population.

In this study, goat milk samples were grouped into a low group and a medium-high group, according to their concentration of αs_1-casein, and according to the gene variant observed, to investigate how the levels of αs_1-casein affected the milk and curd yield. The results showed that 48% of the milk samples contained low levels of αs_1-casein while 52% had a medium-high content of this protein. Six different gene variants were found in the tested goats. 19% of the goats had the gene variant which is responsible for low production of αs_1-casein on both chromosomes, while 24% had the gene variant which is responsible for the high production on both chromosomes. In comparison with earlier studies conducted on the Swedish goat population, the results of this study showed a higher number of goats producing medium-high levels of αs_1-casein. When comparing the results for curd yield, there were no significant differences between the low and medium-high groups. Moreover, it was almost no difference in curd yield between the different gene variants. Interestingly, we observed that when αs_1-casein content was low, two other types of caseins and the total amount of caseins were higher, which may suggest that other types of caseins compensate for low αs_1-casein levels.

The amount of total protein and fat was higher in milk from goats with medium-high levels of αs_1-casein compared to the group with low levels of αs_1-casein. Higher levels of total solids and fat were also found in milk from goats with the gene variant that is associated with the highest level of αs_1-casein, but the total protein content was not significantly different between the gene variants. The fat composition also differed between the αs_1-groups and gene variants. Fat composition of milk is highly affected by factors such as feeding and stage of lactation. However, as these factors were not included in this study it was difficult to draw any conclusions about these results.

It was found that the whey fraction from the group with medium-high levels of αs_1-casein contained higher amounts of proteins. This may suggest that the curds from this group had a weaker structure so that more proteins were lost in the whey and the curd yield decreased. In conclusion, it was found that curd yield was more associated with the total amount of total solids, protein, fat and casein rather than the concentration of αs_1-casein. This may therefore be a better predictor of cheese yield.

Acknowledgements

I would like to give a special gratitude and appreciation to my supervisor Monika Johansson, for providing me with guidance during my work and whose contribution and encouragement, helped me in writing my thesis. I would also like to show my gratitude to the farmers who provided the milk samples. Many thanks to you for participating in this study.

Appendix 1

Table 10. Protein profile and average curd yield of control milk samples (n = 5).

Milk quality traits	Mean ± SD	CV (%)
TWP (% of total protein)	9.40 ± 0.14	1.44
α-La (%)	1.35 ± 0.05	3.77
β-Lg (%)	6.08 ± 0.24	3.92
CN (% of total protein)	84.80 ± 0.91	1.08
αs_1-CN (%)	22.98 ± 0.22	0.94
αs_2-CN (%)	4.43 ± 0.45	10.06
β-CN (%)	43.43 ± 0.72	1.65
κ-CN (%)	13.96 ± 0.34	2.42
Curd yield (g/100g)[1]	61.69 ± 1.07	1.74

α-LA = α-lactalbumin; β-Lg = β-lactoglobulin; CN = casein; CV = coefficient of variation; TWP = total whey protein.

[1]n = 3