

SHORT REPORT

## Genomic analysis of the *CSN2* and *CSN3* loci in two Czech goat breeds

Zuzana Sztankóová<sup>1\*</sup>, Carmela Senese<sup>1,2</sup>, Vladimira Czerneková,  
Gabriela Dudková, Tomáš Kott, Vera Mátlová, Jiri Soldát

<sup>1</sup> Research Institute of Animal Production,  
Přátelství 815, 104 01 Prague 10 – Uhřetěves, Czech Republic

<sup>2</sup> Department of Animal Science, University of Study of Basilicata,  
Via N. Sauro 85, 85100 Potenza, Italy

(Received October 27, 2004; accepted February 24, 2005)

White Short-Haired (WSH) and Brown Short-Haired (BSH) are two protected local goat dairy breeds in Czech Republic. A genetic characterization of both breeds is necessary to preserve them from extinction and to exploit their genetic variation. For this purpose a study was carried out on the genetic polymorphism of the *CSN2* and *CSN3* loci. Genomic analysis was performed by PCR method. Both breeds were characterized by the absence of *CSN2<sup>0</sup>* allele. The *CSN3* locus was found polymorphic, with the *CSN3<sup>A</sup>*, *CSN3<sup>B</sup>* and *CSN3<sup>C</sup>* alleles frequency of 0.15, 0.80 and 0.05 in WSH and 0.52, 0.40 and 0.08 in BSH, respectively.

**KEY WORDS:** *CSN2* / *CSN3* / genetic polymorphism / goat / milk

Investigations on genetic polymorphism of milk proteins were initiated more than 40 years ago and are still continued because of their relationship with quality, com-

---

\*e-mail: sztankoova@seznam.cz

position, and technological traits of milk. In goats, genetic polymorphism has been reported in the *CSN1S1*, *CSN1S2* and in *CSN2 loci* [Martin 1993, Grosclaude *et al.* 1994]. Furthermore, for each of these *loci* the presence has been evidenced of at least one allele related to a “null” content of corresponding protein in the milk [Ramunno *et al.* 1995, 2001]. In several studies based on various techniques, the polymorphism in the caprine *CSN3* gene has been reported [Di Luccia *et al.* 1990, a review], but only recently its genetic variants were characterized [Caroli *et al.* 2001, Yahyaoui *et al.* 2001]. At the moment, with the only exception of preliminary results obtained by Matlova and co-worker (unpublished), the effect of *CSN3* alleles on the quality and technological properties of milk have not been proved. In this report the genetic polymorphism at *CSN2* and *CSN3 loci* is presented in the two protected Czech dairy goat breeds – White Short-Haired and Brown Short-Haired. Future research will cover the *CSN1S1* and *CSN1S2* genes.

### Material and methods

Blood samples for DNA isolation were obtained from a total of 202 goats belonging to White Short-Haired (WSH, n=169) and Brown Short-Haired (BSH, n=33) breeds. DNA was extracted using the Nucleo-Spin blood kit (CLONTECH LABORATORIES).

In order to detect carriers of *CSN2<sup>A</sup>* and *CSN2<sup>0</sup>* alleles related to a “normal” and a “null” level of the corresponding milk proteins, respectively, genomic analysis was performed by allele-specific PCR (AS-PCR) using primers and PCR conditions described by Ramunno *et al.* [1995]. The length of the two allele-specific amplified fragments, containing exon 7 of the gene, is 299 bp. PCR primers for amplification of exon 5 and 6 were also included in the PCR reaction as a positive control for DNA amplification. PCR products were analysed electrophoretically in 3% agarose gel (GIBCO BRL) and stained with ethidium bromide.

Analysis at *CSN3 locus* was performed with PCR-RFLP and Light Cycler analysis. A 459 bp fragment of goat *CSN3* exon 4 was amplified using primers and conditions of PCR described by Yahyaoui *et al.* [2001]. Part of PCR product (10 µl) was digested by *Bse*NI endonuclease at 65°C (FERMANTAS). The digestion products were separated electrophoretically in a 2% agarose gel (GIBCO BRL) stained with ethidium bromide. The digestion of the 459 bp region of *CSN3* exon 4 showed two fragments for allele C and one fragment for alleles A and B. The second part of PCR product was used to distinguish alleles A and B by Light Cycler analysis.

### Results and discussion

Genomic analysis of the *CSN2 locus* revealed the total absence of *CSN2<sup>0</sup>* allele in both breeds. This corroborates the results obtained in other breeds in which the total absence or very low frequencies of *CSN2<sup>0</sup>* allele were found [Ramunno *et al.* 1995]. However, more numerous groups of animals genotyped for *CSN2* in both breeds are

necessary to confirm this phenomenon. Such information could have relevant consequences, since the presence/absence of *CSN2<sup>0</sup>* allele is directly related to the quality and technological properties of milk.

Analysis of *CSN3* locus showed the prevalence of *B* allele in WSH, while of *A* allele in BSH goats. In both breeds allele *C* appeared with very low frequency. These results corroborate those observed in other European goat breeds [Caroli *et al.* 2001, Yahyaoui *et al.* 2003]. Frequencies of genotypes and alleles at the *CSN3* locus are shown in Table 1.

This report submits further arguments for the preservation of the two Czech dairy

**Table 1.** Frequencies of genotypes and alleles at the *CSN3* locus in Czech White Short-Haired (WSH) and Brown Short-Haired (BSH) goat.

Breed	Genotype	No. of animals	Genotype frequency	Allele	Allele frequency
WSH	AA	3	0.02	A	0.15
	AB	43	0.23	B	0.80
	AC	2	0.01	C	0.05
	BB	104	0.62		
	BC	17	0.10		
BSH	AA	7	0.21	A	0.32
	AB	18	0.53	B	0.40
	AC	2	0.06	C	0.08
	BB	3	0.09		
	BC	3	0.09		

goat breeds. Future research developments can be envisaged in the study of the effects of casein haplotype on milk production in goats and cheese-making properties of goat milk.

#### REFERENCES

- CAROLI A., JANN O., BUDELLI E., BOLLA P., JAGER S., ERHARDT G., 2001 – Genetic polymorphism of goat  $\epsilon$ -casein (CSN3) in different breeds and characterization at DNA level. *Animal Genetics* 32 (4), 226-230.
- DI LUCCIA A., MAURIELLO R., CHIANESE L., MOIO L., ADDEO F., 1990 – Kappa casein polymorphism in caprine milk. *Scienza e Tecnica Lattiero-casearia* 41, 305-314.
- GROSCLAUDE F., RICORDEAU G., MARTIN P., REMEUF F., VASSAL L., BOUILLON J., 1994 – Du gène au fromage: le polymorphisme de la caséine  $\alpha$ S1 caprine, ses effets, son évolution. *Productions Animales* 7, 3-19.
- MARTIN P., 1993 – Polymorphisme génétique des lactoprotéines caprines. *Lait* 73, 511-532
- RAMUNNO L., LONGOBARDI E., PAPPALARDO M., RANDO A., DI GREGORIO P., COSENZA G., MARIANI P., PASTORE N., MASINA P., 2001 – An allele associated with a non detectable amount of as2-casein in goat milk. *Animal Genetics* 32, 19-26.

6. RAMUNNO L., MARIANI P., PAPPALARDO M., RANDO A., CAPUANO M., DI GREGORIO P., COSENZA G., 1995 – Un gene ad effetto maggiore sul contenuto di caseina b nel latte di capra. XI Congresso ASPA, Grado, Italy, June 19-22.
7. YAHYAOU M.H., COLL A., SANCHEZ A., FOLCH J.M., 2001 – Genetic polymorphism of the caprine kappa-casein gene. *Journal of Dairy Research* 68, 209-216.

Zuzana Sztankóová, Carmela Senese, Vladimira Czerneková,  
Gabriela Dudková, Tomáš Kott, Vera Mátlová, Jiří Soldát

### Analiza genomowa w *loci CSN2* i *CSN3* kóz dwóch czeskich ras miejscowych

#### Streszczenie

Kozy ras mlecznych – czeskiej krótkoszerstnej białej (WSH) i czeskiej krótkoszerstnej brunatnej (WSB) należą w Republice Czeskiej do ras chronionych. Charakterystyka genetyczna dostarczy dalszego argumentu na korzyść ochrony tych ras i pozwoli wykorzystać w hodowli ich ewentualną genetyczną odrębność. Analizę genomową przeprowadzono metodą PCR. Obie badane rasy charakteryzowały się niewystępowaniem allelu *CSN2<sup>0</sup>*. Locus *CSN3* okazał się polimorficzny, z frekwencją alleli *CSN3<sup>A</sup>*, *CSN3<sup>B</sup>* i *CSN3<sup>C</sup>* odpowiednio 0,15, 0,80 i 0,05 w rasie WSH i 0,52, 0,40 i 0,08 w rasie BSH.