Determining the frequencies of B1, B2, B3 and E alleles of the *CSN1S1* gene and their effects on milk yield and composition in Saanen goats

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Abstract

The aim of the study was to determine the frequencies of B1, B2, B3 and E alleles of the *CSN1S1* gene and their effects on milk yield and composition in the Saanen goat breed. Milk samples were collected to identify milk composition with Fourier transform ınfrared (FTIR) spectroscopy. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to characterize the alleles of *CSN1S1* gene. The allelic frequencies of the B1, B2, B3 and E alleles were 0.927, 0.073, 0.390, and 0.272, respectively. B1 and B2 alleles did not affect milk yield and composition. B3 allele had significant effects on protein, fat, total solid (TS), solid not fat (SNF), casein and lactose percentages, but not on lactose yield. E allele significantly influenced fat and TS percentages of milk in the Saanen goat breed. The protein (3.58%), fat (3.83%), TS (12.06%), SNF (8.60%) and casein percentages (2.80%) were higher in B3/B3 goats than in B3/NB3 (NB3: non-B3) and NB3/NB3 goats. For E allele, NE/NE (NE: non-E) goats displayed higher fat (4.06%) and TS (12.42%) percentages of milk than their E/E and E/NE counterparts. As a result, the potential for improving milk composition by selecting for B3 and E allele may be significant in Saanen goats.

Keywords: *Capra hircus*, PCR-RFLP, SNP, milk production
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Introduction

In ruminants, 80% of milk proteins consist of four types of casein (αS1, β, αS2 and κ) (Ramunno *et al*., 2004). Milk caseins are the main nutritional source for newborns in placental mammals (Caravaca *et al*., 2009). AlphaS1-casein (αS1-CN), beta-casein (β-CN), alphaS2-casein (αS2-CN) and kappa-casein (κ-CN) are expressed in goat milk and encoded by *CSN1S1, CSN2, CSN1S2* and *CSN3* genes, respectively (Martin *et al*., 2002). These are organised as a cluster in a 250 kilobase (kb) genomic DNA segment in chromosome 6 in goats (Martin *et al*., 2002).

The goat *CSN1S1* gene has ~16785 base pairs (bp), including 1138 bp of exonic regions and 15647 bp of intronic regions with a total similarity with the corresponding bovine sequence of about 57% (Ramunno *et al*., 2004). It is known that 18 alleles of *CSN1S1* gene have been identified so far, which are associated with different expression levels of αS1-CN in milk (Küpper *et al*., 2010). These alleles can be grouped in four classes: strong alleles (A, B1, B2, B3, B4, B', C, H, L and M) are associated with 3.5 g/L; intermediate alleles (E and I) are associated with 1.1 g/L; weak alleles (F and G) are associated with 0.45 g/L of αS1-CN content; and null alleles (01, 02 and N) are associated with the absence of αS1-CN in milk (Grosclaude *et al*., 1997; Ramunno *et al*., 2000; Ibeagha-Awemu *et al.,* 2008; Mastrangelo *et al*., 2013). As a result, the characterization of the *CSN1S1* gene is important owing to its relationship with cheese production and milk-processing properties (Kumar *et al*., 2007). While the B1, B2 and B3 alleles originated from single nucleotide substitutions responsible for amino acid replacements, the E allele is characterized by the insertion of a DNA segment between the 124th and the 125th nucleotide of the 19th exon (Jansá-Perez *et al*., 1994; Bevilacqua *et al.,* 2002). The B1, B2 and B3 alleles of *CSN1S1* genes are differentiated from A-type (A, G, H, I, 01, 02) alleles with the G→C transversion at the 22nd nucleotide of exon 10; with the T→C transversion at the eighth nucleotide of exon 4; and with G→A transition occurring at the 14th nucleotide of exon 12, respectively (Acc.No: AJ504710) (Cosenza *et al*., 2008). The E allele consists of the insertion of long interspersed repeated elements (LINE) of 457-458 bp at the 124th nucleotide of exon 19 (Jansá-Perez *et al*., 1994).

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The polymorphisms at αS1-casein locus affect not only the quantity of casein in goat milk, but also its structural and nutritional characteristics and technological properties (Mastrangelo *et al*., 2013). Characterization of these animals for *CSN1S1* variability is important owing to their relationship with milk-processing properties (Kumar *et al*., 2007). The milk composition and coagulation properties are fairly associated with the the amounts of αS1-CN (Clark & Sherbon 2000). Lower percentages of total solid (TS), solid not fat (SNF), fat, protein and casein, and a lower coagulation rate were observed in milks that lacked αS1-CN, compared with milk that contained high amounts of αS1-CN in goats (Clark & Sherbon 2000). Milk from EE goats for the *CSN1S1* gene had a significantly higher curdling rate than milk from homozygote B-type (B-type: B1, B2, B3, B4, C, L) individuals in the cheese-making process (Caravaca *et al*., 2011).

Devold *et al*. (2011) pointed out that the contents of casein were significantly lower in 'null' milks (1.93%) than in milks of the 'strong' group (2.05%) in Norwegian dairy goats. The results obtained by Caravaca *et al.* (2008) in a study on Murciano-Granadina goats showed that BB genotyped goat for *CNS1S1* gene had a higher level of αS1-CN content (8.50 g/L) of milk than EF (6.9 g/L), BF (6.56 g/L) and EE (6.39 g/L) genotyped goats. Another study by these authors indicated that the *CSN1S1* genotype did not affect protein, casein and fat concentrations in Murciano-Granadina goats (Caravaca *et al*., 2009). For milk yield and composition, an investigation of the Sarda breed showed that *CSN1S1* BB goats produced significantly higher protein and casein percentages. In addition, αs1-CN was significantly higher in BB and AB Sarda goats than in AF and BF Sarda goats (Balia, 2013). Dominant effects were found for some genotypes for milk yield (AN and BN), fat yield (AN and BE), protein yield (AN and BE), lactose yield (AN), and TS yield (AN). Unfavourable dominance effects were found for protein contents (AB and AN) and TS contents (AB, AN, and AF) in Alpine, Saanen and Toggenburg goats (Vázquez-Flores *et al*., 2012). Despite the number of studies that determined the frequency of *CSN1S1* alleles, investigations into the effects of the *CSN1S1* gene on milk yield and composition are limited. Furthermore, there is no published information about the effects of each B-type allele on milk yield and composition.

The objective of this study was therefore to evaluate the frequencies of B1, B2, B3 and E alleles of *CSN1S1* gene and determine their effects on milk yield and composition in Saanen goats.

Materials and Methods

The research was carried out on 123 Saanen goats belonging to two herds located in Bursa, Turkey. These herds were reproductively disconnected, so the goats were not related genetically, and were selected at random within the herds. Goats were reared intensively and milked by machine twice a day. Blood and milk samples were collected from selected animals according to ethical procedures. Ethical approval was received from Uludag University (2014-07/02).

In the study, 5 mL blood was collected from the jugular vein using vacutainer tubes containing EDTA as anticoagulant. These blood samples were stored at +4 ºC until they arrived at the genetic laboratory of Faculty of Veterinary Medicine-Uludag University. Genomic DNA was extracted from blood by the phenol-chloroform method (Powell & Gannon, 2002). The amount and purity of the DNA samples were measured with a spectrophotometer according to the ratio of absorbance at 260 and 280 nm-260/280 (Nanodorp-2000c,Thermo Scientific Inc.). DNA samples were stored at −80 ºC until the PCR-RFLP was performed.

Genotyping at the *CSN1S1* locus (B1, B2, B3 and E alleles) was performed by the PCR-RFLP method according to Cosenza *et al*. (2008) and Torres-Vázquez *et al*. (2008). For the B1, B2, B3 and E alleles of *CSN1S1* genes, 311, 310, 231 and 662 bp PCR products were amplified with the established primer sets, respectively (NCBI Acc.No: AJ504710).

For B1, B2, B3 alleles, PCR reaction was prepared in a final volume of 50 µL containing 100 ng/mL genomic DNA, 10 pmol of each primer (B1 allele; F:5' GAAAAGAGAACATGTACTTTG 3', R:5' CATCTTCCTTTTGAATGTACTT 3', B2 allel; F:5'TTCAAATGGAAAAACATTCTCC 3', R:5' GTCAAATGTATAGGTACAGAT 3', B3 allel; F:5' TTAGTTTCCCATTCTTTACTC 3', R:5' GAAGCTCTAACATGATTTGAT 3'), 1.25 U Taq DNA polymerase, 1X Standard Taq Reaction Buffer (10 mM Tris-HCl, 50 mM KCl, pH:8,3), 1.5 mM $MgCl_2$, 0.2 mM dNTP (NEB Biolabs Inc.M03200S, N0447S). PCR amplification conditions consisted of an initial step of 97 ºC for 2 min, followed by 30 cycles of 94 ºC for 45 sec, 55 ºC for 45 sec, 72 ºC for 45 sec and a final step of 72 ºC for 10 min. Differentiation between alleles B1, B2 and B3 was developed by the PCR-RFLP method, according to Cosenza *et al*. (2008). An 8 µL of each PCR product was digested with MnlI for B1, B2 alleles and DdeI restriction enzyme for B3 alleles at 37 ºC for about 16 hours according to their specific prescription. PCR and enzyme digestion products were evaluated by electrophoresis in 2% agarose gel prepared with 0.5X TBE buffer and ethidium bromide. The bands were visualized with the DNr Minilumi imaging system (Cosenza *et al*., 2008).

The PCR reaction for E allele was prepared in a final volume of 50 µL, containing 100 ng/mL genomic DNA, 10 pmol of each primer (F:5' ATG GGA TTG AAA ATT CCA TGC 3', R:5' ATA CTA CTG GAA TTT AGG TA 3'), 1.25 U Taq DNA polymerase, 1X Standard Taq Reaction Buffer (10 mM Tris-HCl, 50 mM KCl,

pH: 8.3), 1.5 mM $MgCl_2$, 0.2 mM dNTP (NEB Biolabs Inc. M03200S, N0447S). PCR amplification conditions included an initial step of 94 ºC for 3 min, followed for 35 cycles of 94 ºC for 50 sec, 59 ºC for 50 sec, 72 ºC for 50 sec and a final step of 72 ºC for 10 min. Similarly to other alleles, 8 µL of each PCR product was digested with MspI restriction enzyme at 37 ºC for about 16 hours according to the prescription. PCR and enzyme digestion products were evaluated by electrophoresis in 2.5% agarose gel, which was prepared with 0.5X TBE buffer and ethidium bromide. The bands were visualized with the DNr Minilumi imaging system (Torres 2008). The milk data (yields and amounts of milk components) were recorded throughout lactation from 67 Saanen goats in Herd 1. After parturition, milk samples were collected in 100 mL sterile sample containers bimonthly during lactation. Samples were transported at +4 ºC to the laboratory within two hours (Balia *et al*., 2013). Total milk yield per lactation was calculated by the Fleischmann method (Trapez II), which was described in International Committee for Animal Recording (ICAR) Guidelines 2014 (Berger & Thomas, 2005). Collected milk samples were analysed for total protein, fat, TS, SNF, total casein and lactose with Fourier transform ınfrared (FTIR) spectroscopy (MilkoScan™ FT1, Foss Electric, Hillerød, Denmark) (Berget *et al*., 2010). As the first step of analyses, genotype frequencies were calculated. Data were processed by Popgene v1.32 software to estimate the genotype frequencies and possible deviation from Hardy-Weinberg equilibrium from Saanen goats (Yeh *et al*., 2000). Statistical analysis was carried out in Minitab 15 statistical software (Minitab 2000) using the general linear model procedure (GLM). The model employed was:

$$Y_{ijklmnopr} = \mu + \beta L_i + A_j + T_k + M_l + B1_m + B2_n + B3_o + E_p + e_{ijklmnopr}$$

where $Y_{ijklmnopr}$ is the dependent variable (milk, protein, fat, TS, SNF, casein, lactose yields and rates),
µ is the general mean,
β is the constant for lactation period,
L_i is the effect of lactation period (i = 101-288),
A_j is the effect of age (j = 1,2,3,4≤),
T_k is the effect of birth type (k = 1,2,3),
M_l is the effect of month of birth (l = February,March),
$B1_m$ is the effect of B1 genotype (m = B1,B1/NB1,NB1),
$B2_n$ is the effect of B2 genotype (n = B2,B2/NB2,NB2),
$B3_o$ is the effect of B3 genotype (o = B3,B3/NB3,NB3),
E_p is the effect of B1 genotype (p = E,E/NE,NE), and
$e_{ijklmnopr}$ is the random error effect.
For all parameters, model effects were declared significant at P <0.05.

Results and Discussion

The PCR products of B1 allele (311 bp) were digested by restriction enzyme MnlI and showed two fragments for allele B1 at 125 and 186 bp. Allele 'non-B1' (NB1) had a single undigested fragment at 311 bp (Figure 1). B2 homozygote individuals for *CSN1S1* gene showed a single undigested fragment of 310 bp, whereas 'non-B2' (NB2) homozygote individuals had two fragments of 77 and 233 bp after the digestion of *MnlI* restriction enzyme (Figure 2). For B3 allele of *CSN1S1* gene single undigested fragment (231 bp) was observed for B3 homozygote individuals, when two fragments of 97 and 134 bp were observed after the digestion of DdeI restriction enzyme for 'non-B3' (NB3) homozygote individuals (Figure 3). A 662 bp fragment was observed in homozygote individuals for E, the 662 and 205 bp fragments were observed in heterozygote individuals, and a 205 bp fragment was observed in homozygote individuals for 'non-E' (NE) alleles (Figure 4).

The genotypic and allelic frequencies of B1, B2, B3 and E alleles at *CSN1S1* locus in the Saanen breed are shown at Table 1. The allelic frequencies of the B1, B2, B3 and E alleles were 0.985, 0.030, 0.291 and 0.216 in Herd 1, and 0.857, 0.125, 0.509 and 0.339 in Herd 2, respectively. B1/B1 genotype was the most frequent in Herd 1 and 2 (97.0%, 73.2%). The homozygote 'non-B1' genotype was not found in Herd 1, and only one individual was found in Herd 2. For B2 allele, the most frequent genotype was homozygote 'non-B2' with the frequencies of 97.01% and 42% in Herds 1 and 2, respectively. The heterozygote individuals and homozygote individuals for B2 did not exist in Herds 1 and 2. Three genotypes (B3B3, B3/NB3, NB3/NB3) were detected for B3 allele in Saanen goats. Although NB3/NB3 was the most frequent (52.24%) in Herd 1, the most prevalent genotype was determined B3/NB3 (48.21%) in Herd 2. The frequencies of homozygote 'non-E' genotype (62.69%, 44.64%) was determined to be higher than the other genotypes in Herds 1 and 2. In general, B1/B1 (86.18%), NB2/NB2 (86.99%), B3/NB3 (42.88%) and NE/NE genotypes (54.47%) were found to be more frequent in the Saanen breed that was investigated. Although Hardy-Weinberg equilibrium and χ^2-values were not estimated owing to the genotypic frequency for B1 and B2 alleles (n <5 in some groups), significant deviation was not observed for the B3 and E alleles in the breed (P >0.05).

The *P*-values obtained for each factor of milk yield and composition are given in Table 2. According to the data, unlike the number of kids born (birth type) and month at birth, the protein, fat, total solid, solid not fat, total casein and milk yield were affected by the age and lactation length. Although genotype influenced milk composition, B1 and B2 alleles of the *CSN1S1* gene did not affect milk yield and composition ($P > 0.05$). Except for lactose yield, B3 allele has a significant effect on protein, fat, TS, SNF, casein and lactose percentage ($P < 0.01$, $P < 0.05$). The B3 allele tended to be significant on milk yield ($P = 0.058$). E allele significantly influenced fat and total solid percentages of milk in Saanen goats ($P < 0.05$).

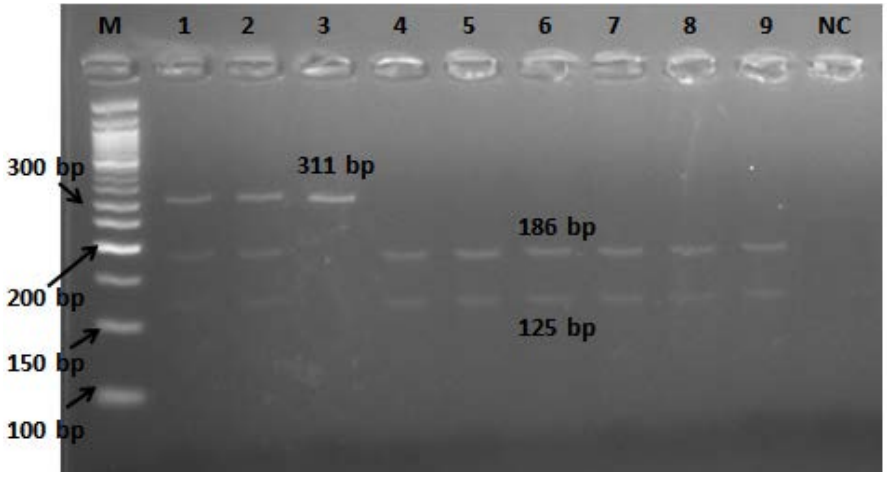

Figure 1 B1 allele of *CSN1S1* gene cut with MnlI restriction enzyme.
The products were electrophoresed on a 2.5% agarose gel (M: marker, columns 1,2: B1/NB1 genotype, column 3: NB1/NB1 genotype, columns 4-9: B1/NB1 genotype, NC: negative control).

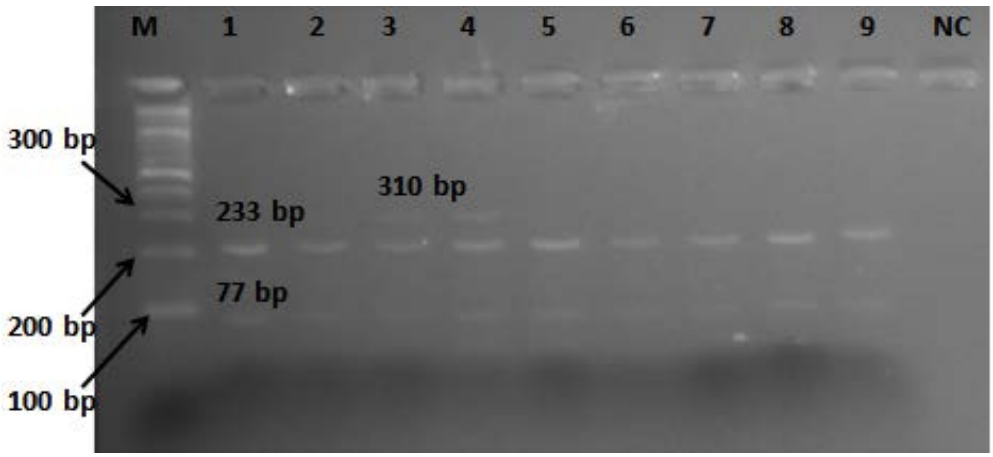

Figure 2 B2 allele of *CSN1S1* gene cut with MnlI restriction enzyme.
The products were electrophoresed on a 2.5% agarose gel (M: marker, columns 1,2,5-9: NB2/NB2 genotype, column 3,4: B2/NB2 genotype, NC: negative control).

Least square means and pooled standard errors of milk yield and compositions according to the *CSN1S1* genotype in the Saanen goat breed are given in Table 3. The protein, fat, TS, SNF and casein percentages were statistical higher in B3/B3 goats than in NB3/NB3 goats. In contrast, the B3/B3 goats had significantly lower lactose percentages than NB3/NB3 goats. Dominance effect was observed for B3 allele for protein, fat, TS, SNF and casein percentages. For fat and TS percentage, NE/NE goats displayed higher fat (4.06%) and TS (12.42%) rates than their E/E counterparts. The fat and total solid percentages were affected by significant dominance effects of the NE allele.

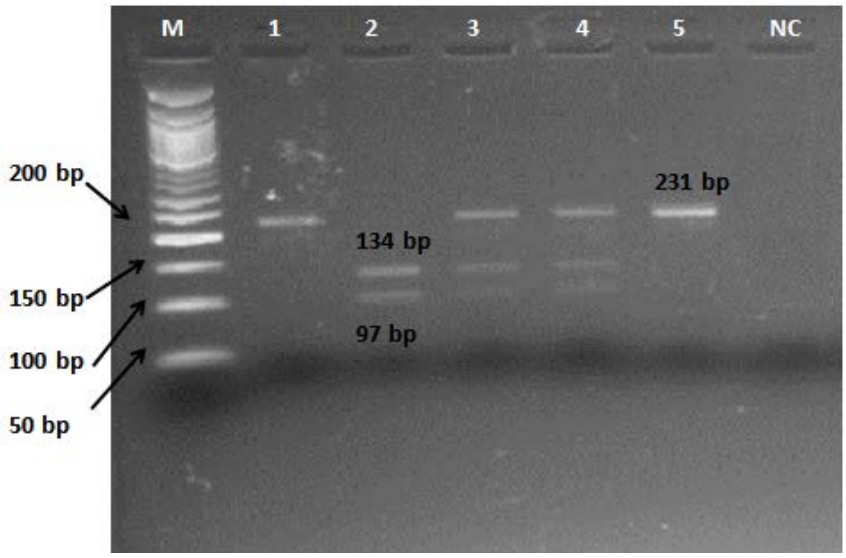

Figure 3 B3 allele of *CSN1S1* gene cut with the DdeI restriction enzyme.
The products were electrophoresed on a 2.5% agarose gel (M: marker, columns 1,5: B3/B3 genotype, column 2: NB3/NB3 genotype, columns 3,4: B3/NB3 genotype, NC: negative control).

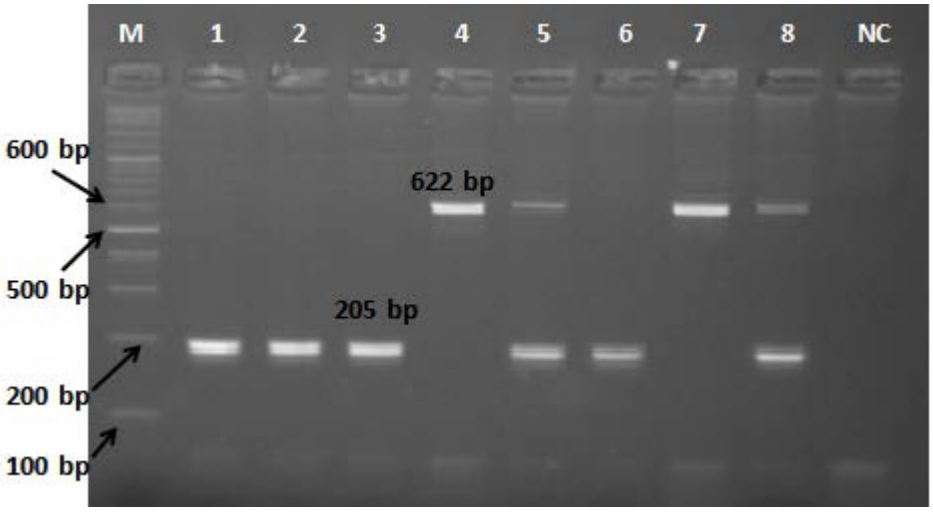

Figure 4 Characterization of allele E of the *CSN1S1* locus.
The amplified fragments of *CSN1S1* were separated on a 2% agarose gel (M: marker, columns 1-3,6: NE/NE genotype, columns 4,7: E/E genotype, columns 5,8: E/NE genotype, NC: negative control).

Analysis of the effects of the *CSN1S1* gene on goat milk and composition is important to determine the selection scheme. The allelic frequencies of the B1, B2 and B3 alleles were 0.985, 0.030 and 0.291 in Herd 1; 0.857, 0.125 and 0.509 in Herd 2; 0.927, 0.073 and 0.390 in total, respectively. Previous studies showed that the 65 allelic frequencies of B were determined as 0.065 in Girgentana dairy goats (Mastrangelo *et al.*, 2013); 0.05 - 0.230 in Indian goat breeds (Kumar *et al*., 2007); 0.234 in Bunte Deutsche Edelziege, and 0.103 in Weiße Deutsche Edelziege (Kupper *et al.*, 2010); 0.080 in Hungarian milk goats (Veress *et al*., 2004); 0.230 in Murciano-Granadina, 0.090 in Malagueña, 0.050 in Alpinee (Jordana *et al*., 1996); 0.590 in Skopelos (Kalamaki *et al*., 2014); 0.893 in Czech Fleckvieh (Kučerová *et al*., 2006); 0.007 in Frisa, 0.008 in Orobica, 0.037 in Verzasca, 0.119 in Camosciata (Caroli *et al*., 2006); 0.148 in the Maltese goat breed (Chessa *et al*., 2003). Allelic frequencies of B* were 0.0 (Maga *et al*., 2014), 0.006 (Grosclaude *et al*., 1987) and 0.110 (Torres-Vázquez *et al*., 2008) in Saanen goats, respectively. The allelic frequencies of B1, B2 and B3 alleles (0.927, 0.073, 0.272) in Saanen goats were not similar to data recorded by Cosenza *et al*. (2008), which was 0.007 for B1, 0.012 for B2 and 0.083 for B3.

Table 1 Genotype and allele frequencies of *CSN1S1* gene in Saanen goat breed

Genotype		Herd 1		Herd 2		Total		Allele	Herd 1		Herd 2		Total	
		n	Genotype frequencies (%)	n	Genotype frequencies (%)	n	Genotype frequencies (%)		n	Allele frequencies (%)	n	Allele frequencies (%)	n	Allele frequencies (%)
	B1/B1	65	97.01	41	73.21	106	86.18	B1	132	0.985	96	0.857	228	0.927
B1	B1/NB1	2	2.99	14	25	16	13.01	NB1[1]	2	0.015	16	0.143	18	0.073
	NB1/ NB1	0	0	1	1.79	1	0.81							
	B2/B2	2	2.99	0	0	2	1.63	B2	4	0.030	14	0.125	18	0.073
B2	B2/NB2	0	0	14	25	14	11.38	NB2[2]	130	0.970	98	0.875	228	0.927
	NB2/NB2	65	97.01	42	75.0	107	86.99							
	B3/B3	7	10.45	15	26.79	22	17.89	B3	39	0.291	57	0.509	96	0.390
B3*	B3/NB3	25	37.31	27	48.21	52	42.88	NB3[3]	95	0.709	55	0.491	150	0.610
	NB3/NB3	35	52.24	14	25	49	39.84							
	E/E	4	5.97	7	12.50	11	8.94	E	29	0.216	38	0.339	67	0.272
E**	E/NE	21	31.34	24	42.86	45	36.59	NE[4]	105	0.784	74	0.661	179	0.728
	NE/NE	42	62.69	25	44.64	67	54.47							

[1] NB1: non-B1, [2] NB2: non-B2, [3] NB3: non-B3, [4] NE: non-E,
Hardy Weinberg equilibrium and χ^2-values were not estimated due to the genotypic frequency for B1 and B2 alleles.
Significant deviation from Hardy-Weinberg equilibrium was not observed for the B3 and E alleles in the investigated breed.
* B3; $\chi^2 = 1.553$; $P = 0.2155$, ** E; $\chi^2 = 0.7285$; $P = 0.3933$.

Table 2 *P*-values obtained for each fixed factor considered in mixed model used to analyse traits of Saanen goat breed

Factors	Traits												
	Milk yield	**Protein %**	**Protein yield, kg**	**Fat %**	**Fat yield, kg**	**TS %**	**TS yield, kg**	**SNF %**		**Casein %**	**Casein yield, kg**	**Lactose %**	**Lactose yield, kg**
Age	0.000	-	0.000	-	0.000	-	0.000	-	0.000	-	0.000	-	0.000
Number of kids born	-	-	-	-	-	-	-	-	-	-	-	-	-
Month at birth	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactation length	0.000	-	0.000	-	0.000	-	0.000	-	0.000	-	0.000	-	0.000
B1	-	-	-	-	-	-	-	-	-	-	-	-	-
B2	-	-	-	-	-	-	-	-	-	-	-	-	-
B3	0.058	0.007	-	0.050	-	0.018	-	0.039	-	0.009	-	0.046	0.038
E	-	-	-	0.033	-	0.027	-	-	-	-	-	-	-

TS: Total solids; SNF: Solid not fat; -: Not present.

B1, B2, B3, E: alleles

Table 3 Least square means and pooled standard errors of milk composition, according to CSN1S1 genotype in Saanen goat breed

		Traits					
	Genotype	**Protein %**	**Fat %**	**TS[1] %**	**SNF[2] %**	**Casein %**	**Lactose %**
B1	B1/B1	3.37	3.53	11.58	8.46	2.62	4.24
	B1/NB1	3.42	3.98	12.09	8.44	2.68	4.12
	NB1/ NB1	-	-	-	-	-	-
B2	B2/B2	3.41	3.70	11.75	8.40	2.66	4.14
	B2/NB2	-	-	-	-	-	-
	NB2/NB2	3.38	3.82	11.92	8.50	2.65	4.22
B3	B3/B3	3.58^{a}	3.83^{a}	12.06^{a}	8.60^{a}	2.80^{a}	4.08^{b}
	B3/NB3	3.49^{a}	3.93^{a}	12.18^{a}	8.59^{a}	2.73^{a}	4.18^{b}
	NB3/NB3	3.12^{b}	3.51^{b}	11.26^{b}	8.17^{b}	2.43^{b}	4.29^{a}
E	E/E	3.29	3.58^{b}	11.51^{b}	8.35	2.56	4.21
	E/NE	3.31	3.64^{b}	11.57^{b}	8.33	2.59	4.20
	NE/NE	3.59	4.06^{a}	12.42^{a}	8.68	2.81	4.13
Pooled SE		0.0372	0.0502	0.0952	0.0489	0.0315	0.0181

TS: Total solid; SNF: Solid not fat; −: Not present.
^{a,b} : New superscript indicates that this group is statistically significantly different from the previous one.

The results of the current study showed that the allelic frequencies of E allele were 0.272 in Saanen goats. These results are not consistent with reports in Bunte Deutsche Edelziege (0.171), Weiße Deutsche Edelziege (0.419) (Küpper *et al*., 2010), Girgentana dairy goats (0.008) (Mastrangelo *et al*., 2013), Murciano-Granadina (0.590), Malagueña (0.650), Alpinee (0.340) (Jordana *et al*., 1996), Hungarian milk goats (0.310) (Veress *et al*., 2004), Maltese (0.057) (Chessa *et al*., 2003), Skopelos (0.070) (Kalamaki *et al*., 2014), Camosciata (0.114), Orobica (0.008) (Caroli 2006), White Shorthair (0.054) and Brown Shorthair (0.085) (Sztankóová *et al*., 2007). Frequencies similar to those of the current study were found only in Frisa (0.200) and Verzasca (0.201) goat breeds by Caroli *et al.* (2006). In the current study, the frequencies of E were lower than those reported by Soares *et al*. (2009) (0.350), Grosclaude *et al.* (1987) (0.410), Torres-Vázquez *et al*. (2008) (0.420) and Maga *et al.* (2014) (0.705) in Saanen goats. The diversity of frequencies may be because the goats come from different origins or from herds that were applied different selection parameters.

The present study showed that protein, fat, TS, SNF, casein and milk yield were affected by age and length of lactation. Contrary to results for goats published by Kominakis *et al.* (2000), lambing month and birth type were not found to be statistically significant for total milk yield. The effects of age on the milk production of goats were similar to data recorded by Mavrogenis *et al*. (1984). In close agreement with Olechnowicz *&* Sobek (2008), no significant influence was found for number of kids born on the quantitative parameters of milk yield. The effects of age and lactation length on milk composition should depend on negative correlation with yield and contents of milk.

Balia *et al.* (2013) pointed out that goats with BB genotypes of the *CSN1S1* gene produced higher protein and casein percentages. Unlike the findings of Balia *et al.* (2013), B1 and B2 alleles of the *CSN1S1* gene did not affect milk yield and composition in the present study. On the other hand, significant effects on protein, fat, TS, SNF, casein and lactose percentage were found by B3 allele (P <0.05). So, the positive effects of genotype on milk yield and composition should be derived from B3 alleles compared with B-type. Screening the herds with B3 genotype instead of B-type of *CSN1S1* gene may be more felicitous. Altough the protein, fat, TS, SNF, casein percentages were determined to be higher in B3/B3 and B3/NB3 than NB3/NB3 genotypes, lactose percentage was seen to be low in B3/B3 and B3/NB3 genotypes (Table 3). An increase of protein, fat, TS, SNF, casein percentages in B3/B3 and B3/NB3 genotype should occur because of dominant effects of B3 allele. Additionally, B3 allele tended to be significant in the milk yield (P = 0.058). A greater number of animals with copious records should be studied to determine the effect of B3 allele on milk yield. An investigation into Murciano-Granadina goats by Caravaca *et al.* (2009) indicated that the protein, casein, lactose and SNF percentages were 5.07%, 3.57%, 2.82%, 4.53%, 8.93% in BB genotyped goats, respectively. The results were similar to the authors' data on Saanen goats with B3/B3 genotype shown in

Table 3. The fat percentage of milk (5.07%) in Murciano-Granadina (Caravaca *et al*., 2009) was found to be higher than in the current study of Saanen goats (3.83%). Balia *et al.* (2013) pointed out that the fat, protein and lactose percentages of milk in Sarda goats with BB genotype was 5.13%, 4.29% and 4.76%, respectively. The fat, protein and lactose percentages of milk in the Saanen goat breed with B3/B3 genotype were found to be lower than specified in Sarda goats by Balia *et al.* (2013). Similar data were recorded for fat (3.4%) and lactose percentages (4.3%) of milk on Saanen, Alpinee and Toggenburg goats with BB by Flores *et al*. (2012). Protein (2.9%) and TS percentages (10.7%) were found to be higher than the current data in Saanen with B3/B3.

Caravaca *et al.* (2009) indicated that fat and SNF percentages were 4.99% and 8.84% in Murciano-Granadina goats with EE genotype. Fat percentage was lower in Saanen goats with EE genotype (as shown in Table 3) than the Murciano-Granadina goat breed (Caravaca *et al.,* 2009). The results of Flores *et al.* (2012) showed that the fat and total solid percentages of milk in Saanen, Alpine and Toggenburg were 3.18% and 10.79%, respectively. Compared with the study of Flores *et al*. (2012), fat and TS percentages were higher in Saanen goats with EE and NE/NE genotypes in the present study. NE allele increased the fat and TS percentages of milk statistically in Saanen goats. NE/NE genotype exhibited higher fat and TS percentages than EE and E/NE genotypes. Positive effects on fat and TS percentages of NE should be based on dominant effects.

Conclusion

In conclusion, the effects of B1, B2, B3 alleles of the *CSN1S1* gene in Saanen goat breeds were reported for the first time in this publication. According to these results, the allelic frequencies of the B1, B2, B3 and E alleles were 0.927, 0.073, 0.390 and 0.272, respectively. Effects of age and lactation length on protein, fat, TS, SNF, casein and milk yield were found to be significant (P <0.05). However, the environmental factors of month at birth and number of kids born did not affect milk composition. The effects on milk of genotypic factors such as B1 and B2 alleles were not significant in the present study. B3 and E alleles, on the other hand, influenced milk composition considerably (P <0.05). The B3 allele tended to have a significant effect on milk yield (P = 0.058). A greater number of animals with copious records should be studied to clarify the effects of B1, B2, B3 and E alleles on milk yield and composition in Saanen goats. The potential for improving milk composition by selecting for B3 and E allele may be significant. Thus B3 and E alleles could be used as alternative selection criteria for milk composition in Saanen goats. Functional traits such as milk production become important for efficient breeding schemes in dairy goats. In addition, utilizing the genotype information of *CSN1S1* gene in selection strategies for milk yield and composition would allow breeders to select production aspects.

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Authors' contributions

FB coordinated the project design and implementation. DTD and SA was in charge of sample collecting and with HS was responsible for laboratory tests (including DNA isolation and PCR-RFLP procedures). All co-authors participated in results, statistics and interpretation. FB and DTD were in charge of writing the manuscript.

Conflict of interest declaration

We wish to confirm that there are no known conflicts of interest associated with the publication of this manuscript We also confirm that this manuscript has been read and approved by all authors and that the order of authors listed in the manuscript has been approved by all of us.

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