

Genetic Polymorphism of Milk Proteins in Hungarian Spotted and Hungarian Grey Cattle: A Possible New Genetic Variant of β -Lactoglobulin

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ABSTRACT

Genetic variants of α_{s1} -, β -, and κ -caseins and of β -lactoglobulin were studied with PAGE and isoelectric focusing in milk samples of 101 Hungarian Spotted and 120 Hungarian Grey cows. Gene frequencies and genotypic frequencies were estimated. Significant differences in gene frequencies between the two breeds were observed at the κ -casein and β -lactoglobulin locus. The variants β -casein C, κ -casein C, and β -lactoglobulin D were not found in Hungarian Grey cows. On immobilized pH gradient, isoelectric focusing gels containing carrier ampholytes, a new unidentified protein band appeared near β -lactoglobulin B in about 10% of the milk samples of Hungarian Grey cattle.

(Key words: milk protein, genetic polymorphism, Hungarian cattle)

Abbreviation key: CA-IEF = isoelectric focusing in the presence of carrier ampholytes, CA-IPG-IEF = isoelectric focusing in immobilized pH gradients in the presence of carrier ampholytes, CN = casein (used with α_{s1} -, α_{s2} -, β -, and κ -), HG = Hungarian Grey, HS = Hungarian Spotted, LG = lactoglobulin (used with β -).

INTRODUCTION

The six major milk protein fractions exist in different allelic forms: α_{s1} -casein (CN) (A, B, C, and D), α_{s2} -casein (A, B, C, and D), β -casein (A¹, A², A³, B, C, D, and E), κ -casein (A, B, C, and E), α -lactalbumin (A, B, and C), and β -lactoglobulin (LG) (A, B, C, D, E, F, G, H, and W). These fractions are controlled by codominant autosomal genes according to the Mendelian laws of inheritance. The various genetic variants differ from each other by only a few amino acid substitutions (3). The occurrence of genetic variants varies among different breeds of dairy cattle (19). Some studies indicate that the genetic variants and the physical and chemical properties of the milk are correlated [for review see (6)]. Breeding for yields of milk, protein, and fat may have altered gene frequencies. Therefore, it is important to study breeds that have undergone little selection or breeds with a small population.

The Hungarian Grey (HG) and the Hungarian Spotted (HS) are the two most characteristic Hungarian breeds. The HG breed originated directly from wild cattle (*Bos primigenius*) and was a famous beef breed in the Middle Ages; until the end of the 19th century, it was the dominant breed of cattle in Hungary. Although threatened by extinction, the HG breed now plays an important role as a genetic resource and as a tourist attraction (1, 2). To our knowledge, the milk protein polymorphisms of this breed have never been examined.

The HS breed arose at the end of the 19th century from HG crossbred with Simmental

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cattle. The HS was developed as a dual purpose breed, providing milk and meat, and, although milk yield has been improved in this breed, its importance as a dairy animal is continually decreasing because of the higher yielding Holstein-Friesian.

The objectives of this study were twofold: 1) to evaluate milk protein genetic polymorphisms in the HG and HS breeds of cattle and 2) to compare the genetic polymorphism in milk proteins of a representative HS population with the HG and Simmental breeds from which it originated.

MATERIALS AND METHODS

Milk samples from 120 HG and 101 HS cows were collected without preservation. Because HG cows are kept wild, they are not used to being handled; thus, milking is very difficult. The HG milk samples were taken from cows of one herd during a roundup. The 101 HS milk samples were randomly selected from a normal commercial dairy herd representative for this breed. The samples were stored at 4°C on the day of sampling and overnight.

Milk protein phenotyping was carried out with three different methods. The HS milk samples were investigated with PAGE and isoelectric focusing in the presence of carrier ampholytes (CA-IEF) and the HG milk samples with CA-IEF and IEF in immobilized pH gradients in the presence of carrier ampholytes (CA-IPG-IEF). None of these methods is capable of resolving all possible milk protein genetic variants in one single run. Polyacrylamide gel electrophoresis was used as a standard system in the Hungarian laboratory. The disadvantage of this method is that it fails to separate the different β -CN A genetic variants. To separate β -CN A³, A², A¹, and κ -CN A from E and C from B, CA-IEF was applied. The CA-IPG-IEF was used for high resolution separation of the genetic variants of β -LG (B from W and C from D) and the characterization of the possible new variant. With this method, only β -LG variants can be separated, whereas the α -lactalbumin and casein variants precipitate at the application point.

The PAGE of casein and whey protein fractions was carried out on 16-cm \times 18-cm \times 1-mm polyacrylamide gels with 17 wells according to the method described by Medrano and Sharrow (13). Alkaline gel electrophoresis

to type casein variants was performed with a nondissociating, discontinuous buffer system: 3.5% (stacking) and 8% (running) polyacrylamide gel containing 4 M urea (Sigma Chemical Co, St. Louis, MO). Whey proteins were typed using a nondissociating, continuous buffer system (14% polyacrylamide gel). Gels were stained with Coomassie brilliant blue R-250 (Serva, Heidelberg, Germany).

For CA-IEF, 124- \times 258- \times .25-mm polyacrylamide gels were prepared according to the method developed by Krause et al. (9). The polymerization solution was made of 9.4 ml of gel stock solution [4.85% (wt/vol) acrylamide (Serva), .15% (wt/vol) NN'-methylene-bisacrylamide (Pharmacia LKB, Bromma, Sweden), 48.05% (wt/vol) urea (Sigma), 15% (wt/vol) glycerol (Sigma)], and .6 ml of the following mixture of carrier ampholytes: 16.6% (vol/vol) Ampholine, pH 2.5 to 4.5 (Pharmacia LKB); 13.3% (vol/vol) Pharmalyte, pH 4.2 to 4.9 (Pharmacia LKB); 11.6% (vol/vol) Pharmalyte, pH 4.5 to 5 (Pharmacia LKB); 11.6% (vol/vol) Pharmalyte, pH 5 to 5.5 (Pharmacia LKB); 25% (vol/vol) Serva-

TABLE 1. Gene frequencies of Hungarian Spotted (HS) and Hungarian Grey (HG) cattle.

	HS		HG		P ¹
	\bar{X}	SD	\bar{X}	SD	
α_{s1} -Casein					
A	
B	.89	.02	.82	.02	NS
C	.11	.02	.18	.02	NS
β -Casein					
A ³	
A ²	.72	.03	.75	.03	NS
A ¹	.21	.03	.23	.03	NS
B	.06	.02	.02	.01	NS
C	.01	.01	NS
κ -Casein					
A	.75	.03	.64	.03	NS
B	.22	.03	.36	.03	P < .05
C	.03	.01	NS
E
β -Lactoglobulin					
A	.47	.03	.20	.03	P < .0001
B	.505	.03	.75	.03	P < .001
D	.025	.03	NS
X	... ²05	.01	...

¹Significance determined by chi-square test.

²Not determined.

lyte, pH 5 to 6 (Serva); and 25% (vol/vol) Ampholine, pH 5 to 8 (Pharmacia LKB). Prefocusing was for 25 min at 4 W (maximum 2000 V and 15 mA); sample focusing was for 60 min at 4 W (maximum 2000 V and 15 mA); and focusing was for 120 min at 5 mA (maximum 2500 V and 20 W). The gels were stained with Coomassie brilliant blue G-250 as described by Krause et al. (9).

The CA-IPG-IEF (9) was carried out in 5% T, 3% C gels (124 × 258 × .5 mm) at pH 5.1

to 5.6 in the presence of 1% (vol/vol) Servalyte, pH 5 to 6 (Serva), according to the method of Krause et al. (9). Sample focusing was for 30 min at 200 V (maximum 1.6 mA and .5 W); focusing was for 60 min at 3000 V (maximum 5 mA and 15 W) and 150 min at 5000 V (maximum 2.5 mA and 15 W). The gels were stained with Coomassie brilliant blue G-250 (Serva).

For PAGE, whole milk samples were defatted by centrifugation at 800 × g at 4°C for 10

TABLE 2. Genotype frequencies of Hungarian Spotted (HS) and Hungarian Grey (HG) cattle.

	HS				HG				P ^{3,4}
	F ¹		E ²		F		E		
	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	\bar{X}	SD	
	(%)								
α_{s1} -Casein									
BB	78	3	79	3	66	3	68	3	NS
CC	1	1	1	1	1	1	3	1	NS
BC	21	3	20	3	34	3	29	3	NS
β -Casein									
A ² A ²	52	3	52	3	55	3	56	3	NS
A ¹ A ¹	4	1	4	1	4	1	6	1	NS
BB	...		<1		...		<1		...
CC	...		<1	
A ² A ¹	29	3	30	3	38	3	35	3	NS
A ² B	9	2	9	2	3	1	3	1	NS
A ² C	1	1	2	1		NS
A ¹ B	3	1	3	1	1	1	1	1	NS
A ¹ C	2	1	1	1		NS
BC	...		<1	
κ -Casein									
AA	54	3	56	3	36	3	41	3	P < .025
BB	4	1	5	1	8	2	13	2	NS
CC	...		<1	
AB	35	3	33	3	56	3	46	3	P < .01
AC	6	2	5	1		P < .025
BC	1	1	2	1		NS
β -Lactoglobulin									
AA	22	3	22	3	5	1	4	1	P < .001
BB	27	3	26	3	56	3	56	3	P < .0001
DD	...		<1	
AB	47	3	47	3	28	3	29	3	P < .01
AD	4	1	2	1		NS
BD	1	1	2	1		NS
XX	... ⁵			<1		...
AX	... ⁵		...		1	1	2	1	...
BX	... ⁵		...		10	2	8	2	...

¹F = Found.

²E = Expected, calculated according to the Hardy-Weinberg law, using estimated allele frequencies.

³Significance determined by chi-square test.

⁴Differences in genotype frequencies of HS and HG were tested.

⁵Not determined.

min. The caseins were separated from whey proteins by isoelectric precipitation (14). The casein and whey protein fractions were stored at -20°C after lyophilization, and samples (3 to 5 μl ; 25 to 40 μg) were loaded. For isoelectric focusing, defatted milk samples were lyophilized and stored at -20°C , and samples (9 to 12 μl ; 50 to 100 μg) were loaded.

The genetic variants of individual caseins and whey proteins were identified by comparison with known genetic variants. Milk samples, casein, and whey protein fractions from individual cows were used as reference samples, which were kindly provided by F. Grosclaude, Institut National de la Recherche Agronomique, Jouy-en-Josas, France, and E.R.B. Graham, South Australian Department of Agriculture, Adelaide, South Australia, Australia.

Direct counting was used to estimate gene frequencies and genotypic frequencies of the milk proteins; to test differences between the two breeds examined, a chi-square analysis was performed.

RESULTS AND DISCUSSION

Properties of milk are influenced by its detailed composition. Thus, any environmental or genetic factors affecting milk composition would also be expected to affect processing properties of milk (12, 15, 18). Three different gel electrophoretic methods were used to phenotype 101 HS and 120 HG cattle for the genetic variants of α_{s1} -, β -, and κ -CN and β -LG.

Table 1 shows the gene frequencies, and Table 2 shows the genotypic frequencies, of

TABLE 3. Gene frequencies in Simmental breed from the literature and as determined by various methods.

	Method and reference				
	SGUE ¹ (8)	PAGE (4)	CAGE ² (5)	PAGE (11)	IEF ³ (17)
α_{s1} -Casein					
A	... ⁴	... ⁴	... ⁴	... ⁵	... ⁴
B	.940	.921	.916	... ⁵	.918
C	.060	.079	.084	... ⁵	.082
β -Casein					
A ¹	... ⁵	.225	... ⁵	... ⁵	.231
A ²	... ⁵	.655	... ⁵	... ⁵	.673
A ³	... ⁵	... ⁴	... ⁵	... ⁵	.001
A	.705	.880	.896	.893	.905
B	.060	.105	.066	.088	.082
C	.235	.015	.038	.021	.013
κ -Casein					
A	... ⁵	.588	.697	.695	.754
B	... ⁵	.412	.303	.305	.225
C	... ⁵	... ⁵	... ⁵	... ⁵	.020
E	... ⁵	... ⁵	... ⁵	... ⁵	.001
β -Lactoglobulin					
A	... ⁵	.450	.478	... ⁵	.498
B	... ⁵	.550	.514	... ⁵	.485
D	... ⁵	... ⁴	.008	... ⁵	.017
Cows tested, no.	242	420 ⁶ 210 ⁷	2262	1557	2626

¹Starch gel urea electrophoresis.

²Cellulose acetate gel electrophoresis.

³Isoelectric focusing.

⁴Not determined or not observed.

⁵Not determined.

⁶Number of cows tested for casein variants.

⁷Number of cows tested for β -lactoglobulin variants.

the different casein and whey protein variants in the breeds of cattle studied.

In the α_{s1} -casein system, the variants B and C were found, and the allelic frequencies were similar to those observed in most western breeds (6). Variant α_{s1} -CN A, which is present in the Holstein breed (16), was absent in HS and HG breeds. The α_{s1} -CN, B occurs 89 and 82% in HS and HG, respectively.

Among the seven known genetic variants of β -CN, A¹ and A² are the most frequent in common breeds. In both breeds examined, the A¹ and A² alleles predominated. The frequencies of β -CN A¹ and A² are 21 and 72% in HS and 23 and 75% in HG breeds, respectively. The frequency of the β -CN B allele was very low, approximately 6% in HS and 2% in HG cows. The β -CN C allele, which is present at low frequencies in several continental breeds, was only found in HS cows (1%). Dairy producers might have conceivably selected indirectly for the B allele of α_{s1} -CN and the A¹

or A² allele of β -CN because selection has been traditionally for high milk and fat yields. Close association between these alleles and the desired yield traits was reported (16). The examined HS population showed no significant differences in the different milk protein gene frequencies compared with those of the Simmental breed, which had the most important role in the development of the HS breed. Table 3 reviews the gene frequencies found in the Simmental breed (4, 5, 8, 11, 17).

For κ -CN, three alleles were found in HS and two in HG. The κ -CN A allele was more frequent in both breeds than the κ -CN B allele. The frequency of κ -CN B was higher in HG (36%) than in HS cows (22%). κ -Casein C occurred in 3% of the studied HS cows. There is at present no reasonable explanation for the more favorable selection for the A allele of κ -CN. The genotypic frequencies of κ -CN AA and AB are significantly different in the two breeds examined, which is in accordance with

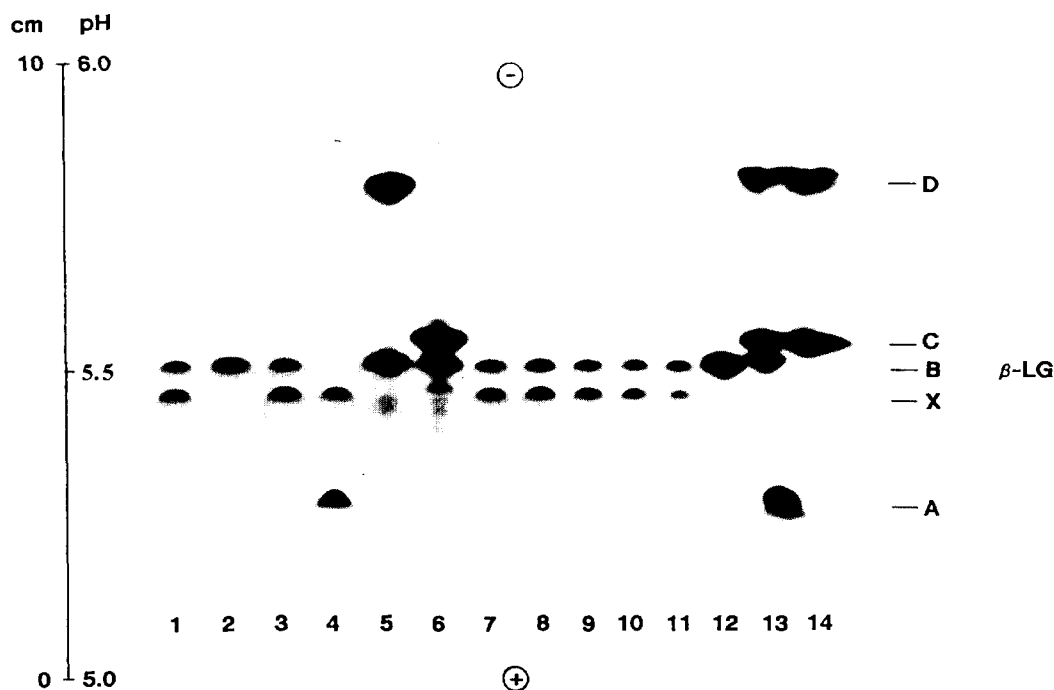


Figure 1. Isoelectric focusing of individual Hungarian Grey β -lactoglobulin (LG) samples in an immobilized pH gradient in the presence of carrier ampholytes (pH 5 to 6) over a separation distance of 10 cm (9). Lanes 1, 3, and 7 to 11: β -LG BX; lane 2: β -LG BB; lane 4: β -LG AX; lane 5: β -LG BD standard; lane 6: β -LG BC standard; lane 12: β -LG BB standard; lane 13: mixed standard containing β -LG AB and CD; and lane 14: β -LG CD standard.

the gradually decreasing frequency of κ -CN A proceeding from northwestern to southeastern Europe (10). Contrary to results for the Simmental breed (4), the variant E of κ -CN was not found in the HS breed. The absence of variants α_{s1} -CN A, β -CN A³, and κ -CN E in HS may be due to the limited number of cows examined.

Figure 1 shows the separation pattern of the various β -LG genetic variants on CA-IPG-IEF gels. In about 10% of the HG cows, we found a new unidentified protein band on CA-IPG-IEF gels. Until identification and sequence analysis, this new protein is indicated as β -LG X. Because only β -LG variants are separated in this narrow pH range (pH 5 to 6), and, because concentration of this protein is equimolar to the other β -LG variants, we assume that this protein is a new genetic variant of β -LG. Its frequency within the population is about 5%. Further characterization and protein sequencing will be undertaken to support our data. In the β -LG locus, gene frequencies were significantly different between the HS and HG breeds. As in Simmental cattle (4, 5, 17), allelic frequencies in HS were nearly equal for the most common A (47%) and B (50%) variants. Variant D, which was found first in the Montbeliarde breed (7), was present in less than 5% of the HS cows in heterozygous forms only. However, in HG the allele B was the predominant genotype (75%), whereas the frequency of the A genotype was 20%. For α -lactalbumin, all cows have the genotype BB.

The observed and expected genotype frequencies (Table 2) showed no significant differences. According to the Hardy-Weinberg law, both populations approximate genetic equilibrium.

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