Identification of the frequency of *CSN1S2* gene alleles and the effects of these alleles and parity on milk yield and composition in Saanen goats

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SUMMARY

The expression of *CSN1S2* gene is known to regulate the α -S2 casein levels in milk composition, moreover is estimated to affect the milk yield and composition in goat. However, the knowledge about the *CSN1S2* gene on milk yield and composition is restricted in Saanen goats. Therefore, this study aimed to specify the effect of *CSN1S2* polymorphisms on milk yield/composition parameters. The genotyping of Saanen goats (n=120) was performed by PCR-RFLP methods. The GLM procedure was prefered as statistical analysis to defined the effect of those variations on investigated traits. The result showed that the AA (26.67%), BB (33.33%) and AB (40.00%) for A-B variant; N* (94.17%), N*0 (5.83%) for D-0 variant; FF (7.50%), NF/NF (65.83%) and F/NF (26.67%) for F variant in Saanen goats. The effect of *A-B* allele of *CSN1S2* gene (P<0.05) and parity (P<0.001) on milk composition yields were found significant in the current study. However, the effect of *A-B* allele on lactation milk yield was determined tended to be significant (P=0.090). Moreover, significant correlations between the milk composition parameters such as fat, protein, TS (total solid), SNF(solid not fat), casein, or lactose were observed (P<0.001). The novel outputs might be useful for developing goat breeding strategies or improving the dairy industry, especially for milk products.

KEY WORDS

Goat, parity, milk yield/composition, Saanen, CSN1S2.

INTRODUCTION

Goat milk has high importance due to the economic, nutritional, and medical properties, also is a good source for the humans who suffers an allergy from cattle milk¹. Proteins are the main nutritional and bioactive components of milk that are produced mainly from mammary gland tissue². The casein which forms the 80 percent of milk protein in ruminants composed of four types as alphaS1-casein (α-S1 casein), alphaS2-casein (α-S2 casein), beta-casein (β -casein) and kappa casein (κ -casein)³. Besides being the most important of allergens in milk, α -S2 casein, which is a kind of phosphoprotein is consisting of 208 amino acids⁴. Although the CSN1S2A and CSN1S2B gene have been established at the casein loci in the human genome, it is known that the α -S2-casein does not exist in human milk⁵. The tolerance to cow milk in humans may be related to the presence of this protein has been thought. Milk caseins, which constitute the primary food source in mammals with placenta, were controlled by CSN1S1, CSN1S2, CSN2, and CSN3 genes that are cumulated at 250-kb DNA fragment in the caprine chromosome⁵. The casein genes were determined polymorphic in the literature⁶⁻⁸. These genotypic diversities have been seen as an essential factor in terms of genetic progression. Moreover, these variations allow selecting the suitable choice for dairy products depending on the genetic basis³.

The *CSN1S2* is an 18.438 nucleotide length gene consisting of 18 exons ranging in size from 21 to 266 nucleotides⁹. Fur-

thermore, the *CSN1S2* gene that is structurally similar to other calcium-sensitive genes due to their organization at the 5' and 3' endings have seven alleles (A, B, C, D, E, F, and 0) in goats⁴. Also, these alleles relevant to three different levels of α -S2 casein in milk, such as normal (2.5 g/L per allele), intermediate (1.5 g/L-per allele), or null (absence of α -S2 casein)³.

The alleles of A(0.400) and F(0.330) of CSN1S2 gene were determined more frequently in Sarda goats; also, the D and E alleles were not found at the investigated flock⁴. On the other hand, the frequency of the N* (N*=A, B, C, and E) and F allele was specified 0.522 and 0.478 in the Czech goats, respectively besides, the heterozygous FN* genotype has the highest frequency in the studied breed7. The daily milk yield and the average protein yield of lactation were determined highest in Czech goats with N*N* genotype; 2.68 ± 0.06 kg and 17.194 ± 0.507 kg, respectively. The most common genotype was determined as AA in Girgentana^{10,11} Arbi "Tunisian" goats¹² and AF in Argentana des Etnas goats¹⁰. However, the A+B+C+E allele frequency was determined 0.635 in Bulgarian dairy goats (Hungarian Milking White, Hungarian Milking Brown, Hungarian Milking Multicolor) with the lowest frequency belongs to 0 alleles (0.146)¹³. In terms of CSN1S2 gene, the variation was noticed in the literature with the different allele and genotype frequencies. Also, the A, B, C, G alleles were identified with the frequencies of 0.920, 0.080, 0.000 and 0.000 respectively in Saanen goats (n = 26) by Chiatti et al.¹⁴. Moreover, the most frequent genotype was found AA (22/26) in that flock. However, the most frequent genotype was found AC (0.300) in Saanen dairy goats (n=20) at another study performed by Grobler et al.¹⁵.

The studies were performed to determine the allele and genotype frequencies of the *CSN1S2* gene that were done in Sarda, Czech (Czech), Girgentana, Argentana des Etnas, Arbi, and Bul-

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garian milkman (Hungarian Milking White, Hungarian Milking Brown, Hungarian Milking Multicolor) goat breeds in summary. Besides, the limited information existed in the literature for Saanen goats in terms of *CSN1S2* gene; to the best of our knowledge, the effect of those variations on milk composition has not been investigated extensively up to now in Saanen goats. However, the *CSN1S2* gene was strongly thought to be effective on milk yield and composition in other species or races. Therefore, this study was carried out to identify the frequency of *CSN1S2* gene alleles and it's the effect on total milk yield and composition in Saanen goats.

MATERIALS AND METHODS

Animal sources and milk analysis

The analysis was performed on 120 purebred Saanen goats belongs to the farm located at Bursa province in the Marmara Region of Turkey. The herd was barned in intensive conditions. All goats were feeding with the same diets and had water ad libitum during the research due to standard commercial practices. The milk data from Saanen goats (the ages range from 1 to 6) were recorded during the lactation period while they were milking twice a day. Also, the samples were collected in sterile sample containers twice a month for evaluation and were transported to the laboratory via the cold chain. The samples were qualified for milk composition such as protein, fat, casein, or lactic acid using infrared spectroscopy by filter technology with Fourier data transformation (FTIR- MilkoScan™ FT1, Foss Electric, Denmark). The experiments were performed in compliance with the ethical requirements with the approval of the local Ethics Committee for Animal Research (2018-04/01).

Genomic analysis

The phenol-chloroform method described by Green and Sambrook¹⁶ was used to extract the DNA from the blood sample. The blood samples (4 mL) were collected from the peripheral blood to the K_3 EDTA tubes and then were reached the laboratory via the cold chain. The SNP markers of the *CSN1S2* gene were preferred from different sources as given in Table 1, and the information of them was confirmed by the databases such as GeneBank or EMBL (https://www.ncbi.nlm.nih.gov/search/all/?term= or http://www.ensembl.org). Genotypes of *CSN1S2* polymorphisms were determined using a polymerase chain reaction and restriction fragment length polymor-

phism method (PCR-RFLP) (3,17). The PCR amplification was performed in a total volume of 25 μ L containing 14.5 μ L dH₂O, 5 μ L Protocol for OneTaq[®] Quick-Load 2X Master Mix with Standard Buffer (M0486), 1 L (0.025 M) of each primer, and 2.5 L of the DNA sample at a concentration of 100 ng/ μ L. Subsequently, the 5 μ L of the amplified product was digested with the corresponding restriction enzyme that the details were given in Table 1. Agarose gel electrophoresis (containing ethidium bromide) was used for separating the PCR and RFLP products at 90-100V for 45 to 60 minutes for visualization. Afterward, the obtained PCR-RFLP fragments were visualized by a gel imaging system (DNr-Minilumi, DNR Bio-Imaging Systems), and the observed patterns were evaluated according to aspects of the authors in the literature.

Statistical analysis

The direct counting method was chosen to identify the genotype frequencies of investigated SNPs. The variations among observed and expected frequencies of genotypes were examined by χ^2 test in order to confirm Hardy-Weinberg Equilibrium (HWE). The statistical analysis were carried out with the general linear model (GLM) procedure of Minitab (Minitab Inc., State College, PA, USA, version 17.1.0) to evaluate the differences between the investigated parameters and individual effects. The stepwise regression model was used to choose the best appropriate model for the investigated factor; thus, the D-0 and F polymorphisms were removed from the model. According to the selected method, the mixed model was applied to established possible significant differences between the genotypes as follow: $Y_{ijk} = \mu + A_i + P_j + e_{ijk}$, where Y_{ijk} symbolized the observed value; μ is the overall mean for each trait; A_i is the fixed effect of A-B alleles of CSN1S2 gene (i=AA, BB, AB); P_k is the fixed effect of parity (k=1th, 2th, 3th, 4th, 5th); e_{ijk} is the random error (The adjusted R² value varies from 53.33 to 60.32 for the studied traits).

The population genetic incidences such as expected heterozygosity (He), effective allele numbers (Ne), and the polymorphism information content (PIC) were estimated by the descriptive data of Botstein et al.¹⁸. In addition, Pearson's correlation coefficient (PCC) was used to evaluate the phenotypic correlation coefficients on the basis of the population. Three groups of PCC were classified as described below: PCC is < 0.25 equal to low correlation, PCC is between 0.25 – 0.50 equal to intermediate correlation, and PCC is > 0.50 equal to high correlation¹⁹. The significance level accepted 0.05 for HWE, for

 Table 1 - Description of primer sequences, PCR conditions, restriction enzymes that were applied for genotyping the polymorphisms of CSN1S2 gene in the current study.

Alleles of CSN1S2 gene	Acc. no.	Primer sequences (from 5 to 3)	PCR amplicon	Restriction enzyme	Genotyping	References
A-B	X65160 (GenBank)	F: GCCATTCATCCCAGAAAG R: CTCTTCATTTGCGTTCCTTA	1,2 kb	Msel	AA: 230, 270, 300 bp BB: 230, 270, 400 bp AB: 230, 270, 300, 400bp	Cosenza et al., 1998
D-0	AJ131370 (EMBL)	F: GACACATAGAGAAGATTC R: CGTTGGGACATTTTATCT	301 bp	Ncol	0: 301 bp D: 62, 133 bp N*:133, 168 bp *N:A,B,C,D,E or F	Ramunno et al., 2001
F	AJ131370 (EMBL)	F: TCTCTTGCCATCAAAACA R: TGGTCTTTATTCCTCTCT	310 bp	Alw26l	FF: 310 bp NF/NF: 131, 179 bp F/NF: 131, 179, 310 bp	Ramunno et al., 2001

Alleles of CSN1S2 gene	Genotype	n	%	He	Ne	PIC	χ 2(HWE)	P(HWE)	Allele frequencies	
A-B	AA	32	26.67		1.9912	0.3739	4,6301	0,031	А	0.467
	BB	40	33.33	0.4978					В	0.533
	AB	48	40.00							
D-0	N*	113	94.17	0.0563	1.0630	0.0547	0,1083	0,742	N*	0.971
	N*0	7	5.83						0	0.029
F	FF	9	7.50	0.3295		0.2752	4,4043	0,036	F	0.208
	NF/NF ⁺	79	65.83		1.4914				NF	0.792
	F/NF	32	26.67							

Table 2 - The population genetic indices and genotypic frequencies of CSN1S2 gene, accordance with HWE.

N*: A,B,C,E or F; NF*: not F, χ^2 (HWE) - Hardy-Weinberg equilibrium χ^2 value, *P < 0.01; P < 0.001 - not consistent with equilibrium. n: number of goats.

He: gene heterozygosity; Ne: effective allele number, PIC: polymorphism information content.

0.001- 0.05 for GLM procedure, moreover the P-value less than 0.10 (P < 0.10) was considered as a tendency in the current study.

RESULTS

The genotypic structure of the investigated population

We investigated the A-B, D-0, and F alleles of CSN1S2 gene in the Saanen population. The distribution of genotype and allele frequencies, moreover, the population genetic parameters including He, Ne, and PIC values of these genotypes with X² and P significance of Hardy-Weinberg equilibrium (HWE) are presented in Table 2. Although the A-B and F variations were not consistent $(P \le 0.05)$, the *D*-0 marker was found compatible with HWE (P=0.742). The He, Ne, and PIC values were observed to varying from 0.0563 to 0.4978; 1.0630 to 1.9912; 0.0547 to 0.3739 respectively by the statistics. The PCR and RFLP results were shown in Figure 1 and 2; according to the results, three genotypes (AA, BB and AB) were determined for A-B marker in the current study, and the AB genotype which was characterized by fragment sizes of 230, 270, 300 and 400 bp was defined as the most frequent in the studied Saanen flock (40.00%). Similarly, the A-*B* allele, the homozygote and heterozygote genotypes were detected for F allele of CSN1S2 in the present study. The genotype frequencies of F marker were 65.83% for NF/NF, 26.67 for F/NF, and 7.50% for FF in Saanen goats. Although three different genotypes were established for A-B and F variations of CSN1S2 gene, there were detected only N* and N*0 genotypes for D-0 with the ratio of 94.17% and 5.83%, respectively. The D allele, which forms the bands of 133 and



Figure 1 - The electrophoresis patterns of PCR products of CSN1S2 gene.



Figure 2 - The RFLP digestion products of *CSN1S2* gene electrophoresed in 2.0-2.5% agarose gels. (L: DNA Ladder-amplicon length 100-1500 bp; NC: Negative control for overall. **D**: The RFLP products of *A-B* variant of *CSN1S2* gene, Line 1, 3: AB; Line 2, 4: AA; Line 5:BB.

E: The RFLP products of *F* variant, Line 1, 3, 4, 5: NF/NF; Line 2: F/NF. F: The RFLP products of *D*/0 variant, Line 1 to 5: N*0).

62 bp, was not existing in this study. Thus the most frequent alleles were found B (0.533) for *A-B*; N^* (0.971) for *D-0* and NF (0.792) for *F* variation that was given in Table 2.

Relationship of *CSN1S2* gene polymorphism with milk yield and composition

The least-square means and standard errors for the CSN1S2 gene (A-B allele) and parity effects on milk yield and composition are given in Table 3. According to results, the A-B allele of CSN1S2 gene had significant effects on protein, fat, TS, SNF, casein, and lactose yield of goat milk (P<0.05). Moreover, this variant had a tendency to be significant on the milk yield parameter in Saanen goat (P=0.090). The AA genotype was correlated with higher fat yield (18.83±1.68 kg) than AB and BB genotype. Similarly, the higher protein, TS, SNF, casein, and lactose yield were observed in animals with AA genotype for A-B marker. On the other hand, significant associations were established between the parity and all investigated traits (P<0.001) in the study. The highest milk yields such as total milk yield, fat, TS, SNF, casein, and lactose belong to the animals at 3th and 4th parity, while the highest value for milk protein was recorded at 3^{th} to $\ge 5^{\text{th}}$ parity. The milk yield and composition parameters seemed to be at the lowest value on the first parity in accordance with the results.

Correlations between the compositions of goat milk

The outputs of Pearson correlation coefficients were given in Table 4. According to the summary of the descriptive data of PCC, the high correlations were detected as expected in the current study. The milk yield was highly correlated with the studied parameters such as protein, fat, TS, SNF, casein, and lactose (P<0.001). Simultaneously, the high level of correlations was indicated significantly between the whole milk composition parameters of Saanen in the present study (P<0.001).

DISCUSSION

The genotypic structure of the investigated population

This research established the relationship between *CSN1S2* alleles, which are a strong candidate for milk traits in various breeds, and milk production parameters in Saanen dairy goats. Seven alleles of the gene, including A, B, D, F, and 0 identified up to now¹⁰, and according to the results, the B was found the most frequent allele (0.533) for *A-B* variant in the current study. These findings are in disagreement with those reported by Sacchi et al.²⁰, who indicated the allele frequencies between 0.014 to 0.175 in Vallasena, Roccaverano, Maltese, Jon-

 Table 3 - Effects of CSN1S2 gene polymorphisms on milk yield and composition in Saanen goats with the levels of significance, least-squares means, and standard errors.

	Genotype	n	Total Milk yield (kg)	Protein (kg)	Fat (kg)	TS** (kg)	SNF⁺ (kg)	Casein (kg)	Lactose (kg)
A-B	AA	6	531.8 ± 44.5	15.90 ± 1.31^{a}	18.83 ± 1.68 ª	59.01 ± 4.92ª	41.66 ± 3.52^{a}	12.48 ± 1.03^{a}	21.67 ± 1.91ª
	AB	29	425.3 ± 21.3	11.95 ± 0.62^{b}	13.69 ± 0.80 ^b	43.37 ± 2.35 ^b	31.00 ± 1.68^{b}	9.45 ± 4.98^{b}	15.87 ± 0.91^{b}
	BB	23	459.0 ± 23.2	$12.73\pm0.68^{\text{ab}}$	14.22 ± 0.87 ^b	45.94 ± 2.56^{ab}	32.92 ± 1.83^{ab}	$9.95\pm5.42^{\text{ab}}$	16.93 ± 0.99^{ab}
	Р		0.090~	*	*	*	*	*	*
Parity	1 th	17	316.2 ± 27.9°	7.52 ± 0.81°	8.54 ± 1.05°	27.40 ± 3.08°	19.92 ± 2.20°	5.89 ± 6.50°	10.34 ± 1.19°
	2 th	12	389.2 ± 32.9^{bc}	10.96 ± 0.96 b	12.79 ± 1.24^{bc}	40.92 ± 3.63^{b}	$29.29 \pm 2.60^{\text{b}}$	8.66 ± 7.67^{b}	15.21 ± 1.41 ^{bc}
	3 th	16	515.1 ± 29.3ª	15.86 ± 0.86 ^a	18.46 ± 1.10^{a}	57.68 ± 3.23ª	40.75 ± 2.31^{a}	12.44 ± 6.83^{a}	20.72 ± 1.25^{a}
	4 th	7	603.6 ± 43.2^{a}	17.88 ± 1.27^{a}	21.59 ±1.62ª	66.10 ± 4.77ª	46.06 ± 3.41^{a}	14.10 ± 1.00^{a}	23.67 ± 1.85^{a}
	≥ 5 th	6	536.1 ± 44.3^{ab}	15.42 ± 1.30^{a}	16.52 ± 1.67^{ab}	$55.09\pm4.89^{\text{ab}}$	39.95 ± 3.50^{ab}	$12.05\pm1.03^{\text{ab}}$	$20.83 \pm 1.90^{\text{ab}}$
	Р		***	***	***	***	***	***	***

N*: A, B, C, E or F, TS**: total solid, SNF+: solid not fat,

***= P<0.001, *= P<0.05, NS=not significant, a.b.c.d.e = Different superscripts within a column indicate significant differences.

~: tended to be significant (P<0.010)
</p>

Table 4 -	The Pearson	correlations	between	the	composition	characteristics	of Saanen milk ¹ .	

Variables	Milk yield	Protein	Fat	TS	SNF	Casein
Protein	0.926					
Fat	0.867	0.948				
TS	0.928	0.988	0.980			
SNF	0.941	0.993	0.952	0.993		
Casein	0.922	0.999	0.956	0.991	0.993	
Lactose	0.950	0.973	0.937	0.983	0.993	0.973

¹All correlations were found statistically significant (P<0.001).

ica, and Garganica goats. Moreover, the present observation conflict with the work of Vacca et al.^{4,12,21} in which the allele frequencies were found 0.06 and 0.366 in Sarda and Tunisian local goat breeds, respectively. The frequency of A allele (0.467) was determined similar to data recorded by Marletta et al.¹⁰, who dedicated the frequency as 0.413 in Argentata dell'Etna goats. On the other hand, the higher values for A were recorded in Girgentana (0.744) by Marletta et al.¹⁰. Previous studies indicate that the D allele of CSN1S2 gene was not detected in local Tunisian breeds12; Vallasena, Roccaverano goats13, and Turkish goats²². In close agreement with the literature, the N* allele was observed the most frequent for D-0 variant (0.971), and the frequencies of 0 were detected 0.029 in the present study. The differences between the allele frequencies of the literature and the current research should be explained by the flock or breed differences. Consistent with the A-B or D-0 variants, the frequencies of F allele exhibited a variation in the literature. According to the literature, the frequency of F allele varies from 0.190 to 0.408 in Vallasena, Roccaverano, Maltase, Jonica, Garganica²⁰; local Tunisian breeds¹²; Hungarian milking goats, Italian goats13; Girgentana11; Guanzhong23, and Sarda goats21 respectively. The frequency of F allele was determined 0.208 in the present study, which is in agreement with Yue et al.²³, who reported the frequency as 0.205 in Xinong Saanen breed. On the other hand, these findings were noticed to be higher than Lan et al.⁶ in Saanen goats (0.087). This could be due to the flock or race variations that have different gene pools, as in other alleles.

The genotype frequency of AA, BB, and AB of A-B variant were found 26.67%, 33.33%, and 40.00% in the study, as given in Table 2. This conclusion is not consistent with data found in the literature by Chiatti et al.14, who indicated the genotype frequencies of AA and AB were 84.62% and 15.38% in Saanen goats. Moreover, the homozygote genotype for B allele for CSN1S2 gene did not exist in that study. Othman and Ahmed²⁴ emphasized that the 0 alleles were not observed in Egyptian goats breeds; all individuals had N*N* genotype for D-0 variant. Our results for N*N* genotype (94.17%) were similar to data recorded by Othman and Ahmed²⁴; however, two different genotypes, including N*0 were found in the present study. The most frequent genotype was detected NF/NF in Saanen goats, which was similar to data recorded by Othman and Ahmed²⁴, Lan et al.²⁵, and Balcioglu et al²⁶. In other respects, the lowest frequency for F variant was identified as FF (13.61%) in close agreement with Lan et al.²⁵, Balcioglu et al.²⁶, and Sztankóová et al.7. The reason for the observed similarities might be due to the genetic resemblance of the flocks.

Relationship of *CSN1S2* gene polymorphism with milk yield and composition

The composition of milk determines several fields, such as the economic value, cheese-making ability, or the raw material selection for the dairy product. The effect of *A-B* variant *of CSN1S2* genotype and parity on these parameters was adjusted significantly in the present study. Vacca et al.²¹ indicated that the impact of *A-B* and *F* allele/variant was found significant on daily milk yield, fat, and protein yield in Sarda goats. The daily milk yield, fat yield, and protein yield of Sarda goats were detected 931.1 g/day, 48.78 g/d, and 39.89 g/d in AA genotyped individuals, respectively. The present findings support the hypothesis of Vacca et al.²¹ that the higher protein yield

(15.90±1.31 kg), fat yield (18.83±1.68 kg), TS yield (59.01±4.92 kg), SNF yield (41.66±3.52 kg), casein yield (12.48±1.03 kg) and lactose yield (21.67±1.91 kg) were observed in goats with AA than AB or BB genotypes. On the other hand, no significant associations were confirmed between the A, F alleles, and physical, chemical, or cytological parameters of Sarda breed by the other study of the identic researcher⁴. The reason for the impact differences between the literature and current results might be arised from the breed dissimilarity. Also, it was thought that the effect of A-B variant of CSN1S2 on casein yield was identified for the first time in Saanen goats in the present study. Prasad et al.²⁷ reported that the milk yield of Jamunapari, Barbari, and Black Bengal goats was the highest in the first parity. On the other hand, the effects of parity were found significant in Maltese goats by Carnicella et al.²⁸, and the highest milk yield was indicated at third (301.3±4.38 kg) and the fourth (302.1±3.99 kg) parity. Unlike the findings of Prasad et al.²⁷ our results consisted of Carnicella et al.28 that the highest milk yields of Saanen goats were found on the 3rd (515.1±29.3 kg) and the 4th (603.6±43.2 kg) parity in the present study. Although Addass et al.29 and Zahraddeen et al.30 emphasized that the impact of parity on TS yield was not significant, our results were similar to recorded by Prasad et al.27, who indicated the highest TS ratio at the 5th parity in Jamura, Bahrani and Black Bengal goats. Prasad et al.²⁷ noted the highest protein value at the 1st to 4th parity in the same research. It was known that the protein yield of milk is an essential criterion for the cheese-making ability for all species. Carnicella et al.²⁸ reported the highest protein ratio at the first parity in Maltese dairy goats. However, Prasad et al.²⁷ observed the higher protein at the 1st to 4th parity. In contrast to Carnicella et al.²⁸, the highest protein yield was found at the 3rd to 5th parity in Saanen goats in the current study. The fat value, which is another significant compound of milk, was detected to be impressed by parity in goats; thus, the highest fat yields were found at 1st 28 or 3rd 29-30 parity in the literature. Similar results with Zahraddeen et al.³⁰ and Addass et al.²⁹ were adjusted in the present study. But also our observations seemed to be a conflict with the work of Carnicella et al. (2008). On the other hand, the highest lactose yield was found on the 3rd 29-30 and 5th parity27 in goats. The present findings support the hypothesis that the highest lactose yields were established at the 3rd and 4th parity in dairy goats. It was well known that milk production increases with age until the peak yield. The reason for the higher milk composition yield to be seen on the 3-4th could be explained by this situation.

Correlations between the compositions of goat milk

Correlation between the milk composition parameters is an important item for the purpose of milk production. Addas et al.²⁹, and Zahraddeen et al.³⁰ indicated that no significant correlations were found between the TS and fat composition of goat milk. Moreover, they reported that the lactose was significantly associated with the TS and fat in Red Sokoto (RS), Sahel (SG), and West African Dwarf (WAD) goat breeds (P≤0.05). However, Prasad et al.²⁷ were found significant correlations between the TS and fat composition of milk. Unlike the previous studies performed in RS, SG, and WAD goats, the observation of the current study was compatible with the work of Prasad et al.²⁷ that the correlations between the TS and fat were significant in Saanen goats. Moreover, our results showed that, as demonstrated in previous studies performed by Addas et al.²⁹,

and Zahraddeen et al.³⁰, significant correlations were observed with the lactose and TS or fat composition. Although the correlations between the lactose yield and TS or protein was defined as significant, no significant association was determined in terms of the correlation between the lactose and fat compound of goat milk by Prasad et al.²⁷. In addition, important correlations were detected between the protein and fat composition in Beetal and Beetal crossbreeds at the same research. The results obtained in the present investigation are in agreement with the findings reported by Prasad et al.²⁷ in terms of correlations between the protein and fat or lactose. We assume that the diversity in the correlations can be caused by different breeds with variable milk composition and yields.

CONCLUSION

As a consequence, goat milk, which is one of the valuable animal protein sources, as well as its beneficial structure and organoleptic properties, is important for both the human health and dairy industry to prevent and be an adjunctive treatment of many diseases. The genetic structure of Saanen goats was identified in terms of *CSN1S2* variants in the present study. The most frequent genotypes were specified as AB for *A-B* variant, N* for *D-0* variant, and NF/NF for *F* variant. Moreover, a wide range of associations between the *CSN1S2* gene (*A-B* allele), parity, and the milk yield and compositions were identified in Saanen goats. The selection of these goats with that favorable genotypes may result in animals with higher milk composition yields; thus, a benefit for management systems in dairy goat breeding and the development of breeding strategies might be achieved.

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1. Warsama L.M., El Zubeir I.E.M. (2015). Comparison of chemical composition of goat milk from farms and individual households in Khartoum State, Sudan, Management of land use systems for enhanced food security: conflicts, controversies and resolutions. Tropentag, Berlin, Germany.

References

- Lima M. J. R., Teixeira-Lemos E., Oliveira J., Teixeira-Lemos L. P., Monteiro A. M. C., Costa J. M. (2018). Nutritional and health profile of goat products: focus on health benefits of goat milk. In Goat Science. InTech. https://doi.org/10.5772/intechopen.70321., 191-194.
- Ramunno L., Cosenza G., Pappalardo M., Longobardi E., Gallo D., Pastore N., Di Gregorio P., Rando A. (2001). Characterization of two new alleles at the goat CSN1S2 locus, Anim Genet, 32: 264-268.
- 4. Vacca G.M., Ouled Ahmed Ben Ali H., Pazzola M., Sanna M., Dettori M.L., Carcangiu V. (2009a). An investigation on allele frequency at the CSN1S2 locus and its relationship with milk parameters in the Sarda goat. J Anim Feed Sci, 18: 628-637.
- Martin P, Szymanowska M., Zwierzchowski L., Leroux C. (2002). The impact of genetic polymorphisms on the protein composition of ruminant milks. Reprod. Nutr. Dev, 42: 433-459.
- Lan X.Y., Chen H., Pan C-Y., Li R-B., Li X-C., Fang X-T. (2005a). Between Polymorphisms of CSN3, CSN1S2 and β-lg Genes and Litter Sizes of Xinong Saanen Dairy Goat. Zhongguo Nong Ye Ke Xue, 38(11):2333-2338.
- Sztankóová Z., Rychtářová J., Kyselová J., Mátlová V., Štípková M., Matějíčková J., Marková M. (2013). Effect of the αs1-, αs2-, β- and κ-casein genotypes on the milk production parameters in Czech goat dairy

breeds. Grant J, 2:76-80.

- Turhan Dincel D., Ardicli S., Samli H., Balci F. (2016). 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. S. Afr. J. Anim. Sci, 46(2): 180-190.
- Groenen M.A.M., Dijkhof R.J.A., Verstege A.J.M., Van Der Poel J.J. (1993). The complete sequence of the gene encoding bovine αs2-casein. Gene 123:187-193.
- Marletta D., Bordonaro S., Guastella A.M., D'urso G. (2004). Genetic polymorphism at CSN1S2 locus in two endangered sicilian goat breeds. J. Anim. Breed. Genet, 121: 52-56.
- Palmeri M., Mastrangelo S., Sardina M., Portolano B. (2014). Genetic variability at cs2-casein gene in girgentana dairy goat breed, Ital. J. Anim. Sci,13: 116-118.
- Vacca G.M., Ouled Ahmed Ben Ali H., Carcangiua V., Pazzolaa M., Dettoria M.L. (2009b). Genetic structure of the casein gene cluster in the Tunisian native goat breed, Small Rumin Res, 87: 33-38.
- Kusza S., Veress G., Kukovics S., Jávor A., Sanchez A., Angiolillo A., Bösze Z. (2007). Genetic polymorphism of αs1- and αs2- caseins in Hungarian milking goats. Small Ruminant Res, 68: 329-332.
- Chiatti F., Chessa S., Bolla P., Caroli A., Pagnacco G. (2005). Casein genetic polymorphisms in goat breeds of Lombardy, Ital J Anim Sci, 4(2):46-48.
- Grobler R., Visser C., Chessa S., Van Marle-Koster E. (2017). Genetic polymorphism of CSN1S2 in South African dairy goat populations, S. Afr. J. Anim. Sci, 47(1): 72-78.
- Green M., Sambrook D.W.J. (2012). Isolation and quantification of DNA. Molecular Cloning: A Laboratory Manual, 4th ed., 1:11-80, Cold Spring Harb. Press. Cold Spring Harb. New York.
- Cosenza G., Rando A., Longobardi E., Masina P., Ramunno L. (1998). A MseI RFLP at the goat αs2-casein gene, Anim Genet, 29: 150-160.
- Botstein D., White R.L., Skolnick M., Davis R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet,.32: 314-331.
- Buyukozturk S. (2002). Basit ve Kısmi Korelasyon, In: Sosyal Bilimler çin Veri Analizi El Kitabı. Ed. Buyukozturk S, 23rd ed., 31-34, Pegem Yayıncılık, Ankara, Turkey.
- Sacchi P., Chessa S., Budelli E., Bolla P., Ceriotti G., Soglia D., Rasero R., Cauvin E., Caroli A. (2005). Casein Haplotype Structure in Five Italian Goat Breeds. J Dairy Sci, 88(4): 1561-1568.
- Vacca G.M., Dettori M.L., Piras G., Manca F., Paschino P., Pazzola M. (2014). Goat casein genotypes are associated with milk production traits in the Sarda breed. Anim Genet, 45(5):723-731.
- Bozkaya F., Mundan D., Karabulut O., Yerturk M., Gurler S., Aral F. (2008). An investigation on the distribution of O and D alleles of the CSN1S2 gene in goat populations raised in southeastern region of Turkey. Small Rumin Res, 78: 193-196.
- 23. Yue X.P., Fang Q., Zhang X., Mao C.C., Lan XY, Chen H., Lei C.Z. (2013). Effects of CSN1S2 Genotypes on Economic Traits in Chinese Dairy Goats. Asian-Australasian journal of animal sciences, 26(7), 911-915. https://doi.org/10.5713/ajas.2013.13018 2013). Effects of CSN1S2 Genotypes on Economic Traits in Chinese Dairy Goats. Asian-Australas J Anim Sci, 26(7):911-915.
- 24. Othman E.O., Ahmed S. (2006). Analysis of Genetic Polymorphisms in the Egyptian Goats CSN1S2 Using Polymerase Chain Reaction. J. Biol. Sci, 6: 238-241.
- 25. Lan X.Y. Chen H., Zhang R-F., Tian Y. Zhang Y-D., Fang X-T., Sun W-B. Lei C-Z, Hu S-R. (2005b) Association of Polymorphisms of CSN1S2 Gene with Average Milk Yield and Body Sizes Indexes in Xinong Saanen Dairy Goat. Xu Mu Shou Yi Xue Bao, 36(4): 318-322.
- Balcioglu M.S., Karsli T., Sahin E., Karabag K. (2016). Genetic polymorphism at CSN1S2 locus in hair goats reared in Antalya. 7(1):2504-2509. VII International Scientific Agriculture Symposium, Jahorina, Bosna Hersek.
- 27. Prasad H.V., Tewari H.K., Sengar O.P. (2005). Milk yield and composition of the beetal breed and their crosses with Jamunapari, Barbari and Black Bengal breeds of goat. Small Rumin Res, 58: 195-199.
- Carnicella D., Dario M., Ayres M.C., Laudadio V., Dario C. (2008). The effect of diet, parity, year and number of kids on milk yield and milk composition in Maltese goat. Small Rumin Res, 77: 71-74.
- Addass P.A., Tizhe A.P., Midau A., Alheri P.A., Yahya M.M. (2013). Effect of genotype, stage of lactation, season and parity on milk composition of goat, in Mubi, Adamawa State, Nigeria. Ann Biol Res, 4(8): 248-252.
- Zahradden D., Butswat I.S.R., Mbap S.T. (2007). Evaluation of some factors affecting milk composition of indigenous goats in Nigeria. Livestock Res Rural Dev, 19(11): 1-6.