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Original article

β-Lactoglobulin polymorphism in ovine breeds: influence on cheesemaking properties and milk composition

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Summary — Milk samples from 340 Merino and 234 Lacha sheep were analyzed by isoelectric focusing in thin gels with carrier ampholytes. Five different β-lactoglobulin (β-Lg) phenotypes (A, B, AB, AC and BC) were observed in milk from the Merino breed, but only three phenotypes were found in milk from the Lacha breed (A, B and AB). Differences in gene frequencies between flocks were observed. The gene frequencies were A: 0.58, B: 0.41, C: 0.01 in milk from the Merino breed and A: 0.47, B: 0.53 in milk from the Lacha breed. Rheological parameters, coagulation time (r), rate of firming (K_{20}) and curd firmness (A_{max}) were determined, and chemical parameters (protein, lactose, fat and total solid content) were studied for the Merino breed; the pH was measured in all samples. No relationship was found between ß-Lg polymorphism and renneting properties in milk from the Merino breed. Protein content and pH were positively correlated with r.

β-lactoglobulin / ovine milk / genetic polymorphism / coagulation

Résumé — Polymorphisme de la ß-lactoglobuline de brebis. Influence sur les paramètres de fabrication du fromage et de la composition du lait. On a analysé des échantillons du lait de 340 brebis Merino et 234 brebis Lacha par focalisation isoélectrique sur gels minces avec ampholytes porteurs. Cinq phénotypes différents (A, B, AB, AC et BC) de β-lactoglobuline (β-Lg) ont été observés dans le lait de la race Merino, et seulement trois dans le lait de la Lacha (A, B et AB). On a constaté des différences dans les fréquences des gènes entre les troupeaux : A : 0,58 ; B : 0,41 ; C : 0,01 pour le lait provenant de la race Merino, et A : 0,47 ; B : 0,53 pour celui de la race Lacha. Pour la race Merino, on a determiné les paramètres rhéologiques: temps de gélification (r), vitesse de raffermissement (K_{20}) et fermeté des gels (A_{max}), et les paramètres chimiques tels que protéine, lactose, matière grasse et teneur en solides totaux. Le pH a été mesuré dans tous les échantillons. On n'a pas trouvé de rapport reliant le polymorphisme de la ß-Lg et les propriétés de coagulation dans le lait de la race Merino. La teneur en protéine et le pH sont corrélés positivement avec r.

β-lactoglobuline / lait de brebis / polymorphisme génétique / coagulation

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INTRODUCTION

Since the initial discovery of the genetic polymorphism of β-lactoglobulin (β-Lg) by Aschaffenburg and Drewry (1955), the genetic polymorphism of milk proteins has been extensively studied in cow's milk. Recently, Grosclaude (1995) summarized the main established facts about the relationship between genetic variants and technological properties; in cattle, the polymorphism of β-Lg has a major effect on the β-Lg content (β-Lg A > B) and an opposite effect on casein number. For cheese production, milk with κ-casein (κ-CN) B is better than milk with κ-CN A. In the goat the polymorphism of α_{S1}-casein (α_{S1}CN) has a strong effect on casein content of milk, α_{S1}CN A > E > F, on the total amount of protein per lactation, and on cheesemaking properties.

Research on the polymorphism of ewe's milk is not yet as extensive as it is on cow's milk. Nevertheless, during recent years considerable advances have been made. Reports on new variants of α_{S2}-casein (Chianese et al, 1993) and the primary structure of A, C and D α_{S1}-casein (Ferranti et al, 1995) have recently been published. In addition, variants A and B are the most common genetic variants of β-Lg. Erhardt (1989) and Erhardt et al (1989) isolated and sequenced a less common type of β-Lg called the C variant. There are several studies concerning the β-Lg genotypes and gene frequencies in different breeds, β-Lg A being more common than β-Lg B (Ng-Kwai-Hang and Grosclaude, 1992). β-Lg C has been found only in the Tajik and Merinoland breeds. In Spanish autochthonous ovine breeds, only the Manchega and Segureña have been studied (López-Gálvez et al, 1994).

There is little information about the relationship between genetic variants and technological properties in ewe's milk, although it is widely used in cheesemaking. Some preliminary studies made on Manchega sheep's milk (López-Gálvez et al, 1993; Martínez-Hens et al, 1993; Pirisi et al, 1995) have shown that genetic polymorphism has an influence on renneting properties and on cheese yield of ewe's milk.

Some of the most particular cheeses in Spain, such as Serena and Torta del Casar, are prepared using vegetal rennet and milk from the Merino breed. Roncal cheese is manufactured with milk from the Lacha breed.

The aim of this paper is to describe the genetic polymorphism of the β-Lg fraction in two Spanish ovine breeds (Merino and Lacha), and to study the implications of the genetic polymorphism of β-Lg on renneting properties.

MATERIALS AND METHODS

Milk samples

Three hundred and forty individual milk samples from Merino sheep (two flocks) and 234 from Lacha sheep (five different flocks) were analyzed. All samples were obtained during the middle of the lactation period. The whey protein fraction was obtained by acidification at pH 4.6 with acetate buffer.

Isoelectric focusing of whey proteins

Polyacrylamide gel isoelectric focusing (PAGIF) was carried out on commercially available plates (Ampholine PAG plates, pH 3.5–9.5, Pharmacia-LKB, Uppsala, Sweden) according to the method of López-Gálvez et al (1994) used to separate β-Lg A and B variants in the ovine Manchega breed. Commercial PAGIF plates were stained with Coomassie blue R-250 (Winter et al, 1977). After PAGIF, whey proteins were transferred to a nitrocellulose membrane according to the procedure of Molina et al (1996). Immunodetection of the bands corresponding to β-Lg was carried out using commercial polyclonal antiserum against bovine β-Lg.

Renneting properties and chemical parameters

Renneting properties of 215 individual milk samples from the Merino breed were measured using Formagraph apparatus (A/SN Föss-Electric, Hellerup, Denmark). A rennet solution was obtained by dissolving 125 mg of rennet containing 85% chymosin and 15% bovine pepsin (CHR Hansen, Copenhagen, Denmark) in 100 mL of 0.2 mol/L acetate buffer. The sample (10 mL) was heated at 35 °C for 30 min, after which 0.2 mL of rennet solution was added. Rheological parameters such as coagulation time (r), rate of firming (K_{20}) and curd firmness (A_{max}) were obtained.

Other chemical parameters, ie, fat, lactose, protein and total solid content were measured using a Milko-Scan 203 apparatus (A/SN Föss-Electric, Hellerup, Denmark) for the Merino breed samples.

Statistical analysis

The allele frequencies in the β-Lg fraction were estimated by direct counting of the phenotypes. Only the predominant band of each allele was considered for phenotyping. To test differences between observed and expected frequencies, a chi-square analysis was performed on the basis of the Hardy–Weinberg law.

A one-way analysis of variance was applied to test the differences between the means of the rheological and chemical parameters for the different phenotypes of β-Lg. The statistical analyses were carried out using Statgraphics program v.5.0 (1989) (Graphic Software Systems, Rockville, MD, USA).

RESULTS AND DISCUSSION

Genetic polymorphism of the β-Lg fraction in the Lacha and Merino breeds

Figure 1 shows the isoelectric focusing pattern of some whey samples containing β-Lg A, B and C; β-Lg C presents a lower isoelectric point (pI = 5.44) than β-Lg A (pI = 5.87). This result agrees with the data on primary sequencing of a new ovine β-Lg C described by Erhardt (1989). This β-Lg C presents a single exchange, Arg → Gln at position 148, which causes the lower pI observed. Bands corresponding to β-Lg were identified by western blotting using anti β-Lg antisera (data not shown).

The isoelectric focusing pattern of the whey proteins frequently shows additional fainter bands. For instance, in lane 4, there is a strong band of β-Lg A and a faint band in the region of β-Lg B. In lane 2, the opposite effect is observed: a fainter band in the region of β-Lg A, this being a strong band in the region of β-Lg B. In cow's milk, a similar phenomenon has been observed. This fact could be attributed to the higher expression of one of the alleles of β-Lg in het-

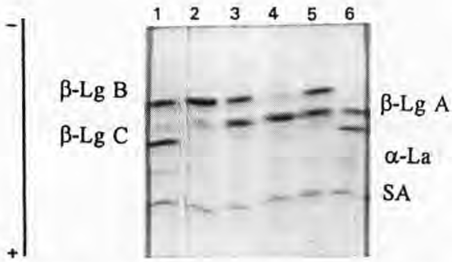

Fig 1. Isoelectric focusing pattern carried out on commercially procured plates polyacrylamide gel (pH = 3.5–9.5) of some whey samples of Merino breed sheep. Lane 1, whey sample containing β-Lg BC; lane 2, whey sample containing β-Lg B; lanes 3 and 5, whey samples containing β-Lg AB; lane 4, whey sample containing β-Lg A; lane 6, whey sample containing β-Lg AC. β-Lg A: β-lactoglobulin A; β-Lg B: β-lactoglobulin B; β-Lg C: β-lactoglobulin C; SA: serum albumin.

Isoélectrophorégramme sur gel de polyacrylamide commercial (pH = 3,5–9,5) de protéines solubles de laits individuels de brebis Merino. Ligne 1 : échantillon contenant β-Lg BC ; ligne 2 : échantillon contenant β-Lg B ; lignes 3 et 5 ; échantillon contenant β-Lg AB ; ligne 4 : échantillon contenant β Lg A ; ligne 6 : échantillon contenant β-Lg AC. β-Lg A : β-lactoglobuline A ; β-Lg B : β-lactoglobuline B ; β-Lg C : β-lactoglobuline C ; SA : sérum albumine.

erozygous form (Braunschweig et al, 1995; Frutos et al, 1996). However, the origin of these bands could not only be due to a different expression of β-Lg, but other proteins could also migrate in the same region under these conditions.

The results show that polymorphism exists for the β-Lg fraction in the milk of the two breeds considered. In the Lacha breed, two genetic variants (A and B) and three phenotypes of β-Lg (AA, BB and AB) could be demonstrated. β-Lg from the Merino breed is more heterogeneous than β-Lg from other Spanish breeds such as Manchega or Segureña (López-Gálvez et al, 1994) so three genetic variants were found: A, B and C; but only five phenotypes were observed by combining two alleles: AA, BB, AB, AC and BC (phenotype CC was not found). The β-Lg C found in milk from the Spanish Merino breed could be similar to the β-Lg C found by Erhardt (1989) in milk from Merinoland and Hungarian Merino breeds, which contain blood from the Spanish Merino.

Table I shows the distribution of genotypes and gene frequencies at the β-Lg locus obtained by PAGIF in the Merino and Lacha breeds respectively. In both Merino breed flocks, AB is the most common β-Lg phenotype. The gene frequency of allele β-Lg A (0.58) was the highest value. The lowest frequencies correspond to the genotypes carrying the allele C of β-Lg. Thus, out of the 340 samples of the Merino breed, only four showed the genotype AC and five the genotype BC. The gene frequency of β-Lg C was only 0.01. In other breeds, such as the Merinoland, the gene frequency of β-Lg C was 0.175 (Erhardt, 1989). When the expected frequencies were calculated on the basis of the Hardy–Weinberg law, good agreement was found between the observed and the expected frequencies.

Similarly, AB is the common β-Lg phenotype in milk from the Lacha breed. The results for the total population show good agreement with the Hardy–Weinberg law, meaning that this population approximates genetic equilibrium. When we compared the observed and expected frequencies within each flock, we obtained chi-square values which were lower than the critical value, except for one of the flocks studied. This flock showed the highest value of phenotype BB (0.68).

Table I. Distribution of β-Lg genotypes and gene frequencies in different sheep flocks of the Merino and Lacha breeds.

Répartition des génotypes de β-Lg et fréquences de gènes de différents troupeaux de brebis des races Merino et Lacha.

Breed	n		*Genotypic frequencies*					*Gene frequencies*			
			AA	*BB*	*AB*	*AC*	*BC*	χ^2	*A*	*B*	*C*
Merino	340	Obs	116	59	156	4	5	1.06	0.58	0.41	0.01
		Exp	114.38	57.15	161.7	5.13	3.62	df = 4			
Lacha	234	Obs	55	68	111	–	–	0.48	0.47	0.53	
		Exp	51.69	68.26	116.58			df = 2			

n: number of samples; χ^2: chi-square value; df: degrees of freedom; obs: observed frequencies; exp: expected frequencies on the basis of the Hardy–Weinberg law.

n : *nombre d'échantillons ;* χ^2 *: valeur de chi carré ; df : degrés de liberté ; obs : fréquences observées ; exp : fréquences attendues selon les lois de Hardy–Weinberg.*

The comparison of gene frequencies in both breeds shows that the frequencies for β-Lg A are higher than those for β-Lg B in milk from the Merino breed. Similar results have been obtained in milk from the Manchega breed (López-Gálvez et al, 1994) and in the majority of ovine breeds studied, β-Lg A is the most common allele (Ng-Kwai-Hang and Grosclaude, 1992). Nevertheless, in Lacha sheep β-Lg B is the most common allele, as in Rhon sheep (Erhardt, 1989).

Relationship between β-Lg polymorphism and technological properties in milk from the Merino breed

Table II shows the mean values and standard deviations of the rheological and chemical parameters studied in Merino breed samples. To avoid the effect of different frequencies of the genetic variants found, a one-way analysis of variance was performed using only the phenotypes A, B and AB. No statistical differences ($P < 0.05$) were found on the studied renneting properties (r, K_{20}, A_{max}) with the genetic variants of β-Lg. Similarly, no statistical differences related to the genetic polymorphism of β-Lg were found in the composition of Merino milks. Nevertheless, López-Gálvez et al (1993) found a better rate of curd firming in Manchega ewe's milk containing β-Lg AA, and Martínez-Hens et al (1993) found differences between the values of total protein and casein content in milks with a different β-Lg genetic variant. They found that the BB homozygous form presented the lowest protein and casein content values.

Table II. Milk composition and renneting properties in milks from the Merino breed with different β-Lg variants.

Composition du lait et paramètres rhéologiques de laits individuels de brebis Merino avec des phénotypes différents de β-Lg.

		β-Lg Genotype									
		AA n = *67*		*AB* n = *100*		*BB* n = *41*		*AC* n = *3*		*BC* n = *4*	
		Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Fat	(%)	6.15	1.58	6.24	1.39	6.03	1.63	4.21	1.06	5.28	1.86
Protein	(%)	5.75	0.80	5.74	0.69	5.73	0.83	5.46	0.31	6.10	0.60
Lactose	(%)	5.03	0.39	5.05	0.46	5.17	0.37	4.94	0.42	4.74	0.52
TS	(%)	17.4	1.57	17.5	1.33	17.4	1.50	15.3	0.96	16.5	1.57
pH		6.65	0.15	6.65	0.14	6.63	0.14	6.65	0.12	6.77	0.07
r	(min)	15.8	5.65	15.4	6.53	16.1	6.79	14.7	3.39	15.9	3.64
K_{20}	(min)	2.7	0.77	2.6	0.87	2.7	1.01	4.1	1.42	3.2	0.75
A_{max}	(mm)	59.0	7.81	59.4	7.30	58.3	7.66	61.7	5.51	58.9	4.62

n: number of samples; mean: mean value; SD: standard deviation; TS: total solids; r: coagulation time; K_{20}: rate of firming; A_{max}: curd firmness.

n : *nombre d'échantillons ; mean : valeur moyenne ; SD : déviation standard ; TS : solides totaux ;* r : *temps de gélification ;* K_{20}, *temps de raffermissement ;* A_{max} *: fermeté des gels.*

These results are similar to those found in cow's milk, where the effect of genetic polymorphism of β-Lg on renneting properties is controversial. In many cases, results from different studies are not comparable for various reasons including population size, breed of the animals, or frequency of the genetic variants under consideration (Ng-Kwai-Hang, 1995). Genetic polymorphism of other proteins directly involved in the coagulation process, like κ-CN, are also related to renneting properties in cow's milk, although the mechanism involved is still not clear (Jakob, 1994). Probably the same process takes place in ovine milk, and it is important to study the genetic polymorphism of the casein and whey fraction together.

Although some authors have tried to find a relationship between pH and protein polymorphism, no statistical differences were found in pH values with the β-Lg phenotypes in any breed. Nevertheless, when the correlation matrix (data not shown) is established between the renneting properties and the physicochemical parameters studied, pH and protein content are positively correlated with coagulation time (0.72 and 0.53 respectively, $P < 0.01$) (fig 2). Analogously, pH is also correlated with K_{20} (0.57; $P < 0.01$). Similar results have been obtained by Delacroix-Buchet et al (1994), who showed a positive correlation between pH and protein content with coagulation time (0.70 and 0.41, respectively) in milk from the Lacaune ovine breed. pH is one of the main factors which affects the renneting properties. As occurs in cow's milk, pH was found to be related to protein content (correlation coefficient, 0.42).

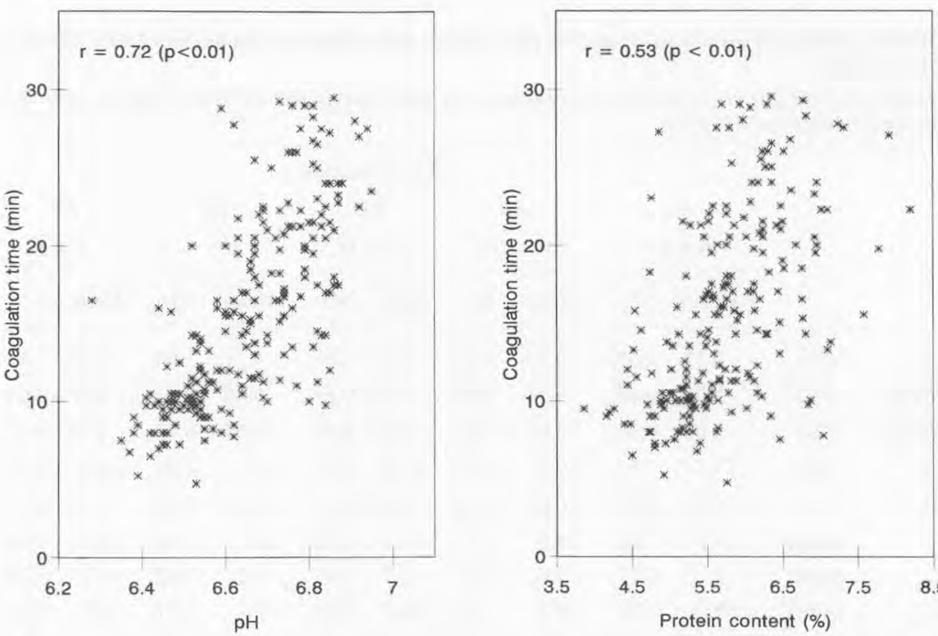

Fig 2. Coagulation time versus pH and protein content in milk from Merino breed sheep. *r*: correlation coefficient; *P*: significance level.

Temps de gélification en fonction du pH et taux protéique du lait de brebis Merino ; r *: coefficient de corrélation ;* P *: niveau de signification.*

Based on these results, the effect of the genetic polymorphism of β-Lg on renneting properties of milk is still under discussion, and it is probable that the renneting properties depend more on the concomitant changes in milk composition and pH.

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