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ORIGINAL ARTICLE

Extreme frequencies of the α_{s1}-casein "null" variant in milk from Norwegian dairy goats— implications for milk composition, micellar size and renneting properties

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Abstract Caprine α_{s1}-casein polymorphism is one of the key factors which determines important technological properties of milk, such as rennetability and cheese yield. The indigenous Norwegian goat breed is characterized by a remarkably high frequency (>70%) of a "null" variant of α_{s1}-casein unique to this population. This mutation, referred to as the exon 12 D allele, involves a single base deletion in exon 12 of the α_{s1}-casein gene. The aim of this study was to provide more detailed

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information on how α_{s1}-casein polymorphism affects composition and rennet coagulation properties of milk from Norwegian dairy goats. Class of α_{s1}-casein significantly affected milk composition; crude protein, casein and pH. The mean casein micelle size was larger in milks containing α_{s1}-casein "null" variant when compared to "strong" milks; in contrast, the heat-induced changes in micelle size appeared to be least pronounced in this group. Compositional differences between "strong" and "null" milks may explain these differences. α_{s1}-Casein class had a significant effect on gel strength recorded 30 min after the addition of rennet, with the "strong" milks giving the greatest firmness. However, a considerable fraction of the milk samples in this study were unable to form a strong clot, regardless of the α_{s1}-casein variant. These results indicate that environmental and compositional factors not considered in this study may be important in the curd formation process.

Keywords Caprine milk · α_{s1}-casein · Composition · Micellar size · Rennet coagulation

摘要 – 挪威奶山羊乳中 α_{s1}–酪蛋白"零" 型变异体呈现高频率 – 影响着乳组成、胶束大小及凝乳特性。 山羊 α_{s1}-酪蛋白遗传多态性是决定乳重要技术特性(如凝乳特性和干酪产量) 的重要因素之一。来自本土挪威山羊品种的乳非常独特、这个群体中α_{s1}-酪蛋白"零" 型变异体出现的频率高达 70% 以上。参照第 12 外显子 D 等位基因、这个突变体涉及到第 12 外显子中单个碱基的缺失。本研究目的在于提供、关于α_{s1}-酪蛋白遗传多态性对挪威乳山羊乳组成和凝乳特性影响更为详尽的信息。 α_{s1}-酪蛋白的类型显著影响着乳组成、粗蛋白、酪蛋白和 pH。与含有 αs1-酪蛋白"强"型变异体的乳相比、含"零"型变异体的乳、形成的酪蛋白胶束的平均直径更大、且由热诱导对胶束尺寸的影响更小。包含"强" 型和"零" 型变异体的乳组成的差异可以解释上述区别。α_{s1}-酪蛋白类型对在加入凝乳酶 30 分钟后的胶强度具有显著的影响。含有"强" 型变异体的乳具有最大的硬度。然而、本研究中、无论含有何种 α_{s1}-酪蛋白变异体类型、乳样中相当大比例不能形成强的凝块。这些结果表明本研究中没有考虑的环境和组成因素、可能对凝块的形成过程起到重要作用。

关键词 山羊奶 · αs1-酪蛋白 · 乳组成 · 胶束大小 · 酶凝乳

1 Introduction

The polymorphism at the caprine α_{s1}-casein locus has received considerable interest over the past years due to its impact on quality and important technological properties of the milk. The basis for these compositional and technological variations is a highly variable content of α_{s1}-casein of the milk caused by considerable differences in casein synthesis (Marletta et al. 2007).

To date, as many as 18 different variants of caprine α_{s1}-casein have been identified, each associated with a specific protein concentration in the milk (Caroli et al. 2007; Park et al. 2007). Based on the concentration of α_{s1}-casein present in the milk, the variants are commonly organized into four classes: (1) "strong" variants (A, B1, B2, B3, B4, Bk, C, H, L and M) synthesized at a high level (3.5 $g \cdot L^{-1}$ per allele), (2) "intermediate" variants (E and I) synthesized at a intermediate level (1.1 $g \cdot L^{-1}$ per allele), (3) "weak" variants (F and G) synthesized at a low level (0.45 $g \cdot L^{-1}$ per allele) and (4) "null" variants (01, 02 and N) not associated with protein synthesis (for review see (Küpper et al. 2010; Marletta et al. 2007)).

In addition to a higher content of α_{s1}-casein, milks containing these "strong" variants ("strong" milks) are characterized by higher content of total protein, milk fat, calcium, higher Ca-ion activity, lower pH and smaller casein micelles when compared to their "intermediate", "weak" or "null" counterparts. Moreover, "strong" milks have more favourable cheese making properties (rennet coagulation properties, cheese yield and quality) (Clark and Sherbon 2000a, b; Pierre et al. 1998a) and are less prone to lipolysis and off-flavours (Chilliard et al. 2003) than milks of other α_{s1}-casein classes.

In Europe caprine milk is mainly used for cheese manufacture, and in most economically significant breeds frequencies of "strong" and "intermediate" α_{s1}-casein alleles dominate (for review see (Marletta et al. 2007)). The occurrence of "weak" and "null" variants is reported to be very low in most European breeds, except from the Spanish Canaria and the Italian Frisa breeds in which the gene frequency of α_{s1}-casein "null" is 20% and 10%, respectively (Caroli et al. 2006; Jordana et al. 1996). Moreover, a frequency of 23% of the "weak" F allele has recently been observed in the Italian Garganica breed (Albenzio et al. 2009). The situation regarding frequencies of α_{s1}-casein variants in Norwegian caprine milk is strikingly different. In a study by Hayes et al. (2006) where 39 SNPs of the 4 casein genes were typed in 436 bucks, the "null" variant, unique to the Norwegian goat population, was found at a very high frequency (0.72). This particular mutation arising from a deletion in exon 12 of the α_{s1}-casein gene (CSN1S1) and referred to as the D allele (referred to as allele 1 of exon 12 in Hayes et al. (2006)) was found to decrease contents of protein and fat of the milk (Ådnøy et al. 2003; Dagnachew 2009).

The aim of this study was to provide more in-depth analysis on how this Norwegian "null" variant of α_{s1}-casein affects other aspects of milk composition (contents of protein, casein, calcium and magnesium, pH, Ca-ion activity), mean size of casein micelles and rennet coagulation properties.

2 Materials and methods

2.1 Milk samples

Milk samples were obtained from 254 goats from nine farms in three different geographical regions of Norway. Herds 1 (69 animals), 2 (74 animals) and 3 (12 animals) were located in the North; Herds 4 (20 animals) and 5 (12 animals) in the South-West; Herds 6 (20 animals), 7 (20 animals), 8 (19 animals) and 9 (8 animals) in the South-East. The goats, aged 1–5 years,were of the Norwegian Dairy Goat breed, a landrace of ancient origin.

Milk samples from the animals belonging to the Herds 1, 2, 6 and 7 were collected once. To study changes in milk composition and renneting properties with time, milk samples from the animals in Herds 3, 4, 5, 8 and 9 were repeatedly collected three or four times in intervals of 2 or 3 weeks.

A sample of 40 mL was taken from each goat during the morning milking. The samples were preserved by addition of 0.02% (w/v) sodium azide and defatted by centrifugation at 3,000× *g* as previously described (Devold et al. 2000). Measurements of pH, Ca-ion activity, mean micellar size and renneting properties and preparation of

the non-casein nitrogen fraction were performed on fresh milk. The milk samples were kept at −20°C for further use.

2.2 Milk composition

The levels of total nitrogen (TN) and non-casein nitrogen (NCN) were determined using the Kjeldahl method (Kjeltec 1035 Analyser, Tecator, Höganäs, Sweden). Casein nitrogen was calculated as the difference between TN and NCN. The content of crude protein (CP) and casein (CN) was obtained using the conversion factor of 6.38, for N to protein (IDF – International Standard (IDF 20-5) & Milk – Determination of nitrogen content – Part 5: Determination of protein-nitrogen content 2001).

The NCN fraction was obtained from the skimmed milk by isoelectric precipitation of the caseins, essentially as described by Aschaffenburg and Drewry (Aschaffenburg and Drewry 1959) using smaller volumes of milk and reagents and a premixed solution of acetic acid, sodium acetate and water (1:1:4 v/v). In brief, 4 g of milk were mixed with 6 g of the acetic acid-sodium acetate-water solution to obtain a pH of 4.6. The NCN fraction was separated from the precipitated caseins by centrifugation at 3,000×*g* for 20 min at room temperature.

Milk pH and Ca-ion activity were measured using a pH and ion meter (Radiometer, Copenhagen, DK) equipped with a combined pH electrode or an ion selective electrode and a calomel reference electrode, respectively. The apparatus was calibrated using calcium-containing standard solutions (Holt et al. 1981). The contents of total calcium and magnesium were measured by atomic absorption (Devold et al. 2000).

2.2.1 Identification of α_{s1}-casein variants by isoelectric focusing

The genetic variants of α_{s1}-casein in milk from individual animals were identified by isoelectric focusing (IEF) using ultrathin (0.3 mm) urea polyacrylamide gels according to a modified method of Erhardt (1989). A mixture of carrier ampholytes was chosen to give maximum resolution of the caprine α_{s1}-casein complex; Ampholine, narrow range pH 3.5–5.0, Pharmalyte, narrow range pH 4.2–4.9 and Pharmalyte narrow range pH 5.0–6.0 (Amersham Biosciences, Uppsala, Sweden) were used at a volume ratio of 3:4:1. The protein load was increased threefold from that of the original protocol to obtain a more reliable identification of “weak” and “null” variants of α_{s1}-casein. The gels were stained with Coomassie Brilliant Blue R-250.

The α_{s1}-casein variants were identified by the use of reference samples of known identity; AA, BF, CC, EE or 00 (kindly provided by Prof. F. Grosclaude, INRA, France) and Norwegian “null” or F-like (selected samples from Herds 1 and 2) (Ådnøy et al. 2003).

2.2.2 Identification of caseins by MALDI peptide mass fingerprinting

Proteins were separated by IEF and visualized by staining with Coomassie Brilliant Blue. Identification of dissected protein bands by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was carried out

essentially as described in Devold et al. (2006). Resulting tryptic peptides were desalted and concentrated on a Zip-tip column (Millipore, MA, USA) and successively dried on the MS target in presence of acidified α-cyano-4-hydroxycinnamic acid and trifluoroacetic acid. MALDI MS spectra of the peptide mixtures were obtained in positive reflector-ion mode on a Voyager DE PRO mass spectrometer (Applied Biosystems, MA, USA). Monoisotopic peptide masses were assigned and used in database searches against the NCBI and Swissprot databases.

2.2.3 Genotyping of α_{s1}-casein variants by PCR

The goats from Herds 1 and 2 were genotyped using a PCR method that allowed identification of three alleles in exon 12 of CSN1S1: here termed D, G and A. This method allowed identification of Norwegian "null" and F-like α_{s1}-casein variants (encoded by the D and G alleles, respectively) (Ådnøy et al. 2003). The exon 12 A allele is found in all α_{s1}-casein variants other than "null" and F-like. DNA for genomic analysis was isolated from blood samples according to Rogne et al. (1987). The PCR analysis was peformed as previously described by Ådnøy et al. (2003). In brief, a typical 40 PCR cycles was performed in a PCR mixture containing: 10 μL 10 X PCR buffer (500 mmol·L^{-1} KCl, 200 mmol·L^{-1} Tris-HCl, 1.5 mmol·L^{-1} MgCl2, pH 8.4 and 0.01% gelatin), 0.2 mmol·L^{-1} of each dNTP, 2.5 U AmpliTaq DNA polymerase, 40 pmol of each primer, 50 ng DNA and H2O to a final volume of 100 μL. Denaturation; 1 min at 95°C, annealing; 45 s at 58°C, extension; 2 min at 73°C. Sequencing was carried out with ABI PRISM®

BigDye™ Terminator Cycle Sequencing Ready Reaction Kit and the automatic DNA-sequencer Model 377 (PE Applied Biosystems, Wellesley, MA, USA).

2.3 Mean size of native and heated casein micelles

The mean size of native and casein micelles in unheated (raw) and heated milks was determined by photon correlation spectroscopy on skimmed milks as previously described by Devold et al. (2000). The effect of heat treatment on mean micelle size was measured after heating the skim milk samples at 70, 80 and 90°C for 30 min. Prior to particle size analysis, samples were diluted in simulated milk ultrafiltrate (Jenness and Koops 1962) which had been filtered through a 0.2 μm filter (Millipore, Bedford, MA, USA). Size measurements were performed using either the Coulter Model N4MD apparatus (Coultronics, Hialeah, FL, USA) or the ZetaSizer 3000 HS (Malvern Instruments, Malvern, UK).

2.4 Rennet coagulation properties

Rennet coagulation properties were measured using the Formagraph method (Foss Electric A/S, Hillerød, DK). Chymosin (Naturen Standard 190, Chr. Hansen Laboratorium, Hørsholm, DK) was diluted in acetate buffer (1:50) and added to milk at a level of 20 μL·mL^{-1}. Four parameters were derived from the resultant traces: rennet clotting time (RCT; time from addition of rennet to starting point of curd formation), curd firming times (K12, K20; time from starting point of curd

formation until curve widths of 12 or 20 mm width, respectively) and gel strength (A30; width of curve 30 min after rennet addition).

2.5 Statistical analyses

For statistical evaluation, the milk samples were divided into four classes: "strong", "intermediate", "weak" and "null" based on their IEF "α_{s1}-casein profile"; e.g. all homozygous "strong" samples and heterozygous samples containing one "strong" genetic variant were merged into the "strong" class.

Some samples exhibited poor renneting properties and did not coagulate. Other samples coagulated but did not reach the gel strengths of 12 and/or 20 mm. In cases where no coagulation was observed after the 30 min experimental period a RCT of 50 min was used for further evaluation of the results. Samples that did not reach K12 and K20 were given fixed values of 20 and 40 min, respectively.

The response variables were analysed by using the mixed model procedure (proc mixed) of SAS (SAS release 8.3, SAS Institute, Cary, NC, USA), based on the following model:

$$y = \text{class of } \alpha_{s1} - \text{cn} + \text{age of goat} + \text{day and herd} + \text{goat} + \text{residual}.$$

The explanatory fixed effects included were: group of α_{s1}-casein: "strong", "intermediate", "weak" and "null"; age of goat, either 1, 2, 3 or 4 or >4 years; day and herd: test day in herd (4 different test days, 9 herds). The random effects were goat+residual.

Differences between treatment means were considered significant when resultant P values were >0.05.

Proportions of samples in each α_{s1}-casein class without proper coagulating properties (i.e. samples in which RCT, K12 and K20 were not achieved during the measurement period as described above) were compared using Fisher's Exact test SAS (SAS release 8.3, SAS Institute, Cary, NC, USA). Significance was set at the 5% level (two-tailed P). Regarding the animals in Herds 3, 4, 5, 8 and 9 from which samples were repeatedly collected, milks from the first sampling were included in this dataset.

3 Results and discussion

3.1 α_{s1}-casein polymorphism and milk composition

Our results showed a remarkable high frequency (70%) of homozygous "null" goats. These results are in agreement with the previously published results of Hayes et al. (2006). Frequencies "strong", "intermediate" and "weak" goats in our study were 17%, 2% and 11%, respectively. α_{s1}-casein variants in milk samples from Herds 1 and 2 were determined by both IEF and PCR methods. The results based on the latter method were particularly noteworthy; animals devoid of the exon 12 D allele ("null" allele) were found at very low frequencies (2%). Frequencies of individuals homozygous and heterozygous for this allele were 73% and 25%, respectively (Ådnøy et al. 2003).

Isoelectric focusing protein profiles of a selection of caprine milks with different genetic variants of α_{s1}-casein are presented in Fig. 1. From this figure it is evident

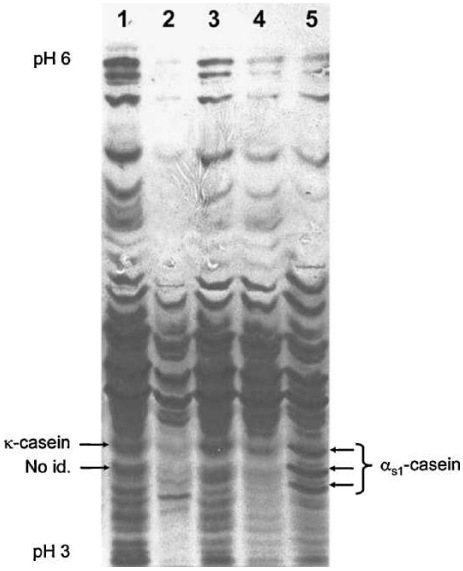

Fig. 1 IEF protein profiles of caprine milks with different α_{s1}-casein variants (genotypes are given in parentheses). Lanes 1 and 3: Atypical α_{s1}-casein "null" milks (00), Lane 2: α_{s1}-casein F-like milk (F0), Lane 4: Typical α_{s1}-casein "null" milk (00) and Lane 5: α_{s1}-casein A-like milk (X0)

that some milk samples exhibited an atypical band pattern; in the "null" milks (genotype 00) seen in Lanes 1 and 3 high concentration of proteins appeared in the α_{s1}-casein area. The samples shown in Lanes 2, 4 and 5 represent F-like, "null" and A-like variants (genotype F0, 00 and X0), respectively. MALDI peptide mass fingerprinting was used to identify two protein bands taken from one of the atypical "null" milks (Lane 1). No traces of α_{s1}-casein were detected in these bands. Analysis of the band co-migrating with the upper band of α_{s1}-casein A (highest pI) revealed the presence of κ-casein. The identity of a second protein band corresponding to the α_{s1}-casein A middle band could not be established. The intensities of these non-α_{s1}-casein protein bands were highly variable between samples (data not shown). IEF profiles of most "strong" milks in this study resembled the A variant of α_{s1}-casein standard. Such "A-like" variants (Lane 5 in Fig. 1) may correspond to "new" protein variants (encoded by α_{s1}-casein haplotype 4) recently identified in the Norwegian goat population (Hayes et al. 2006; Pellerud 2006).This particular haplotype found at a frequency of 6% and with a positive impact on protein content in the milk was suggested as the "link" between the A variant and the Norwegian "null" variant (referred to as haplotype 1).

Mean values for compositional factors, casein micelle size and rennet coagulation characteristics of the different α_{s1}-casein classes and statistical analysis of these data are given in Table 1. These results showed that class of α_{s1}-casein ("null", "weak", "intermediate" and "strong") had a statistically significant effect on contents of CP and casein, pH, Ca-ion activity (P=0.06), content of magnesium, mean size of native micelles and gel strength (A30).

In the present study the contents of CP and casein were significantly lower in "null" milks than in milks of the "strong" group. In a recent study the exon 12 D allele of α_{s1}-casein was found to reduce the content of protein in Norwegian caprine milk (Dagnachew 2009). These observations are in good agreement with previous

Table 1 Effect of class of α_{s1}-casein on milk composition, mean micellar size and rennet coagulation properties. Significant effects of casein class are indicated by $P<0.05$ (bold letters). Results are given as mean values (ls means)

		Number of animals	Herds	P value	Class of genetic variants of α_{s1}-casein			
					Strong	Intermediate	Weak	Null
Milk composition	Crude protein (%)	234	1–6, 8, 9	0.038	2.98[a]	3.19[ab]	2.94[b]	2.87[b]
	Casein (%)	234	1–6, 8, 9	0.002	2.05[a]	2.05[ab]	1.92[ab]	1.93[b]
	pH	254	All	0.001	6.66[a]	6.75[ab]	6.70[b]	6.73[b]
	Ca-ion activity (mM)	222	1, 2, 4, 6–8	0.064	2.78[a]	2.75[ab]	2.34[ab]	2.37[b]
	Ca (mmol·L^{-1})	163	1, 2, 7	0.543	25.29	25.00	23.97	24.95
	Mg (mmol·L^{-1})	163	1, 2, 7	0.014	6.22[a]	7.12[a]	6.21[b]	6.47[b]
Mean size of casein micelles	Native micelles (nm)	222	1, 2, 4, 6–8	0.001	**207[a]**	**195[ab]**	**222[b]**	**216[b]**
	Heated micelles (nm)	59	4, 6, 8	0.471	261		243	250
	Difference in size (nm)	59	4, 6, 8	0.326	20		10	17
Rennet coagulation properties	RCT (min)	202	1–4, 8, 9	0.131	7.3	8.1	13.1	10.7
	K12 (min)	202	1–4, 8, 9	0.128	4.2	3.6	6.4	6.1
	K20 (min)	202	1–4, 8, 9	0.248	11.2	11.5	12.1	15.5
	A30 (mm)	202	1–4, 8, 9	0.001	**29.1[a]**	**24.8[ab]**	**25.2[ab]**	**21.5[b]**

[a,b] New letter indicates that this group is statistically significant from the previous one.

results (Clark and Sherbon 2000a; Grosclaude et al. 1987; Pierre et al. 1998b; Pirisi et al. 1994; Schmidely et al. 2002). In these aforementioned studies the difference in casein content between "weak" and "strong" milks was smaller than the actual difference in their α_{s1}-casein content (i.e. ~3 g per allele L^{-1} between homozygous A and F milks). Grosclaude and Martin (1997) pointed out that this may be due to an increased production of other caseins, such as β-casein in low α_{s1}-casein milks. These observations conflict with the work of Chanat et al. (1999),who reported that intracellular transport of casein in secretory cells was impaired in milks devoid of α_{s1}-casein.

The difference in casein content between "strong" and "null" milks in our study (1.2 g·L^{-1}, respectively) was less pronounced than that reported in the aforementioned studies. The high frequency of heterozygous "null-strong" α_{s1}-casein goats in the "strong" group as confirmed by the PCR analysis of the animals in Herds 1 and 2 may explain this trend. The contents of CP for the "strong" and "null" classes were 2.98% and 2.87%, respectively. No effect of class of α_{s1}-casein was seen on the content of NCN (i.e. difference between CP and casein content). These results may indicate that the reduced rate of α_{s1}-casein synthesis in "null" goats is compensated for by synthesis of other caseins and not by other nitrogen-containing compounds such as whey proteins.

Our results showed that the pH in milk of the α_{s1}-casein "strong" group was significantly lower than that of "null" milks. Hence, our results are in keeping with

previous findings in that α_{s1}-casein polymorphism was correlated with milk pH (Ambrosoli et al. 1988; Pierre et al. 1998b; Pirisi et al. 1994). However, the link between α_{s1}-casein polymorphism and calcium content distribution in the milk was less clear; both the fraction of Ca (i.e. total amount, soluble content and/or ion activity) seemed to be affected, as well as the magnitude of these variations between "strong" and "weak" milks among the reports (Ambrosoli et al. 1988; Pierre et al. 1998b; Pirisi et al. 1994; Schmidely et al. 2002). No difference was found in total calcium content between "strong" and "null" milks in our study. Thus, the higher Ca-ion activity observed in "strong" milks is explained by their lower pH compared to their "null" counterparts. When pH is reduced, the equilibrium between insoluble colloidal calcium phosphate associated with casein micelles and soluble complexes is shifted towards the latter (Fox and McSweeney 1998). A pH shift in bovine milk similar to the difference between "null" and "strong" milks in our study (6.73 vs. 6.66) gave an increase in Ca-ion activity corresponding to the differences observed between these milks (2.37 vs. 2.78 mmol·L^{-1}) (Lin et al. 2006).

3.2 α_{s1}-casein polymorphism and mean size of casein micelles in unheated and heated milks

Our results showed that individual milk samples devoid of α_{s1}-casein ("null" class) were characterized by larger micelles than α_{s1}-casein "strong" milk samples. These differences were statistically significant and in good agreement with previous studies (Pierre et al. 1995, 1998a, 1999; Tziboula and Horne 1999), all of which concluded that the mean size of casein micelles increased as the level of α_{s1}-casein decreased. Caseinmicelles in caprine milks with a low content of α_{s1}-casein have been described as more highly hydrated than those from milks having a high content of this protein (Mora-Gutierrez et al. 1997). This may in part explain some of the differences in mean size between casein micelles from milks with "strong" and "weak" variants of α_{s1}-casein.

Previously reported differences in mean size between α_{s1}-casein "null" and "strong" micelles were much more pronounced than those reported in the current study (216±26 vs. 207±35 nm); 280 vs. 200 nm (Pierre et al. 1995) and 260 vs. 220 nm (Pierre et al. 1998c). These previous studies included only three α_{s1}-casein "null" goats, compared to the present study including 150 homozygous α_{s1}-casein "null" goats. The smaller differences in mean size observed in our study are probably related to the smaller differences in casein content between "strong" and "null" milks compared to the studies mentioned above in which the variation in casein content between "strong" and "null" milks was much more pronounced.

The studies by Pierre et al. (1995, 1998c) concluded that the content of α_{s1}-casein was negatively correlated to the mean size of casein micelles in caprine milks.

To date, no information on the effect of heat treatment on the mean size of caprine casein micelles has been reported. In this study, the milk samples were heated at 90°C for 30 min. No changes in micellar size were observed when milk samples were heated at lower temperatures, such as 70 and 80°C (data not shown). There was no significant relationship between class of α_{s1}-casein and the mean size of heated casein micelles

(P=0.47). However, the heat-induced changes were smaller in "null" than in "strong" micelles (10 vs. 17 nm).

3.3 α_{s1}-casein polymorphism and rennet coagulation properties

The results in Table 1 showed that class of α_{s1}-casein significantly affected the gel strength (A30) with the "strong" milks giving firmer gels than the "null" milks.

It is generally accepted that α_{s1}-casein polymorphism has a strong impact on renneting properties of the milk with "strong" milks giving firmer gels and higher cheese yield than milks with "weak" or "null" α_{s1}-casein variants. However, much remains uncertain regarding the nature and magnitude of this relationship (the type of renneting property to be affected, as well as the magnitude of variations). In a recent study by Albenzio et al. (2009) no significant differences in coagulation properties were observed between two groups of goats characterized by the highest frequency of strong or weak alleles at CSN1S1 locus. In a study of Ambrosoli et al. (1988) RCT was significantly affected by α_{s1}-casein variant ("high type" vs. "low type"). Pirisi et al. (1994) found only minor differences in the RCT between the milks of two groups of goats classified "high" and "zero" using a turbidimetric method.

In the studies by Clark and Sherbon (2000a, b) the presence of "strong" α_{s1}-casein variants positively influenced K20, however, the authors concluded that rennet coagulation properties were better correlated to contents of casein and α_{s1}-casein than to the actual variant of α_{s1}-casein. In the studies described above, longer RCTs were observed with "strong" milks, thus they conflict with our results which showed the opposite trend.

The composition of "strong" milks (lower pH, higher content of casein and calcium and higher Ca-ion activity) in our study was more favourable regarding renneting properties compared to "null" milk samples. These findings correspond with previously published results (Ambrosoli et al. 1988; Clark and Sherbon 2000a; Pierre et al. 1998a; Pirisi et al. 1994) and may in part be responsible for the better rennet coagulation properties of milks of the "strong" α_{s1}-casein group. By manipulating these compositional factors Remeuf et al. (1995) considerably improved the rennet coagulation properties of caprine milk containing "weak" α_{s1}-casein variants. Adjustments of pH and protein content proved to be the most successful approach.

Some of the milk samples in the current study had poor coagulation properties and did not give recordable Formagraph values of K12 and K20. The proportions of such

Table 2 Proportion of milk samples (given in%) in each α_{s1}-casein class in which gel strengths of K12 and K20 were not achieved during the Formagraph measurement

Rennet coagulation property not achieved	Proportion of samples per class of α_{s1}-casein				P value*
	Strong	Intermediate	Weak	Null	
K12	2.8	0	0	4	ns
K20	5.6	0	6.7	11.4	ns

ns: not significant

samples in each class of α_{s1}-casein are shown in Table 2. A considerable fraction of samples regardless of group failed to form strong gels (K20); these samples accounted for 11.4% and 5.6% in the "null" and "strong" groups, respectively. However, the proportions of poor renneting samples were not significantly different between α_{s1}-casein classes. It should be noted that this analysis was done on a dataset including milks from the first sampling in cases where samples were repeatedly collected. It should be noted that in these cases of repeated sampling, the proportions of poor renneting samples remained constant as stage of lactation increased.

4 Conclusion

Norwegian Dairy Goats are exceptional in having a remarkable high frequency of an α_{s1}-casein "null" allele. This allele is found at high frequencies in homozygous (70%) and heterozygous forms, and goats carrying only "non-null" α_{s1}-casein alleles are found at very low frequencies. The low frequencies of the latter in the "strong" α_{s1}-casein class in this study may explain the smaller than expected differences in milk composition and renneting properties between our "strong" and "null" milks. The potential for improving milk composition and thereby its coagulation properties by selecting for "strong" α_{s1}-casein variants may be significant.

Further research on Norwegian goats' milk should focus on the phenotypic differences between the recently identified casein haplotypes many of which are unique to this national goat population. Emphasis should be on compositional factors which are likely to influence quality of the milk and products made therefrom, such as the contents of total casein and individual caseins, rate lipolysis and content of free fatty acids.

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