

Casein micelle size in relation with casein composition and α_{s1} , α_{s2} , β and κ casein contents in goat milk

Alice Pierre*, Françoise Michel, Yvon Le Graët, Laurence Zahoute

Laboratoire de recherches de technologie laitière, Inra, 65, rue de Saint-Brieuc,
35042 Rennes cedex, France

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Abstract — The relationship between micelle size and casein micelle composition was studied on 21 individual goat milks from animals homozygous for α_{s1} casein variants A, B₂, C, E, F and O. A large variation in milk composition was obtained, as α_{s1} secretion levels varied from 7.2 g·kg⁻¹ in A milks to none in O milks. Mean micelle size (MMS), determined by photon correlation spectroscopy, varied between samples from 192 nm to 287 nm. This was explained by the different aspects of the histograms of casein distribution according to the size, determined from transmission electron microscopy data, which showed a maximum either in the low diameter range (20–130 nm) or in the large diameter region (130–260 nm), or even intermediary figures with a bimodal distribution. The caseins, α_{s1} CN, α_{s2} CN, β CN and κ CN were determined in milks from nitrogen matter determinations ($N \times 6.38$) and RP-HPLC analysis of casein. Polynomial relations were calculated between micelle size and milk compositional parameters. MMS was correlated on one side to the α_{s1} CN and κ CN levels in milks (g·kg⁻¹) and, on the other side, to the proportions of α_{s1} CN %, α_{s2} CN % and β CN % in total casein. These polynomial relations allowed the prediction of the mean micelle size in milks from the casein levels, with a 15 % accuracy. Monofactorial correlations also showed a significant effect of α_{s1} CN ($r = -0.77$), but not any of κ CN. Mineral composition of milks was determined, calcium by atomic absorption spectrophotometry and phosphorus, by a colorimetric method. Goat milks were characterized by a constant colloidal inorganic P level (12.4 (SD = 1.7) mmol·kg⁻¹). In contrast, colloidal Ca (Cac), SerineP and total colloidal P (Pc) were correlated to the total casein content. The ratio Cac/Pc was the most constant parameter in goat milks, amounting 1.22 (SD = 0.05), presumably characterizing a unique mode of association of caseins in milk. No significant correlation was obtained between the colloidal Ca and P levels in milks and the size of micelles. © Inra/Elsevier, Paris.

goat milk / casein / micelle size / α_{s1} casein/ colloidal calcium and phosphorus

Résumé — Taille des micelles du lait de chèvre en relation avec la composition de la caséine et les teneurs en caséines α_{s1} , α_{s2} , β et κ dans le lait. La relation entre la taille des micelles de caséine et la composition de la fraction caséine des laits a été étudiée sur 21 laits individuels provenant de

* Correspondence and reprints. E-mail: apierre@labtechno.roazhon.inra.fr

chèvres homozygotes pour les variants de la caséine α_{s1} , A, B₂, C, E, F ou O, de manière à obtenir une grande variation de composition des laits. En effet, à ces variants correspondent des niveaux de sécrétion différents de la caséine α_{s1} , de 7,2 g·kg⁻¹ pour le variant A à une absence totale pour le variant O. La taille moyenne des micelles a été mesurée par spectrophotométrie de corrélation de photons ; la distribution de la caséine selon le diamètre micellaire a été déterminée par microscopie électronique à transmission. La teneur en caséine totale et la composition de la caséine ont été obtenues à partir du dosage de la matière azotée totale ($N \times 6,38$) et de l'analyse de la caséine par chromatographie liquide à haute pression en phase inverse. Les minéraux colloïdaux, calcium et phosphore, ont été dosés respectivement par spectrophotométrie d'absorption atomique et par une méthode colorimétrique. La taille moyenne des micelles dans les laits variait de 192 à 287 nm. La variation a été expliquée par l'aspect des histogrammes de distribution de la caséine en fonction de la taille, qui montrait, selon le lait, soit un maximum pour des valeurs faibles (20–130 nm), soit un maximum pour des valeurs élevées (130–260 nm), soit encore une distribution bimodale. Une relation polynomiale a été calculée, reliant la taille moyenne des micelles (MMS) et les paramètres de composition des laits (α_{s1} CN, α_{s2} CN, β CN et κ CN). MMS était significativement corrélée d'une part à la teneur en α_{s1} CN et κ CN des laits, d'autre part à la proportion de α_{s1} CN %, α_{s2} CN % et β CN % dans la caséine totale. Ces relations ont permis de déterminer la taille micellaire moyenne des laits de chèvre à partir de leurs teneurs en caséines α_{s1} , α_{s2} , β et κ , avec une incertitude de 15 %. Les corrélations monofactorielles ont aussi été calculées, montrant un effet significatif de la teneur en α_{s1} CN ($r = -0,77$), mais non de la κ CN. La composition minérale des laits de chèvre était caractérisée par une teneur constante en phosphate inorganique colloïdal (12,4 [SD = 1,7] mmol·kg⁻¹), quelle que soit la teneur en caséine totale, et par des teneurs variables en calcium colloïdal (Cac), en P lié aux sérines et en P colloïdal (Pc) qui étaient corrélées à la teneur en caséine totale du lait. Le rapport Cac/Pc est apparu comme un paramètre invariant, ayant une valeur de 1,22 (SD = 0,05) dans les laits de chèvre, identique à la valeur déjà déterminée dans le lait de vache. Ce rapport semble caractériser un mode d'association unique des caséines dans les micelles des laits. Aucune relation significative entre minéraux et taille micellaire n'a été mise en évidence. © Inra/Elsevier, Paris.

lait de chèvre / caséine / taille de micelle / caséine α_{s1} / calcium colloïdal / phosphore colloïdal

1. INTRODUCTION

Casein in milk is structured in micelles, the size of which is dependent on mammalian species [1]. Casein micelle composition includes, in addition to minerals, different caseins, α_{s1} , α_{s2} , β and κ casein for the most part, the relative proportions of which are fairly constant within species except in colostrum, but variable between species. Many investigations have been done on cow milk to determine the relation between micelle size and casein composition. κ CN content was found to be in inverse relation to mean micelle size, in cow milks from various breeds [17] and in cow milks containing different κ CN variants [12]. In addition, the κ CN proportion in artificial micelles made from bovine caseins was

found related to micelle size in the same way [23]. Among goat milks, a difference in mean micelle size was reported in relation to α_{s1} CN variants [20, 24]; consequently, α_{s1} CN could also be a factor of variation of micelle size.

Goat milk is characterized by a high genetic variability of nature and quantity of caseins, stemming mainly from α_{s1} CN casein with about 14 variants already identified [13]. The α_{s1} CN variability results not only in the substitution or deletion of one or two amino acids (A, B₂ and C variants), but also in large deletions of the sequences. In the F variant, for example, the segment 59 to 95, containing a SerP cluster, is deleted. The α_{s1} CN variability also induces variation in the secretion levels [6]. In goat milks its content varies from

7.2 g·kg⁻¹ milk for the A variant, to none for the O variants. From the comparison of A and O goat milks, Pierre et al. [18] have observed differences in casein micelle size distribution, which explained differences in mean micelle size: the A milks were characterized by a narrow distribution of micelle sizes centered on a low diameter range while O milks had a broad distribution up to large diameters. Micelle size variation may therefore be related to the α_{s1} CN level in milk.

Such goat milks, having a casein composition varying to a larger extent than cow milks, were expected to be a good model to study the relation between casein composition and micelle size. It is the purpose of this paper to study the size characteristics of micelles (mean micelle size and size distribution of casein), and their chemical composition (casein proportions and colloidal Ca and P) in individual goat milks, from animals being homozygous for the various α_{s1} CN variants, and to search for the relations between size and composition.

2. MATERIALS AND METHODS

Goat milks were provided by Station d'Amélioration Génétique des Animaux de Toulouse (Inra). They were from 21 goats, homozygous for the A, B₂, C, E, F, O alleles of α_{s1} CN. Lactation numbers were the same (2nd) as well as lactation stages (180–215 d).

Determination of total nitrogen (TN) and of pH 4.2 soluble nitrogen (SN), extracted by the Rowland procedure [22], were made by Kjeldahl analysis (N × 6.38). Casein (CN) yield was calculated as: CN = TN - SN. The pH was chosen at 4.2 for the separation of SN, because it corresponds to the isoelectric pH of goat casein in milk, as determined in preliminary experiments. Phosphorus was determined by a photometric method [5] and calcium by atomic absorption spectrophotometry (Spectra A 300, Varian, Palo Alto, Ca, USA). Analyses of Ca and P were on total milk (T) and on ultrafiltrates, obtained either at the natural pH of milk (UF), or after a 1N-HCl addition up to pH 5.0 (UF 5.0). Ultrafiltration was carried on through CF25 Centricon membranes (Amicon, Paris, France) at 20 °C

and it involved a centrifugation step (750 g, 45 min). The following fractions were then calculated: colloidal P (Pc) and colloidal Ca (Cac), as the difference [T-UF]; inorganic colloidal P (Pic), as [UF 5.0 - UF]; Serine P (SerP), as [Pc - Pic].

HPLC chromatography (Varian 5 000, Palo Alto, Ca, USA) allowed the separation of individual components in total casein. Reverse phase separation on a C4 column – 4.6 mm diameter, 150 mm length (Vydac Interchim, Montluçon, France) was achieved using as eluents, (A) 0.1 % trifluoroacetic acid (TFA) in water, and (B) 0.096 % TFA in acetonitrile/water (80/20, v/v). Separation was obtained in 30 min on the column at 40 °C, with a flow rate of 1 mL·min⁻¹ and eluent B increasing from 37 to 53 % [18]. Sample preparation involved separation of casein from milk at 30 °C by precipitation at pH 4.2. Complete description of experimentals could be found elsewhere [18]. Proportions of the various caseins in total casein were obtained from the peak areas on the HPLC profiles, corrected by the specific relative absorbance of each casein calculated from the results of Jaubert [11]: κ CN, 0.934; α_{s2} CN, 0.877; α_{s1} CN, 0.810; β CN, 1.0. The contents of the caseins in milk were calculated from the measured proportions combined with the total casein content of milk estimated by N analysis.

Average casein micelle size was determined by photon correlation spectroscopy (PCS) on skimmed milks. Measurements were performed with a Coulter N4MD apparatus (Coultronics, Hiialeah, FL, USA), equipped with a He Ne laser light (632.8 nm), with the following experimental conditions: scattering angle 90°, temperature 20 °C, viscosity 1.002 cP and refractive index of the solvent 1.333 (water), measure time 600 s, sample time 4.5 μ s. From the measured autocorrelation function of the scattered light was calculated an intensity-weighted average diffusion coefficient. It allowed the calculation of an average diameter corresponding to the size of a spherical particle with an equivalent diffusion coefficient. Experimental methods were the same as already reported [18].

Histograms of micelle size distribution were determined on 11 of the milks only, chosen amongst those having the highest and lowest individual casein content relative to α_{s1} CN, α_{s2} CN or κ CN in order to increase any differences. Observations of milk micelles were made on a Philips CM12 apparatus (Philips Industrie, Bobigny, France), on a transmission mode, with a 80 kV voltage. Milk samples were skimmed

as already described, then diluted 1/250 in 0.2 mol·L⁻¹ sodium cacodylate buffer at pH 7.4. An aliquot was dropped on a collodion-treated carbon grid (300 mesh, 3 mm diameter). After 30 s, excess sample was removed on a filter paper and the grid was immersed in a 2% (w/v) uranyl acetate solution for 2 min. The grid was then drained and air dried. Histograms were calculated from transmission electron micrographs (TEM) according to Schmidt et al. [23]. From the plates obtained, the number fractions of micelles in 14 size classes of 20 nm width were determined by counting random fields. About 1100 individual diameters of particles were measured for each milk. All the micelles present on the plates were measured. Results were treated as described by Schmidt et al. [23] to obtain the volume moment average diameter. Micelles of diameter ≥ 130 nm are referred to here as "large micelles". The relative volume fraction of large micelles was calculated from TEM data. Assuming a constant micelle density, the volume distribution is the same as the casein mass distribution.

Polynomial analysis was a multivariate analysis of the least square type.

3. RESULTS

3.1. Size of casein micelles in milks

3.1.1. Mean micelle size

Mean micelle sizes in milks were determined by PCS. The results are grouped in *table I* according to the α_{s1} CN variants in milks. Three groups of milks, A, (B₂ + C), (E + F + O) can be distinguished, differing significantly in mean micelle size ($P = 0.05$), in spite of the small number of milks in each group. Such results are consistent with those of Remeuf [20].

3.1.2. Casein micelle size distribution

Histograms of casein micelle size distribution were obtained from TEM. Typical histograms of milks of each variant group are presented in *figure 1*. Comparing A milks and O milks, a large difference in casein micelle size distribution was observed. In two of the A milks (out of three) the distribution was centered on small

Table I. Mean micelle size (MMS), obtained by PCS, in goat milks according to their α_{s1} casein variants. Minimum and maximum values are the lowest and highest values obtained for individual milks inside a same variant group.

Tableau I. Taille micellaire moyenne (MMS), obtenue par PCS, dans les laits de chèvre contenant différents variants de la caséine α_{s1} et valeurs minimale et maximale obtenues pour des laits individuels à l'intérieur d'un même groupe de variants.

α_{s1} CN variant	Number of samples	MMS: Mean micelle size (nm)		
		Mean	Minimum value	Maximum value
A	6	221	192	236
B ₂	1	247	–	–
C	2	248	248	249
E	6	264	241	287
F	3	266	255	271
O	3	262	255	283

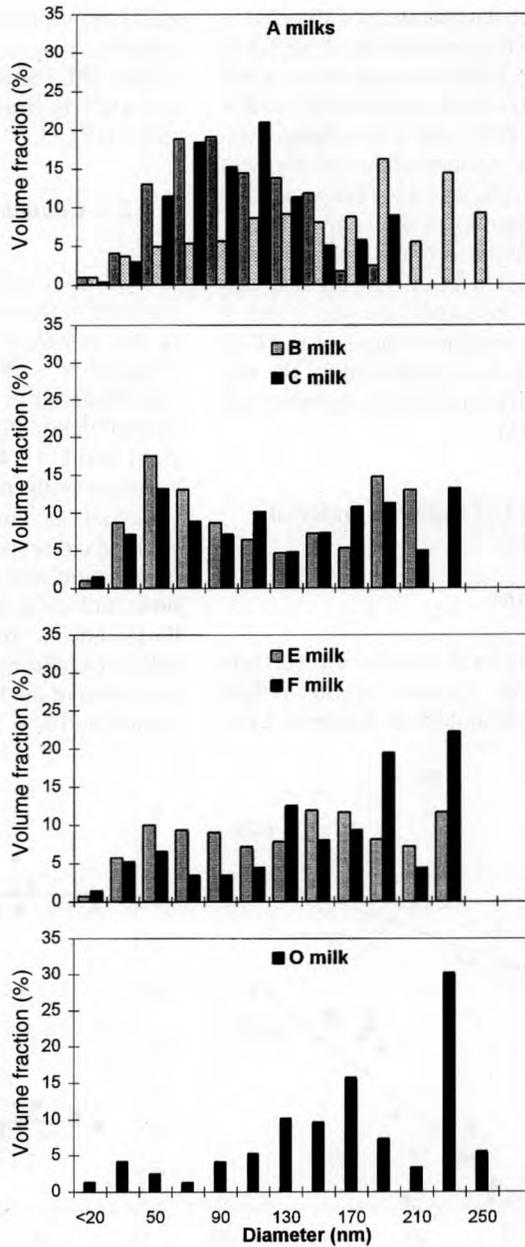


Figure 1. Casein distribution according to micelle size (from TEM) in individual goat milks with different α_{s1} CN variants: 3 different A variant milks; one milk only for each of the other variants.

Figure 1. Histogrammes de distribution de la caséine selon la taille micellaire (obtenue par TEM) dans des laits individuels de chèvre contenant des variants différents de la caséine α_{s1} .

diameter values (20–130 nm), as already reported [18], while in O milk, most of the casein was in large micelles (≥ 130 nm). Milks with B₂, C, E and F variants showed intermediate profiles, some of the milks hav-

ing a bimodal distribution. Thus, the profiles progressively changed from A to O milks; the small micelle peak decreased in E and F milks, and disappeared in O milk. The large micelle peak increased up to a maximum in

O milk in which it accounted for about 80 % of the casein. The proportion of casein in large micelles (TEM) was found correlated with the mean micelle size in the milks, determined by PCS, with a correlation coefficient $r = 0.76$. An individual variability of micelle distribution was also observed inside a same variant group, as shown by the comparison of histograms of the three different A milks reported in *figure 1*. A bimodal distribution was observed for one of the A milks (looking similar to those of the E or F milks, which was confirmed by the statistical comparison of these distributions ($r_{CA, F} = 0.91$).

3.2. Amount of colloidal material in milks

3.2.1. Caseins

Total casein level in milks varied from 17.1 to 33.1 $\text{g}\cdot\text{kg}^{-1}$ (*figure 2a*), due to their different α_{s1} CN and β CN contents. Con-

trarily, no variation of κ CN and α_{s2} CN contents was related to the total casein content (*figure 2b*). The average amounts of α_{s2} CN and κ CN in milks were respectively 4.4 (SD = 0.8) $\text{g}\cdot\text{kg}^{-1}$ and 4.5 (SD = 0.5) $\text{g}\cdot\text{kg}^{-1}$.

3.2.2. Colloidal Ca and P

Colloidal phosphorus (Pc) levels are reported in *figure 3a* as a function of total casein, as well as the levels of its two main components, SerP and Pic. An increase in Pc was observed in relation with the amount of SerP, while Pic remained at a quite constant level, 12.4 (SD = 1.7) $\text{mmol}\cdot\text{kg}^{-1}$, whatever be the total casein content in milk. Colloidal calcium (Cac) also increased with the total casein content (*figure 3b*) and was highly correlated with Pc ($r = 0.97$). Cac/Pc ratio had quite the same value in all the milks and amounted 1.22 (SD = 0.05). Contrarily, Cac/Pic increased from 1.69 to 2.24, as a result of the Cac variation, with a mean value Cac/Pic = 1.94 (SD = 0.14).

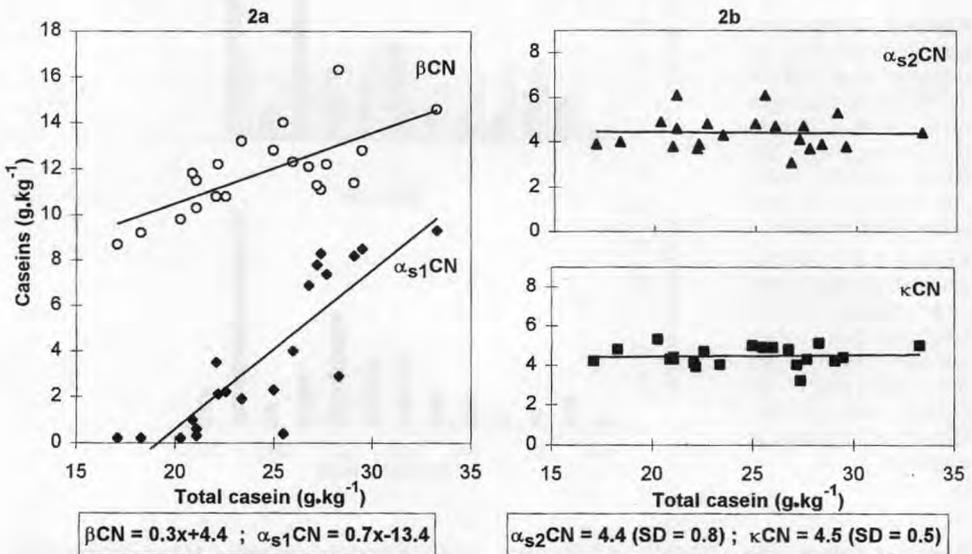


Figure 2. Levels of caseins in individual goat milks having different total casein contents. $\text{g}\cdot\text{kg}^{-1}$ ($n = 21$). **2a:** α_{s1} CN and β CN. **2b:** α_{s2} CN and κ CN.

Figure 2. Teneurs en caséines des laits individuels de chèvre ayant différentes teneurs en caséine totale. $\text{g}\cdot\text{kg}^{-1}$ ($n = 21$). **2a:** α_{s1} CN et β CN. **2b:** α_{s2} CN et κ CN.

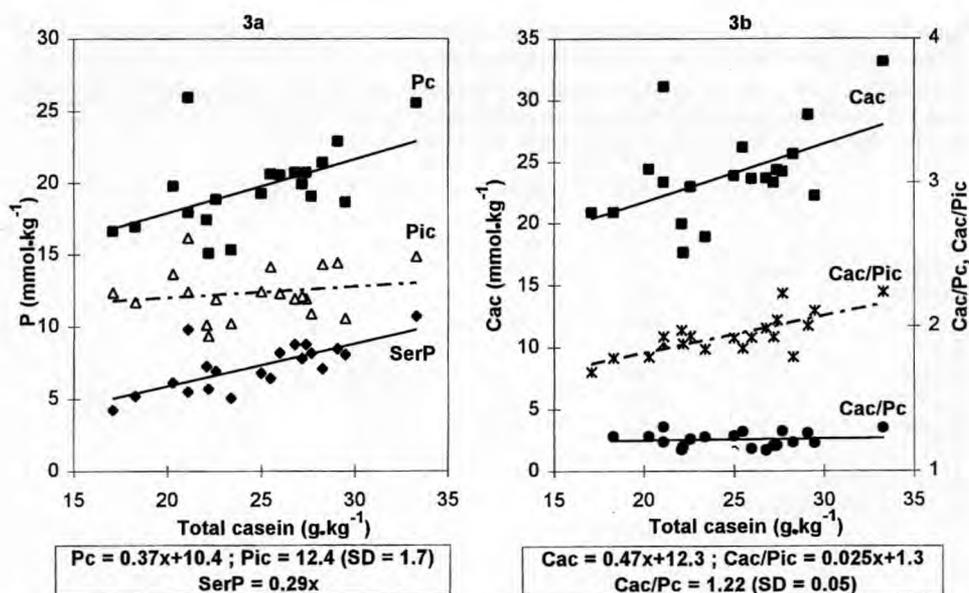


Figure 3. Colloidal minerals in individual goat milks ($\text{mmol}\cdot\text{kg}^{-1}$). **3a.** Colloidal P (Pc), inorganic colloidal P (Pic), serine P (SerP). **3b.** Colloidal Ca (Cac) and molar ratios, Cac/Pc and Cac/Pic ($n = 20$).

Figure 3. Minéraux colloïdaux dans les laits individuels de chèvre ($\text{mmol}\cdot\text{kg}^{-1}$). **3a.** P colloïdal (Pc), P inorganique colloïdal (Pic), P des phosphosérines (SerP). **3b.** Ca colloïdal (Cac) et rapports molaires, Cac/Pc et Cac/Pic ($n = 20$).

3.3. Composition of casein micelles

3.3.1. Proportions of caseins and minerals

As a result of the variation in the amounts of the caseins in milks, a wide variation in their relative proportions in total casein was observed: κCN (12 % to 26 %), $\alpha_{s2}\text{CN}$ (11 % to 29 %), $\alpha_{s1}\text{CN}$ (1 % to 30 %), βCN (39 % to 58 %). Also varied the colloidal Ca and P fractions (expressed as $\text{mmol}\cdot\text{g}^{-1}$ of total casein): Cac/CN (0.75 to 1.48), Pc/CN (0.65 to 1.23), Pic/CN (0.36 to 0.76).

3.3.2. Correlations between caseins proportions and colloidal Ca and P

Correlation coefficients between micellar constituents were calculated and are report-

ed in *table II*. The large variation of $\alpha_{s1}\text{CN}$ % induced negative correlations with other caseins, as the variables were linked, leading to a correlation coefficient in the range $r = -0.80$. In the mineral fraction, Cac/CN and Pic/CN were highly correlated ($r = 0.95$), according to the relation: $\text{Cac/CN} = 1.49 \text{ Pic/CN} + 0.22$. In this relation, the value of the slope, 1.49, corresponded to the mean value of the Ca/P ratio in the colloidal inorganic calcium phosphate of the milks and the constant, 0.22 represented the average amount of Ca directly bound to casein in the milks, $0.22 \text{ mmol}\cdot\text{g}^{-1} \text{ CN}$ [8]. Between caseins and minerals, the highest correlation coefficients were obtained between $\alpha_{s2}\text{CN}$ % and Pic/CN ($r = 0.86$) and between $\alpha_{s2}\text{CN}$ % and Cac/CN ($r = 0.82$). κCN % was positively correlated with Pic/CN ($r = 0.78$). The negative correlation coefficient obtained between $\alpha_{s1}\text{CN}$ % and Pic/CN

Table II. Correlation coefficients between casein proportions (% of total casein) and colloidal Ca and P ($\text{mmol}\cdot\text{g}^{-1}$ CN) in the micelles of individual goat milks having different α_{s1} CN variants ($n = 20$).

Tableau II. Coefficients de corrélation entre les proportions des caséines (pourcentage de la caséine totale) et les teneurs en calcium et phosphore colloïdaux ($\text{mmol}\cdot\text{g}^{-1}$ CN) dans les micelles de caséine des laits de chèvre contenant différents variants de la caséine α_{s1} ($n = 20$).

	α_{s1} CN %	α_{s2} CN %	β CN %	κ CN %	Pic/CN	Pc/CN
α_{s2} CN %	-0.80					
β -CN %	-0.79	ns				
κ CN %	-0.85	0.67	0.49			
Pc/CN	-0.58	0.80	ns	0.63	-	
Pic/CN	-0.76	0.86	ns	0.78	-	
Cac/CN	-0.61	0.82	ns	0.65	0.95	0.97
Cac/Pic	0.79	-0.58	-0.64	-0.70	-0.63	-

ns: not significant ($P = 0.05$).

ns : non significatif ($P = 0,05$).

($r = -0.76$) was in agreement with the results of Schmidt et al. [23].

3.4. Relation between micelle size and composition of casein

3.4.1. Correlations

Correlation coefficients between size parameters of casein micelles (mean micelle size, casein in large micelles) and casein or mineral levels in milks ($\text{g}\cdot\text{kg}^{-1}$) or caseins proportions in micelles (% total casein), are reported in *table III*. In relation to casein levels in milks ($\text{g}\cdot\text{kg}^{-1}$), the higher correlations were observed with total casein and α_{s1} CN. No correlation was observed with κ CN. Most of the coefficients related to casein in large micelles are higher than those of mean micelle size. Considering now casein proportions (% of total casein), the higher correlations with size parameters were obtained with α_{s1} CN % ($r = -0.73$ and -0.86), and also with α_{s2} CN % ($r = 0.61$ and 0.86). κ CN % showed a positive coefficient with MMS ($r = 0.46$). When the partial correlation, meaning the correlation at a constant α_{s1} CN proportion, was calculated between size and κ CN %, the coefficient

obtained was $r = -0.35$, not significant at $P = 0.05$, but only at $P = 0.20$. In *figure 4* is presented the relation between mean micelle size and casein proportions. In the hypothesis of a linear relation, regression coefficients were calculated which explained 53 % of the variance when related to α_{s1} CN, and respectively 37 % for α_{s2} CN, 44 % for β CN and 21 % for κ CN. The amount of casein in large micelles was presented in *figure 5* as a function of the α_{s1} CN proportion and of the Pic/CN ratio. α_{s1} CN % explained 74 % of the total variance (*figure 5a*). In relation with the Pic/CN variation (*figure 5b*), the curve showed a biphasic course, casein in large micelles increased for Pic/CN values from 0.42 up to about 0.55 and, for the higher values of the ratio, a plateau was reached corresponding to 80 % of casein in large micelles. Other colloidal mineral fractions in milks were not highly correlated with micelle size parameters, neither considering their proportions nor their contents in milks (*table III*).

3.4.2. Polynomial regressions

Polynomial relations were also calculated between micelle size parameters and the

Table III. Correlation coefficients between size parameters (Mean micelle size, MMS, obtained by PCS; casein in large micelles, ≥ 130 nm, from TEM) and caseins and colloidal minerals ($\text{g}\cdot\text{kg}^{-1}$ and percentage of casein) in individual goat milks with different α_{s1} CN variants; n : number of samples.

Tableau III. Coefficients de corrélation entre les paramètres de taille micellaire, taille micellaire moyenne (MMS), obtenue par PCS, et proportion de la caséine dans les micelles de taille ≥ 130 nm (TEM) et la composition (caséines et minéraux colloïdaux) dans les laits individuels de chèvres ayant des variants de la caséine α_{s1} différents; n : nombre d'échantillons.

	MMS: Mean micelle size (nm)	Casein in large micelles (%)	Total casein ($\text{g}\cdot\text{kg}^{-1}$ milk)
$\text{g}\cdot\text{kg}^{-1}$ milk	$n = 21$	$n = 11$	$n = 21$
Total casein	-0.72	-0.69	-
α_{s1} CN	-0.77	-0.86	0.85
α_{s2} CN	ns	ns	ns
β CN	ns	ns	0.70
κ CN	ns	ns	ns
Percentage of total casein			
α_{s1} CN %	-0.73	-0.86	0.82
α_{s2} CN %	0.61	0.86	-0.71
β CN %	0.66	ns	-0.52
κ CN %	0.46	0.73	-0.82
Pic/CN	0.43	0.77	-0.72
$\text{mmol}\cdot\text{kg}^{-1}$ milk	$n = 20$		$n = 20$
Pc	-0.61	ns	0.53
Pic	ns	ns	ns
Cac	-0.54	ns	0.57
Cac/Pic	-0.64	-0.75	0.75

ns: not significant ($P = 0.05$).

ns : non significatif ($P = 0,05$).

α_{s1} , α_{s2} , β , κ casein levels in milks. Their characteristics are reported in table IV. Two different relations were calculated, one from the caseins contents ($\text{g}\cdot\text{kg}^{-1}$) of the milks, the other from the casein proportions (%). The results show that the α_{s1} CN and κ CN contents of milks were significantly in relation with micelle size. The two coefficients were negative, the higher being ascribed to κ CN (-20.4). Considering now casein relative proportions, α_{s1} CN %, α_{s2} CN % and β CN % had significant coefficients, all three positive. κ CN % showed no significant relation.

4. DISCUSSION

4.1. Micelle size

Profiles of TEM histograms of casein distribution versus micelle diameter varied according to the milks. Casein in goat milks seemed to be preferentially distributed either in small micelles, or in large micelles, or even, in various relative proportions among these two populations according to the milk, leading to bimodal or widely varying distributions. The prevalence of one on the other explained the variations observed in

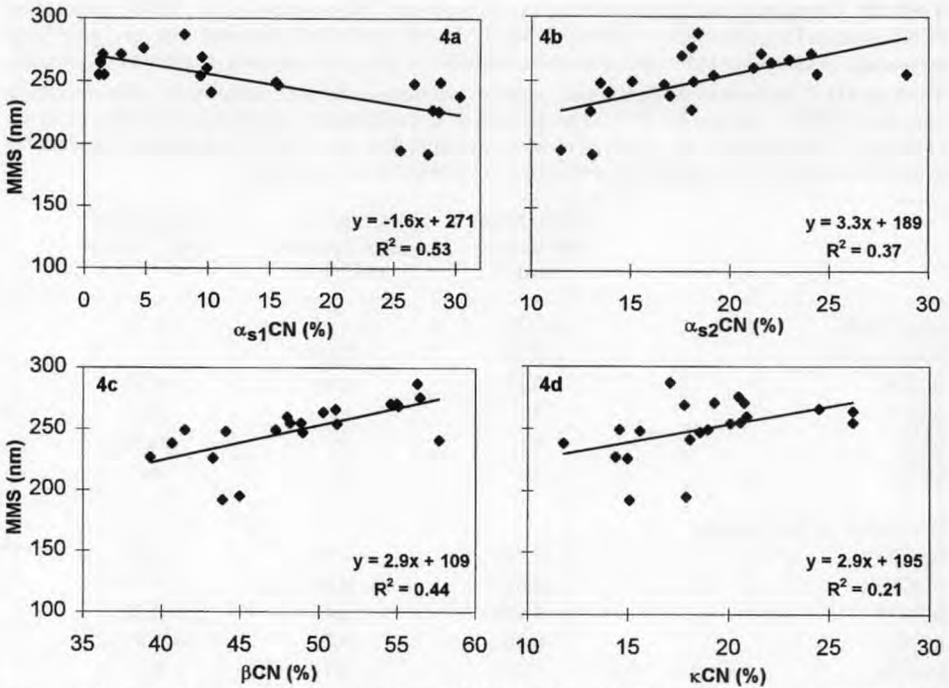


Figure 4. Relation between mean micelle size (MMS), obtained by PCS, and the proportion of the different caseins in the total casein of individual goat milks ($n = 21$). **4a.** α_{s1} CN. **4b.** α_{s2} CN. **4c.** β CN. **4d.** κ CN.

Figure 4. Relation entre la taille micellaire moyenne (MMS), obtenue par PCS, et la proportion des différentes caséines dans la caséine totale de laits individuels de chèvre ($n = 21$). **4a.** α_{s1} CN. **4b.** α_{s2} CN. **4c.** β CN. **4d.** κ CN.

the mean micelle size (MMS). These variations of MMS were in fact better correlated to the levels of α_{s1} CN and κ CN in the milks, than to the α_{s1} CN genetic variant itself.

Mean micelle size (MMS) was found in relation with casein micelle composition according to the following relations:

$$(a) \text{MMS1 (nm)} = -6.4 \alpha_{s1}\text{CN} - 20.4 \kappa\text{CN} + 364 ;$$

$$(b) \text{MMS2 (nm)} = 0.95 \alpha_{s1}\text{CN \%} + 3.9 \alpha_{s2}\text{CN \%} + 3.5 \beta\text{CN \%}.$$

These equations allowed the determination of MMS with about 15 % accuracy. MMS were calculated for the 21 goat milks using these equations, and the results plotted against the experimental data obtained by

PCS measurements (*figure 6*). The relations obtained explained respectively 78 and 62 % of the variance. Consequently, the equations allow one to calculate an estimated value for mean micelle size in goat milk from its casein content or its casein composition.

In equation (a), relative to casein contents, two caseins intervene, α_{s1} CN and κ CN. The α_{s1} CN content was found negatively correlated to micelle size, which has never been reported up to now. This was confirmed by the value of the monofactorial correlation coefficient between micelle size and α_{s1} CN (*table III*) which was also negative ($r = -0.77$). It might be a specific feature of goat milks, but, more likely, the large variation of α_{s1} CN content in the goat

Table IV. Polynomial relations between mean micelle size (MMS), measured by PCS, and casein composition in goat milks (number of samples: 21).**Tableau IV.** Relations polynomiales entre la taille micellaire moyenne (MMS), obtenue par PCS et la composition de la caséine des laits de chèvre (nombre d'échantillons : 21).

Y	x coefficients relating to:						
	Caseins/g·kg ⁻¹ milk				Constant	F	r ²
	α_{s1}	α_{s2}	β	κ			
(a) Mean micelle size (MMS1)	-6.4	ns	ns	-20.4	364	29	0.76
SD	1	-	-	6	27		
	Casein % of total casein				Constant	F	r ²
	α_{s1}	α_{s2}	β	κ			
(b) Mean micelle size (MMS2)	0.95	3.9	3.5	ns	0	1 282	0.99
SD	0.3	1.1	0.5	-	-		

F: F-value; r²: regression coefficient; SD: standard deviation; ns: not significant ($P = 0.05$).

F: valeur de F; r²: coefficient de régression; SD: écart type; ns: non significatif ($P = 0,05$).

milks studied allowed to highlight and quantify the relation, which did not appear in cow milk, as the variation of its α_{s1} CN level is lower. The coefficient ascribed to κ CN in equation (a), is also negative and has the higher value. This result agrees well with the data already obtained on cow milk: κ CN content of individual milks was reported to be inversely related to micelle size [12, 17]. The coefficient relating to κ CN, -20.4, seems valid for milks others than goat milk. As an example, the results obtained by Gutiérrez-Adán et al. [7] on the milks of transgenic mice bearing the bovine κ CN gene can be quantitatively explained by the variation in their bovine κ CN contents. In figure 7 is reported, as a function of the bovine κ CN contents of the mice milks, on one side the experimental mean micelle sizes (MMS) measured by these authors, and, on the other side, the mean micelle sizes (MMS-calc) calculated from the experimental size determined in normal mice milk, then modified as a function of the bovine κ CN content, according to the -20.4 κ CN

relation. The difference between the two relations is not significant ($P = 0.05$), meaning that the calculation is a good prediction of the sizes.

In another respects, it is noteworthy that the monofactorial correlation between the κ CN level and micelle size was not significant (table III), contrary to equation (a).

In equation (b), in which intervene the relative proportions of the caseins (%) in goat milks, micelle size was significantly related to the proportions of caseins other than κ CN, the most to β CN %, due to its high coefficient and to its large proportion in milk, and, to a lesser extent, to α_{s1} CN % and α_{s2} CN %. No significant effect of κ CN on the size was observed, when κ CN was expressed as a percentage. More, the monofactorial correlation between size and κ CN % gave a positive hardly significant coefficient ($r = 0.46$). Only after calculation of the corresponding partial correlation of κ CN % at a constant α_{s1} CN %, a negative coefficient for κ CN % became apparent. So, it

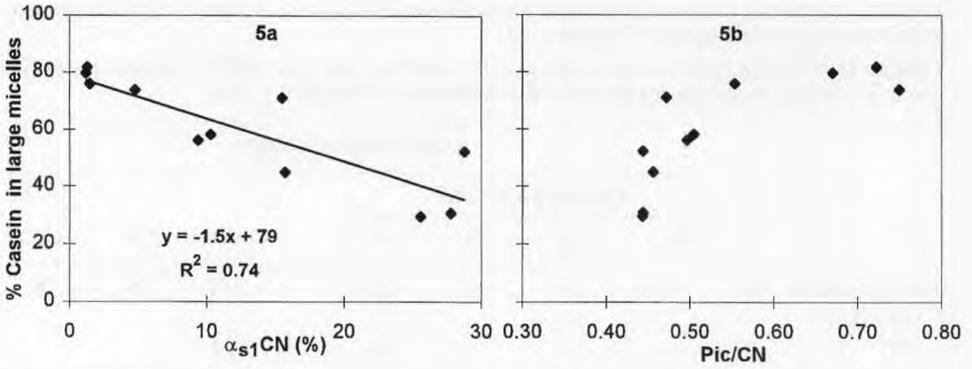


Figure 5. Relation between the proportion of casein contained in large micelles (≥ 130 nm, TEM) and the proportion of α_{s1} CN (a) and the Pic/CN ratio (b), in the total casein of individual goat milks ($n = 11$).

Figure 5. Relation entre la proportion de caséine incluse dans les grosses micelles (≥ 130 nm, TEM) et la proportion de caséine α_{s1} (a) et le rapport Pic/CN (b) dans la caséine totale de laits individuels de chèvre ($n = 11$).

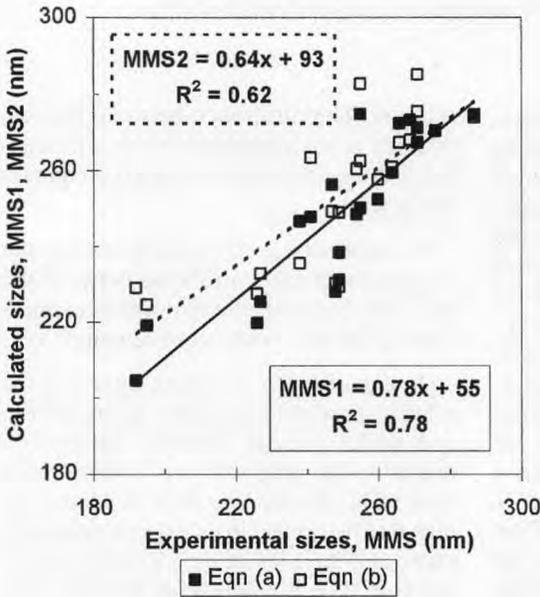


Figure 6. Relation between the mean micelle sizes experimentally measured by PCS (MMS) and the calculated sizes obtained either from equation (a) involving casein levels in milk, (MMS1), or from equation (b) involving casein proportions, (MMS2). Diameters in nm ($n = 21$).

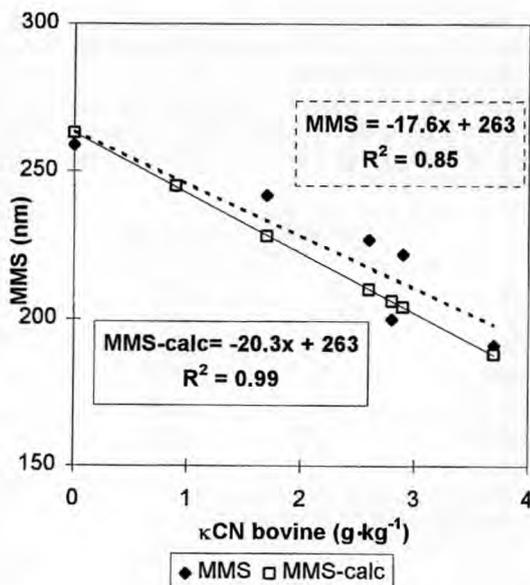
Figure 6. Relation entre les tailles micellaires obtenues par PCS (MMS) et les tailles calculées à partir de l'équation (a) faisant intervenir les teneurs en caséine (MMS1), et de l'équation (b) faisant intervenir les proportions des caséines (MMS2). Diamètres en nm ($n = 21$).

seems that the weight of the α_{s1} CN level, having the greater range of variation in goat milks matched with a passably high coefficient, totally masked or modified the weight of the κ CN variation, in spite of its higher coefficient. A negative coefficient for κ CN %

would be in agreement with the results obtained either in cow milk or in goat milk comparing the composition of the small and large micelles of individual milks [2–4, 14, 15, 19, 24]. Schmidt et al. [23] also demonstrated that the size of micelles (artificial

Figure 7. Mean micelle sizes in the milks of transgenic mice, as a function of their bovine κ CN content. MMS, experimental results from Gutiérrez-Adán et al. [7]; MMS-calc., micelle sizes calculated from the experimental size determined in normal mice milk, then modified as a function of the bovine κ CN content of the mice milk, using the -20.4κ CN coefficient.

Figure 7. Taille moyenne des micelles de caséine en fonction de la teneur en κ CN dans des laits de souris transgéniques. MMS exp., résultats expérimentaux de Gutiérrez-Adán et al. [7]; MMS-calc., tailles calculées à partir de la valeur expérimentale obtenue dans le lait de souris normale et corrigée en fonction de la teneur en caséine κ bovine des laits, en utilisant le coefficient -20.4κ CN.



micelles) was inversely related to κ CN proportions. However, conditions of their experimentation were such (variation of κ CN proportions at a constant casein level in the suspension) that there was an 'one-to-one' relation between κ CN concentrations and κ CN proportions. Thus, from their results, it was not possible to conclude which of the two was the efficient parameter. In contrast, when individual milks were studied, not only κ CN concentration varied, but also, and independently, their total casein concentration.

Considering micelle size in relation with colloidal minerals, no main correlation was obtained, as already reported for cow milk casein micelles by Schmidt et al. [23], apart the relation reported between casein in large micelles and Pic/CN .

4.2. Micelle composition

The wide range of variation observed in the casein levels and in their proportions was however compatible with the edifica-

tion of micelles in all the goat milks, as observed by TEM. Micelles appeared, in every case, as constituted of granular aggregates of various sizes, similar to those already described by Richardson et al. [21] as well in goat milk, as in cow milk and ewe milk.

The levels of colloidal mineral in goat milks showed many similarities with that of cow milk at a same total casein content (table V). Some of the parameters determined were found varying according to the total casein content in goat milks, as SerP, Pc and Cac, while others remained constant in all the milks, as Pi (12.4 (SD = 1.7) $\text{mmol}\cdot\text{kg}^{-1}$), or Cac/Pc, 1.22 (SD = 0.05). The constant Cac/Pc ratio observed in either cow or goat milk, confirmed the main role of calcium phosphate during casein aggregation into micelles [8, 9, 16].

4.3. Conclusion

The unique structural appearance of micelles assorted of a same mineral composition could mean that a same mineral

Table V. Composition of micellar calcium phosphate in cow milk and in a goat milk having the same total casein content (25.8 g·kg⁻¹).

Tableau V. Composition du phosphate de calcium micellaire dans le lait de vache et dans un lait de chèvre ayant la même teneur en caséine totale (25,8 g·kg⁻¹).

	Cow milk*	Goat milk**
Cac	21.2	24.0
Pc	17.3	19.6
Pic	10	12.4
SerP	7.3	7.2
Cac/Pic	2.13	1.94
Cac/Pc	1.23	1.22

* From [10] and [25]; ** from figure 3.

* Voir [10] et [25]; ** voir figure 3.

structure could be involved in the aggregation of caseins into micelles in all the milks, whatever the species and whatever the proportions of the caseins.

Contrarily, the preferential edification of low size or large size micelles, leading to different mean micelle sizes, seemed to be dependent on the α_{s1} CN and κ CN levels in milks. It is suggested that the α_{s1} CN and κ CN levels in milks could be the influent factors on micelle size regulation, or at least, be in close relation with them, although our experiments have not demonstrated they were causal. However, it is not yet possible to explain the results observed from the present knowledge on goat milk.

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