Genetic polymorphism at the *CSN1S1* gene in two Czech goat breeds

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ABSTRACT: The genetic polymorphism of the *CSN1S1* (casein *alpha-S1*) locus was investigated in two endangered Czech goat breeds (White Shorthair and Brown Shorthair). These breeds are kept mainly for their good dairy performance. Genetic characterization of the *CSN1S1* locus contributes to the knowledge of the genetic structure of these two endangered breeds. The study was performed on 498 goats (333 White and 165 Brown Shorthair goats) by means of different polymerase chain reactions (PCR). We detected *A** (associated with normal content of protein), *E*, *F* and *01* alleles. The analysis showed a prevalence of *CSN1S1 F* (0.658; 0.597) and *CSN1S1 A** (0.269; 0.303) alleles. In both breeds, the frequency of occurrence of *E* and *01* alleles was very low: *E* (0.054; 0.085) and *01* (0.019; 0.015), respectively. No population followed the Hardy-Weinberg equilibrium, the value of polymorphic information content (PIC) being 0.426 in White and 0.472 in Brown Shorthair goats. Moreover, the test of population differences (P = 0.130) showed no significant differences between White and Brown Shorthair goats. This genetic peculiarity makes the preservation of the population of both breeds worthwhile.

Keywords: goat; *CSN1S1*; allele frequency; biodiversity

Study of the genetic polymorphism of goat casein genes (α_{S1}-, β-, α_{S2}- and κ-casein) has aroused a considerable research interest because goat casein polymorphisms are related to dairy performance. These genes significantly affect the chemical, physical, technological and nutritional quality of goat's milk (Martin et al., 2002).

CSN1S1 (casein *alpha-S1*) is the main calcium-sensitive casein in ruminant milk, distinguished by high qualitative and quantitative genetic variation. The goat *CSN1S1* gene is an excellent example for demonstrating that a major proportion of the variability observed in the casein *alpha-S1* content of goat's milk is due to the presence of autosomal alleles at a single structural level locus. So far at least 17 alleles ($A, B_1, B_2, B_3, B_4, C, D, E, F, G, H, I, L, M, N, 01$ and 02) which are associated with different levels of *CSN1S1* expression in the milk have been identified.

On the basis of the milk content of casein *alpha-S1*, the *CSN1S1* variants can be classified into 4 groups: strong alleles ($A, B_1, B_2, B_3, B_4, C, H, L,$ and M) producing almost 3.5 g/l of casein *alpha-S1* each; intermediate alleles (*E* and *I*; 1.1 g/l); weak alleles (D, *F* and *G*; 0.45 g/l); and null alleles (0_1, 0_2, and *N*) producing no α_{S1}-casein (Grosclaude et al., 1987; Mahé and Grosclaude, 1989; Leroux et al., 1990, 1992; Jansà-Pérez et al., 1994; Rando et al., 1998; Ramunno et al., 2000; Bevilacqua et al., 2002; Cosenza et al., 2003; Ramunno et al., 2005).

The objective of this study was to evaluate genetic polymorphism in the *CSN1S1* gene and to provide information for a program of conservation and improvement in two endangered goat breeds (White and Brown Shorthair goats) raised in the Czech Republic.

MATERIAL AND METHODS

Blood samples were collected from 333 White Shorthair goats and 165 Brown Shorthair goats randomly chosen from the national flock. Genomic

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DNA was extracted from the blood using ABI PRISM 6100 analysis (Nucleic Acid Prep. Station, Applied Biosystem Co.) according to standard protocol and was typed using the analyses described below (Table 1). The amplification products and the restriction patterns were made visible on agarose gel (PCR-agarose, Top-Bio, CR) in TBE buffer stained with ethidium bromide.

White Shorthair (WSH) and Brown Shorthair (BSH) goats are national breeds raised mainly for their good dairy performance. Both breeds are characterized by very good fertility, early maturity and a high level of milk production.

The WSH breed was developed between 1900 and 1930 by the crossbreeding of white local goats with Saanen bucks imported from Switzerland to improve milk production, namely in Moravian regions. The WSH breed is classified as belonging to the Saanen group. During the same period, coloured local goats of North Bohemia were improved by crossbreeding with German (Harz) and Swiss (brown Alpine) bucks. The BSH is classified as belonging to the Chamois group. Both breeds have been maintained as separate populations since 1927. In 1995 they were recorded in the National Program on Genetic Resources as potentially endangered species (www.schok.cz – Sheep and Goat Breeders Association).

Statistical analysis

PowerMarker data analysis software (Liu and Muse, 2005) was used to estimate allele and genotype frequencies and polymorphic information content and to verify the Hardy-Weinberg equilibrium and population differentiation test in WSH and BSH goat breeds.

RESULTS AND DISCUSSION

For our molecular analysis we typed four genetic variants: *"A*"* genetic variant which is associated with the normal content of protein (*A* = A, B* and *C), E, F* and *01*. Each of these variants corresponds to a different level of the *alpha-S1* casein content in milk (see above the Introduction). We observed the following genotypes: *A*A*, A*F, EF, FF, 01A*, 01E* and *01F*. The results of the genetic polymorphism at the *CSN1S1* locus in our two endangered goat breeds (WSH and BSH) are reported in Table 2.

The analysis of the *CSN1S1* locus showed a prevalence of *F* allele, associated with a low level of protein content in the milk of both breeds (WSH and BSH). Allele *A**, which is associated with a high level of protein synthesis casein *alpha-S1*, was present at intermediate frequency. *E* and *01* allele were identified at a very low frequency in both breeds. Comparison of our results with those available in the literature showed a similarity to the Alpine and Saanen breeds characterized by a high frequency of *F* allele. However, in the Alpine and Saanen breeds, allele *E* shows a higher frequency relative to our observed results (Grosclaude et al., 1987; Tadlaoui Ouafi et al., 2002; Veress et al., 2004).

The most common genotype was A**F* followed by *FF* genotype and *EF* genotype in the WSH and BSH breed, respectively. Another genotype in WSH was *01F*, followed by *A*A** and *01E* with a very low frequency. Genotype *01A** was not detected in the WSH breed compared to the BSH. In the case of the BSH breed, genotype *A*A** had a slightly higher frequency than in the WSH breed, followed by genotypes *01E* and *01A**, which were present at a very low frequency. Genotype *01F* was not detected in the BSH compared to the WSH. Moreover, in our work we did not observe the genotypes *0101, A*E* or *EE* in either of the goat breeds.

On the basis of our results, the genetic structure in both Czech goat breeds corresponds to a low quantitative variability of casein *alpha-S1* content, which is associated with strong and weak heterozygous genotypes (*A*F*) and weak homozygous genotypes (*FF*), followed by medium and weak heterozygous genotypes (*EF*) (see above, the Introduction). Similar results were recorded in the

Table 1. Analyses applied to the DNA samples for genotyping

Locus	Level	Method	Identification of alleles	Reference
CSN1S1	DNA	PCR-RFLP	*A*, F*	Ramunno et al. (2000)
		AS-PCR[1]	*E*, non *E*	Jansà-Pérez et al. (1994), Rando et al. (1998)
			01	Sztankóová et al. (2006)

A = A, B* and *C*; AS-PCR[1] = Allele Specific-PCR

Table 2. Genotype distribution and allele frequencies at the *CSN1S1* locus in the White (WSH) and Brown (BSH) Shorthair goat breed

	WHS			BSH		
Genotype	No.	frequency	SD	No.	frequency	SD
*A*A**	6	0.018	0.008	7	0.042	0.014
*A*F*	167	0.502	0.008	84	0.509	0.014
*A*E*	–	–	–	–	–	–
EE	–	–	–	–	–	–
EF	32	0.096	0.003	25	0.152	0.006
FF	115	0.345	0.010	44	0.267	0.016
0101	–	–	–	–	–	–
*01A**	–	–	–	2	0.012	0.003
01E	4	0.012	0.002	3	0.018	0.004
01F	9	0.002	0.002	–	–	–
Total	333	1.000	65		1.000	
Allele						
A*		0.269	0.0140		0.3030	0.022
E		0.054	0.0008		0.0850	0.014
F		0.658	0.0140		0.0597	0.021
01		0.019	0.0005		0.0150	0.006

*A** = *A*, *B* and *C*

Alpine and Saanen breeds (Grosclaude et al., 1987; Tadlaoui Ouafi et al., 2002; Veress et al., 2004).

The *CSN1S1* locus was tested for the Hardy-Weinberg equilibrium and characterised by observed (H_{obs}) and expected (H_{exp}) heterozygosity and polymorphic information content (PIC) in both goat populations. The WSH and BSH populations did not follow the Hardy-Weinberg equilibrium at the *CSN1S1* locus and showed medium heterozygosity (H_{obs} equals 0.636 (WSH) and 0.690 (BSH); H_{exp} equals 0.491 (WSH) and 0.544 (BSH), and PIC 0.426 and 0.472, respectively). The "population differentiation test" showed that no significant differences (P = 0.130) existed between WSH and BSH in allele frequencies at the *CSN1S1* locus.

In goats, the *CSN1S1* gene has an important effect on the protein content of goat milk and a smaller effect on total protein yield. Sanchez et al. (2005) predicted that the *CSN1S1* gene could be used as a selection criterion to improve these traits, with the main advantage being that genotypes can be determined at birth in males and females, while the above-specified characteristics are observable only in females after kidding. Manfredi et al. (2000) showed that the *CSN1S1* genotypes of males used for artificial insemination (AI) were partial predictors of the results of progeny tests for protein characteristics, and for these reasons this polymorphism is presently being used for the pre-selection of young males entering into progeny tests in the Alpine and Saanen selection schemes. Whether or not and how the information about the *CSN1S1* genotype will be used in practice will depend on selection objectives and allele frequencies in the studied populations.

At present, the White Shorthair and Brown Shorthair goats are classified as endangered breeds and have been recorded in the National Programme on Genetic Resources. This study provides the first characterization ever by means of genetic markers in these breeds.

Further studies of *CSN1S2*, *CSN2* and *CSN3* caseins are needed for more precise genetic characterization which could be utilized in breeding schemes aiming at the improvement of the quality of processed milk and cheese yield of Czech dairy goat breeds.

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