

## Polymorphism of the $\alpha$ s1-casein, $\kappa$ -casein and $\beta$ -lactoglobulin genes in the Hungarian Milk Goat

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### Abstract

The type and frequency of goat milk protein alleles have not yet been studied in Hungary. Here we report the initial, partial characterization of allelic polymorphisms of  $\alpha$ s1-,  $\kappa$ -casein and  $\beta$ -lactoglobulin genes in a herd of Hungarian Milk Goat based on published RFLP-PCR and AS-PCR methods. Experiments are in progress to confirm and extend the caprine milk protein genotype data and to evaluate its influence on milk quality.

**Keywords:** Polymorphism,  $\alpha$ s1-casein,  $\kappa$ -casein and  $\beta$ -lactoglobulin genes

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### Introduction

In the last one and half decades the number of goats has increased significantly in Hungary. The economic impact of the goat sector is not yet significant, but the potential of this small ruminant has been recognized by the Ministry of Agriculture and Rural Development. The Hungarian Central Statistical Office registered 100000 goats in 2002, of which 50000-55000 were ewes. Three imported (Alpine, Saanen, Boer) and four local goat breeds are found on goat farms. The four local breeds are the Hungarian Improved goat, Hungarian Milk White goat, Hungarian Milk Brown goat and the Hungarian Milk Multicolour goat. The last three Hungarian breeds constitute 75% of the total population (Kukovics, 2001).

Traditionally, goat milk is more important than goat meat in Europe. Goat cheese is an emerging product, which is becoming more and more popular with the Hungarian customer. The long-term aim is to combine traditional selection methods with marker associated selection (MAS). Genetic variants of goat milk caseins and whey components were published and some of them have a significant impact on milk quality (Grosclaude *et al.*, 1987; 1994). The most prominent is caprine  $\alpha$ s1-casein. The CSN1S1 locus is characterized by seven alleles associated with at least four quantitative levels of the corresponding protein: strong (A,B,C); intermediate (E); weak (D,F) and nil (0) (Grosclaude *et al.*, 1987). From this it follows that the frequency of the  $\alpha$ s1-casein alleles has already been examined in several goat breeds in several countries (Grosclaude *et al.*, 1987; Jordana *et al.*, 1991; Ramunno *et al.*, 1991; Grosclaude *et al.*, 1994). It was confirmed that more cheese could be produced from milk (associated with the casein  $\alpha$ s1: A, B, C alleles) with a high casein content, but the structure of the cheese made from this high casein type of milk has changed and its aroma was found to be atypical. In France, milk protein polymorphism assays have been adopted since 1996 in selection programmes for dairy goats (Pierre-Quere, 1995).

$\kappa$ -casein is essential for micelle formation and stabilization, and influences the manufacturing properties of milk. Cheese-making is based on the cleavage of the  $\kappa$ -casein Phe-Met peptide bond by enzymes or heat. Recent studies on goat  $\kappa$ -casein showed that the  $\kappa$ -casein gene is highly polymorphic (Caroli *et al.*, 2001; Yahyaoui *et al.*, 2001; Angiolillo *et al.*, 2002; Jann *et al.*, 2003). The  $\beta$ -lactoglobulin is the major whey protein in ruminants and is also present in the milk of other mammals but not in the milk of humans and rodents (D'Andrea *et al.*, 2001).

The type and frequency of the caprine milk protein alleles have not been studied in Hungary. These initial experiments were performed with the assistance of A. Sanchez and co-workers at Universitat Autònoma de Barcelona, Faculty of Veterinary Medicine, Department of Animal and Food Science, Bellaterra, Spain.

## Materials and Methods

Individual blood samples were collected from two herds (Ceglédbercel, Káva). Fifty Hungarian Milk Multicolour goats from Káva, 27 Hungarian Milk Brown goats, 13 Hungarian Milk Multicolour goats and 19 Hungarian Milk White goats from Ceglédbercel were used in the evaluation. The milk protein genotypes of 109 unrelated animals were determined. No differentiation was made between the above-mentioned local goat types.

The DNA was isolated by standard methods (Bösze *et al.*, 2000). Partial characterization of  $\alpha$ s1-,  $\kappa$ -casein and  $\beta$ -lactoglobulin alleles was performed as published by Ramunno *et al.* (1991), Amills *et al.* (1996), Yahyaoui *et al.* (2000) and Yahyaoui *et al.* (2001).

Concurrent with the milk protein genotyping, milk protein, lactose, milk fat concentrations and somatic cell counts were determined on the individual milk samples.

## Results and Discussion

For  $\alpha$ s1-casein, genotyping was restricted to the E and F alleles, which were found to be the most frequent ones in other breeds. Those alleles have average (E) to low effects (F) on the milk  $\alpha$ s1-casein content and thus have a considerable economic impact. The frequencies of  $\alpha$ s1-casein E and F alleles were found to be much lower in Hungarian Milk goats compared with the French, Italian, Spanish goats (Table 1).

**Table 1** Allele frequencies of  $\alpha$ s1-casein alleles in Hungarian Milk Goats and in references goats

Breeds (numbers)	A+B+C+D+0 alleles	E allele	F allele	References
Alpine (213)	0.14	0.34	0.41	Grosclaude <i>et al.</i> (1987)
Alpine (80)	---	0.35	0.59	Ramunno <i>et al.</i> (1991)
Saanen (159)	0.07	0.41	0.43	Grosclaude <i>et al.</i> (1987)
Saanen (70)	0.03	0.46	0.46	Ramunno <i>et al.</i> (1991)
Canaria (74)	0.28	0.20	-	Jordana <i>et al.</i> (1991)
Hungarian Milk (109)	0.61	0.08	0.31	

The low frequency of the E allele with an intermediate content of  $\alpha$ s1-casein and of the F-allele with a reduced level of  $\alpha$ s1-casein in the Hungarian Milk goat are in contrast to the predominance of those two  $\alpha$ s1-casein alleles in the milk of Saanen and Alpine breeds. Experiments are in progress to determine the frequencies of the remaining five  $\alpha$ s1-casein alleles (A, B, C, D and 0).

**Table 2** Comparison of allele frequencies of A+B and C alleles of the  $\kappa$ -casein gene in Hungarian Milk goats and in Spanish and French goats

Breeds (numbers)	A+B alleles	C allele	References
Malaguena (17)	1	0	Yahyaoui <i>et al.</i> (2001)
Payoya (11)	1	0	Yahyaoui <i>et al.</i> (2001)
Canaria (48)	0.99	0.01	Yahyaoui <i>et al.</i> (2001)
Murciano-Granadina (38)	0.99	0.01	Yahyaoui <i>et al.</i> (2001)
Saanen (33)	0.89	0.11	Yahyaoui <i>et al.</i> (2001)
Hungarian Milk (109)	0.85	0.15	

The method published by Yahyaoui *et al.* (2001) did not enable us to differentiate between the A and B alleles of  $\kappa$ -casein. The frequency of the C allele of  $\kappa$ -casein was almost the same as that in the Hungarian Milk goat, but not to that in the Saanen breed (Table 2). The impact of the  $\kappa$ -casein allelic variants on milk quality remains to be established.

**Table 3** Allele frequencies of -60C and -60T alleles of  $\beta$ -lactoglobulin gene in Hungarian Milk goats and in Spanish goats

Breeds (numbers)	-60C allele	-60T allele	References
Murciano Granadina (69)	0.86	0.14	Yahyaoui <i>et al.</i> (2000)
Canaria (42)	1	-	Yahyaoui <i>et al.</i> (2000)
Payoya (11)	0.73	0.27	Yahyaoui <i>et al.</i> (2000)
Malaguena (18)	0.75	0.25	Yahyaoui <i>et al.</i> (2000)
Saanen (20)	0.73	0.27	Yahyaoui <i>et al.</i> (2000)
Hungarian Milk (109)	0.88	0.12	

Up to date 109 Hungarian Milk goats have been genotyped for  $\beta$ -lactoglobulin promoter polymorphism, based on the method published by Yahyaoui *et al.* (2000). The frequency of the -60T allele of  $\beta$ -lactoglobulin gene was found to be lower in the Hungarian Milk goat than in the Saanen breed (Table 3). The influence of the promoter polymorphism on  $\beta$ -lactoglobulin gene expression levels has to be evaluated.

## Conclusions

The limited number of animals and restricted methods applied so far did not enable us to draw firm conclusions. We are planning to evaluate the CSN1S2 and CSN2 alleles and extend the characterization for all known CSN1 and CSN3 alleles in order to determine the casein haplotypes in the predominant Hungarian Milk goat breed. The direct relationship between the allelic variants of CSN1S1, CSN1S2 and CSN2 genes and casein content, which further influence the physico-chemical properties of milk, could be utilized in breeding schemes aiming at the improvement of milk processing quality and cheese yields of the Hungarian Milk goat breeds.

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