# Relation Between $\alpha_{S1}$-Casein Content and Coagulation Properties of Milk from Swedish Dairy Goats

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**Abstract:** High frequency of Swedish Landrace goats are the carriers of a mutation, where the affected goats are not able to produce $\alpha_{S1}$-casein ($\alpha_{S1}$-CN) in the milk. As a consequence the lack of this protein is connected to lower cheese yield. The aim of this study was thus to determine the relationship between the $\alpha_{S1}$-CN content and coagulation properties of milk from Swedish Landrace goats. Milk samples from 62 dairy goats from two herds were analysed. As many as 44% goats showed low or no production of $\alpha_{S1}$-CN. Only 24% of the investigated goats showed high production of this protein. The coagulation properties of milk from the dairy goats were clearly influenced by the concentration of $\alpha_{S1}$-CN. The milk from low level $\alpha_{S1}$-CN animals resulted in 15% longer coagulation time and 60% weaker gels compared to the gel firmness of the high expressing group. The coagulation time was strongly associated with the pH of the milk ($p<0.001$), where the milk with low $\alpha_{S1}$-CN content had higher pH. Further, goat milk with low levels of $\alpha_{S1}$-CN was shown to have significantly lower total protein ($p<0.05$) compared to milk from high expressing $\alpha_{S1}$-CN goats.

**Keywords:** $\alpha_{S1}$-casein, capillary zone electrophoreses, coagulation time, gel firmness, rheology, Swedish Landrace goats.

## 1. INTRODUCTION

Goats are among the oldest domesticated animals. Humans have consumed their milk for thousands of years as the goat herding tradition dates back to 8000 BC. Nowadays, the world goat population is the third largest after cattle and sheep [1]. Goat milk products are an important source of nutrients and stand for a part of staple diet. Goat milk consumption is predominant especially in developing countries, however, in Europe the goat milk and caprine foods, mostly cheeses, are perceived as high quality products.

In Scandinavia, Norway is the leading country with respect to goat farming. In Sweden today, there is a new trend with growing interest in organic and local production, where goat cheeses are highly valued and requested. The production of the local goat milk products are likely to increase in the years to come, which is supported by the fact that the number of goats in Sweden in the last decade nearly tripled to approximately 12000 goats.

Sweden, unlike Mediterrean countries, has only four goat breeds: Jämt goat, Göinge goat, Lapp goat and Swedish Landrace. The first three mentioned breeds are very rare and it is only Swedish Landrace *(Capra hircus)* that is of economical importance. Swedish Landrace is a medium size breed, used primarily for the small-scale local milk and cheese production [2].

The most valuable goat milk component is protein. In caprine milk, as in other domesticated ruminants, casein accounts for approximately 80% of total proteins [3]. The casein fraction can be further divided into $\alpha_{S1}$-casein, $\alpha_{S2}$-casein, β-casein and κ-casein. Both quantitative and qualitative content of the protein fraction is influenced by environmental, nutritional, physiological and most importantly genetic factors. The $\alpha_{S1}$- casein gene (CSN1S1) is polymorphic which leads to the existence of multiple variants of $\alpha_{S1}$-CN fractions [4]. Several variants of the CSN1S1 gene have been identified and associated with different content of synthesis of $\alpha_{S1}$-CN in caprine milk. These variants have been classified into the high, medium, low and non expression type (null or zero variant) [5]. The higher the content of $\alpha_{S1}$-CN, the better the cheese yield [6, 7].

Thus, the ability of goats to produce milk with high content of $\alpha_{S1}$-CN is of economical importance for goat cheese producers. Since bucks from Norway have been used in the breeding of Swedish goats, the Swedish Landrace goats are closely related to the Norwegian Landrace. The Norwegian Landrace goats have been shown to belong to a population, which contain a high number of goats producing low concentration of $\alpha_{S1}$-CN. The frequency of the null-variant has in several studies been estimated to over 70%

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[8, 9]. The consequence of the use of Norwegian bucks in the Swedish goat breeding is that as much as 65% of an investigated Swedish goat population produced very little $\alpha_{S1}$-CN, if any [10]. The aim of this work was to determine the relation between the different levels (low, medium and high) of $\alpha_{S1}$-CN content and coagulation properties measured with a rheometer.

## 2. MATERIAL AND METHODS

### 2.1. Animals

The study was conducted with 62 goats (Swedish Landrace) from two herds belonging to Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden and Löfsta Goat Farm in Vallentuna, Sweden. The goats were all in the early part of lactation (week 6-12), when a milk sample (50 ml) was taken from each goat during the morning milking. Only milk from healthy animals was sampled. The samples had 17% bronopol (2 µl/ml) added in order to stop the deterioration process of the milk and were stored at 4ºC until analysis. All samples were also defatted at 3000 x **g** at 4ºC for 10 min before analysis.

### 2.2. Content of $\alpha_{S1}$-CN and Total Protein Composition

The protein profile of the goat milk was analysed by capillary zone electrophoresis (CZE). Electro-migration of the goat milk proteins was carried out with a CZE (G-1600AX, Agilent Technologies Co., SE-164 94, Kista, Sweden), controlled by Chemstation software version A 10.02. Separations of the proteins were performed using unfused silica standard capillary, 50 µm inner diameter, 40 cm active length (Chrom Tech, SE-195 30, Märsta, Sweden). The relative concentration of the $\alpha_{S1}$-CN compared to the other caseins were calculated and classified as low (0-6.9%) medium (7-14.9%) and high (15-25%), as described by [10].

The total protein of the goat milk samples were analysed in duplicates the same day after milking by a mid-infrared (MIR) spectroscopy method (MIRIS AB, Uppsala, Sweden).

### 2.3. Rheological Analyses

Elastic modulus G´and viscous modulus G´´ of the milk samples were continuously measured using a Bohlin CVOR-150-900 rheometer (Malvern Instruments Nordic AB, Uppsala, Sweden). The rheometer was equipped with a cup and a concentric cylinder, 25 and 28 mm in diameter respectively and at a height of 40 mm. Temperature was controlled by a peltier element. Aliquots of milk (12 ml) were equilibrated to 35 °C in a water bath for 10 min. The pH at 35 ºC was measured before rheological analysis of each sample. Chymosin (Chy-max Ultra, Chr. Hansen A/S, Hørsholm, Danmark) was added at a concentration of 0.075 IMCU/ml milk. Each sample was subsequently vortexed before being poured into the cup. Gel forming was followed for 30 min with an oscillation frequency of 1 rad/s and a strain of 0.01, which was well within the linear viscoelastic region of milk gels [11]. Coagulation time (Ct) was measured from the point of the enzyme addition until reaching the 1 Pa value. Measurement frequency was set to 8 s. Gel firmness (G´) of the developing gel was plotted against time after 20 minutes ($G_{20}$).

### 2.4. Statistical Analyses

Analysis of variance and the effects of the $\alpha_{S1}$-CN content on coagulation time and gel firmness were performed on the Statistical Analysis System version 9.3 (SAS Institute, Cary, NC, USA) and ANOVA software using MIXED and GML procedure. Values of $\alpha_{S1}$-CN content were log-transformed to improve distribution. Statistical analyses were performed based on the three different levels (low, medium and high) of $\alpha_{S1}$-CN content. A generalized linear model (GLM) was used with the option "Duncan" to estimate the significance of the milk traits. Relation between investigated variables was examined using Pearson´s (on log-transformed date) and Spearman´s (on original data).

## 3. RESULTS

### 3.1. Distribution of $\alpha_{S1}$-CN in the Goat Population and Total Protein Content

Of the 62 analysed animals, 27 (44%) showed low or non- production of $\alpha_{S1}$-CN content, 33% were medium expressing and 24% were high expressing animals (Table **1**). The mean values of the total protein content are presented in Table **1**. Milk from goats with low $\alpha_{S1}$-CN content had 17% lower amount of total protein ($p<0.05$) compared to the other groups.

**Table 1. Protein composition of Swedish Landrace goats. Average values in milk with low (0-6.9%), medium (7-4.9%) and high (15-25%) $\alpha_{S1}$-casein ($\alpha_{S1}$-CN) content.**

| Content of $\alpha_{S1}$-CN (%) n | Total protein (%) |
|---|---|
| Low 27 | $2.93 \pm 0.30^{b}$ |
| Medium 20 | $3.20 \pm 0.38^{a}$ |
| High 15 | $3.39 \pm 0.44^{a}$ |

Mean ± standard error
Means within a column with different superscripts differ significantly ($p<0.05$).

### 3.2. Coagulation Properties and pH

Both the coagulation time and the gel firmness were influenced by the $\alpha_{S1}$-CN content. The coagulation time for the low level was 15% longer than the group with high levels of $\alpha_{S1}$-CN (Table **2**). The milk with low $\alpha_{S1}$-CN content also showed 60% lower gel firmness ($p<0.01$) compared to the milk with high $\alpha_{S1}$-CN (Table **2**). When coagulation time was correlated with $\alpha_{S1}$-CN content, pH and interaction between $\alpha_{S1}$-CN and pH, only the pH had a significant impact on the coagulation time ($p<0.001$) (data not shown). Both $\alpha_{S1}$-CN content ($p<0.05$) and interaction between $\alpha_{S1}$-CN and pH ($p<0.05$) had a significant influence on gel firmness (data not shown).

**Table 2. Coagulation properties and pH of milk from Swedish Landrace goats. Average coagulation time (Ct), gel firmness after 20 minutes ($G_{20}$), and pH in milk with low (0-6.9%), medium (7-4.9%) and high (15-25%) $\alpha_{S1}$-casein ($\alpha_{S1}$-CN) content.**

| | Content of $\alpha_{S1}$-CN | | | |
|---|---|---|---|---|
| | **Low** | **Medium** | **High** | **p** |
| Ct (s) | 905 ± 5 | 780 ± 6 | 767 ± 7 | Ns |
| $G_{20}$ (Pa) | 18 ± 4 | 28 ± 4 | 44 ± 6 | ** |
| pH | 6,56 ± 0,02 | 6,52 ± 00,02 | 6,51 ± 0,02 | Ns |

Mean ± standard error
**p<0.01, Ns = non-significant

**Table 3. Correlation between $\alpha_{S1}$-casein content, coagulation time, pH and gel firmness according to Pearson´s (above diagonal) and Spearman´s (below diagonal). $\alpha_{S1}$-CN = $\alpha_{S1}$-casein; Ct = coagulation time; $G_{20}$ = gel firmness after 20 minutes.**

| | Content of $\alpha_{S1}$-CN | Ct | pH | $G_{20}$ |
|---|---|---|---|---|
| Content of $\alpha_{S1}$-CN | | -0.29 * | -0.26** | 0.43*** |
| Ct | -0.24 * | | 0.58*** | -0.67*** |
| pH | -0.27* | 0.61*** | | -0.68*** |
| $G_{20}$ | 0.42*** | -0.83*** | -0.71*** | |

Correlation performed on logarithmic transfer of $\alpha_{S1}$-casein content
Significance levels at *p<0.05, **p<0.01, ***p<0,001 are indicated

To determine dependence between $\alpha_{S1}$-CN content, pH, coagulation time and gel firmness, Pearson´s and Spearman´s correlation efficiencies were calculated. From these results it was shown that content of $\alpha_{S1}$-CN was negatively related to coagulation time and pH and positively related to gel firmness (Table **3**). However, correlation between $\alpha_{S1}$-CN, coagulation time and pH, although significant, was quite week. The highest correlation coefficient was obtained for coagulation time and gel firmness (r = - 0.83) suggesting that the coagulation time was the most important factor in determination of gel firmness.

## 4. DISCUSSION

The amount of $\alpha_{S1}$-CN in goat milk is crucial for the cheese yield and for the economy of goat cheese producers. Whereas studies of $\alpha_{S1}$-CN have been carried out in Europe since the early 1980s, research connected to $\alpha_{S1}$-CN in milk from dairy goats in Sweden has been very limited. Therefore, the results from this study could be useful not only for goat cheese producers, but also for the breeding program of Swedish goats. Here, we report the correlation between $\alpha_{S1}$-CN content, coagulation time and gel firmness in milk from Swedish Landrace goats.

To study the correlations between the $\alpha_{S1}$-CN content and coagulation properties, we sampled two herds where we knew that there were several high $\alpha_{S1}$-CN producing goats in addition to low and medium producing. Of the investigated goats (62 animals) the ratio was 44, 32 and 24% with low, medium and high $\alpha_{S1}$-CN content, respectively. In this way we had enough with milk samples from each group to run statistical analyses.

The quantity of total protein from Swedish goats was generally lower than in other commercially used breeds [12]. However, in our study the lower amount of total protein was significantly correlated with the low $\alpha_{S1}$-CN content (p<0.005) (Table **1**). These findings agree with those reported by [12] and [7] who found that the content of total protein in goat milk is directly related to higher content of $\alpha_{S1}$-CN. Even more, [7] reported that total protein, amount of $\alpha_{S1}$-CN and coagulation time was positively correlated. Though, the influence of $\alpha_{S1}$-CN on coagulation time has been a point of a debate in the literature. Unlike, [3] who reported elongated coagulation time for the milk with high $\alpha_{S1}$-CN content, we found that the low levels of $\alpha_{S1}$-CN results in longer coagulation time and weaker gels (Table **2**). The coagulation time was though not significant (p>0.005) when the goats were classified as three different levels (low, medium and high) and compared with each other. When classification into levels was eliminated and overall amount of $\alpha_{S1}$-CN in all analysed milk samples was taken into consideration, the results showed a significant correlation between those parameters (data not shown). Thus, in order to obtain more precise results of statistical analysis, the number of goat milk samples need to be enlarged.

Nevertheless, our findings regarding the low levels of $\alpha_{S1}$-CN connecting to longer coagulation time and weaker gels were in agreement with [7] who showed that goat milk that lacked $\alpha_{S1}$-CN had poorer coagulation properties (longer coagulation time and weaker gels) than milk that contained $\alpha_{S1}$-CN. Regarding gel strength, [9] also reported that milks with high $\alpha_{S1}$-CN content had significant higher gel firmness. This was in accordance with our results, where the low-type milk formed significantly (60%) weaker gel (Table **3**). This means that the high level of $\alpha_{S1}$-CN is positively correlated with the amount of total protein and can be also related to increases in gel firmness and thereby cheese yield [7].

For the future there is a need for improvement of cheese-milk quality of Swedish dairy goats by increasing the $\alpha_{S1}$-CN content. It would be beneficial from the economical point of view, since the higher the protein content, the higher the cheese yield.

## CONCLUSION

From the findings of this study it can be concluded that the coagulation properties of goat milk is highly dependent on $\alpha_{S1}$-CN content. If the content of this protein is high in the milk, the higher the amount of the total protein, the lower the pH, the shorter the coagulation time and the better the gel firmness.

## CONFLICT OF INTEREST

The authors confirm that this article has no conflict of interest.

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