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Casein genetic polymorphisms in goat breeds of Lombardy

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RIASSUNTO – Polimorfismo genetico delle caseine in razze caprine allevate in Lombardia. *E' stato condotto uno studio della variabilità genetica delle caseine in sei razze caprine allevate in Lombardia. La tipizzazione, condotta a livello proteico mediante isolettrofocalizzazione, è preliminare ad un approccio molecolare dettagliato su un numero più limitato di soggetti, scelti in base a particolari fenotipi caseinici.*

KEY WORDS: casein, goat, genetics, polymorphisms.

INTRODUCTION – In the last years, the genetic polymorphism of goat caseins has raised a considerable research interest due to relationships with milk quality and technological properties (Martin *et al.*, 2002). The four caseins, α_{s1} -casein (CSN1S1), β -casein (CSN2), α_{s2} -casein (CSN1S2), and k-casein (CSN3), are coded by a cluster of genes (Ferretti *et al.*, 1990; Threadgill and Womack, 1990). Due to the tight association occurring among casein genes, the estimation of the relationship between casein variants and milk traits can be improved by considering the entire casein haplotype instead of single gene typing, as already shown in cattle (Boettcher *et al.*, 2004). Caprine casein typing, however, requires great analytical effort due to the high polymorphism shown, as well as to the presence of quantitative variations. Phenotype screening at the protein level provides useful preliminary information on the genetic polymorphisms occurring at the DNA level. The aim of this work was to screen the casein variation of goat breeds reared in Lombardy by the use of the isoelectrofocusing (IEF). The investigation was preliminary to a more accurate typing, performed at the DNA level, on a selected sample for breeding of goat lines devoted to specific productions (cheese making or fresh milk with nutritional properties).

MATERIAL AND METHODS – Milk samples were collected from the following goat breeds: Saanen (SA), Camosciata (CA), Verzaschese (VZ), Orobica (OR), Frisa (FR), and Bionda dell'Adamello (BA). Sampling was performed in different flocks of Lombardy, characterised for the presence of local breeds and for the official recording of goats' productions. Casein phenotyping was carried out by isoelectric focusing (IEF) according to Caroli *et al.* (2001).

RESULTS AND CONCLUSIONS – The phenotype frequencies at CSN1S1, CSN1S2, and CSN3 are shown respectively in table 1, 2, and 3. At IEF level, typing of CSN1S1 allows to define three levels of expression of this casein: 0 (including genotypes with null or weak alleles), 2 (including genotypes with two strong alleles), and 1 (other genotypes). A more detailed definition of the two variants carried by each individual needs a complex molecular approach analysing several polymorphisms, as described by Sacchi *et al.* (2005). However, the preliminary IEF typing provided useful information on the CSN1S1 variation in Lombardy goats. In par-

ticular, animals with low CSN1S1 level still represents a high percentage of the population (68%), and are mainly concentrated in Orobica breed (84%). Cosmopolite breeds and Verzaschese show rather high frequency of animals carrying both strong CSN1S1 alleles (SA: 27%; CA: 22%; VZ: 18%).

Table 1. Distribution of animals per breed as a function of CSN1S1 level.

CSN1S1 (level)	BA	SA	CA	FR	VZ	OR	Total
0	10	9	15	124	71	648	877
1	5	10	34	103	65	99	317
2	1	7	14	8	29	24	86
Total	16	26	63	235	165	771	1280

As far as CSN1S2 is concerned, IEF allows identifying four variants associated to a normal CSN1S2 content: CSN1S2*A, B, C, and G (table 2). The last allele was identified at the protein level by IEF (Erhardt *et al.*, 2002). Recently, it's most probably coincidence with CSN1S2*E allele, characterised at the DNA level (Lagonigro *et al.*, 2001), has been widely discussed (Sacchi *et al.*, 2005), and the importance of casein variant identification taking into account also the phenotypic expression has been focused. The G variant, except one heterozygous animal (AG) in CA and OR respectively, occurred in Frisa breed. Frequencies of CSN1S2 variants per breed are shown in table 4. A clear predominance was found for CSN1S2*A, ranging from 0.81 to 0.93. The B variant occurred in all breeds, but with noticeable differences in its incidence, ranging from 0.01 (VZ) to 0.19 (BA). The C variant was found with the highest frequency (0.09) in VZ.

Table 2. Distribution of animals per breed as a function of CSN1S2 phenotype.

CSN1S2	BA	SA	CA	FR	VZ	OR	Total
AA	10	22	55	157	133	638	1018
AB	6	4	2	49	3	85	150
AC			4	11	26	28	69
AG			1	11		1	13
BB			1	2		11	14
BC				3		3	6
CC					1	1	2
CG				1			1
GG				2			2
Total	16	26	63	236	163	767	1275

Table 3. Distribution of animals per breed as a function of CSN3 IEF phenotype.

CSN3	BA	SA	CA	FR	VZ	OR	Total
AA	12	22	54	199	135	441	867
AB	3	4	8	35	27	266	343
BB	1					58	59
Total	16	26	62	234	162	765	1269

Recently, extensive polymorphism was shown in goat CSN3 coding gene. A total of 13 polymorphic sites were identified in domestic goat (Jann *et al.*, 2004) allowing the identification of 14 DNA alleles corresponding to 11 protein variants. At the protein level, only two variants can be identified by IEF, because all the CSN3 variants found in domesticated goat so far cluster in two groups on the basis of the isoelectric point (Prinzenberg

et al., 2005). The phenotype distribution shown in table 3 is, therefore, relative to these two IEF variants, which could also be named as A^{IEF} and B^{IEF} to avoid confusions with CSN3 alleles detectable at the DNA level, as proposed by Prinzenberg *et al.* (2005). The predominant IEF variant was CSN3*A^{IEF} in all breeds (table 4), with frequency ranging from 0.75 (OR) to 0.94 (CA). The rather high frequency of CSN3*B^{IEF} in OR (0.25) is remarkable, and could allow to assess relationships with productive traits. An interesting difference has been already suggested by Chianese *et al.* (2000) between the two CSN3 IEF variants, as B^{IEF} seems to be associated with higher milk casein content than A^{IEF}. If this result will be confirmed by the future development of the present investigation, local breeds like Orobica, as well as Bionda dell'Adamello, could be valorised exploiting B^{IEF} variant effects.

Table 4. Frequencies of CSN1S2 and CSN3 IEF variants in the different breeds.

IEF Variant	BA	SA	CA	FR	VZ	OR
CSN1S2*A	0.81	0.92	0.93	0.82	0.90	0.91
CSN1S2*B	0.19	0.08	0.03	0.12	0.01	0.07
CSN1S2*C	0.00	0.00	0.03	0.03	0.09	0.02
CSN1S2*G	0.00	0.00	0.01	0.03	0.00	0.00
CSN3*A ^{IEF}	0.84	0.92	0.94	0.93	0.92	0.75
CSN3*B ^{IEF}	0.16	0.08	0.06	0.07	0.08	0.25

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