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Goat's milk proteins and technological properties

The nature of $\beta$-casein heterogeneity in caprine milk

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Summary — The heterogeneity of some caprine $\beta$-casein patterns was studied using gel electrophoresis at alkaline pH, isoelectric focusing on polyacrylamide gel, immunoblotting with polyclonal antibodies against $\beta$-casein and electrospray mass spectrometry. It was demonstrated that the origin of this heterogeneity depended on multiple phosphorylation of the peptide chain giving 4P, 5P and 6P forms. Caprine $\alpha_s$-casein was also found in 3 phosphorylated forms, 7P, 8P and 9P. Individual caprine milks which did not contain the $\beta$-casein fraction were also identified, as were milks containing reduced amounts of this protein. Using comparative assays on the aptitude of individual milks to coagulate, it was demonstrated that $\beta$-null milks presented longer rennet coagulation times than normal milks and that curd firmness was consistently poorer.

$\beta$-casein / polymorphism / heterogeneity / discrete phosphorylation

Résumé — Hétérogénéité de la caséine $\beta$ caprine. Une électrophorèse à pH alcalin et focalisation isoélectrique sur gel de polyacrylamide suivie d'une coloration spécifique avec des anticorps polyclonaux dirigés contre la caséine $\beta$ ont permis de détecter et d'identifier un certain nombre de variants génétiques de la caséine $\beta$ caprine. L'hétérogénéité de cette protéine a été étudiée par spectrométrie de masse en Electrospray, ce qui a permis de mettre en évidence la présence de 3 formes de caséine $\beta$ à 4, 5 ou 6 groupements phosphate par mole de protéine. La présence de formes de caséine $\alpha_s$ caprine à 7, 8 ou 9 groupements phosphate a aussi été mise en évidence. Des laits individuels dépourvus de caséine $\beta$ ou à faible teneur en caséine $\beta$ ont été retrouvés dans des populations locales italiennes ou chez certaines races caprines. L'aptitude à la coagulation de ces laits est faible. Les laits sans caséine $\beta$ ont un temps de prise beaucoup plus long et ils donnent des caillés plus mous par rapport aux laits ayant une teneur normale en caséine $\beta$.

caséine $\beta$ / polymorphisme / hétérogénéité / phosphorylation partielle
INTRODUCTION

Caprine β-casein, when analyzed via electrophoresis at alkaline pH and isoelectric focusing on polyacrylamide gel (Addeo et al, 1988) shows 2 electrophoretic bands, β₁ and β₂, with similar intensities. On the basis of amino-acid composition and phosphate content (Richardson and Creamer, 1974), the differences in electrophoretic mobility of caprine β-caseins have been attributed to different amounts of phosphate residues, *i.e.* 6 and 5 per mol β₁- and β₂-caseins, respectively. Partial studies of the primary structure carried out on these proteins have shown there is a high homology with ovine β-casein (Pelissier, personal communication). An electrophoretic band doublet corresponding to β₁- and β₂-casein bands has been systematically observed in individual samples. Recently, studies have been carried out showing that variants profiles of caprine β-casein have been observed, in some of which the β-casein bands are apparently not present (Dall'Olio et al, 1989; Moio et al, 1989; Grosclaude et al, 1992). Milks containing reduced amounts of β-casein have also been described (Ramunno et al, 1992). Since a variable number of bands in the β-casein area have been observed in some individual caprine casein samples using PAGE at alkaline pH (Chianese et al, 1992c) the existence of a β-casein polymorphism was suspected. In some cases, this heterogeneity appeared similar to that exhibited by ovine β-casein, known to be the most heterogeneous of the homologous milk fractions from other animal species (Chianese et al, submitted). In the case of human milk, the origin of the heterogeneity of β-casein has been attributed to the multiple phosphorylation of the protein chain, the number of phosphate groups per mol protein ranging from 0 to 5 (Greenberg and Groves, 1979). In the case of ovine casein the nature of the heterogeneity demonstrated by immunochecmical techniques was attributed to the multiple phosphorylation of β-casein from 1P to 7P (Chianese et al, submitted). This study presents some evidence of caprine β-casein heterogeneity determined by β-casein chain phosphorylation and by genetic polymorphism. Finally goats producing no β-casein or reduced content of this protein have been identified.

MATERIAL AND METHODS

About 800 samples of individual goat milks from different Italian breeds and local populations were analyzed. From these, ~ 20 samples were selected on the basis of some variation in the electrophoretic profile of casein. Casein was prepared from skimmed milk following the procedure described by Aschaffenburg and Drewry (1959).

**Fractionation of whole caprine casein by fast protein liquid chromatography (FPLC)**

Fractionation of whole caprine casein was carried out by fast protein liquid chromatography (FPLC, Pharmacia LKB, Uppsala, Sweden) on Q-Sepharose 26 x 300 mm following the procedure described by Andrews et al (1985).

The chemical composition of elution buffer A was 20 mM Tris–HCl at pH 7.0, 4.5 mol/l urea, 0.1% 2-mercaptoethanol. Buffer B consisted of 20 mmol/l Tris–HCl at pH 7.0, 4.5 mol/l urea, 0.35 mol/l NaCl, 0.1% 2-mercaptoethanol. Two g whole caprine casein dissolved in 40 ml buffer A were injected into the column and a linear gradient from 0 to 100% B in 1 000 ml was applied at a flow rate of 4 ml/min. After complete dialysis against distilled water, the pooled fractions corresponding to each peak were freeze-dried.

**Electrophoresis**

Vertical polyacrylamide gel disc electrophoresis (Disc-PAGE) was carried out at pH 8.6 using vertical electrophoresis apparatus (GE2/4L5,
Pharmacia LKB, Uppsala, Sweden) at 200 V and 6°C for 7 h with a gel consisting of a 3.1% T and 6.4%C stacking gel on a 6.6%T and 2.4%C separation gel, as described by Chianese et al (1992a).

**Polyacrylamide gel isoelectric focusing (PAGIF)**

Isoelectric focusing on a thin-layer polyacrylamide gel (PAGIF) was carried out as described by Trieu-Cuot and Gripon (1981). The 2.5–6.5 pH gradient was obtained by mixing Ampholine (Pharmacia LKB, Uppsala, Sweden) 2.5–5.0, 4.5–5.4 and 4–6.5 in a ratio 1:0.9:0.7 by volume.

**Immunoblotting**

Immunodetection of the PAGIF patterns with polyclonal antibodies raised in rabbit against bovine K-, ß-, αs1- and αs2-casein was carried out following a previously described procedure (Chianese et al, 1992a).

**Densitometry**

Densitometry evaluation of the PAGIF patterns was performed with an LKB 2202 UltroScan Laser Densitometer interfaced with an LKB 2222 UltroScan XL laser densitometer and Gel Scan XL 2.0 integration software on an IBM-AT computer.

**DNA analysis**

DNA samples from goats producing ß-casein or no ß-casein were analyzed according to the Southern method using 15 endonucleases (PstI, TaqI, HindIII, EcoRI, EcoRV, PvuII, BamHI, RsaI, BglII, BstEII, HaellI, HindII, SacI, XbaI and DraI) and bovine ß-casein cDNA as probe.

**Thromboelastographic analysis**

The clotting behaviour of individual milk samples with a different ß-casein content (null, intermediate and normal amounts) was determined by Formagraft (Foss Electric, Este, Italy). Three empirical parameters were recorded: the RCT, time elapsed from the addition of rennet until 1 mm was reached; k20, the time from RCT until an amplitude of 20 mm was reached (1/k20 is related to the velocity of curd formation); A30, the amplitude in cm measured after RCT, represents a figure proportional to curd firmness. Forty determinations were carried out on the milk from 3 individual animals on the same day throughout the lactation period.

**Electrospray mass spectrometry**

Electrospray mass spectrometry (ESMS) analysis of the chromatographic fractions and whole casein samples was performed on BIO-Q triple-quadrupole apparatus (VG, Manchester, UK). Protein samples were dissolved in H2O CH3 CN CH3COOH (50:50:1, v:v:v) (25–50 pmol in 10 μl solvent) and injected into the ion source at a flow rate of 2 ml/min; the spectra were scanned from 1 700 to 700 U at 10 s/scan. Mass scale calibration was carried out using the multiple charged ions of a separate introduction of myoglobin. All ESMS molecular masses are shown as average values.

**RESULTS AND DISCUSSION**

**Polyacrylamide gel electrophoresis at alkaline pH**

Figure 1 represents the gel electrophoresis at alkaline pH of some individual caprine whole casein samples. In samples 4 and 5 there were only 2 ß-casein electrophoretic bands with similar intensities, corresponding to ß1 and ß2-caseins. However, in other individual samples of caprine casein, there were more ß-casein bands. Sample 6 contained 1 additional band apart from ß1- and ß2-caseins. Samples 1 and 8 showed a heterogeneity higher than that apparent in sample 6. Sample 7 contained a more intense ß1-casein band than the ß2-casein
Fig 1. Polyacrylamide gel disc electrophoresis at pH 8.6 of 8 individual samples of whole caprine casein. Lanes 2 and 3, β-casein null types; lanes 4 and 5, reference samples containing 2 β-casein bands; lane 7, sample with different intensity of β1 and β2-casein; lanes 1, 6 and 8, samples showing highest heterogeneity in the β-casein area.

Identification of casein fractions by isoelectric focusing and immunoblotting

In order to unambiguously identify the nature of these electrophoretic bands, fractionation of a whole individual caprine casein sample was performed via anion exchange chromatography. Figure 2 shows the elution pattern on a Q-Sepharose column using the FPLC system. PAGIF analysis of the 6 fractions recovered from the corresponding peaks F1-F6, is shown in figure 3. All the isolated fractions appeared to be composed of several bands, so that it was necessary to define the origin of this heterogeneity. Immunoblotting of the PAGIF gel in figure 3, with immunosera against κ-, β-, and αs1 + αs2 fractions was used to detect κ-casein in fractions F1, F2, F3 and F4 (fig 4), β-casein in fractions F4, F5 and F6 (fig 5), and a mixture of αs1 and αs2-caseins in fractions F5 and F6 (fig 6). These results led to the identification of the different casein fractions in the electrophoretic profile of whole caprine casein in figure 3; more specifically, they were used to label the β-casein fraction consisting of 3 bands with different pIs. Figure 7 shows the PAGIF analysis of some individual samples of whole caprine casein with the identification of β-casein bands. Samples 2 and 3 show 2 faint β-casein bands and samples 1, 4, 5, 6, 7 and 8 present 2 main β-casein bands and 1 additional band of
Fig 2. Chromatographic separation on Q-Sepharose of 2 g whole individual caprine casein sample analyzed in figure 1, lane 5.

Chromatographie sur colonne de Q-Sepharose (2,6 x 30 cm) de 2 g de caséine entière caprine. Gradient linéaire de 0,1 à 0,35 M de NaCl. Le résultat de l’électrophorèse en gel de focalisation isoélectrique est donné sur la figure 3.

Fig 3. Polyacrylamide gel isoelectric focusing with pH gradient 2.5–6.5 of fractions F1–F6 separated by Q-Sepharose chromatography (see fig 2); W: whole casein. 

Électrophorèse par focalisation isoelectrique en gel de polyacrylamide dans le gradient de pH 2,5–6,5 des fractions F1–F6 issues de la séparation par chromatographie sur colonne d’échange d’anions sur la figure 2. W: caséine entière caprine.

Fig 4. Immunoblotting of the gel isoelectric focusing from figure 3 after incubation with anti-κ-casein serum following the procedure outlined by Chianese et al (1992a).

Coloration spécifique avec des anticorps polyclonaux dirigés contre la caséine κ selon le protocole expérimental décrit par Chianese et al (1992a) des bandes électrophorétiques séparées par électrophorèse en focalisation isoélectrique dans la figure 3. La numérotation est la même que sur la figure 3.
secondary importance. The $\beta_1$-casein was more intense than the $\beta_2$-casein band in sample 7.

However, it was not sufficient to identify each band in the individual samples of caprine casein with an altered electrophoretic mobility due to one or more amino-acid substitutions.

Immunoblotting was used to establish the origin of the difference observed in the PAGIF patterns and identify the electrophoretic $\beta$-casein variants occurring in individual casein samples. In comparison with the traditional Coomassie staining this is a highly specific procedure when used to establish the origin of the electrophoretic bands.

Fig 5. Immunoblotting of the gel isoelectric focusing from figure 3 after incubation with anti-$\beta$-casein serum following the procedure outlined by Chianese et al (1992a). Coloration spécifique avec des anticorps polyclonaux dirigés contre la caséine $\beta$ selon le protocole expérimental décrit par Chianese et al (1992a) des bandes électrophorétiques séparées par électrophorèse en focalisation isoélectrique sur la figure 3. La numérotation est la même que sur la figure 3.

Fig 6. Immunoblotting of the gel isoelectric focusing from figure 3 after incubation with a mixture of anti-$\alpha_{s1}$-casein and anti-$\alpha_{s2}$-casein sera (1:1, v:v) following the procedure outlined by Chianese et al (1992a). Coloration spécifique avec des anticorps polyclonaux dirigés contre les caséines $\alpha_{s1}$ et $\alpha_{s2}$ selon le protocole expérimental décrit par Chianese et al (1992a) des bandes électrophorétiques séparées par électrophorèse en focalisation isoélectrique sur la figure 3. La numérotation est la même que sur la figure 3.

Fig 7. Polyacrylamide gel isoelectric focusing in the pH gradient 2.5–6.5 of the 8 individual caprine whole casein samples from figure 1 after staining with Coomassie G. Samples are numbered as in figure 1. Électrophorèse par localisation isoélectrique en gel de polyacrylamide dans le gradient de pH 2.5–6.5 des 8 échantillons individuels de caséine entière caprine. Coloration avec Coomassie G. La numérotation est la même que sur la figure 1.
Immunoblotting of individual casein samples

Figure 8 shows the immunoblotting of the gel plate in figure 7 with polyclonal antibodies specific against β-casein. This confirmed that the most frequently encountered whole casein profile contained 2 main β-casein bands (samples 4 and 5); but at least 2 less intense bands are also visible. Another profile shows 3 equally intense main bands (sample 6). In yet other profiles (samples 1 and 8), there are 2 equally intense main bands and 2 other secondary bands. In the immunoblotting profile of β-casein there are usually 23 main bands, as well as 2 or 3 components recognized by the antibodies raised against β-casein. Samples 2 and 3 did not contain β-casein. In order to identify the heterogeneity of the β-casein bands a study was carried out by electrospray mass spectrometry.

Electrospray mass spectrometry

The chromatographic fractions containing β-casein were further purified on the same Q-Sepharose column with a shallower saline gradient, and then used as reference material to determine the molecular weight of β-casein components in each individual milk. Fractions F4, F5 and F6 produced the mass spectra shown in figure 9. The molecular mass (MW) of 2 components in mixture in F4 was 23 697.45 ± 1.35 and 23 776.67 ± 2.46 respectively, that in F5 23 775.65 ± 1.22 and finally that in F6 23 856.55 ± 5.65. Three β-casein components were thus identified in the whole individual caprine casein sample, accounting for 23 697.45 ± 1.35, 23 776.67 ± 2.46 and 23 856.55 ± 5.65 Da. The mass difference between the different β-casein components can be accounted for by the presence of a different number of phosphate residues (Δm = 80) in the same protein. Accordingly, a third phosphorylated β-casein component (MW 23 697.45 ± 1.35) containing 4 phosphate groups/mol protein was identified in addition to β1, an β2-casein, containing 6 and 5 phosphates respectively. This fact confirms the results obtained using immunoblotting, which had revealed the presence of 3 β-casein components recognized by specific antibodies in the same sample (lane 6, fig 8).

The ES mass spectra relative to 5 individual samples of caprine caseins are shown in figures 10 to 14. The molecular masses of the β-casein components are shown in table 1 together with the relative percentage of each peak in the total β-casein fraction. The exact measurements of the molecular masses provide a straightforward identification of the particular components of the β-casein components in the individual milks. The molecular mass of each component detected in the individual samples and the corresponding phospho-
Table I. Measured molecular masses of caprine β-casein in individual milk samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>3P</th>
<th>4P</th>
<th>5P</th>
<th>6P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23792.84 ± 5.26 (43)</td>
<td>23877.28 ± 1.52 (57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>23610.08 ± 3.20 (1.7)</td>
<td>23694.49 ± 1.63 (6.3)</td>
<td>23772.87 ± 0.92 (47)</td>
<td>23851.83 ± 1.28 (44.7)</td>
</tr>
<tr>
<td>3</td>
<td>23775.05 ± 4.18 (45)</td>
<td>23857.97 ± 1.91 (55)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>23769.09 ± 4.51 (47)</td>
<td>23847.19 ± 3.06 (53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>23774.72 ± 1.92 (28)</td>
<td>23854.00 ± 1.56 (72)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figures in parentheses indicate the relative percentage of each component in the total β-casein fraction.

Les valeurs entre parenthèses indiquent le pourcentage relatif de chaque composé dans la fraction totale de caséine β.

These results indicate that: i) in the samples 2, 3, 4 and 5 (figs 11–14), β-casein consists of 2 components of similar molecular weight and 2 phosphorylation levels, 5P and 6P. The relative abundance of these components is similar in all the samples examined except one (sample 5 in table I) in which the relative level of 5P is lower and that of 6P higher; ii) the presence of a β-casein variant in sample 1 was suspected due to its lower mobility de-

Fig 9. Transformed electrospray mass spectra of fractions F4-F6 separated by Q-Sepharose chromatography (see fig 2). The multiple charged ion spectra of the casein fraction were transformed to a real mass scale. The results indicate the presence of 3 distinct β-casein forms (see text).
Fig 10. Transformed electrospray mass spectra of the individual sample of whole caprine casein analyzed in figure 1, lane 6.
Spectre de masse transformé obtenu par la technique d'électrospray à partir de l'échantillon numéro-té 6 sur la figure 1.

Fig 11. Transformed electrospray mass spectra of the individual sample of whole caprine casein analyzed in figure 1, lane 1.
Spectre de masse transformé obtenu par la technique d'électrospray à partir de l'échantillon numéro-té 1 sur la figure 1.
Fig 12. Transformed electrospray mass spectra of the individual sample of whole caprine casein analyzed in figure 1, lane 4.
Spectre de masse transformé obtenu par la technique d'électrospray à partir de l'échantillon numéro-té 4 sur la figure 1.

Fig 13. Transformed electrospray mass spectra of the individual sample of whole caprine casein analyzed in figure 1, lane 7.
Spectre de masse transformé obtenu par la technique d'électrospray à partir de l'échantillon numéro-té 7 sur la figure 1.


**Quantitative β-casein polymorphism**

As shown previously, the ES mass spectra relative to whole caprine casein samples ordinarily present a series of β-casein peaks ranging from ~23610 to 23877 Da. The mass spectrum in figure 15 does not contain any β-casein component, as previously revealed by PAGE analysis and immunoblotting (sample 2 in figs 1 and 8).

Figure 16 shows a PAGIF analysis of 3 individual casein samples from a doe female offspring couple (lane 1 and 3 respectively) compared to that of an individual reference milk (lane 2). The densitometric evaluation of β-casein in these PAGIF patterns produced the following figures: 0% in the doe's milk (lane 1), 19.5% in the female offspring's milk (lane 3) compared to 33% total casein bands in the normal reference milk (lane 2). This means that goats
producing no β-casein are homozygous for a null allele (β-Cn0) whilst each individual heterozygous at this locus produces = half as much β-casein as a normal individual. Table II shows the distribution of β-casein variants in individual milks from caprine breeds and local ecotypes. It was observed that only 9 individuals out of a total of 737 carried the β-casein null character. The allelic frequency of the β-null type is = 0.10 in goats from local ecotypes and the genotypic frequency observed is consistent with the figures expected according to Hardy-Weinberg's law. The genomic region of a β-casein gene in DNA samples obtained from goats producing milk with or without β-casein, showed identical restriction patterns with 15 endonucleases. This means that, as opposed to the results obtained for the caprine αs1-casein null-allele (Di Gregorio et al, 1989), the missing caprine β-casein gene expression is not determined by genomic DNA rearrangements which may be revealed by Southern blotting using 15 endonucleases.

**Discrete phosphorylation of caprine αs1-casein**

The spectrum in figure 15 shows a new series of peaks with molecular masses that differ from those of β-casein. The mass difference between 3 consecutive peaks can be accounted for by the presence of a casein fraction with a different number of phosphate residues (Δm = 80). Considering that caprine αs1-casein contains 8P and presents an MW 23363.49 Da as calculated from its amino-acid sequence (Bri-
### Table II. Genotypic distribution and allelic frequencies of caprine β-casein in local ecotypes and breeds reared in Italy.

Distribution génotypique et fréquence allélique de caséine β caprine chez des écotypes locaux et chez des races en Italie.

<table>
<thead>
<tr>
<th>Goat breed</th>
<th>No of animals</th>
<th>Genotypic distribution</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal (β-Cn A/A)</td>
<td>Null (β-Cn O/O)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal</td>
<td>Null</td>
</tr>
<tr>
<td>Garganica</td>
<td>54</td>
<td>46</td>
<td>0</td>
</tr>
<tr>
<td>Maltese</td>
<td>85</td>
<td>76</td>
<td>1</td>
</tr>
<tr>
<td>Local Ecotypes</td>
<td>428</td>
<td>348</td>
<td>8</td>
</tr>
<tr>
<td>Saanen</td>
<td>80</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>Camosciata</td>
<td>90</td>
<td>90</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig 16. Polyacrylamide gel isoelectric focusing with pH gradient 2.5–6.5 of 3 individual samples of whole caprine casein selected according to the β-casein content. 1: null; 2: normal; 3: intermediate.

Electrophorèse par focalisation isoélectrique en gel de polyacrylamide dans le gradient de pH 2,5–6,5 de 3 échantillons individuels de caséine entière caprine choisis selon le contenu de caséine β. 1) lait dépourvu de caséine β; 2) lait ayant un contenu normal de caséine β; 3) lait ayant un contenu intermédiaire de caséine β.
Table III. Measured molecular masses of caprine $\alpha_{s1}$-casein components with different phosphate amounts in individual milks lacking $\beta$-casein.

Détermination des masses moléculaires de la caséine $\alpha_{s1}$ caprine à différents contenus en phosphate (P) dans un échantillon de caséine dépourvue de caséine $\beta$.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\alpha_{s1}$-casein</th>
<th>7P</th>
<th>8P</th>
<th>9P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23287.62 ± 5.43 (17.2)</td>
<td>23378.10 ± 9.18 (47.8)</td>
<td>23455.91 ± 7.18 (35)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>23289.83 ± 2.83 (44.1)</td>
<td>23368.48 ± 5.26 (35)</td>
<td>23437.23 ± 11.90 (20.9)</td>
<td></td>
</tr>
</tbody>
</table>

Figures in parentheses indicate the relative percentage of the component in the total $\alpha_{s1}$-casein.

Les valeurs entre parenthèses indiquent le pourcentage relatif de chaque composé dans la fraction totale de caséine $\alpha_{s1}$.

casein correspond to the same protein chain with different degrees of phosphorylation, which explains the different isoelectric points shown by each of these components. The different intensities of each of these protein bands depend on the percentage of each protein form containing different numbers of phosphate groups present in individual samples. Taking into account that discrete phosphorylation was observed in ovine and human $\beta$-casein, it can be inferred that the co-existence of casein forms with different degrees of phosphorylation is common to all milks and that it may depend on a hierarchy of the sites susceptible to phosphorylation. According to Mercier (1981) this may be determined by the presence in the casein fraction of primary sites which strongly accept phosphate and secondary sites which are worse acceptors of phosphate groups.

Clotting properties of milks

Results on clotting of caprine milks selected according to $\beta$-casein types indicate that the $\beta$-casein null type apparently has a longer clotting time (15–25 min) than the milk containing the highest amounts of $\beta$-casein (4–7 min) (table IV). Curd firmness was found to be significantly affected by the content of $\beta$-casein: the lower the content, the lowest the firmness (table IV).

Table IV. Lactodynamometric parameters derived from renneting milk with different amounts of $\beta$-casein.

Paramètres lactodynamographiques obtenus à partir de la coagulation des laits individuels à contenus différents de caséine $\beta$.

<table>
<thead>
<tr>
<th></th>
<th>Null type</th>
<th>Intermediate</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCT (min)</td>
<td>15–25</td>
<td>4.5–10</td>
<td>4.5–7</td>
</tr>
<tr>
<td>$k_{30}$ (min)</td>
<td>Not measurable</td>
<td>2–7</td>
<td>3–7</td>
</tr>
<tr>
<td>$A_{30}$ (min)</td>
<td>Not measurable</td>
<td>22–35</td>
<td>27–44</td>
</tr>
</tbody>
</table>
CONCLUSION

These preliminary assays indicate that in order to obtain a more complete picture of the genetic structure of goat breeds and populations, it is necessary to simultaneously consider the $\alpha_{\text{s1}}$- and $\beta$-casein loci. Moreover, the within-breed diffusion of males carrying the $\beta$-null character appears to be detrimental both to a balanced equilibrium among the casein fractions of milk and to the use of milk for cheesemaking.

ACKNOWLEDGMENT

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