Renneting properties of milk from individual ewes: influence of genetic and non-genetic variables, and relationship with physicochemical characteristics

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Summary. The physicochemical characteristics and the renneting properties of milks from Lacaune ewes are reported. The ewes belonged to divergent genetic lines termed 'low' or 'high' and were selected on a combination of fat and protein yield. The 179 second to fifth parity ewes were sampled three times during lactation at about 50, 135 and 175 d in milk. Physicochemical and renneting variables were principally influenced by days in milk and somatic cell count, whereas β-lactoglobulin genotype, parity and divergent lines had relatively few effects. Fat and protein content increased throughout lactation, whereas rennet gel firming rate decreased. Salt distribution and micellar characteristics were also significantly affected by days in milk. Milks with high somatic cell count were characterized by high values of pH and soluble nitrogen content and rennet coagulation properties were significantly affected at $> 3 \times 10^5$–5×10^5 cells/ml milk. Divergent lines had a strong effect on milk yield, but no effect on milk composition consistent with the selection criteria. Gel firming rate was slightly higher in milks from high yield ewes. Protein content, Ca concentration, pH and micelle characteristics were the main factors related to variations in the renneting properties of the milks.

In France most ewes' milk is produced in the Roquefort area in the mountainous Massif Central plateau by the native Lacaune breed, and it is mainly processed into Roquefort blue cheese. Over the last 25 years, the Lacaune population has become a dairy type, with the implementation of an efficient breeding programme and modern husbandry methods using new feeding practices. Since 1987, the selection criterion in dairy Lacaune sheep has moved from milk yield alone to both milk yield and milk composition. Our aim was to verify whether this new goal might stabilize the renneting behaviour of milk.

Factors influencing rennetability have been studied in bovine milk (Grandison *et al.* 1984*a*, *b*, 1985; Okigbo *et al.* 1985; Remeuf *et al.* 1991) but less so in ewes' milk and to an even lesser extent in caprine milk (Remeuf *et al.* 1989). For ewes' milk, correlations between the main physicochemical variables such as pH and fat and protein contents, and renneting properties such as clotting time, gel firming rate and

gel strength have been investigated in few studies (Ubertalle *et al.* 1990; Duranti & Casoli, 1991; Manfredini *et al.* 1992; Delacroix-Buchet *et al.* 1994). Ovine milk, which contains more fat, protein and minerals than bovine or caprine milk, is characterized by a shorter clotting time and stronger curd firmness.

The main objectives of the present study were to quantify the effects of some factors that might influence the composition and renneting properties of milk, and to study the relationship between the physicochemical characteristics of the milk and its renneting properties. The effects of β-lactoglobulin genotype, genetic merit for milk fat and protein yield, days in milk, parity and somatic cell count (SCC) were studied. Analysing the results for these factors allowed us to investigate the relationship between the physicochemical characteristics of milk and rennet coagulation properties.

Materials and methods

Animals

The individual milk samples were provided from an experimental flock (INRA, Domaine de La Fage, Roquefort, France) in which a divergent selection experiment had been carried out since 1987. The ewes were bred from either low or high genetic merit rams taking part in the Lacaune breeding programme. The selection criterion was a combination of fat and protein yield, estimated according to an animal model (Barillet *et al.* 1992). According to the genetic correlations between milk traits (Barillet, 1985), such divergent selection provided two lines of females, designated 'low' and 'high' with an important genetic gap for milk yield between them ($\sim 2{\cdot}5$ genetic standard deviations after three generations), but quite a stable genetic level for fat and protein contents. These lines allowed us to look for stability or possible changes in manufacturing properties of milk for cheesemaking related to the actual Lacaune breeding scheme. Other animals characterized by an intermediate milk yield merit were also sampled and will be referred to as 'medium' line animals.

A total of 179 ewes were involved: 112, 44 and 23 for the high, low and medium lines. They were divided into two blocks of 89 and 90 animals. Each ewe was sampled three times during lactation, after 48, 132 and 174 d in milk for the first block, and 55, 139 and 182 d in milk for the second block. Because of early drying-off of several of the ewes, the sampled ewes decreased from $n = 179$ at time 1 to $n = 161$ at time 2 and $n = 158$ at time 3.

The 179 ewes were all multiparous, in their second (parity 2) or third or later parity (parity 3+), and were artificially inseminated. The average number of daughters per sire was 2·2 with a maximum of 10.

Milk samples

The samples were collected at the morning milking. Each was divided into three parts and sent to the laboratories taking part in the study.

The samples for fat and N analyses were refrigerated at 4 °C and analysed on the same day at Société des Caves, Roquefort. Other samples were treated with NaN_3 (0·4 g/l) and phenylmethylsulphonyl fluoride (0·045 g/l), held at 20 °C and analysed a day later at LGMPA, Grignon for micellar characteristics, salts equilibria and renneting properties. All milk samples were brought up to 30 °C before analysis, shaken slowly to mix and held for 1 h at 20 °C. All determinations were in duplicate and carried out on whole milk except for calcium and phosphorus concentrations, which were measured on skim milk.

Somatic cell counts

Somatic cell counts (SCC) were determined in the Laboratoire Interprofessionel Laitier (F-15000 Aurillac, France) with a Fossomatic instrument (N. Foss & Co, DK-2900 Hellerup, Denmark). Milk samples were classified into four groups according to their SCC: $< 1 \times 10^5$, 1×10^5–3×10^5, 3×10^5–5×10^5 and $> 5 \times 10^5$ cells/ml.

Identification of β-lactoglobulin genetic polymorphism

Milk protein variants were identified by electrofocusing according to the method of Seibert *et al.* (1985) in the Laboratoire de Génétique Biochimique INRA (F-78352 Jouy-en-Josas, France).

For the French Lacaune breed the only significant protein polymorphism concerns the β-lactoglobulin loci with two alleles, A and B (Barillet *et al.* 1993). The frequencies of the genotypes AA, AB and BB in the milks studied were respectively 39·7, 44·7 and 15·6 %.

Chemical analyses and physicochemical determinations

The pH of milk samples was checked at 20 °C 1 h after their arrival in each laboratory, in order to verify that they had been preserved satisfactorily. As milk pH has a strong influence on renneting, it was included as a factor in the statistical analysis of coagulation properties.

Milk fat content was determined by the acidobutyrometric method of Gerber (Norme Française no. VO4-210). Total protein, casein and whey protein contents were calculated after determination of total, soluble and non-protein N by the Kjeldahl method. The soluble N content was determined after isoelectric precipitation of casein (Rowland, 1938). Non-protein N was determined after precipitation of protein with trichloroacetic acid (120 g/l).

Total and diffusible Ca were determined fluorometrically with a Corning Calcium Analyzer 940 (Corning, Halstead CO9 2DX, UK). Total Ca was measured on skim milk defatted by centrifugation at 1500 ***g*** and 20 °C for 20 min. Diffusible calcium was titrated on ultrafiltered whey obtained as follows: 20 ml skim milk was coagulated at 35 °C by addition of 0·25 μg 1:10000 rennet (Laboratoire Granday-Roger, F-21200 Beaune, France). The coagulum was cut crosswise with a spatula 30 min after renneting. The whey was drained and filtered through ashless Whatman no. 41 paper, 1 h after cutting. Then a whey sample was ultrafiltered with a 3000 Da cut-off Centripep Concentrator (Amicon, Beverly, MA 01915, USA) at 1800 ***g*** and 20 °C for 2×1 h.

Total and diffusible inorganic phosphorus (P_i) were measured colorimetrically (International Dairy Federation, 1967) on 120 g trichloroacetic acid/l filtrates of skim milk and ultrafiltered whey respectively.

Diffusible salt concentrations were determined as g/l whey ultrafiltrate and converted to g/l whole milk by multiplying by the factor $(1-y/930)\,(1-x/1000)$, where x and y were respectively casein and fat concentrations in whole milk (White & Davies, 1958). Colloidal Ca and P_i were obtained by the difference between total and diffusible concentrations.

Mean micelle diameter was determined with an N4 Coultronics Particle Analyzer (Coulter Electronics, Miami, FL 33116-9015, USA) as described by Remeuf *et al.* (1989). Micelle mineralization was estimated by the colloidal Ca:casein ratio and expressed in mg/g.

Rennet coagulation properties

Rennet coagulation characteristics were determined with a Formagraph apparatus (N. Foss & Co) on whole milk without pH standardization (Zannoni & Annibaldi, 1981). All samples were equilibrated at 30 °C for 30 min before addition of 0·266 ml/l 1:10000 rennet previously prepared by dilution in 25 mM-piperazine-HCl buffer, pH 5·5.

The renneting properties i.e. rennet clotting time (RCT), time to achieve 30 mm firmness (k_{30}) and gel firmness at twice the clotting time (A_{2RCT}) were measured on the Formagraph tracing. Gel firming rate (GFR) was calculated by $GFR = 30/k_{30}$, and expressed in mm min^{-1}. For the duration of the study, repeated measurements performed on a standard milk, reconstituted from skim milk powder supplied by Station Expérimentale Laitière INRA (F-39801 Poligny, France) allowed us to check the repeatability of the method.

Statistical analyses

The statistical analyses were based on least squares techniques using the general linear model procedures of SAS (SAS, 1989) together with other specific software developed by the INRA Genetics Department.

The measurements were analysed by a mixed model (model 1) using a restrictive maximum likelihood method, and including the following factors: days in milk (three levels), β-lactoglobulin genotype (three levels, AA, AB, BB), parity (two levels, 2 and 3+), genetic line (three levels, high, medium, low), SCC subclass (four levels), pH subclass (seven levels, only for renneting properties) and ewe (the random effect of individual animals).

Model 1 allowed us to calculate the repeatability ρ_i for a given trait between successive measurements for the same ewe at different days in milk as

$$\rho_i = \frac{\sigma^2_{ei}}{\sigma^2_{ei} + \sigma^2_{ri}},$$

where σ^2_{ei} and σ^2_{ri} were respectively the estimated variances of the random effect of the ewe and of the residual effect for the character i (Minvielle, 1990). The repeatability ρ_i (in the range 0–1) allowed us to investigate the genetic variation of the traits measured.

Relationships between physicochemical characteristics and renneting properties of milk were investigated as follows.

(1) Using model 1 we calculated the phenotypic correlations $R\rho_{(1,2)}$ between renneting variables (trait 1), and physicochemical variables (trait 2) using the equation

$$R_{\rho(1,2)} = \frac{\sigma_{e1,2} + \sigma_{r1,2}}{(\sigma^2_{e1} + \sigma^2_{r1})(\sigma^2_{e2} + \sigma^2_{r2})},$$

with $\sigma_{e1,2}$ and $\sigma_{r1,2}$ the estimated covariances of the ewe effect and of the residual effect between traits 1 and 2. Thus the phenotypic correlations are adjusted for the fixed effects included in model 1.

(2) The relative importance of pH and other physicochemical effects accounting for the variation of rennet coagulation properties was estimated by comparing the values of r^2 and the SD of the residual error for three other models including the following factors: model 2, days in milk, genetic line, SCC subclass and ewe; model 3, days in milk, genetic line, SCC subclass, pH subclass and ewe; model 4, days in

milk, genetic line, SCC subclass, pH subclass, ewe and a physicochemical factor X as covariable.

RESULTS

Influence of genetic and non-genetic factors of variation on the physicochemical characteristics and renneting properties of milk

Table 1 contains the average values of physicochemical characteristics and rennet coagulation properties for the individual milk samples, and indicates the statistical significance of the effects included in model 1. Tables 2 and 3 give the values of least-square means for each significant factor.

The milk composition variables and the renneting properties were almost all highly influenced by days in milk. As shown previously for bulk milks (Pellegrini *et al.* 1994), there was a considerable change in ewes' milk composition during the first 140 d in milk, after which the physicochemical characteristics remained relatively more stable. The fat content increased up to the end of the lactation, whereas protein content increased only from time 1 to 2, in agreement with Barillet (1985). The content and partition of milk salts were also affected by days in milk with an increase in total Ca and a decrease in diffusible Ca and P_i. Casein micelle mean diameter was higher at the end of lactation, whereas mineralization decreased. In addition the renneting properties changed during lactation (Table 3), since rennet clotting time increased with days in milk whereas the gel firming rate tended to decrease.

There was no significant effect of β-lactoglobulin genotype for most physicochemical characteristics and rennet coagulation properties. We noted only a small difference in the diffusible Ca content between the AA and BB variants, and in the diffusible P_i content between AB and the other variants.

A few variables were influenced by parity. Milk fat content increased slightly with age when we compared ewes in second lactation and later parities, and the milk of young adults seemed to be characterized by smaller micelles and slightly higher diffusible Ca.

As expected from the selection design, the ewes belonging to the three genetic lines could be distinguished by their average milk yield (Table 2), but not by their milk composition. Only non-protein N and diffusible salt content appeared to be affected by lines. Renneting properties were also related to milk yield merit (Table 3) with a shorter rennet clotting time and a higher gel firming rate for milk of high line ewes.

SCC was negatively correlated with milk yield (Table 2), as reported for cattle (Munro *et al.* 1984). The difference in milk yield between ewes with SCC $> 5 \times 10^5$ cells/ml and $< 1 \times 10^5$ cells/ml was $\sim 14\,\%$ of the average milk yield. Soluble protein content increased with SCC and the difference between the two extreme SCC subclasses was nearly 20 % (Table 2). Concentration and percentage of diffusible Ca tended to be lower for higher SCC milks. *RCT* increased significantly ($P < 0{\cdot}05$) for milks with high SCC and gel firming rate decreased as the SCC increased, with a significant threshold of 3×10^5–5×10^5 cells/ml (Table 3).

Individual repeatability coefficients were calculated for each variable (Table 1). In agreement with previous studies, the repeatability of fat and protein content was medium as was that of *RCT*. The repeatability coefficients for gel firming rate and gel firmness were higher. With respect to the other physicochemical characteristics, it is notable that variables related to salt composition and partition were the most repeatable traits with most coefficients between 0·6 and 0·7, emphasizing the individual genetic variation for these traits.

Table 1. *Means, standard deviations and statistical significance of the fixed effects included in the analysis model and repeatability for physicochemical components and renneting properties measured on individual ewes' milks*

Variable, expressed as g/l whole milk except where otherwise stated	Mean ($n = 498$)	SD	Probabilities associated with effects: Days in milk	β-Lactoglobulin genotype	Parity	Divergent lines	SCC subclass	Repeatability
pH	6·69	0·13	< 0·01	< 0·01	< 0·01	NS	< 0·01	ND
Milk yield, ml	948	506	< 0·01	NS	NS	< 0·01	0.01	0·58
Fat	73·3	14·6	< 0·01	NS	< 0·01	NS	NS	0·36
Total N × 6·38	57·2	7·8	< 0·01	NS	0·04	NS	NS	0·43
Protein N × 6·38	54·4	7·8	< 0·01	NS	0·04	NS	NS	0·43
Casein N × 6·38	44·0	6·5	< 0·01	NS	0·02	NS	NS	0·48
Soluble protein N × 6·38	10·4	1·97	< 0·01	NS	NS	NS	< 0·01	0·35
Non-protein N × 6·38	2·83	0·34	< 0·01	NS	NS	0·04	NS	0·56
Casein, % of total protein	80·96	2·35	< 0·01	NS	NS	NS	< 0·01	0·50
Total Ca	2·09	0·23	< 0·01	NS	NS	NS	NS	0·60
Diffusible Ca	0·40	0·06	< 0·01	< 0·01	0·03	< 0·01	< 0·01	0·66
Diffusible Ca, % of total Ca	19·2	3·06	< 0·01	0·03	NS	< 0·01	< 0·01	0·66
Colloidal Ca	1·69	0·21	< 0·01	NS	NS	NS	NS	0·60
Total P_i	0·95	0·13	NS	NS	NS	NS	< 0·01	0·63
Diffusible P_i	0·27	0·08	< 0·01	< 0·01	NS	< 0·01	NS	0·52
Diffusible P_i, % of total P_i	28·5	6·6	< 0·01	< 0·01	NS	< 0·01	NS	0·33
Colloidal P_i	0·68	0·1	< 0·01	NS	NS	NS	NS	0·47
Micelle mean diameter, nm	196·5	25·4	< 0·01	NS	0·01	NS	NS	0·48
Micelle mineralization, mg/g	38·7	4·1	< 0·01	0·04	NS	NS	NS	0·62
Rennet clotting time, min	20·7	6·3	< 0·01	NS	NS	< 0·01	< 0·01	0·25
Gel firming rate, mm min^{-1}†	5·34	1·72	< 0·01	NS	NS	0·04	< 0·01	0·41
Gel firmness, mm†	57·3	5·5	< 0·01	NS	NS	NS	NS	0·51

SCC, somatic cell count; NS, no significant effect; ND, not determined.
† Defined in the text.

Relationships between physicochemical composition and renneting properties

The effect of pH variations on the renneting properties of milk could be estimated from the values of least-square means for each pH subclass (Table 3). Rennet clotting time and gel firming rate were highly dependent on milk pH, but gel firmness was not.

The relationships between milk composition and rennet coagulation properties were investigated by computing the phenotypic correlations between these two kinds of variables (Table 4). Phenotypic correlations permit estimation of the relationship between two variables, after adjustment for the effects included in model 1, such as pH, that may induce indirect correlations between the variables. These phenotypic correlations showed that *RCT* and *GFR* were inversely related, whereas *GFR* and A_{2RCT} were positively correlated, in agreement with Delacroix-Buchet *et al.* (1994). The main physicochemical variables that were correlated with *RCT* ($P < 0·05$) were total and soluble protein, casein, P_i and % diffusible Ca. Gel firming rate was

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Table 2. *Least-square means† for physicochemical variables measured on individual milks that vary significantly with factors in statistical Model 1‡*

Variable, expressed as g/l whole milk except where otherwise stated	Days in milk			β-Lactoglobulin genotype			Parity		Divergent lines			Somatic cell count subclass‡			
	48–55	132–139	174–192	AA	AB	BB	2	3+	Low	Medium	High	1	2	3	4
Milk yield, ml	1374^{a}	621^{b}	513^{c}		NS			NS	696^{a}	834^{b}	978^{c}	933^{a}	846^{b}	800^{b}	765^{b}
pH	$6{\cdot}74^{a}$	$6{\cdot}70^{b}$	$6{\cdot}67^{c}$	$6{\cdot}70^{a}$	$6{\cdot}67^{b}$	$6{\cdot}73^{a}$	$6{\cdot}68^{a}$	$6{\cdot}72^{b}$		NS		$6{\cdot}66^{a}$	$6{\cdot}70^{b}$	$6{\cdot}72^{b,c}$	$6{\cdot}74^{c}$
Fat	$60{\cdot}5^{a}$	$78{\cdot}9^{b}$	$83{\cdot}3^{c}$		NS		$72{\cdot}7^{a}$	$76{\cdot}2^{b}$		NS				NS	
Total N × 6·38	$49{\cdot}1^{a}$	$62{\cdot}0^{b}$	$62{\cdot}1^{b}$		NS			NS		NS		$57{\cdot}0^{a}$	$57{\cdot}3^{a}$	$57{\cdot}9^{a,b}$	$58{\cdot}9^{b}$
Protein N × 6·38	$46{\cdot}3^{a}$	$59{\cdot}4^{b}$	$59{\cdot}2^{b}$		NS			NS		NS				NS	
Casein N × 6·38	$37{\cdot}0^{a}$	$47{\cdot}8^{b}$	$47{\cdot}4^{b}$		NS			NS		NS				NS	
Soluble protein N × 6·38	$12{\cdot}3^{a}$	$14{\cdot}2^{b}$	$14{\cdot}6^{b}$		NS			NS		NS		$9{\cdot}9^{a}$	$10{\cdot}6^{b}$	$11{\cdot}4^{c}$	$11{\cdot}8^{c,d}$
Non-protein N × 6·38	$2{\cdot}90^{a}$	$2{\cdot}59^{b}$	$2{\cdot}87^{a}$		NS			NS	$2{\cdot}87^{a}$	$2{\cdot}69^{b}$	$2{\cdot}82^{a}$			NS	
Casein, % of total protein	$80{\cdot}1^{a}$	$80{\cdot}4^{b}$	$80{\cdot}1^{a}$		NS			NS		NS		$81{\cdot}6^{a}$	$80{\cdot}7^{b}$	$79{\cdot}5^{c}$	$79{\cdot}0^{c,d}$
Total Ca	$1{\cdot}96^{a}$	$2{\cdot}19^{b}$	$2{\cdot}10^{c}$		NS			NS		NS				NS	
Diffusible Ca	$0{\cdot}41^{a}$	$0{\cdot}40^{a}$	$0{\cdot}37^{b}$	$0{\cdot}40^{a}$	$0{\cdot}40^{a}$	$0{\cdot}38^{b}$	$0{\cdot}40^{a}$	$0{\cdot}39^{b}$	$0{\cdot}38^{a}$	$0{\cdot}42^{b}$	$0{\cdot}38^{a}$	$0{\cdot}41^{a}$	$0{\cdot}39^{b}$	$0{\cdot}40^{a,b}$	$0{\cdot}37^{c}$
Diffusible Ca, % of total Ca	$20{\cdot}9^{a}$	$18{\cdot}3^{b}$	$17{\cdot}9^{b}$	$19{\cdot}2^{a}$	$19{\cdot}4^{a}$	$18{\cdot}5^{b}$		NS	$18{\cdot}7^{a}$	$20{\cdot}1^{b}$	$18{\cdot}3^{a}$	$19{\cdot}6^{a}$	$19{\cdot}0^{b}$	$19{\cdot}4^{a,b}$	$18{\cdot}2^{c}$
Colloidal Ca	$1{\cdot}54^{a}$	$1{\cdot}79^{b}$	$1{\cdot}71^{c}$		NS			NS		NS				NS	
Diffusible P_i	$0{\cdot}30^{a}$	$0{\cdot}24^{b}$	$0{\cdot}26^{c}$	$0{\cdot}26^{a}$	$0{\cdot}28^{b}$	$0{\cdot}25^{a}$		NS	$0{\cdot}29^{a}$	$0{\cdot}24^{b}$	$0{\cdot}26^{c}$			NS	
Diffusible P_i, % of total P_i	$31{\cdot}5^{a}$	$25{\cdot}1^{b}$	$26{\cdot}7^{c}$	$27{\cdot}1^{a}$	$28{\cdot}9^{b}$	$27{\cdot}4^{a,b}$		NS	$29{\cdot}5^{a}$	$26{\cdot}2^{b}$	$27{\cdot}7^{b}$			NS	
Colloidal P_i	$0{\cdot}64^{a}$	$0{\cdot}70^{b}$	$0{\cdot}69^{b}$		NS			NS		NS				NS	
Micelle mean diameter, nm	172^{a}	213^{b}	204^{c}		NS		193^{a}	200^{b}		NS				NS	
Micelle mineralization, mg/g	$41{\cdot}7^{a}$	$37{\cdot}5^{b}$	$36{\cdot}5^{c}$	$39{\cdot}2^{a}$	$38{\cdot}5^{b}$	$38{\cdot}1^{b}$		NS		NS				NS	

NS, no significant effect.
† Least-square means are given only when the effect was significant.
‡ The model and somatic cell counts are described in the text.
^{a,b,c,d} Values in the same line without a common superscript letter were significantly different: $P < 0{\cdot}05$.

Table 3. *Least-square means † for coagulation variables measured on individual milks that vary significantly with factors in the statistical Model 1 ‡*

Factor of variation	Rennet clotting time, min	Gel firming rate, mm min^{-1}	Gel firmness, mm
Days in milk			
48–55	18·8[a]	5·21[a]	53·7[a]
132–139	25·2[b]	5·20[a]	60·1[b]
174–192	26·8[c]	4·71[b]	59·4[b]
Divergent lines			
Low	24·9[a]	4·84[a]	
Medium	23·3[b]	4·99[b]	NS
High	22·7[b]	5·28[b]	
SCC subclass, cells/ml			
$< 10^5$	21·6[a]	5·41[a]	
$10^5 - 3 \times 10^5$	22·3[a]	5·18[a]	NS
$3 \times 10^5 - 5 \times 10^5$	24·2[b]	4·96[a, b]	
$> 5 \times 10^5$	26·4[c]	4·61[b]	
pH class			
< 6·55	16·3[a]	7·04[a]	
6·55–6·60	19·4[b]	5·96[b]	
6·60–6·65	20·4[b]	5·75[b]	
6·65–6·70	22·2[c]	4·97[c]	NS
6·70–6·75	25·2[d]	4·53[d]	
6·75–6·80	28·3[e]	3·7[e]	
> 6·80	33·5[f]	3·32[f]	

SCC, somatic cell counts; NS, no significant effect.
† Least-square means are given only when the effect was significant.
‡ The model is described in the text.
[a, b, c, d, e, f] Values in the same column without a common superscript letter were significantly different: $P < 0{\cdot}05$.

Table 4. *Phenotypic correlations between coagulation characteristics and physicochemical variables*

	Phenotypic correlations†		
	Rennet clotting time	Gel firming rate	Gel firmness
Rennet clotting time	1		
Gel firming rate	−0·50	1	
Gel firmness	0·28	0·56	1
Milk yield	−0·24	NS	−0·22
Fat	0·23	0·12	0·31
Total N	0·44	0·30	0·70
Protein	0·44	0·30	0·70
Casein	0·39	0·35	0·73
Soluble protein	0·41	NS	0·28
Casein as % of total	−0·10	0·30	0·32
Total Ca	0·14	0·24	0·30
Diffusible Ca	−0·18	0·24	NS
Diffusible Ca as % of total	−0·20	NS	−0·25
Colloidal Ca	0·18	0·32	0·42
Total P_i	0·31	NS	NS
Diffusible P_i	0·14	−0·21	NS
Diffusible P_i as % of total	NS	−0·18	−0·12
Colloidal P_i	0·22	NS	NS
Micelle mean diameter	0·28	−0·32	−0·13
Micelle mineralization	−0·23	NS	−0·31

NS, no significant correlation.
† Given only when the correlations were statistically different from zero.

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Table 5. *Analyses of variance for renneting properties according to different models including most relevant physicochemical variables*

Model†	Rennet clotting time, min		Gel firming rate, mm min^{-1}		Gel firmness, mm	
	r^2	STD error	r^2	STD error	r^2	STD error
Model 2	38·1	3·38	40·0	0·92	57·6	2·63
Model 3	68·0	2·59	61·1	0·77	58·7	2·59
Model 4 with X = protein	74·1	2·45	64·7	0·74	76·6	1·98
Model 4 with X = casein	72·9	2·51	66·4	0·72	77·7	1·97
Model 4 with X = total Ca	70·0	2·57	66·0	0·71	64·5	2·37
Model 4 with X = diffusible Ca	71·4	2·49	64·2	0·73	58·7	2·59
Model 4 with X = colloidal Ca	71·1	2·50	64·5	0·74	64·7	2·36
Model 4 with X = total Pi	71·1	2·60	62·4	0·76	58·7	2·60
Model 4 with X = micelle mean diameter	71·9	2·48	61·9	0·79	58·7	2·59

STD error, standard deviation of the residual error.
† The models are described in the text.

principally influenced by casein content and average micelle diameter. Finally, A_{2RCT} was highly correlated with protein, casein and colloidal calcium contents.

In order to quantify the influence of the main physicochemical characteristics on milk coagulating properties, independently of pH, analyses of variance including pH and the most significant physicochemical variables were performed. Values of r^2 and SD of error for the different models relating to rennet clotting time, gel firming rate and gel firmness are presented in Table 5. These results confirmed that pH was prominent in explaining the variations of rennet clotting time and gel firming rate. Whereas model 2, especially the days in milk factor, explained $\sim 40\,\%$ of the variation in these properties, the introduction of pH into the equations (models 3 and 4) increased the r^2 values and decreased the error. On the other hand, the introduction into the model of other physicochemical factors such as protein or casein contents as covariate led to a limited enhancement of the model's efficiency. The main factor responsible for gel firmness variation was casein content: the r^2 was increased markedly over that for model 2 by the introduction of casein content as a covariate (model 4 *v.* models 2 and 3).

DISCUSSION

Effects of genetic and non-genetic factors of variation on physicochemical and coagulation variables

The mean values obtained for milk composition and physicochemical characteristics such as nitrogen fractions, mineral equilibria and micelle properties were consistent with previous results (Assenat, 1985; Pellegrini *et al.* 1994). The average SCC level reached $2{\cdot}2 \times 10^5$ cells/ml, a lower value than usually encountered for flocks in that region. Delacroix-Buchet *et al.* (1994) obtained a shorter mean rennet clotting time, 12·5 min, and a faster gel firming rate, 8·33 mm min^{-1}. A lower average milk pH value, 6·55, and a higher operating temperature, 32 °C, might explain the observed differences with our results. The average gel strength was similar in the two studies.

Days in milk had a strong influence on almost all milk physicochemical characteristics and coagulation properties. These results were in good agreement with previous results on bulk milks (Pellegrini *et al.* 1994), and individual milks (Delacroix-Buchet *et al.* 1994).

The change in milk composition with SCC was in accord with other studies carried

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out on ovine (Duranti & Casoli, 1991; Manfredini *et al.* 1992) and bovine milk (Schultz, 1977; Kitchen, 1981; Munro *et al.* 1984). Our results indicated that the content of diffusible Ca was significantly lower in high cell count milk, which was in agreement with Tallamy & Randolph (1970) for bovine milk. This might be related to higher pH values for milk with high SCC.

The influence of SCC on renneting properties was consistent with observations in cows' milk: an increase in SCC led to a decrease in milk coagulability. We observed a threshold value of $\sim 5 \times 10^5$ cells/ml above which *RCT* and *GFR* were significantly modified, in agreement with the studies of Politis & Ng-Kwai-Hang (1988) who proposed a similar threshold value for coagulability of bovine milk. However, the number of milks we analysed and the range of SCC were probably not sufficient to establish definitely a SCC threshold for renneting properties.

As expected from the selection criteria, divergent lines influenced the milk yield level significantly but had little effect on milk composition. The effect of divergent lines on salt equilibria might reflect different concentrations of citrate, not measured in the present work, with which calcium is mainly associated (Holt, 1985).

Finally our results allowed us to estimate the individual repeatability (which is the upper limit of heritability) for each variable measured. For a given variable, this value is estimated from several measurements recorded for the same ewe, at different days in milk. The repeatabilities for milk yield, milk fat and milk protein were in the range of published values if we take into account that the present recording interval was higher than the usual monthly test (Barillet, 1985). New information was obtained on several variables without published repeatability, such as micellar characteristics or salts equilibria, which were highly repeatable. The repeatability of *RCT* was lower than that reported by Delacroix-Buchet *et al.* (1994). On the other hand our repeatability coefficients for gel firming rate and gel firmness were close to those obtained by this group.

Relationships between physicochemical characteristics of milks and their rennetability

The strong influence of pH on clotting time and gel firming rate was in agreement with previous results in ewes' milk (Duranti & Casoli, 1991; Manfredini *et al.* 1992; Delacroix-Buchet *et al.* 1994). The lack of influence of pH on gel firmness is in accord with Delacroix-Buchet *et al.* (1994).

The positive correlation between protein content and *RCT* was also in agreement with other studies on ewes' milk (Duranti & Casoli, 1991; Manfredini *et al.* 1992; Delacroix-Buchet *et al.* 1994), and correlations of gel firming rate and gel firmness with casein content were in agreement with the results of many studies carried out on bovine (Garnot *et al.* 1982; Storry & Ford, 1982; Storry *et al.* 1983) and caprine milks (Remeuf *et al.* 1989).

The correlation of total Ca content with gel firming rate and coagulum firmness is consistent with the functional role assigned to Ca in the rennet coagulation mechanism (Zittle, 1970; Dalgleish, 1982). The negative correlation between *RCT* and % diffusible Ca indicates that highly diffusible Ca increased the rate of micelle aggregation and reduced rennet clotting time, as suggested by Van Hooydonk *et al.* (1986). Furthermore, the influence of P_i content on *RCT* might be explained through complexing or release of Ca^{2+} (Shalabi & Fox, 1982).

The negative correlation between gel firming rate and average micelle diameter was in agreement with the results of Niki & Arima (1984) on bovine milk, suggesting that smaller micelles increase gel firming rate. The negative correlation between micelle mineralization and gel firmness is more surprising as the opposite is usually

expected. Our hypothesis is that this correlation was probably indirect: casein content varied much more than colloidal Ca which caused variations of colloidal Ca:casein inversely to casein, and thus inversely to gel firmness.

The effects of genetic and non-genetic breeding factors on rennet coagulation properties of individual milks might indirectly reflect the influence of these factors on milk composition. Thus, days in milk had a marked influence on milk casein content, which could be related to concomitant variations in gel firmness. The increase of *RCT* with days in milk, adjusted for the pH effect, might be explained by the increase in protein content and/or the decrease in % diffusible Ca. As far as gel firming rate is concerned, its decrease with lactation stage might be related to the increase in micelle diameter, and to the reduction in % diffusible Ca.

The effect of divergent lines on rennet clotting time, which tended to be longer when the milk yield merit was lower, might be related to salt equilibria since the main difference between milks from each divergent line concerned salts constituents and their distribution. However, our results did not permit clear identification of the variables involved.

Lastly, the study of the SCC effect on rennet coagulation properties showed that *RCT* was affected by SCC independently of pH. Changes in milk composition that are associated with an increase in SCC, more particularly the increase in soluble protein content and the decrease in % diffusible Ca, might be responsible for these changes. Similarly, the gel firming rate of milks with an SCC $> 5 \times 10^5$ cells/ml was significantly lower than that of other milks. Thus, over this threshold value, the milk composition variables influencing the rennetability appeared to be affected so that the coagulability was significantly lowered.

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