Genetic effects of α_{s1}-casein locus on estimated cheese yields in Italian Alpine and Saanen goats

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ABSTRACT: Cheese yield in dairy goat is influenced by milk fat and protein content and also by α_{s1}-casein polymorphisms. The A allele at this locus is associated to better cheese yield due to smaller micellar casein, whereas the E allele seems to negatively affect milk technological properties. We evaluated the genetic effects of *CSN1S1* alleles and genotypes on two estimated soft cheese yields, calculated from individual production data. Our results confirm the significant differences of *CSN1S1* alleles on estimate cheese yield and on fat and protein content of goat milk. A allele showed the highest positive effect on all the parameters recorded and calculated. The lowest effect of the F allele on cheese yield and goat dairy traits was confirmed. The E allele showed a significant negative allele substitution effect for cheese yield and protein %.
Keywords: dairy goat; α_{s1}-casein; genetics

Introduction

Several studies showed the association of α_{s1}-casein polymorphisms with quantitative and qualitative traits of milk production (Martin et al., 2002; Zeng et al., 2007). The α_{s1}-casein locus (*CSN1S1*) is highly polymorphic in goat, and at least 18 different variants have been identified so far (Devold et al., 2010). These can be grouped into 4 classes considering the milk content of α_{s1}-casein: "strong alleles" (A, A', B1, B2, B3, B4, B', C, H, L, and M); "intermediate alleles" (E and I alleles); "weak alleles" (F and G) L; "null alleles" (01, 02 and N) producing no α_{s1}-casein (Martin et al., 1999; Caroli et al. 2007).

The polymorphisms of goat α_{s1}-casein were widely studied and allowed production improvements especially in cosmopolitan breeds, where bucks are selected also according to their *CSN1S1* genotype. Genetic selection in all these years aimed to improve the frequencies of the alleles associated to better cheese yield especially in Alpine and Saanen breeds, which are mainly selected in France. Cheese yield is commonly calculated by breeders and breeders association only from milk yield, fat and protein contents, even if this trait is influenced by *CSN1S1* genotype. In particular, the A allele is associated to better cheese yield due to smaller micellar casein (Remuef, 1993), whereas the E allele seems to negatively affect milk technological properties (Martin et al. 1999).

In order to analyze the recent selective indications, we evaluated the allelic substitution effects and the dominance effects of *CSN1S1* alleles and genotypes on estimated soft cheese yields and single traits used to calculate cheese yields themselves.

Materials and Methods

The research was carried out on 216 Alpine and 105 Saanen goats, intensively reared in 8 farms spread all over Lombardy, in northern Italy.

Phenotypic data. For the 321 goats analyzed, individual production data were available: milk yield (kg/d, MILY), fat (%, FAT) and raw protein content (%, wt/wt, PRO). Production data were measured monthly during lactations of year 2011 (ca. 8 measurements for each animal) and were provided by the Lombardy Regional Breeders Association (ARAL).

Genotypic data. Genotype analysis at the *CSN1S1* locus was performed on genomic DNA, according to different protocols: the A, B, F and N alleles were detected using the PCR-RFLP (PCR-Restriction Fragment Length Polymorphism) assay described by Ramunno et al. (2000, 2005); afterwards the 01 allele was distinguished from the A allele according to the AS-PCR (Allele Specific-PCR) described by Cosenza and colleagues (2003); E allele was distinguished from the B one according to Dettori et al. (2009), with the same type of PCR.

Statistical analysis. Theoretical soft cheese yield production as a function of α_{s1}-casein genotypes was estimated for a subset of 314 animals. 7 goats, carrying N or 01 allele, were excluded from the analysis because these alleles resulted underrepresented. Soft cheese yields were estimated using the equation proposed by Zeng et al. (2007) and the one used by regional breeders association (personal communication, 2014), respectively:

$$CY1 = 5.72 \times FAT + 0.29 \times TP + 0.76$$
$$CY2 = 0.9 \times FAT + 3.8 \times PRO + 1.47$$

where CY = cheese yield (kg cheese/ 100 L milk);

FAT = fat %, TP % = total protein %, PRO = protein %.

The mean value of the single data recorded monthly on each animals were used for FAT, TP and PRO.

The following ANOVA models were used to analyze the genotype effect on soft cheese yields, milk yield, protein and fat %:

$$y_{ijkl} = m + CSN1S1_i + Breed_j + Farm_k(Breed_j) + Goat_l + e_{ijkl}$$

where y_{ijkl} = the variable (CY1, CY2, FAT, PRO, MILY); m = overall mean; $CSN1S1_i$ = fixed effect of the ith genotype (n.=10 genotypes) ; $Breed_j$ = fixed effect of the jth breed (Saanen, Alpine); $Farm_k(Breed_j)$ = j^{th} breed nested within k^{th} farm; $Goat_l$; = random effect of the lth goat; e_{ijkl} = random residual effect. To determine the allelic substitution and the dominance effects of A, B, E and F alleles, the $CSN1S1_i$ effect in the above model was modified as follow. To analyze the allelic substitution effect at the $CSN1S1$ locus we assigned, according to Dagnachew et al., 2011:

1= if the locus is homozygous for the ith allele
0= if the locus is heterozygous for the ith allele
-1=if there is no the ith allele at the locus

To analyze the dominance effect at the CSN1S1 locus we assigned:

1= if the locus is heterozygous
0=if the locus is homozygous for any of the 2 alleles

All the statistical analysis were carried out with the software JMP of the SAS Institute (JMP 9.0.2, 2010).

Results and Discussion

In the Alpine breed, the frequencies of the alleles A, B, E, and F at the $CSN1S1$ locus resulted 0.61, 0.7, 0.19 and 0.11 respectively. In the same order, the allelic frequencies for Saanen were 0.35, 0.08, 0.40 and 0.15 respectively, with a significant difference at chi square test (P<0.001) between breeds for A and E allele. Genotypes EE, EF, BE and AA resulted significantly different at chi square test (P<0.001) between breeds. In the Alpine, allele A is the most frequent, whereas in Saanen breed, the allele E is reported with the highest frequency.

To better understand the role of genetic variants, we evaluated the theoretical soft cheese yields according to the two formulas expressed in material and methods, starting from the mean values of fat and protein recorded for each animal. The estimate proposed by Zeng and colleagues (CY1) mainly rewards fat % in milk, vice versa the one proposed by ARAL (CY2) emphasize protein %. Both the estimate cheese yields resulted significantly affected by breed, farm and genotype in the sample analyzed in present work (P<0.001). The Least Square Mean (LSM) values for CY1 and CY2 for each genotype and for milk yield, fat and protein % are presented in table 1 and 2 respectively. Considering CY1 as variable, a significantly higher production was observed for the AA genotype in respect to the EF and FF genotypes (P<0.05 at Tukey's HSD test). Also for CY2,

AA genotype showed the highest LSM, significantly different from AF, BF, EF, EE and AE. It is worth mentioning that semen companies propose as the best animals for the artificial insemination (AI), bucks homozygous for strong (AA and BB) and intermediate alleles (EE), but also the heterozygous BE and AE.

Table 1. LSM (± SE) of estimate cheese yields by CSN1S1 genotypes

Genotype	nº	LSM ± SE [1]	
		CY1, kg/100L milk	CY2, kg/100L milk
AA	94	22.09 ±0.26 [a]	18.46 ± 0.13 [a]
AB	23	21.13 ± 0.46 [ab]	18.22 ± 0.23 [ab]
AE	80	21.15 ± 0.26 [ab]	17.80 ± 0.13 [bd]
AF	45	21.09 ± 0.34 [ab]	17.38 ± 0.17 [bcd]
BB	6	21.33 ± 0.88 [ab]	18.15 ± 0.44 [abcd]
BE	6	20.75 ± 0.88 [ab]	17.24 ± 0.44 [abcd]
BF	6	19.19 ± 0.88 [ab]	16.33 ± 0.44 [cd]
EE	32	21.69 ± 0.40 [ab]	17.37 ± 0.20 [bcd]
EF	17	19.98 ± 0.54 [b]	16.75 ± 0.27 [c]
FF	5	18.64 ± 0.98 [b]	16.93 ± 0.49 [abcd]

Table 2. LSM (± SE) of milk yield, fat (FAT) and protein (PRO) content by CSN1S1 genotypes

Genotype	LSM ± SE [1]		
	MILY, kg/d	FAT, %	PRO, %
AA	2.99 ± 0.08	3.57 ± 0.05 [a]	3.63 ± 0.03 [a]
AB	3.09 ± 0.14	3.40 ± 0.08 [ab]	3.60 ± 0.05 [ab]
AE	3.12 ± 0.08	3.41 ± 0.04 [ab]	3.49 ± 0.03 [bc]
AF	3.16 ± 0.10	3.41 ± 0.06 [ab]	3.38 ± 0.04 [cd]
BB	3.29 ± 0.26	3.44 ± 0.15 [ab]	3.57 ± 0.10 [abcd]
BE	3.14 ± 0.26	3.35 ± 0.15 [ab]	3.36 ± 0.10 [abcd]
BF	3.05 ± 0.26	3.08 ± 0.15 [ab]	3.18 ± 0.10 [cd]
EE	2.99 ± 0.12	3.51 ± 0.07 [ab]	3.35 ± 0.04 [cd]
EF	3.06 ± 0.16	3.21 ± 0.09 [b]	3.26 ± 0.06 [d]
FF	3.17 ± 0.29	2.98 ± 0.17 [b]	3.36 ± 0.11 [abcd]

[1]Different letters show significant differences (P<0.05) to Tuckey Kramer HSD test

LSM for milk yield did not show any statistical difference between genotypes (table 2). Fat % showed the same statistical differences observed for CY1 due to the great importance of fat content in the formula proposed by Zeng and colleagues. There is not a full correspondence between CY2 and protein %, even if the significant differences between AA, AE and EE genotypes were confirmed.

For the allele substitution effects, reported in table 3, a significantly higher effect (P<0.001) was observed for

the A allele in both cheese yields, respectively 0.70 and 0.63 kg of cheese/100 L of milk and the lowest significant effect (P<0.001) for the F allele (-1.05 and -0.80 kg of cheese/100 L of milk). Moreover, for the CY2 parameter also the E allele showed a significant (P<0.01) negative effect (-0.36 kg of cheese/100 L of milk). The AE genotype showed a strong negative dominance effect (-0.90 kg of cheese/100 L of milk) on CY1, while for the AF and EF genotypes a significantly negative dominance effect on CY2 was observed. As for the LSM, fat% showed the same significant allele substitution and dominance effects for CY1. Similar genetic trends for A and E allele are reported for fat and protein % by Vasquez-Flores et al., 2012.

The trend of the allele substitution effects for fat and protein % resulted similar to, respectively, CY1 and CY2. Finally, for the dominance effect, fat% showed specular significant effect to CY1, whereas for protein % a similar effect to CY2 was observed, with the only difference in the BF genotype (table 3 and 4).

Conclusion

Our results confirm the significant differences of *CSN1S1* alleles on estimate cheese yields and fat and protein content of goat milk. A allele shows the highest positive effect on both cheese yield estimates and also on fat and protein content. Also the lowest effect on goat dairy trait of the F allele is confirmed. The E allele shows a significant negative allele substitution effect for cheese yield and protein %. It is worth mentioning that the EE and AE genotypes determine a penalizing cheese yield, when protein % is the key factor of estimation. In this case selective indication should be carefully evaluated, as the rewarding system of goat semen assigns the same value to bucks homozygous for strong (AA) and intermediate alleles (EE) and for the heterozygous ones (AE).

Table 3. Allele substitution (α) and dominance (d) effect (mean ± SE) of cheese yield estimates (CY1, CY2)

		CY1, kg/100L milk			CY2, kg/100L milk		
		r^2	Effect	P	r^2	Effect	P
α	A	0.40	0.70 ± 0.19	<0.001	0.46	0.63 ± 0.1	<0.001
	B	0.38	-0.37 ± 0.31	N.S.	0.39	0.01 ± 0.17	N.S.
	E	0.37	-0.13 ± 0.21	N.S.	0.41	-0.36 ± 0.11	<0.01
	F	0.40	-1.05 ± 0.29	<0.001	0.44	-0.80 ± 0.15	<0.001
d	AB	0.26	-0.70 ± 0.51	N.S.	0.30	-0.09 ± 0.27	N.S.
	AF	0.38	-0.71 ± 0.46	N.S.	0.44	-1.02 ± 0.23	<0.001
	AE	0.32	-0.90 ± 0.32	<0.01	0.38	-0.32 ± 0.17	N.S.
	BE	0.38	-1.37 ± 0.10	N.S.	0.54	-0.50 ± 0.05	N.S.
	BF	0.19	-0.85 ±1.25	N.S.	0.65	-0.96 ± 0.53	N.S.
	EF	0.33	-1.37 ± 0.72	N.S.	0.36	-0.75 ± 0.32	<0.05

Table 4. Allele substitution (α) and dominance (d) effect (mean ± SE) fat (FAT) and protein (PRO) content

		FAT, %			PRO, %		
		r^2	Effect	P	r^2	Effect	P
α	A	0.40	0.12 ± 0.03	<0.001	0.44	0.14 ± 0.02	<0.001
	B	0.38	-0.07 ± 0.06	N.S.	0.36	0.015 ± 0.04	N.S.
	E	0.37	-0.02 ± 0.04	N.S.	0.39	-0.09 ± 0.02	<0.001
	F	0.40	-0.18 ± 0.05	<0.001	0.41	-0.17 ± 0.03	<0.001
d	AB	0.26	-0.12 ± 0.09	N.S.	0.30	0.01 ± 0.06	N.S.
	AF	0.37	-0.11 ± 0.09	N.S.	0.42	-0.24 ± 0.05	<0.001
	AE	0.32	-0.16 ± 0.05	<0.01	0.39	-0.05 ± 0.04	N.S.
	BE	0.38	-0.24 ± 0.17	N.S.	0.55	-0.07 ± 0.11	N.S.
	BF	0.44	-0.14 ± 0.21	N.S.	0.74	-0.22 ± 0.10	<0.05
	EF	0.33	-0.23 ± 0.12	N.S.	0.36	-0.14 ± 0.07	<0.05

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