Short communication: The unusual genetic trend of α_{S1} -casein in Alpine and Saanen breeds

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ABSTRACT

Genetic variation at the α_{S1} -casein locus (*CSN1S1*) is recognized as being crucial in the selection of dairy goats for cheese yield. At this locus, the existence of alleles that have strong, intermediate, weak, and null favorable effects on cheese yield and curd firmness is well known. Selection for alleles that have a strong favorable effect has been deliberately carried out, especially in France. In fact, the importance of α_{S1} -casein in selection was recently confirmed in the selling policies of semen, where bucks are marketed according to their genotypes. We evaluated genotypes and alleles frequencies at the α_{S1} -casein locus in 491 Italian Saanen and Alpine goats and compared them with previous data to investigate their evolution over the past decade. We also estimated soft cheese yield in a subset of the most represented genotypes to quantify the economic importance of considering the genetic trend of α_{S1} -casein genotype frequencies. We found a significant increase in frequency of the allele with the strongest favorable effect, A (+12 and +13%), and of the intermediate allele E (+17 and +7%) in Saanen and Alpine goats, respectively. Surprisingly, the frequency of the strong allele B decreased strikingly over time (–12% in Saanen, –6% in Alpine from 2004 to 2012). This is consistent with the current marketing of semen, in that bucks that are homozygous for strong (AA and BB) and intermediate alleles (EE) and even heterozygous for these alleles (BE and AE) are considered equal. It is worth noting that this practice strongly penalizes the best breeders that have flocks composed almost entirely of goats that are homozygous for strong alleles. For heterozygous goats, we estimated an economic loss of €85 and €215 per goat per lactation, respectively, for AE and BE, compare with AA and BB genotypes. The marketing of buck semen should clearly differentiate these 2 alleles to ensure the best economic genetic progress at this locus.

Key words: genetic trend, α_{S1} -casein allele, goat, cheese yield

Short Communication

Over the last 30 yr, selection in goats has been directed toward identification of genetic components related to milk protein composition. Starting with the pioneering work of Grosclaude and colleagues (1987), α_{S1} -casein has received particular attention. α_{S1} -Casein, coded by the single autosomal gene *CSN1S1*, is one of the most important milk proteins in goat, although, in this species, it represents on average the lowest amount of the total casein content. The genetic variants of the *CSN1S1* affect casein, protein, and fat contents, as well as cheese yield and quality (Park, 2007). The 18 known alleles are grouped into 4 classes that are associated with the α_{S1} -casein content in milk: the strong alleles (A, A', B₁, B₂, B₃, B₄, B', C, H, L, and M) produce almost 3.5 g/L of α_{S1} -casein each, the intermediate alleles (E and I) produce 1.1 g/L, the weak alleles (F and G) produce 0.45 g/L, and the null alleles (O₁, O₂, and N) produce no α_{S1} -casein (Martin et al., 1999; Bevilacqua et al., 2002; Caroli et al., 2007). Until now, genetic selection has focused on improving the frequencies of the alleles associated with the greatest casein production, especially in France and in the Alpine and Saanen breeds. The aim is to increase the aptitude of these breeds for cheese making. Despite the production improvements in these breeds, where bucks are also selected according to their *CSN1S1* genotype, the genetic potential of goats has not been exploited as much as in other species and deserves further attention.

To explore the effects of genetic selection on α_{S1} -casein in the Italian Saanen and Alpine breeds, we evaluated how the frequencies of the major *CSN1S1* allelic variants have changed over the past 10 yr in the 2 breeds. Moreover, we assessed the association of the most frequent genotypes with estimated soft cheese yield to evaluate recent trends and determine whether further selection improvement is still possible.

Blood samples were collected from 363 Alpine (347 goats and 16 bucks) and 132 Saanen (126 goats and 6

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bucks), reared in 9 farms across northern Italy. In all 9 farms, the goats were reared under intensive systems, thus providing better feeding control. Animals were milked twice daily and their reproduction was mainly based on natural mating. For 322 goats, individual production data were also available: milk yield (kg/d) and fat and raw protein contents (% wt/wt) were determined by using the infrared method (Foss Milkoscan FT 6000; Foss, Hillerød, Denmark), and urea content (mg/dL) was determined using the pH-differential technique (ISO 14637; ISO, 2006). Monthly production data in 2010 and 2011 (around 12 measurements for each animal) were supplied by the Lombardy Regional Breeders Association (ARAL) together with pedigree information (sire and dam) for 364 animals. Artificial insemination must have been moderately used because 20% of these goats had French ancestors. In total, 119 out of 130 sires had daughters on just one farm, and the other sires had progeny in no more than 2 farms. The sires had 3.5 ± 3.3 daughters (mean $\pm$ SD) and only 3 of them had more than 10 daughters (22 at maximum).

Genomic DNA was extracted using the commercial kit ReliaPrep Blood gDNA Miniprep System (Promega, Madison, WI) starting from 200 μ L of whole blood. Genotype analysis at the *CSN1S1* locus was performed on genomic DNA according to different protocols and methods: the *CSN1S1**A group (0₁ allele is here classified as A), B group (E allele is here classified as B), F, and N alleles were investigated using the PCR-RFLP assay described by Ramunno et al. (2000, 2005). The 0₁ allele was distinguished from the A group according to the allele specific (AS)-PCR described by Cosenza et al. (2003, 2008), and the E allele from the B group according to Dettori et al. (2009).

To evaluate the effect of α_{S1} -casein genotypes on cheese-making, theoretical soft cheese yield was estimated for a subsample of 212 animals from 7 farms where the genotypes with the highest frequencies (AA, AE, AF, and EE) were distributed homogeneously. Soft cheese yield was estimated using the equation proposed by Zeng et al. (2007) and based on the results of specific cheese-making trials:

$$CY = 5.72 \times F + 0.29 \times TP + 0.76$$

$$(r^2 = 0.81),$$

where CY = cheese yield (kg of cheese/100 L of milk); F = fat %; TP % = total protein %, calculated starting from raw protein (RP; % wt/wt) net of NPN as follows:

$$TP = RP \times (100 - NPN)/100;$$

NPN, in turn, was calculated using the following model (L. Rapetti, unpublished data):

$$NPN (\% \text{ of total N}) = 0.247 \times \text{urea (mg/dL)} + 3.637$$

$$(r^2 = 0.82, \text{ root mean square error} = 0.94, P < 0.001).$$

A χ^2 test was applied to analyze the distribution of genotypic and allelic frequencies using the statistical software JMP of SAS (JMP 9.0.2, 2010; SAS Institute Inc., Cary, NC). The following mixed model was used to analyze the associations between genotype and soft cheese yield:

$$y_{ijklmno} = m + CSN1S1_i + \text{Breed}_j + \text{Farm}_k(\text{Breed}_j) \\ + \text{Goat}_l + \text{Lac}_m + \text{Sire}_n + b(\text{DIM}) + e_{ijklmno},$$

where $y_{ijklmno}$ = cheese yield (kg of cheese/100 L of milk) estimated for each monthly production datum; m = overall mean; $CSN1S1_i$ = fixed effect of the i th genotype (AA, AE, AF, EE); Breed_j = fixed effect of the j th breed (Saanen, Alpine); $\text{Farm}_k(\text{Breed}_j)$ = j th breed nested within k th farm (1, ..., 5); Goat_l = random effect of the l th goat; Lac_m = fixed effect of the m th order of lactation (1, 2, ≥ 3); Sire_n = random effect of the n th sire; $b(\text{DIM})$ = covariate for days in milk; and $e_{ijklmno}$ = random residual effect.

The sire and the goat itself were fitted as random effects to account for paternal half-sib relationship, as suggested by Strucken et al. (2011).

We compared the allele frequencies measured in the present work with those reported in 2 studies conducted in 2005 (S. Chessa, unpublished data; Budelli et al., 2005) on 99 Saanen goats and 88 Alpine goats sampled on farms other than those of the present work (Table 1). Allele frequencies observed in our research showed statistically significant differences (χ^2 test, $P < 0.001$) compared with those of 8 yr before. We observed increases in the frequency of the strong allele A (+12 and +13%) and the intermediate allele E (+17 and +7%) and a reduction in frequency of the weak allele F (−18 and −12%) in Saanen and Alpine breeds, respectively. The most unexpected result was the marked decrease in frequency of the strong allele B (−12 in Saanen and −6% in Alpine; Table 1).

Our results are consistent with those reported in previous work where frequencies at this locus recorded in 1985 and 1994 were compared. In that work, Grosclaude et al. (1994) highlighted an increase in the frequency of the A allele in both breeds, a slight decrease of the B allele, a predominance of the E allele in Saanen, and a reduction of the F allele in Alpine breeds.

Our observation is consistent with recent trends in buck selection (Caprine Genetic Contract 2013; www.aral.lom.it). Semen companies propose that the best animals for AI are bucks that are either homozygous for

Table 1. Allele frequencies at the α_{S1} -casein gene (*CSN1S1*) locus and estimated content variation of α_{S1} -casein in Saanen and Alpine goats

Allele	Saanen		Alpine		Estimated content variation of α_{S1} -CN (g/L)	
	2004 (n = 97)	2012 (n = 132)	2004 (n = 88)	2012 (n = 359)	Saanen	Alpine
A	0.23	0.35	0.43	0.56	+ 0.42	+ 0.46
B	0.18	0.06	0.21	0.15	-0.42	-0.21
E	0.26	0.43	0.11	0.18	+ 0.19	+ 0.08
F	0.32	0.14	0.23	0.11	-0.08	-0.05
N	0.01	0.00	0.01	0.00	—	—
O ₁	0.00	0.02	0.01	0.00	—	—

strong (AA and BB) and intermediate alleles (EE) or heterozygous (BE and AE). Thus, bucks with AA, AE, or EE genotypes at the α_{S1} -casein locus are marketed in the same way, even though their theoretical production of α_{S1} -casein is 7, 4.6, and 2.2 g/L, respectively. This could explain the great increase of *CSN1S1**E observed in both breeds. The decrease in frequency of the B allele cannot be disregarded because genetic loss proceeding in this direction will be relevant, considering the increase of an intermediate allele at the expense of this strong allele. It has been suggested that precise genotyping was not carried out to differentiate the B from the E allele due to the higher costs and complexity required for this analysis, at both the protein and DNA levels. The reduction of the B allele could also be due to its lower frequency in the French breeds (Grosclaude et al., 1994), from which most selected bucks and semen used in Italy derive.

To better evaluate the consequences of buck selection, we calculated the expected variation of α_{S1} -casein content (g/L) in the 2 compared time periods, multiplying the theoretical production value associated with every allele by its frequency (Table 1). In Alpine goats, we observed an increase of 0.56 g/L for each animal, and 90% of this increase was linked to animals carrying strong alleles. The Alpine breed showed a gain of 7% (+13% of A allele, -6% of B allele) in strong alleles, resulting in greater production of α_{S1} -casein (+0.50 g/L for each goat), accompanied by a slight increase in the intermediate allele that translated to +0.06 g/L per animal. In comparison, in Saanen goats, the variation of α_{S1} -casein production was less relevant (only +0.22 g/L for each goat) and was entirely due to reduction of the F allele frequency (-18%) and an increase of the E allele frequency (+17%). The differences observed could be interpreted as more efficient selection for the alleles associated with greater α_{S1} -casein content in the Alpine compared with Saanen breed. This hypothesis is also supported by the lack of Hardy-Weinberg equilibrium (χ^2 P -value = 0.005) in Alpine goats, unlike the Saanen

breed, which did show Hardy-Weinberg equilibrium at this locus.

The A allele is associated with a smaller micellar size and consequently better cheese yield (Remuef, 1993), whereas the E allele seems to negatively affect the technological properties of milk (Martin et al., 1999). Therefore, the higher frequency of the A allele observed in the Alpine breed should yield milk with higher cheese-making aptitude compared with milk produced by Saanen goats. Caravaca and colleagues (2011) reported significantly better curd-firming properties for goats homozygous for *CSN1S1**E than for *CSN1S1**B in the Murciano-Granadina breed. Those authors attributed the discrepancies to physiological and genetic differences between the breeds analyzed.

To better understand the role of genetic variants in cheese-making, we evaluated the theoretical soft cheese yield associated with different α_{S1} -casein genotypes. We considered only the 4 most represented genotypes (AA, EE, AE, and AF), which were well distributed in the 5 farms. Cheese yield and distribution of the 4 genotypes are reported in Table 2. Estimated cheese yield was significantly affected by breed ($P < 0.001$) and by genotype ($P = 0.003$). Alpine goats showed higher ($P < 0.001$) cheese yield production (Table 3), probably because of a significantly higher milk fat concentration in Alpine than in Saanen (3.48 vs. 3.24%; $P < 0.001$), which is known to result in higher cheese yield (Guo et al., 2004).

Table 2. Least squares means ($\pm$ SE) of cheese yield according to α_{S1} -casein gene (*CSN1S1*) genotype with number of samples genotyped (n)

Genotype	n	Cheese yield (kg/100 L of milk)
AA	79	22.28 $\pm$ 0.30 ^a
EE	29	21.40 $\pm$ 0.42 ^{ab}
AE	70	21.21 $\pm$ 0.29 ^b
AF	34	20.52 $\pm$ 0.41 ^b

^{a,b}Means with different superscript letters are significantly different ($P < 0.05$) by Tukey's honestly significant difference (HSD) test.

Table 3. Least squares means ($\pm$ SE) of milk production, fat and protein percentages, and cheese yield, according to breed with number of samples genotyped (n)

Breed	n	Milk yield (kg of milk/d)	Fat (%)	Total protein (%)	Cheese Yield (kg/100 L of milk)
Saanen	68	2.99 $\pm$ 0.08	3.22 $\pm$ 0.05	3.34 $\pm$ 0.04	20.16 $\pm$ 0.26
Alpine	144	3.18 $\pm$ 0.08	3.64 $\pm$ 0.05	3.38 $\pm$ 0.04	21.47 $\pm$ 0.45
<i>P</i> -value		NS	<i>P</i> < 0.001	NS	<i>P</i> < 0.001

The AA genotype showed significantly higher cheese production than the AE or AF genotypes. The EE and AE genotypes were associated with reductions in yield of 0.88 and 1.07 kg of cheese/100 L of milk, respectively.

The importance of considering the α_{S1} -casein genotype in selection is easily understood if we analyze, for instance, the statistical difference observed ($P < 0.05$ at Tukey's honestly significant difference test; Table 2) between goats carrying AA and AF or AE genotypes from an economic point of view. Assuming, in direct selling, a medium commercial price in the Italian market of €14/kg for soft goat cheese and a medium production of 568 L of milk per animal per lactation, the difference between AA and AF or AE genotypes in cheese yield (1.76 kg or 1.07 kg of cheese per 100 L of milk) results in an economic loss/gain of €140 or €85 per goat per lactation (1.76 or 1.07 kg $\times$ €14 $\times$ 5.68 L). Just one weak allele instead of a stronger allele can cause a consistent economic loss for the farmer. For the BB and BE genotypes, it is possible to suppose only a purely indicative economic gain/loss of €215, because the small number of goats carrying these genotypes in our sample does not allow a reliable estimate. It is worth noting that our soft cheese yield calculation was biased because we did not consider a direct measure of micellar size. Theoretically, an unbiased measurement could increase further the effects observed for our different genotypes.

The role of the α_{S1} -casein allelic evolution should be carefully considered in general improvements of goat breeding. A deeper analysis of the other caseins, determination of casein haplotypes, and association studies could provide further information of the real effects of casein variants on milk quality. Particular attention should be paid to sire choice, especially with AI. Semen of bucks carrying at least one strong allele (A or B) should not receive the same genetic evaluation as animals carrying an intermediate allele (E). Even if the cheese yield of animals with AA and EE genotypes is not significantly different, using AI semen from males carrying the E allele would increase the frequency of AE heterozygotes, and consequently goats, resulting in an important decrease in production (AE –1.07 kg of cheese/100 L of milk compared with AA). This is espe-

cially true for farms with a high prevalence of AA, BB, and AB α_{S1} -casein genotypes, where an increase of the AE genotype could significantly decrease the dairy's performance.

Although the polymorphisms in α_{S1} -casein were established 20 yr ago and their importance in selection of dairy goat breeds is known, the analysis required for complete genotyping of the locus and associated costs do not allow all farmers to access this information when choosing bucks. To overcome limitations in genotyping, an improvement of genomic technologies with a more accurate identification of α_{S1} -casein alleles would be useful.

REFERENCES

- Bevilacqua, C., P. Ferranti, G. Garro, C. Veltri, R. Lagonigro, C. Leroux, E. Pietrolà, F. Addeo, F. Pilla, L. Chianese, and P. Martin. 2002. Interallelic recombination is probably responsible for the occurrence of a new α_{S1} -casein variant found in the goat species. *Eur. J. Biochem.* 269:1293–1303.
- Budelli, E., S. Chessa, F. Chiatti, P. Bolla, and A. Caroli., 2005. Variabilità degli aplotipi caseinici nella razza caprina Camosciata. *Sci. Tecn. Latt. Cas.* 56:205–220.
- Caravaca, F., J. L. Ares, J. Carrizosa, B. Urritia, F. Baena, J. Jordana, B. Badaoui, A. Sánchez, A. Angiolillo, M. Amills, and J. M. Serradilla. 2011. Effects of α_{S1} -casein (*CSN1S1*) and κ -casein (*CSN3*) genotypes on milk coagulation properties in Murciano-Granadina goats. *J. Dairy Res.* 78:32–37.
- Caroli, A., F. Chiatti, S. Chessa, D. Riganese, E. M. Ibeagha-Awemu, and G. Erhardt. 2007. Characterization of the casein gene complex in West African goats and description of a new α_{S1} -casein polymorphism. *J. Dairy Sci.* 90:2989–2996.
- Cosenza, G., R. Illario, A. Rando, P. Di Gregorio, P. Masina, and L. Ramunno. 2003. Molecular characterization of the goat *CSN1S1**01 allele. *J. Dairy Res.* 70:237–240.
- Cosenza, G., A. Paucillo, D. Gallo, L. Colimoro, A. D'Avino, A. Mancusi, and L. Ramunno. 2008. Genotyping at the *CSN1S1* locus by PCR-RFLP and AS-PCR in a Neapolitan goat population. *Small Rumin. Res.* 74:84–90.
- Dettori, M. L., G. M. Vacca, V. Carcangiu, M. Pazzola, M. C. Mura, and A. M. Rocchigiani. 2009. A reliable method for characterization of the goat *CSN1S1* E allele. *Livest. Sci.* 125:105–108.
- Grosclaude, F., M. F. Mahe, G. Brignon, L. Distasio, and R. Jeunet. 1987. A Mendelian polymorphism underlying quantitative variations of goat alpha-s1-casein. *Genet. Sel. Evol.* 19:399–412.
- Grosclaude, F., G. Ricordeau, P. Martin, F. Remeuf, L. Vassal, and J. Bouillon. 1994. From gene to cheese: Polymorphism of alphas1-casein in goat milk, its effects and development. *Prod. Anim.* 7:3–19.
- Guo, M., Y. W. Park, P. H. Dixon, J. A. Gilmore, and P. S. Kindstedt. 2004. Relationship between the yield of cheese (Chevrè) and chemical composition of goat milk. *Small Rumin. Res.* 52:103–107.

- ISO (International Standardization Organization). 2006. ISO 14637: Milk. Determination of urea content. Enzymatic method using difference in pH (Reference method). ISO, Geneva, Switzerland.
- Martin, P., M. Ollivier-Bousquet, and F. Grosclaude. 1999. Genetic polymorphism of casein: A tool to investigate casein micelle organization. *Int. Dairy J.* 9:163–171.
- Park, Y. W. 2007. Impact of goat milk and milk products on human nutrition. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 2 (081). CAB International, Wallingford, UK.
- Ramunno, L., G. Cosenza, M. Pappalardo, N. Pastore, D. Gallo, P. Di Gregorio, and P. Masina. 2000. Identification of the goat *CSN1S1*(F) allele by means of PCR-RFLP method. *Anim. Genet.* 31:342–343.
- Ramunno, L., G. Cosenza, A. Rando, A. Pauciullo, R. Illario, D. Gallo, D. Di Berardino, and P. Masina. 2005. Comparative analysis of gene sequence of goat *CSN1S1* F and N alleles and characterization of *CSN1S1* transcript variants in mammary gland. *Gene* 345:289–299.
- Remuef, F. 1993. Influence du polymorphisme genetique de la caseine S_1 caprine sur les caracteristique physico-chimiques et technologiques du lait. *Lait* 73:549–557.
- Strucken, E. M., D. J. de Koning, S. A. Rahmatalla, and G. A. Brockmann. 2011. Lactation curve models for estimating gene effects over a timeline. *J. Dairy Sci.* 94:442–449.
- Zeng, S. S., K. Soryal, B. Fekadu, B. Bah, and T. Popham. 2007. Predictive formulae for goat cheese yield based on milk composition. *Small Rumin. Res.* 69:180–186.