Relationships of growth hormone gene and milk protein polymorphisms to milk production traits in Simmental cattle

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Summary. The importance of milk proteins and the positive effect of administration of growth hormone (GH) on milk production, and the presence in some dairy cattle lines of greater GH concentrations prompted us to examine the presence of restriction fragment length polymorphism at the GH gene using the restriction enzyme *Taq*I and to investigate associations between this polymorphism in Simmental cows and bulls, as well as milk protein variants in Simmental cows, and milk production traits. Blood and milk were sampled from 279 Italian Simmental cows and semen was collected from 148 bulls of the same breed. Two fragment bands, denoted A and B, of 6200 and 5200 bp respectively, were examined and three patterns, AA, AB and BB, were found in both animal samples. All variants previously reported in other studies, for κ, β, and α_{s1}-caseins, and β-lactoglobulin, were found in the cows' samples. For the cows' samples, a BLUP (Best Linear Unbiased Predictor) analysis of results was performed using a REML (Restricted Maximum Likelihood) program and known heritabilities, whereas for bulls we have performed a General Linear Model analysis. The effect of GH gene polymorphism, using *Taq*I restriction enzyme, on milk production traits was not significant, but bulls of BB pattern had a higher breeding value for milk yield than AA bulls ($P < 0.05$). For the κ-casein genotypic effects, cows of AB genotype gave milk with 1.53 ± 0.70 g/kg less fat than cows of AA genotype. In addition, breeding values for milk protein content were significantly higher in BB bulls, with 0.87 ± 0.32 and 0.71 ± 0.34 g/kg more milk protein than AA and AB bulls respectively. Thus, our results revealed a GH gene polymorphism and indicated significant effects of milk protein polymorphisms on milk production traits in the Italian Simmental breed.

Studies of genetic polymorphisms at the nucleotide level brought a promising way to improve milk quality and quantity, especially by making conventional breeding much more powerful and efficient. This will be achieved primarily by

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making possible the identification at birth of interesting genotypes, before performance recording, and incorporation of marker information in conventional selection.

It is now well established that growth hormone (GH) plays an essential role in lactation processes. Several authors (Grigsby & Trenkle, 1986; Peel & Bauman, 1987; Reinecke *et al.* 1993) reported an association between some features of GH secretion and genetic values in cattle. There are also correlations between milk traits and polymorphisms at the GH gene (Høj *et al.* 1993; Lucy *et al.* 1993; Parchury *et al.* 1993). Milk protein polymorphisms have also been extensively studied (see review by Jacob, 1993), and effects of κ-casein and β-lactoglobulin variants on milk components, milk yield and cheese manufacturing have been reported (Bovenhuis *et al.* 1992; Cowan *et al.* 1992).

Typing animals for all the genes encoding a polygenic trait such as milk production would be a tremendous and impractical alternative. Thus, it appears more realistic to focus on only a few genes having effects that account for a significant part of the genetic variation in milk production traits. The purpose of the present study was to investigate associations between milk production traits and the GH gene as well as milk protein polymorphisms in cattle.

MATERIALS AND METHODS

Animals

Blood (50 ml), taken by jugular venepuncture on to EDTA, and milk (10 ml) were sampled from 279 Italian Simmental cows from the same herd. Semen (five straws) was also obtained from 148 commercially available registered Italian Simmental bulls.

Birth dates of cows were from February 1982 to August 1990; 80% were born after January 1987. They had their first calving at 24–34 months of age, and were daughters of 91 Italian Simmental bulls. About half the cows were distributed among half-sib families of 3–16 (per family) and 11% among daughter groups of two cows.

Pedigree information and 305 d lactation milk production records for cows and November 1994 BLUP (Best Linear Unbiased Predictor) evaluation with information on κ-casein genotypes for bulls, including production of milk, milk fat, and milk protein and percentages of milk fat and milk protein, were obtained from the milk registration codes of the Italian breeder association Associazione Nazionale Allevatori Bovini Di Razza Pezzata Rossa Italiana. Table 1 gives the means and SD of the milk production traits of the samples.

Growth hormone gene polymorphism

Genomic DNA was extracted from blood for the cows as described by Sneyers *et al.* (1994) and from semen following the procedure of Lucy *et al.* (1993).

The probe was produced in our laboratory as follows. A 1505 bp *Bam*HI–*Sma*I fragment, which covers the bovine GH gene from base 7 of exon 1 to base 21 of exon 5 described by Gordon *et al.* (1983), was amplified by polymerase chain reaction from bovine genomic DNA and cloned in plasmid pBluescript KS$^+$. Methods of ligation, transformation of *Escherichia coli* (line JM 105), plasmid amplification and plasmid purification followed the methods of Sambrook *et al.* (1989). The insert was excised from the vector by digestion with *Bam*HI and *Sma*I restriction enzymes, separated from plasmid DNA by agarose gel electrophoresis, purified by use of a commercial kit (Gene Clean, Westburg SA, NL-3830 Leiden, The Netherlands) and used as a probe after verification of the nucleotide sequence.

Table 1. *Values of 305 d milk production traits at first lactation of 279 cows and of milk production breeding traits of 148 bulls of the Italian Simmental breed*

Trait	Bulls		Cows	
	Mean	SD	Mean	SD
Milk, kg	+66	314	4560	931
Fat, kg	NA	NA	223·6	52·6
Protein, kg	NA	NA	197·2	38·2
Fat, g/kg	−0·13	1·40	49·03	4·73
Protein, g/kg	−0·03	1·03	43·25	2·35

NA, breeding values of bulls for milk fat and protein yields were not available.

Southern blot analysis of DNA, digested by *Taq*I restriction enzyme and hybridized with a GH gene probe, was performed as described by Sneyers *et al.* (1994).

Milk protein variants

α_{s1}-, β- and κ-caseins and β-lactoglobulin variants were determined by urea-PAGE from milk samples. The electrophoresis was carried out in a Mini Protean apparatus (Bio-Rad, Richmond, CA 94804, USA). A polyacrylamide separation gel (16 g/l, containing 25 g bisacrylamide/l) was prepared in 190 mM-Tris-citrate–7 M-urea, pH 8·0. The stacking gel was composed of 57 g polyacrylamide/l containing 27 g bisacrylamide/l in 63 mM-Tris-borate, pH 8·4. Samples were prepared by adding 250 μl skim milk, 250 μl purified water and 1 ml Poulik's modified buffer (9·8 M-urea–100 mM-Tris, adjusted to pH 6·8 by adding citric acid) in the presence of 20 μl β-mercaptoethanol. Gels were stained with Coomassie blue R250 (Bio-Rad) in perchloric acid (700 g/l) and destained in acetic acid (50 ml/l).

Statistical analysis

Cows. The statistical analysis was carried out using a REML (Restricted Maximum Likelihood) program denoted by MTDFREML (multiple-trait derivative-free REML; Boldman *et al.* 1993) that incorporates a sparse matrix package, SPARSPAK (Chu *et al.* 1984).

An animal model, taking into account relationships between animals, was used to test possible associations between restriction fragment length polymorphism (RFLP) at the GH gene, using the *Taq*I restriction enzyme (GH–*Taq*I), and milk production traits at the first lactation. Dependent variables included 305 d productions of milk, fat and protein, and contents of milk fat and milk protein.

$$y_{ijkl} = \mu + s_i + b_1(c_{ijkl} - \bar{c}) + b_2(c_{ijkl} - \bar{c})^2 + m_j + g_k + u_l + e_{ijkl}, \tag{1}$$

where y_{ijkl} is the record of cow l, μ a constant common to all observations, s_i the fixed effect of year–season class of calving i ($i = 1, 2, 3, \ldots, 17$ for February 1984 to August 1984, September 1984 to January 1985, February 1985 to August 1985, …, February 1992 to August 1992 respectively), c_{ijkl} the calving age in days of cow $ijkl$, $\bar{c}$ the mean calving age, b_1 and b_2 the linear and quadratic regression coefficients of age at calving, m_j the fixed effect of month of calving j ($j = 1, 2, \ldots, 12$), g_k the fixed effect associated with the GH–*Taq*I pattern k, u_l the random genetic effect of cow l assumed to be normally distributed as $\text{N}(0, \mathbf{A}\sigma_u^2)$, where $\mathbf{A}$ is the numerator relationship matrix and e_{ijkl} the random residual effect of observation $ijkl$, also assumed to be normally distributed as $\text{N}(0, \mathbf{I}\sigma_e^2)$, where $\mathbf{I}$ is the identity matrix $n \times n$ (n is the number of observations) and σ_e^2 is the residual variance.

For milk protein polymorphisms, we have used another animal model in order to distinguish between the effects of the different polymorphisms. Indeed, casein genes are closely linked (Grosclaude *et al.* 1973), and estimates of casein genotypic effects using the model in eqn (1) might be influenced by effects of linked casein genes because of linkage disequilibrium. Statistical models in which milk protein genotypic effects were adjusted for effects of other milk protein genes have been used previously (Ng-Kwai-Hang *et al.* 1984; Gonyon *et al.* 1987; Haenlein *et al.* 1987; Bovenhuis *et al.* 1992). The second model used in the present work was as follows.

$$y_{ijklmno} = \mu + s_i + b_1(c_{ijklmno} - \bar{c}) + b_2(c_{ijklmno} - \bar{c})^2 + m_j$$
$$+ \alpha_{s1}\text{-Cn}_k + \beta\text{-Cn}_l + \kappa\text{-Cn}_m + \beta\text{-Lg}_n + u_o + e_{ijklmno}, \qquad (2)$$

where $y_{ijklmno}$, μ, s_i, $c_{ijklmno}$, $\bar{c}$, b_1, b_2, m_j, u_o, *and* $e_{ijklmno}$ have meanings corresponding to those in eqn (1), $\alpha_{s1}\text{-Cn}_k$ is the fixed effect of α_{s1}-casein genotype k, $\beta\text{-Cn}_l$ the fixed effect of β-casein genotype l, $\kappa\text{-Cn}_m$ the fixed effect of κ-casein genotype m and $\beta\text{-Lg}_n$ the fixed effect of β-lactoglobulin genotype n.

To obtain the numerator relationship matrix for both models, we considered pedigree information, including sires, dams, paternal grandparents and maternal grandparents. A total of 1015 Simmental animals, including ancestors without records, were involved in the analyses.

BLUP analyses were performed for both models, and solutions were obtained using, as final values, estimates of genetic additive and residual variances of the Italian Simmental cattle population from Alps & Averdunk (1984); no iterations were made and each trait was analysed separately. Heritabilities in the Simmental cattle population were 0·23, 0·39 and 0·46 for milk yield, fat content and protein content respectively (Alps & Averdunk, 1984). For milk fat and protein yields we used a heritability value of 0·25. Analyses and comparisons of patterns or estimates of genotype effects were performed as described by Boldman *et al.* (1993).

In order to investigate for sensitivity of GH–*Taq*I pattern estimates, we have performed analyses similar to those above for both models, using heritability values greater and smaller by 0·10 than the ones cited previously. We used this test because of the apparently restrictive size and structure of the cows' samples: if the patterns were confounded with the sires, the pattern effect estimates would strongly depend on the heritability, and consequently it would be impossible to separate the effects of the patterns from those of the sires.

Bulls. The effects of GH–*Taq*I RFLP and κ-casein genotypes on breeding values (BLUP evaluation) for milk production traits was tested by the General Linear Model procedure of the SAS software program (SAS, 1989) according to the linear model

$$Y_{ij} = \mu + g_j + e_{ij},$$

where Y_{ij} is the breeding value of the bull i for milk fat or protein contents, or milk yield, μ the mean of all observations, g_j the fixed effect of GH–*Taq*I pattern or κ-casein genotype j and e_{ij} the random residual term.

RESULTS

GH–Taq*I and milk protein polymorphisms*

Frequencies of the GH–*Taq*I patterns and the milk protein genotypes are listed in Tables 2 and 3 respectively. Two restriction fragment bands of 6200 and 5200 bp, denoted by A and B, were revealed using *Taq*I enzyme and a GH probe (Fig. 1); three GH–*Taq*I patterns, AA, AB and BB, were found.

Table 2. *Incidence of GH-*Taq*I patterns† in Simmental cows and bulls*

(Values are percentages with numbers of animals in parentheses)

	Genotype		
	AA	AB	BB
Cows	85·3 (238)	14·0 (39)	0·7 (2)
Bulls	79·7 (118)	18·2 (27)	2·0 (3)

† Fragments generated using *Taq*I restriction enzyme on the growth hormone probe.

Table 3. *Incidence of milk protein genotypes in Simmental cows*

(Values are percentages with numbers of cows in parentheses)

Genotype	Frequency	Genotype	Frequency
κ-Casein		β-Casein	
AA	59·9 (167)	AA	81·3 (226)
AB	33·3 (93)	AB	14·0 (39)
BB	6·8 (19)	AC	1·8 (5)
		BB	2·9 (8)
α_{s1}-Casein		β-Lactoglobulin	
BB	78·7 (218)	AA	36·2 (101)
BC	20·9 (58)	AB	39·4 (110)
CC	0·4 (1)	BB	24·4 (68)
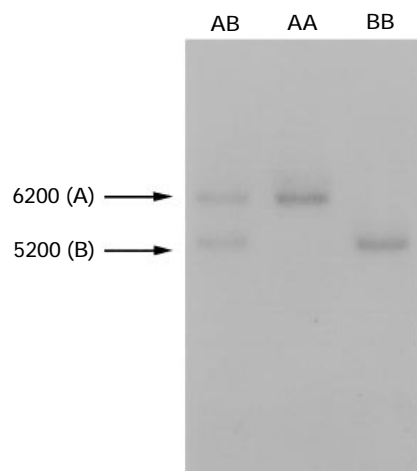
Fig. 1. Patterns generated using the restriction enzyme *Taq*I on the growth hormone gene found in Simmental cows and bulls. The sizes of digested fragments are on the left, and the patterns are at the top. Fragment length (in bp) was estimated relative to the DNA size markers, lambda/*Hin*dIII and ϕX174 DNA/*Hae*III fragments.

Three genotypes AA, AB and BB for κ-casein and β-lactoglobulin were revealed. Moreover, the cows exhibited AA, AB, AC or BB β-casein genotypes and BB, BC or CC α_{s1}-casein genotypes. In the bulls' samples, κ-casein genotype frequencies were 55·4, 35·1 and 9·5 % for AA, AB and BB respectively.

*GH–*Taq*I pattern effect on milk production traits*

Comparison between least square means, for bulls, and estimates, for cows, of GH–*Taq*I patterns are presented in Tables 4 and 5 respectively. The patterns effect on milk production traits was not significant (overall test). However, in the bulls'

Table 4. *Differences between least square means of GH–TaqI patterns† and κ-casein genotype effects on 305 d milk production breeding values of Simmental bulls*

(Values are estimates or least square means±SE)

Comparison	Milk, kg	Fat, g/kg	Protein, g/kg
	GH–*Taq*I		
AA *v.* AB	-55 ± 72	-0.13 ± 0.32	0.16 ± 0.22
AA *v.* BB	$-382 \pm 185*$	1.17 ± 0.83	0.59 ± 0.58
AB *v.* BB	-327 ± 193	1.30 ± 0.87	0.43 ± 0.61
	κ-Casein		
AA *v.* AB	71 ± 59	-0.17 ± 0.26	-0.16 ± 0.20
AA *v.* BB	166 ± 96	0.40 ± 0.42	$-0.87 \pm 0.32**$
AB *v.* BB	95 ± 100	0.56 ± 0.43	$-0.71 \pm 0.34*$

Fragments using *Taq*I restriction enzyme on the growth hormone gene.
Differences were significant: $*P < 0.05$, $**P < 0.01$.

Table 5. *Differences between estimates of GH–TaqI pattern and milk protein genotype effects on 305 d first lactation milk production traits in Simmental cows†*

(Values are estimate differences±SE)

Comparison	Milk, kg	Fat, g/kg	Fat, kg	Protein, g/kg	Protein, kg
	GH–*Taq*I				
AA *v.* AB	138 ± 172	1.35 ± 0.92	14.20 ± 10.28	-0.10 ± 0.43	4.30 ± 7.23
	κ-Casein				
AA *v.* AB	$271 \pm 132*$	$1.53 \pm 0.70*$	$22.93 \pm 7.77**$	-0.39 ± 0.33	10.19 ± 5.54
AA *v.* BB	-61 ± 252	0.97 ± 1.33	3.64 ± 14.69	-1.04 ± 0.63	-9.83 ± 10.58
AB *v.* BB	-332 ± 249	-0.56 ± 1.32	-19.29 ± 14.53	-0.65 ± 0.62	$-20.02 \pm 10.44*$
	β-Casein				
AA *v.* AB	-98 ± 172	-0.01 ± 0.91	-7.09 ± 10.06	-0.11 ± 0.43	-7.30 ± 7.23
	α_{s1}-Casein				
BB *v.* BC	-130 ± 147	-1.07 ± 0.78	-12.34 ± 8.56	-0.05 ± 0.37	-6.53 ± 6.17
	β-Lactoglobulin				
AA *v.* AB	-58 ± 139	$-1.68 \pm 0.75*$	-10.83 ± 8.19	-0.24 ± 0.34	-1.83 ± 5.85
AA *v.* BB	58 ± 164	-0.10 ± 0.87	3.75 ± 9.56	0.02 ± 0.41	3.64 ± 6.87
AB *v.* BB	116 ± 152	$1.58 \pm 0.81*$	14.59 ± 8.93	0.26 ± 0.38	5.47 ± 6.42

† Genotype groups with few cows are not presented.
Differences were significant: $*P < 0.05$, $**P < 0.01$.

samples, milk yield for the BB pattern was significantly higher than for AA by 382 ± 185 kg ($P < 0.05$). The sensitivity test showed that effect estimates of patterns were only slightly dependent on a 0·10 variation of heritabilities; such a change in heritability values led to a modification of 0·11 to 6·45% in pattern estimates.

Milk protein polymorphisms effects

In the bulls' samples, the effect of κ-casein polymorphism on breeding values for milk protein content was significant; bulls had milk protein content values greater by 0.87 ± 0.32 and 0.71 ± 0.34 g/kg than for AA and AB bulls respectively (Table 4).

Results for milk protein effects in the cows' samples are presented in Table 5; κ-casein genotypes had a significant effect on milk fat yield and content in addition to milk yield. Cows of the AA genotype produced 22.93 ± 7.77 kg, 1.53 ± 0.70 g/kg and 271 ± 132 kg more fat yield, milk fat content and milk yield respectively, compared with the cows carrying the AB κ-casein genotype. In addition, BB cows gave higher milk protein yields than AB cows ($P < 0.05$).

For neither β-casein nor α_{s1}-casein genotypes did the analyses reveal any significant effect on the milk traits investigated. However, cows' groups of AC and BB β-casein genotypes and the cows' group of CC α_{s1}-casein genotype were not considered because of the low numbers in these groups. The β-lactoglobulin polymorphism effect was significant only for milk fat content, with the AB genotype giving 1.68 ± 0.75 and 1.58 ± 0.81 g/kg more fat than AA and BB respectively ($P < 0.05$).

A variation of heritability values by 0.10 affected milk proteins genotype estimates only very slightly, with a change ranging from 0.08 to 8.35%.

<div align="center">DISCUSSION</div>

*GH–Taq*I *polymorphism*

Rocha *et al.* (1992) and Cowan *et al.* (1989) reported fragments of GH–*Taq*I in Holsteins that seemed to be identical to those detected in the present study. The fragments denoted in the present study by A and B very probably correspond to the fragments of 6.5 and 5.5 kb respectively found by Cowan *et al.* (1989) or, more nearly, to those of 6.15 and 5.2 kb respectively reported by Rocha *et al.* (1992). Cowan *et al.* (1989) reported that animals demonstrating the 6.5 kb pattern were homozygotes for one allele, and those showing the 6.5, 5.5 kb pattern were heterozygotes with one chromosome carrying this allele, and the other a similar allele with an insertion or deletion of ~ 1 kb between the $3'$ *Eco*RI and *Taq*I sites. The frequencies of GH–*Taq*I patterns in the present study are not, apparently, different from those observed by Rocha *et al.* (1992) in Holsteins. Moreover, selection has probably worked indirectly against the less frequent B allele.

The observation that BB bulls have a superior milk yield breeding value should be considered with some reservation, because of the very low numbers of BB pattern in the analysis, but deserves further study. Using the polymerase chain reaction–RFLP technique and digestion of the polymerase chain reaction product with the restriction enzyme *Alu*I, Zhang *et al.* (1993) revealed two alleles, L and V, responsible for alternative forms of bovine GH with a Leu or Val residue at position 127. They found substantial variation of the frequency of these alleles among eight evaluated cattle breeds ; one allele was three times as common in beef breeds as in Holsteins. Lucy *et al.* (1993) reported a significantly higher predicted transmitting ability for milk yield for VV genotype Jersey cows, but no significant effect was found in their samples of Holstein, Guernsey, Ayrshire or Jersey bulls. Moreover, Schlee *et al.* (1994) observed no significant effect of this L/V polymorphism on milk trait breeding values of 221 Simmental bulls.

Milk proteins polymorphisms

The milk proteins variants observed in our study have already been reported by several authors (Ng-Kwai-Hang *et al.* 1984 ; Gonyon *et al.* 1987 ; Haenlein *et al.* 1987 ; Aleandri *et al.* 1990 ; Bovenhuis *et al.* 1992). However, we failed to detect the C β-casein and D β-lactoglobulin variants reported by Graml *et al.* (1985, 1986) in a sample of 2262 Fleckvieh cows, probably because of our lower sample size.

The observed effect of κ-casein genotypes on milk fat agrees with the findings of Cowan *et al.* (1992), who reported a decrease in probable transmission for fat content (0.079%) and fat yield for cows inheriting the κ-casein B allele compared with those with the A variant in Holstein cattle. In other studies, the AA genotype was found to be a favourable κ-casein genotype for fat content (Ng-Kwai-Hang *et al.* 1984) and Bovenhuis *et al.* (1992) noted an association between the B allele and lower fat yield.

This allele was also found to enhance protein content, as in our bulls' samples (Ng-Kwai-Hang *et al.* 1984; Gonyon *et al.* 1987; Aleandri *et al.* 1990; Bovenhuis *et al.* 1992), and protein yield (Ng-Kwai-Hang *et al.* 1984, 1990; Aleandri *et al.* 1990; Cowan *et al.* 1992). The effect of κ-casein genotypes on milk yield was found to be significant in some studies (Gonyon *et al.* 1987; Lin *et al.* 1989; Bovenhuis *et al.* 1992) but not in others (McLean *et al.* 1984; McLean, 1987; Aleandri *et al.* 1990; Cowan *et al.* 1992).

For β-casein genotypes effects, our results are in accord with those of Geldermann *et al.* (1985), Haenlein *et al.* (1987) and Aleandri *et al.* (1990). However, others have reported significant effects of β-casein genotypes, although for different milk traits. In Holsteins, Ng-Kwai-Hang *et al.* (1984) found significant genotypic effects on milk, fat and protein yields and on fat but not protein content, while in the study of Gonyon *et al.* (1987) the only significant effect observed was on protein content. Another conflicting result was that of Bovenhuis *et al.* (1992), who found a significant effect of β-casein genotypes on milk yield, protein content and yield, and fat content.

Our results on the effect of the α_{s1}-casein genotype are in conflict with studies that found significant effects (Ng-Kwai-Hang *et al.* 1984; Haenlein *et al.* 1987; Aleandri *et al.* 1990). However, they agree with the results of Graml *et al.* (1986) for the Fleckvieh breed, Gonyon *et al.* (1987) and Bovenhuis *et al.* (1992) who also found no significant effect on milk production traits.

For β-lactoglobulin polymorphism, significant effects on milk fat content have been reported (Ng-Kwai-Hang *et al.* 1984; Graml *et al.* 1985; Haenlein *et al.* 1987; Aleandri *et al.* 1990), but BB was the favourable genotype instead of AB, as in our study.

Most of the studies cited in this discussion have used a multigene model for analysing data for milk protein genotypic effects. Nevertheless, few of them have produced similar findings with respect to the significance and size of genotypic effects. Some studies did not consider relationships between animals in the analysis (Ng-Kwai-Hang *et al.* 1984, 1986; Aleandri *et al.* 1990) and others accounted only for the relationship between animals through sires (Graml *et al.* 1985, 1986; Gonyon *et al.* 1987; Haenlein *et al.* 1987). Kennedy *et al.* (1992) demonstrated that ordinary least-squares estimates are potentially biased if the gene has a real effect and if directional selection for the trait of interest has been practised. Most dairy cattle populations have been selected and there will be relationships between animals. Thus using an animal model seems to be a more suitable method for estimating genotypic effects.

The degree of association between polymorphisms at known loci and quantitative traits depends on the degree of linkage between the polymorphic loci and an eventual quantitative trait locus, and so is influenced by linkage disequilibrium mainly because of selection. Moreover, similar results on polymorphism effects, from different studies, might indicate a direct influence or the presence of a highly linked quantitative trait locus and it is very likely that this applies to the κ-casein genetic polymorphism with milk protein content. Indeed, Bovenhuis *et al.* (1992) came to a similar conclusion after comparing their findings with those reported in other studies.

In conclusion, the results of the present study revealed GH–*Taq*I polymorphism in Simmentals with a probable relationship to milk yield, and demonstrated the effects of milk protein genotypes on milk production traits. Taking other studies into account, this latter is especially valid for κ-casein genetic polymorphism and milk protein content and so may provide interesting implications for selection since the favourable κ-casein allele B is still relatively rare. Increasing the sample size to

include more animals of rare genotypes and profiles, such as the GH–*Taq*I BB profile, and extending investigation to other genes of the somatotropic and lactogenic axes may provide more interesting findings.

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