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Genetic variants of alpha$_{s1}$-CN in goat milk: breed distribution and associations with milk composition and coagulation properties

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Abstract

One dairy goat herd of mixed breeds was investigated for frequency of genetic variants of alpha$_{s1}$-casein (α_{s1}-CN) and relationships between the genetic variants, milk composition, coagulation properties, and breed. Milk composition (percent total solids, solids-non-fats (SNF), fat, protein, casein, and α_{s1}-CN), genetic variants of α_{s1}-CN, and coagulation properties (coagulation time, coagulation rate, and curd firmness) were determined for the milk of 93 individual goats. The elution order of the α_{s1}-CN genetic variants by reverse-phase high pressure liquid chromatography was D, F, (E and B3), C, A, B2 or B1. The frequencies of individual variants, in descending order, were F (52.7%), E (18.3%), A (9.7%), B1 or B2 (4.8%), O and D (4.8% each), C (2.7%), and B3 (2.2%). The most common allele combinations were F/F (37.6%), F/E (10.8%), and E/E (10.8%). Milks containing at least one 'high type' genetic variant (α_{s1}-CN A, B1, B2, B3, or C) contained higher percent total solids, SNF, protein, and α_{s1}-CN than milks that contained only 'low type' variants (α_{s1}-CN F or D) or were homozygous for the null variant (α_{s1}-CN O/O). Alpha$_{s1}$-CN genetic variants were not highly correlated with coagulation properties. Milk from Nubians was more likely to contain a high type genetic variant than Alpine milk.

Keywords: Goat milk; Cheese; Coagulation; Alpha$_{s1}$-casein; Genetic variants

1. Introduction

By the early 1980s seven genetic variants of caprine α_{s1}-CN had been identified (Boulanger et al., 1984). Soon after that, it was recognized that the genetic variants could be grouped into categories based on levels of synthesis of α_{s1}-CN in goat milk (Grosclaude et al., 1987). Grosclaude et al. (1987) showed that 'high type' variants A, B, and C were associated with higher amounts of α_{s1}-CN (approximately 3.6 g/l); 'intermediate type' variant E was associated with intermediate amounts (approximately 1.6 g/l); and 'low type' variants D and F were associated with low amounts (approximately 0.6 g/l) of α_{s1}-CN in goat milk. Milk which was homozygous for variant O, the 'null type', lacked α_{s1}-CN. The authors suggested that each individual variant played an additive role in dictating the synthesis of α_{s1}-CN. For instance, the F/F combinations were found to yield significantly less α_{s1}-CN than E/F, and both yielded significantly less α_{s1}-CN than A/F and A/E combinations (Grosclaude et al., 1987).

In the late 1990s, three additional α_{s1}-CN genetic variants were identified (Martin and Addeo, 1996).

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Variant G, like D and F, was found to be associated with low amounts of α_{s1}-CN in milk. Additionally, variant B, originally thought to be one variant, was found to exist in three different forms; B1, B2, and B3, which are all associated with relatively high synthesis of α_{s1}-CN.

To date, ten genetic variants of the protein alpha$_{s1}$-casein (α_{s1}-CN) have been identified in goat milk (α_{s1}-CN A, B1, B2, B3, C, D, E, F, G and O) (Boulanger et al., 1984; Grosclaude et al., 1987; Brignon et al., 1989; Martin and Addeo, 1996). Thus, one of 55 possible allelic combinations of genetic variants may exist in the milk of an individual goat. This fact has led several researchers to investigate how genetic variants may influence milk composition and functional properties (Boulanger et al., 1984; Grosclaude et al., 1987; Ambrosoli et al., 1988; Brignon et al., 1989; Barbieri et al., 1995; Martin and Addeo, 1996).

For cow milk, genetic variants of caseins and whey proteins are associated with milk yield, composition, and processing properties (McLean et al., 1984; Marziali and Ng-Kwai-Hang, 1986; Ng-Kwai-Hang et al., 1986). Goat milk with high levels of α_{s1}-CN has been found to have better milk composition, including total solids, fat, protein, casein, phosphorus, and lower pH than milks with low levels of α_{s1}-CN (Grosclaude et al., 1987; Ambrosoli et al., 1988; Barbieri et al., 1995). In addition, goat milk which contained high levels of α_{s1}-CN was found to have an elongated coagulation time but a desirable faster coagulation rate and firmer curd than milk with low α_{s1}-CN (Ambrosoli et al., 1988; Clark and Sherbon, 2000). Ryniewicz et al. (1996) reported that protein, casein, dry matter and curd quality were higher in milk of goats characterized by high type variants of α_{s1}-CN. Pierre et al. (1996) reported that goat milk with A/A type α_{s1}-CN had a higher total nitrogen and higher fat level than milk with O/O type α_{s1}-CN; cheeses made from A/A type milk were more firm and had higher yields; and goaty volatile aroma compounds were also lower in A/A cheeses.

It has been recommended that selection of animals be based on genetic combinations that lead to optimal cheese production rather than higher protein or fat content in the milk (Aleandri et al., 1990). Further, it has been estimated that genetic differences account for nearly 25% of the variation in milk yield and approximately 50% of the variation for fat, protein and solids-non-fats (SNF) content of cow milk (Gonyon et al., 1987). If these concepts hold for goat milk, the potential for improvements in cheese characteristics using genetic variants of α_{s1}-CN may be significant. Thus, it is of interest to establish whether genetic variants of α_{s1}-CN are related to superior milk composition, coagulation properties and economic traits. This study was designed to examine the effect of genetic variants of α_{s1}-CN on goat milk composition and coagulation properties, as well as examine relationships between US dairy goat breeds and genetic variants.

2. Materials and methods

2.1. Milk collection

Milk samples were collected from 93 individual dairy goats between June–August of 1994 and in May, June, October and November of 1996. Sampling was done during the regular morning (05.00 h) or evening (17.00 h) milking. All milk samples were collected from Side Hill Acres, Candor, NY; a herd composed of about 200 dairy goats representing Alpine, LaMancha, Nubian, Oberhasli, Saanen, Toggenburg breeds and some crosses. Herd feeding and health conditions were good. Goats were randomly selected for milk collection based on stanchion position. Each sample was collected mid-way through the milking process by removing inflations and hand-milking approximately 50 ml into individual Whirlpak® bags which were then placed on ice. Samples were transported to Cornell University where milk composition and coagulation properties were analyzed that afternoon (if morning collection) or refrigerated and analyzed the following morning (if previous evening collection).

2.2. Milk composition

Total solids were determined in duplicate by oven drying (AOAC, 1990c). Fat content was determined in duplicate using the Mojonnier ether extraction method (AOAC, 1990b). SNF were calculated by subtraction. The Acid Orange 12 dye binding method (AOAC, 1990a), modified for use with a Bausch & Lomb Spectronic 20 (Rochester, NY) and a Bausch & Lomb short path length flow through cuvet (0.299 mm), was

used to determine the true protein content of milk (pers. commun., Udy Corp., 1991). The AO12 dye binding method for percent true protein was calibrated against the Kjeldahl Direct method for determination of percent true protein (AOAC, 1991). Casein content of 55 samples (milk collected in 1996 only) was determined by first precipitating the casein with acetic acid and sodium acetate, then substituting the whey protein filtrate for milk in the modified AO12 dye binding method.

2.3. Coagulation properties

Coagulation properties were measured based on Bohlin et al. (1984). Due to equipment availability, coagulation properties of only 75 individual goat milk samples were analyzed in two sets of experiments, separated by 18 months. Coagulation time, coagulation rate and curd firmness were measured on goat milk samples using a bob (#100 004) and cup (#100 087) assembly unit and 1.064 g cm torsion bar in a Bohlin VOR Rheometer (Bohlin Reologi Incentive Group, Cranbury, NJ). An IBM PS/Value Point computer (IBM, Armonk, NY) controlled the system.

The procedure used for analyzing coagulation properties of 45 samples tested in the summer of 1994 is as follows. First, 500 μl (6 ml/kg) MD088 Ezal® lactic culture (Marschall products, Groupe Rhone-Poulenc, Dange Saint-Romain-France) were added to a 25 ml sample of goat milk maintained at 35°C. After 10 s, calf rennet (Chr. Hansen, Inc., Milwaukee, WI) diluted 1:10 (to obtain 0.1 ml/kg milk) was added to the milk and mixed by inversion for 15 s. After 30 s, 15 ml of the milk mixture was poured into the Bohlin cup, and the bob was lowered below the surface of the milk. An air-diverting funnel was secured around the bob and cup to minimize surface dehydration. At 120 s, oscillation was initiated. The Bohlin Rheometer was set at 1 Hz frequency and 1% amplitude. Measurements including complex modulus (G^*, Pa), storage modulus (G', Pa), loss modulus (G'', Pa), phase change (delta), range (% deflection), and viscosity of the sample were made at 60 s intervals for 3900 s.

Thirty goat milk samples were collected and analyzed in the fall of 1996. The analysis procedure was the same as that used in 1994, except that the samples were maintained at 38°C and coagulated with 300 μl culture and microbial rennet (Chr. Hansen Inc., 100% strength chymosin from Aspergillus niger). These differences were controlled in the statistical analysis.

2.4. Isolation and identification of genetic variants of α_{s1}-CN

Casein was isoelectrically precipitated from whole goat milk. First, skim milk was separated from whole milk by centrifugation in a Sorvall RC-5B (DuPont Co., Wilmington, DE) at $2500\times g$ (4250 rpm) and 30°C for 20 min. The resulting skim milk was diluted with an equal volume of distilled water and 10% acetic acid. After mixing, 1 M sodium acetate was added, and the mixture was centrifuged at $500\times g$ (1800 rpm). The casein pellicle was dispersed in distilled water and dissolved in 0.2 N NaOH. Samples were coagulated and washed a total of four times, ending with washed casein. Individual isoelectrically precipitated casein samples were frozen until reverse-phase high pressure liquid chromatography (RP-HPLC) was performed on the samples.

Alpha$_{s1}$-CN was separated from the other caseins and identified using an RP-HPLC method adapted from Jaubert and Martin (1992). Lyophilized standards from whole goat casein were supplied by M. -F. Mahe (INRA Laboratoire de Genetique Biochimique, Centre de Jouy-en-Josas, France). Before analysis, the standard (10 ppm) or sample (100 ppm) was reduced with 10 mM dithiothreitol in a 40°C Versa-bath® (Fisher Scientific, Pittsburgh, PA) for 1 h, then diluted with the same volume of 0.10% trifluoroacetic acid and filtered through a 0.45 mm Millipore (Bedford, MA) filter. Due to the fast degradation of trifluoroacetic acid, all isoelectrically precipitated casein and lyophilized standards were prepared (denatured, diluted, and filtered) within 2 h of the HPLC run to minimize ionic species degradation and baseline drift.

Mobile phase solvent A, 0.10% trifluoroacetic acid (Sigma, St. Louis, MO) in HPLC grade water (∼pH 2.00) and solvent B, 0.096% trifluoroacetic acid in 80% acetonitrile (∼pH 1.80) were prepared daily to minimize degradation of trifluoroacetic acid. Fresh solvents were dispensed into smaller flasks that were tempered to 40°C in the Versa-bath® and continuously flushed with high purity grade helium gas.

Two Waters 501 HPLC pumps were controlled by a Waters Automated Gradient Controller (Waters

division of Millipore, Milford, MA). A linear gradient was run from 42 to 58% solvent B for 42 min. The Waters 484 Tunable Absorbance Detector gain was set at 0.005 absorbance units full scale and the eluent absorbance was continuously monitored at 214 nm. A Hewlett Packard 3392A integrator (Palo Alto, CA) recorded the results. Chromatography was run on a Phenomenex (Torrance, CA) reverse-phase C4 column (Atlantis 5 mm C4 300 A, 4.6 mm×25 cm) and guard column (Atlantis 5 mm C4 300 A, 4.6 mm×30 mm). A Rheodyne (Cotati, CA) model 7010 sample injector was used to deliver a 20 ml sample onto the column. A Powerstat voltage control unit (Superior Electric Co., now carried by Warner Electric Ann Arbor, MI), attached to heating tape (Glas-col Apparatus Co. Terre Haute, IN) insulated with flame-resistant tape, was used to maintain the column at 40°C for optimal separation of peaks.

Retention times and peak areas of each casein peak of known standards and samples were entered into a Microsoft Excel™ (Redmond, WA) spreadsheet. Retention times were calculated for each peak, relative to β-CN. Due to genetic variation, zero, one, or two peaks could be present in the α_{s1}-CN region. By the use of standards and comparison with previously published results for caprine caseins (Jaubert and Martin, 1992), the peaks were identified. Individual α_{s1}-CN peak areas were added to obtain a value of total α_{s1}-CN. The value was then compared to the sum of all casein peaks, total CN peak area, to obtain a value of α_{s1}-CN relative to total CN for statistical analysis.

The elution order of α_{s1}-CN genetic variants was determined by conducting RP-HPLC analysis on different combinations of known standards, and observing the resultant chromatograms. Retention times (relative to β-CN) and peak areas (relative to total CN) of the test samples were compared to those of known standards to determine the genetic variant(s) present in each sample.

Milks that lacked peaks in the α_{s1}-CN region were considered homozygous α_{s1}-CN O/O. Samples with single peaks were presumed homozygous (i.e. F/F, E/E, A/A) since double peaks in other chromatograms confirmed that separation of heterozygous variants (i.e. F/D, E/A, D/C, etc.) was adequate and repeatable. In one case, the peak area ratio did not agree with the expected outcome (a low amount of α_{s1}-CN was found when the relative retention time of the peak suggested C/C). Because variant O does not give a corresponding peak, the presence of a null variant allele was presumed to account for the lowered synthesis.; therefore the sample was considered C/O. Although the elution times of variants B3 and E were the same, B3 and E were distinguished on the basis of peak area ratios. Because the synthesis rate of α_{s1}-CN is higher for the variant B3 than E (Grosclaude et al., 1987; Brignon et al., 1989), a peak was considered B3 if the amount of α_{s1}-CN was high, or E if the amount of α_{s1}-CN was intermediate.

2.5. Statistical analysis

Pearson correlation coefficients were determined using SAS statistical software (SAS/STAT®, 1989). A variant of the Kruskal–Wallis non-parametric test for one-way ANOVA by ranks was used to detect differences between breeds, controlling for month of collection and linear and quadratic terms (Zar, 1996). Because milk components exhibited a curvilinear trend over months, the quadratic month term was included in the analysis. A general linear model (GLM) was used to analyze 'null', 'intermediate' and 'high' group differences (Minitab, 1991). Differences were considered significant when resultant p-values were less than 0.05.

One-way ANOVA was used to determine differences between milk components and coagulation properties in response to genetic variant combinations (Minitab release 8, Minitab, Inc., State College, PA). When the individual genetic variant combinations were tested for significant differences, few were found due to the small sample size in each category, so genetic variants were grouped into categories for statistical analysis. Null samples were considered one category, 'null' and low-type variants composed a second category, 'low'. Variant combinations that included an intermediate-type variant but no high-type variant made up a third category, 'intermediate'. The final category included milks that contained any high-type variant, 'high'. The Pearson Chi-square and pairwise comparison tests were used to test for homogeneity among the six US dairy goat breeds and two cross-breed combinations. Differences were considered significant when resultant p-values were less than 0.05.

3. Results and discussion

3.1. Identification of genetic variants

Although the 10 genetic variants of caprine α_{s1}-CN (α_{s1}-CN A, B1, B2, B3, C, D, E, F, G, and O) are similar (Boulanger et al., 1984; Grosclaude et al., 1987; Brignon et al., 1989; Martin and Addeo, 1996), differences in protein structure and hydrophobicity enable isolation and identification of most variant combinations using RP-HPLC (Jaubert and Martin, 1992). Standards of α_{s1}-CN were combined in various ratios to determine RP-HPLC elution order. Fig. 1(a), (b) and (c) show a limited number of the results of this process to minimize journal space utilization. Peaks are labeled to indicate the genetic variant represented. Fig. 1(a) shows α_{s1}-CN standards E and A, injected in a 2:1 volume ratio. Fig. 1(b) shows standards α_{s1}-CN E and C injected in a 2:1 volume ratio. Fig. 1(c) shows the α_{s1}-CN C, B, and F standard preparations injected at 1:2:2 volume ratio.

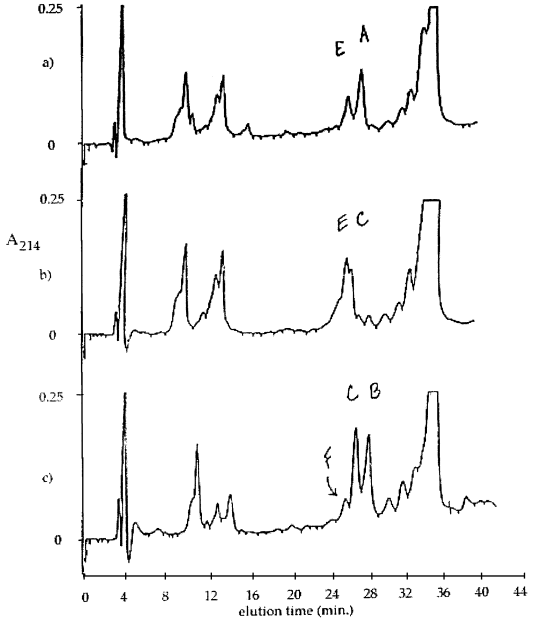

Fig. 1. Separation of α_{s1}-CN genetic variants from whole goat casein by RP-HPLC. (a) α_{s1}-CN E and A (2:1 volume), (b) α_{s1}-CN E and C (2:1 volume), (c) α_{s1}-CN F, B and C (2:2:1 volume). Variants labeled accordingly.

Under the RP-HPLC experimental conditions, variant C eluted after F and before B; variant A eluted between variants C and B; variant C eluted after E; and variant E eluted between F and B. Over the entire testing period, the average retention times of individual α_{s1}-CN genetic variants (relative to total CN) remained within 1.7% of duplicates in chromatograms of standards. A standard for variant α_{s1}-CN D was not available. Peaks eluting before α_{s1}-CN F were assumed to be α_{s1}-CN D, since previous research suggested this to be the case (Brignon et al., 1989). Variant G has been reported to be associated with low synthesis, but frequency and elution information have not been reported (Martin and Addeo, 1996). In addition, no α_{s1}-CN G standard was available. Thus, for the purposes of this work, variant G was not included in the discussion.

The order of RP-HPLC elution of the α_{s1}-CN genetic variants: D, F, (E and B3), C, A, and (B1 or B2), confirms and adds more detailed evidence of the order of elution established by Jaubert and Martin (1992). Although variants E and B were inseparable by Jaubert and Martin (1992), we were able to separate what we think to be variant B1 or B2, from variant E. At the time the Jaubert and Martin (1992) study was conducted, no published research had distinguished between α_{s1}-CN B1, B2, and B3, and the single variant was called B. It is now known that variants α_{s1}-CN B1 and B2 differ by a single amino acid substitution and a phosphorylation in variant B2 (Martin and Addeo, 1996). In addition, variants B3 and E co-elute because they are structurally the same, except that variant E contains a genomic insertion that results in reduced synthesis of α_{s1}-CN (Perez et al., 1994; Martin and Addeo, 1996). When α_{s1}-CN E and α_{s1}-CN B/F standards were co-injected, our experimentation successfully separated two variants of different synthesis levels, so it was ascertained that the 'α_{s1}-CN (B/F)' standard provided by INRA was α_{s1}-CN (B2/F) or (B1/F), rather than α_{s1}-CN (B3/F). Because Jaubert and Martin (1992) were unable to separate α_{s1}-CN B and E, it is likely that the 'α_{s1}-CN B' they reported was actually α_{s1}-CN B3. Variants α_{s1}-CN B1 and B2 could not be distinguished by the conditions used in our research since only one peak was present in the B1/B2 region. Alternatively, only one of the two variants, B1 or B2, was present in the herd studied.

Table 1
Alpha$_{s1}$-casein (α_{s1}-CN) genetic variant frequencies found in 93 individual dairy goats from one herd[a]

Variant combination	Number of animals	Frequency (%)
F/F	35	37.6
F/E, E/E	10 each variant combination	10.8 each
F/A	6	6.5
F/D	5	5.4
O/O	4	4.3
F/B2 and E/A	3 each variant combination	3.2 each
F/C, D/A, B2/B3, and A/A	2 each variant combination	2.2 each
D/E, D/C, E/B2, O/C, B2/B2	1 each variant combination	1.1 each
C/A, B3/A, F/B3, and B2/A	1 each variant combination	1.1 each

[a] Genetic variants B1 and B2 were not separable by the method used in this research. For the purposes of this table, samples that could have included B1 or B2 are called B2.

3.2. Genetic variant frequencies

Table 1 summarizes the α_{s1}-CN genetic variant frequencies found in the 93 individual dairy goats surveyed. Twenty-two different genetic variant combinations were found in the herd. The most common genetic variant combinations found were F/F (37.6%), F/E (10.8%), and E/E (10.8%). Only 4.3% of the animals were homozygous for the null variant O/O. Homozygous and heterozygous combinations composed of only high type variants were found with low frequency in the herd (A/A and B2/B3, 2.2% frequency; C/A, B3/A, B2/A, and B2/B2, 1.1% frequency).

Table 2 shows the frequency of individual α_{s1}-CN genetic variants in the herd, listed by breed. The F variant predominated in the herd (52.7%), and in every breed, particularly in Toggenburg and Alpine breeds (83.3 and 54.1%, respectively). Variant E was found at intermediate levels (18.3%) overall and in most breeds in the herd. Nubians and LaManchas had a significant representation of variant A (25 and 20.6%, respectively). Milk from crossbred does had a high percentage of the null variant (18.2%) because of two Saanen×Alpine crosses which were homozygous for the null variant. Two Nubian×Alpine crosses contributed to the 13.6% representation of the A variant in the cross category. Pearson Chi-square analysis showed that milk from Nubians was more likely to contain high type variants than milk from Alpines. Even though the Nubian breed had the highest frequency of the A variant, it is important to note

Table 2
α_{s1}-CN genetic variant frequencies found in 93 dairy goats from one US herd, compared by breed[a]

Variant	Frequency of individual variant (%), by breed							
	All breeds (total)	A	L	N	O	S	T	C
A	9.7	2.7	20.6	25.0	0.0	9.1	5.6	13.6
B2	4.8	6.8	2.9	0.0	0.0	9.1	5.6	0.0
B3	2.2	1.4	2.9	8.3	0.0	4.6	0.0	0.0
C	2.7	1.4	2.9	8.3	0.0	0.0	0.0	9.1
D	4.8	8.1	2.9	8.3	0.0	0.0	0.0	4.6
E	18.3	20.3	17.6	0.0	50.0	31.9	5.6	13.6
F	52.7	54.1	50.0	41.7	50.0	45.5	83.3	41.0
O	4.8	5.4	0.0	8.3	0.0	0.0	0.0	18.2
n	93	37	17	6	2	11	9	11

[a] A: Alpine, L: LaMancha, N: Nubian, O: Oberhasli, S: Saanen, T: Toggenburg, C: crossbred does include four N×A, one L×A, three S×A, one S×O, one N×O, and one A×L×N×S. Genetic variants B1 and B2 were not separable by the method used in this research. For the purposes of this table, samples that could have included B1 or B2 are called B2. Variant G was not determined.

Table 3
α_{s1}-CN genetic variant frequencies reported in the literature, compared to present study[a]

Variant	Frequency of individual variant (%), by report			
	(a)	(b)	(c)	(present study)
A	8–10	10.5	8.0	9.7
B[b]	89–90	6.5	4.7	7.0
C	1–3	0.5	2.0	2.7
D	N/R	N/R	4.7	4.8
E	89–90	37.5	39.8	18.8
F	N/R	42.0	47.3	52.2
O	N/R	3.0	4.7	4.8

[a] (a) Boulanger et al., 1984: Saanens and Alpines, France; (b) Grosclaude et al., 1987: Saanens and Alpines, France; (c) Martin and Addeo, 1996: Saanens and Alpines, France and Italy; (present study): all breeds and some crosses, one herd, USA.

[b] For the purposes of this table, samples that could have been B1, B2 or B3 are called B. Method (a) could not separate variants B and E. Methods (b) and (c) did not separate variants B1, B2, and B3. Present study could not separate variants B1 and B2; N/R: not reported.

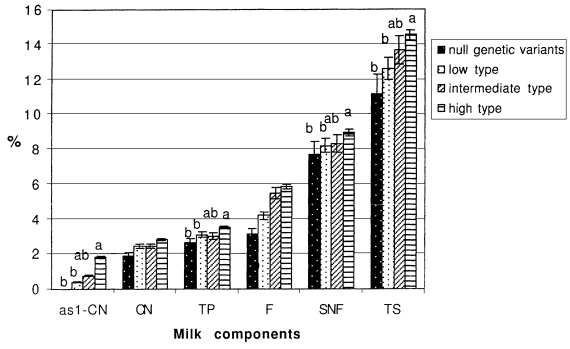

Fig. 2. Effect of genetic variant types upon alpha$_{s1}$-CN (α_{s1}-CN), casein (CN), protein (TP), fat (F), solids-non-fat (SNF) and total solids (TS) in goat milk. 'Null' includes O/O variant only; 'low' includes only F and/or D variants; 'intermediate' includes E variant; 'high' includes A, B1, B2, B3 and/or C variant. a, b: different letters on columns indicate significant differences exist within a component.

that the null variant was not absent from the Nubian breed. High type variants B3 and C were found with the lowest frequency in the herd.

Table 3 is a summary of α_{s1}-CN genetic variant frequencies, including a comparison of the present work to values found by several other researchers (Boulanger et al., 1984; Grosclaude et al., 1987; Martin and Addeo, 1996). The values obtained in our research were similar to values reported in France and Italy where only Alpine and Saanen breeds were tested. The most notable difference is that a lower frequency of variant E was found in the United States herd than in European goats (18.3 in US compared to 30–40% in France and Italy). This suggests that a herd of Saanens and Alpines may be more likely to contain a higher frequency of the E variant than a more mixed breed herd, as was the case in the present study.

3.3. Genetic variants of α_{s1}-CN, milk composition, and coagulation properties

Average percent values for all milk components were lowest for milks that were homozygous for the null α_{s1}-CN variant and were highest for samples containing a high type genetic variant in combination with any other type of variant (Fig. 2). Milk that contained at least one high type variant had significantly higher total solids, SNF, protein, and α_{s1}-CN than samples that contained only low type variants or were homozygous for the null α_{s1}-CN variant. Although percent fat and casein in null variant milk were lower than fat and casein in milk containing at least one high type genetic variant, there were no significant differences due to high variability or small sample size within those categories. The intermediate type variant E appeared to improve milk composition over null variant milk composition, but differences were not significant.

The current work found no significant differences between coagulation properties of milks containing different genetic variants, but trends were notable (Fig. 3). Milk that contained at least one high type variant tended to have higher coagulation rate, curd firmness and coagulation time than milk devoid of α_{s1}-CN. Previous work showed that milk components (Ambrosoli et al., 1988; Barbieri et al., 1995; Pierre et al., 1996; Clark and Sherbon, 2000), and α_{s1}-CN specifically (Ambrosoli et al., 1988; Pierre et al., 1996; Ryniewicz et al., 1996), improve coagulation properties. Although α_{s1}-CN genetic variants were not strongly related to coagulation properties in this study, goat milk that lacked α_{s1}-CN contained significantly lower percentages of milk components and tended to have poorer coagulation properties than milk containing high type α_{s1}-CN variants.

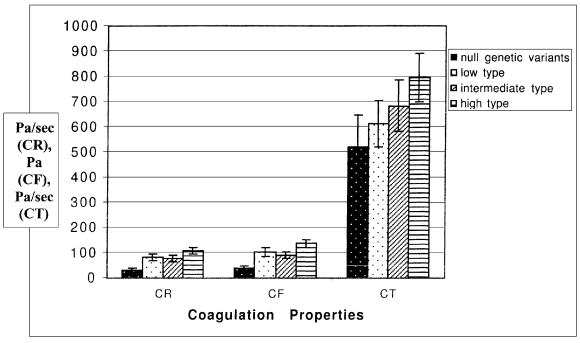

Fig. 3. Effect of genetic variant types upon coagulation rate (CR, Pa/s), curd firmness (CF, Pa) and coagulation time (CT, s) of goat milk. 'Null' includes O/O variant only; 'low' includes only F and/or D variants; 'intermediate' includes E variant; 'high' includes A, B1, B2, B3 and/or C variant.

The results of this work suggest considering a breeding program that selects against animals carrying null variants and selects for animals carrying high type variants of α_{s1}-CN if improving milk composition and enhancing cheese-making potential are goals. However, α_{s1}-CN genetic variant information is not often known and is difficult to acquire. Selecting for animals that produce high solids may be an adequate alternative to measuring α_{s1}-CN since percent total solids, SNF and protein are highly correlated with curd firmness and coagulation rate (Clark and Sherbon, 2000).

Selecting particular breeds may also play a role in improving cheese-making potential. In this study, milk from Nubians was more likely to contain a high type variant than milk from Alpines. In addition, Nubians have been shown to exhibit the best combination of milk composition and coagulation properties (Clark and Sherbon, 2000). Increasing the number of Nubians in a herd may increase the prevalence of high type variants in the herd, enhance the composition and coagulation properties of goat milk, and ultimately increase cheese yields and economic returns.

4. Conclusions

The RP-HPLC method used in this research was able to separate caseins and α_{s1}-CN genetic variants, and enabled determination of relative quantities of those components in goat milk. The elution order of α_{s1}-CN under the conditions of this research was D, F, (E and B3), C, A, and (B1 or B2). The distribution of individual α_{s1}-CN genetic variants in 93 individual goats of different breeds sampled from one herd was F (52.7%), E (18.3%), A (9.7%), B1 or B2 (4.8%), O and D (4.8% each), C (2.7%), and B3 (2.2%). These frequencies were similar to previous research done in France and Italy with Saanen and Alpine breeds. Low and intermediate type α_{s1}-CN genetic variants were found to predominate in the herd. The most common genetic variant combinations found were F/F (37.6%), F/E (10.8%), and E/E (10.8%). Milk from the Nubian breed was more likely to contain high type genetic variants than milk from Alpines. Goat milks which were homozygous for the null variant of α_{s1}-CN (O/O, devoid of α_{s1}-CN) had lower percentages of total solids, SNF, fat, protein, and casein than milk samples that contained a high type genetic variant. Null type milk appeared to have poorer coagulation properties than high type milk, but differences were not significant. Breeding programs that select for animals that produce milk containing high type α_{s1}-CN genetic variants (if known) or high solids is recommended if cheese making is the primary objective.

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