

Evaluation of some environmental and genetic factors (*CSN3* and *AGPAT6* gene) on milk yield and composition in Saanen goats

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ABSTRACT

This study was designed to determine the effect of some environmental factors on milk yield and composition in Saanen goats and the effect of *CSN3* and *AGPAT6* gene polymorphisms on milk production traits. Saanen goats were genotyped for milk traits, and the samples were collected during the lactation, and the milk yield/compositions of each goat were specified. In terms of the *CSN3* and *AGPAT6* genes, the genotypes were defined by PCR-RFLP. The milk yield (MY), lactation length (LL), protein, fat, total solid (TS), solid not fat (SNF), casein, lactose rates and yields were as follows; 388.9 ± 17.5 kg, 243 ± 5.81 days, $3.29 \pm 0.05\%$, $3.59 \pm 0.07\%$, $11.60 \pm 0.13\%$, $8.43 \pm 0.06\%$, $2.57 \pm 0.04\%$, $4.31 \pm 0.03\%$, 11.21 ± 0.47 kg, 12.79 ± 0.64 kg, 40.95 ± 1.82 kg, 29.53 ± 1.31 kg, 8.82 ± 0.37 kg, 15.40 ± 0.74 kg respectively. The determined genotype and allele frequencies of *CSN3* and *AGPAT6* gene were as follows; FF (%99.20), MF (%0.80), F (0.996), M (0.004); GG (%12), GC (%43.20), CC (%44.80), G (0.336), C (0.664). The impacts of lactation length on all milk yield parameters (protein, fat, TS, SNF, casein, lactose, MY) ($P < 0.001$); the age on all milk yield parameters ($P < 0.001$) and the protein, fat, SNF, casein rates ($P < 0.05$), the TS rate and LL ($P < 0.01$) were found to be significant. The birth type was not found to significantly affect any milk yield parameters. Although Saanen goats showed the variation for the *AGPAT6* gene (g.9263C>G), the herd was found to be monomorphic (FF) for the *CSN3* gene. The effect of the *AGPAT6* gene on milk traits in Saanen goats was determined not to be statistically significant. Environmental factors, such as maternal age and lactation length, were found to significantly affect some milk traits in Saanen goats. So consideration of factors such as maternal age or lactation length could be useful for improving breeding strategies for dairy goats.

Key words: milk yield/composition; PCR-RFLP; Saanen goats; *CSN3*; *AGPAT6*

Introduction

Goat products such as milk are some of the important factors contributing to the economy of farmers living in developing countries (BOSEWELL et al., 2018). In recent years, interest in goat's milk products has also increased in developed countries in Europe and America (SELVAGGI et al., 2014). This increase may be

associated with economic value due to the high prices of dairy products, as well as their beneficial ingredients (SELVAGGI et al., 2014).

The average milk yield of Saanen goats ranges from 204-1095 kg per lactation (BOICHARD et al., 1989; BELICHON et al., 1998; TZIBOULA-CLARKE, 2003; DAMIAN et al., 2008; TORRES-

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VÁZQUEZ et al., 2009; KESENKAS et al., 2010; TOLU et al., 2010; ULUTAS et al., 2010; NORRIS et al., 2011; BOLACALI and KUCUK, 2012; AKTAS et al., 2012; ISHAG et al., 2012). Similarly, like milk yield, it was noticed that the composition of milk varies between herds. Although the protein content of milk, which influences its cheese-making properties and is an important factor for the dairy industry, was reported as 2.84% in Saanen goats by DAMIAN et al. (2008). This ratio was estimated as 3.56% by NORRIS et al. (2011). The milk yield and composition are known to be affected by environmental factors, such as: breed (KOMINAKIS et al., 2000; GOETSCH et al., 2011), year (HAMED et al., 2009; KOMINAKIS et al., 2000; OLECHNOWICZ and SOBEK, 2008), season (GOETSCH et al., 2011; HAMED et al., 2009; CIAPPESONI et al., 2004), parity (HAMED et al., 2009; KOMINAKIS et al., 2000; GOETSCH et al., 2011; OLECHNOWICZ and SOBEK, 2008; CIAPPESONI et al., 2004), and birth type (HAMED et al., 2009; KOMINAKIS et al., 2000; GOETSCH et al., 2011; OLECHNOWICZ and SOBEK, 2008; CIAPPESONI et al., 2004; GOONEWARDANE et al., 1999; KETTO et al., 2014). These factors were investigated in Zaraibi goats (HAMED et al., 2009), Skopelos goats (KOMINAKIS et al., 2000), Polish White improved goats (OLECHNOWICZ and SOBEK, 2008), Czech White Shorthaired goats (CIAPPESONI et al., 2004), Nubian goats (GOONEWARDANE et al., 1999), Norwegian x Small East crossbreds (KETTO et al., 2014) and Alpine goats (CREPALDI et al., 1999).

In Saanen goats, few studies have been performed about the effects of environmental factors such as season, year, age, lactation stage/length or parity on milk production traits (ISHAG et al., 2012; TORRES-VÁZQUEZ et al., 2009; ZOA-MBOÉ et al., 1997; NORRIS et al., 2011; MIOČ et al., 2008). Also, the results of the investigated effects differ from each other. In contrast to MIOČ et al. (2008), ISHAG et al. (2012), and NORRIS et al. (2011), the effect of age on MY, fat, and protein percentage was found to be significant by ZOA-MBOÉ et al. (1997) and TORRES-VÁZQUEZ et al. (2009). On the other hand, the effect of birth type on lactation traits in Saanen goats has only

been investigated by BOLACALI and KUCUK (2012). Furthermore, the effect of lactation length on these traits has only been analyzed by ISHAG et al. (2012).

The κ -casein gene (*CSN3*) located on caprine chromosome 6 contains five exons, and most of the coding sequences of the mature protein (162 aa) are present in exon 4 (YAHYAOUÏ et al. 2001; 2003). (Gene ID:100861231). The polymorphism of the caprine *CSN3* gene that substitutes Val119 to Ile119 was first reported by MERCIER et al. (1976). The gene was extremely polymorphic with 21 identified alleles (A, B, B', B'', C, C', D, E, F, G, H, I, J, K, L, M, N, O, P, Q, and R; PRINZENBERG et al., 1999 and 2005; CAROLI et al., 2001; YAHYAOUÏ et al., 2001; JANN et al., 2004; GUPTA et al., 2009). All these variants are separated into two groups; AIEF (A, B, B', B'', C, C', F, G, H, I, J, L) and BIEF (D, E, K, M, N, O, P, Q, R) according to isoelectric points (PRINZENBERG et al., 2005). CHIANESE et al. (2000) reported that the BIEF group of *CSN3* variants might be related to the high casein content in milk. The effect of the *CSN3* gene on protein and casein contents was studied in Murciano-Granadina goats (CARAVACA et al., 2009); Camosciata, Frisa, Orobico, Verzasca goats (CHIATTI et al., 2007); and Norwegian dairy goats (DAGNACHEW et al., 2011). According to the results obtained, the effects of the investigated alleles were found to be statistically significant. Similar to the previous studies, SZTANKÓOVÁ et al. (2009) indicated that the alleles of B, C, and D of the *CSN3* gene affected the protein content of Czech dairy goats milk. However, polymorphisms or effects of the *CSN3* gene have been investigated in various goat breeds, while studies on Saanen goats are limited. Moreover, the F and M alleles of the *CNS3* gene were not studied sufficiently, while predominantly the alleles of A and B were investigated. PRINZENBERG et al. (2005) studied the existence of *CSN3**M (Gen Bank accession no: AY428577) and *F (Gen Bank accession no: AY090466) alleles in Saanen goats. The M allele of the *CSN3* gene differs from two nonsynonymous mutations (G→A transition at position 384, resulted in Asp90→Asn90 amino acid changes; C→T transition at position 550 resulted in

Val145→Ala145 amino acid changes) from the F allele (PRINZENBERG et al., 2005).

The *AGPAT* enzyme family contained in Lysophosphatidic acid acyltransferase (LPAAT) has eight members, known as *AGPAT1*, 2, 3, 4, 5, 6, 7, and 8 (YE et al., 2005). The *AGPAT6* gene (also known as GPAT4) located on caprine chromosome 27, has 14 exons (Gen Bank ID. NC_022319.1). BEIGNEUX et al. (2006) indicated that *AGPAT6*, used in triglyceride biosynthesis, is expressed in the epithelial breast tissue in mice. The milk of mice had *AGPAT6* -/- genotype, and was found to be free of diacylglycerol and triacylglycerol; the breast epithelium did not develop, and it was noticed that intracellular fat droplets were smaller in those animals (BEIGNEUX et al., 2006). As a result, it was concluded that the *AGPAT6* gene plays a key role in milk fat production in the breast epithelium. BIONAZ and LOOR (2008) emphasized that *AGPAT6* affected the fat content of milk by regulating the fat synthesis in the Holstein breed. The g.9263C>G mutation occurred in the 4th exon of the *AGPAT6* gene, resulting in a synonymous genetic code with threonine “ACC(Thr)>ACG(Thr)” at position 195 aa of *AGPAT6* (HE et al., 2011). They reported that the frequencies of CC, GC, and GG genotypes were 0.694, 0.198, and 0.108 g.9263C>G mutation in Xinong Saanen ve Guanzhong goats. According to that study, the milk fat/protein content and milk yields varied depending on CC, GC, and GG genotypes, and individuals with GG and GC genotypes had significantly higher milk yield and composition. On the other hand, the significant effects of the *AGPAT6* gene on milk protein composition were studied by MIKAILAITĖ (2017), and it was claimed that individual Lithuanian dairy goats that had CG genotype (g.9263C>G) produced higher milk protein yield. However, studies of *AGPAT6* gene polymorphisms are limited in the literature.

Besides the limited data on candidate genes' (such as *CSN3* and *AGPAT6*) effects on milk yield and composition in Saanen goats, to the best of our knowledge, the wide range studies about milk traits, including milk SNF, lactose, or casein yields of Saanen goats are insufficient. Thus, this study aimed to determine the effects of some environmental factors such as age, lactation length, and birth type on the milk yield and composition of Saanen

goats. Another objective of the current study was to estimate the frequencies of *CSN3* (550 C→T) and *AGPAT6* (g.9263C>G) gene polymorphisms in Saanen goats and their effects on milk yield and composition.

Materials and methods

Animals. The data were obtained from Saanen goats (n = 125) in the Southern Marmara Region of Turkey. The goats belonged to two independent herds (Farm1 and Farm 2) located in Bursa, Turkey. The latitude and longitude of Farm 1 (Herd1) and Farm 2 (Herd2) are 40 °13'51.5"N 28 °52'31.0"E and 40 °02'47.5"N 28 °51'44.4"E respectively. The goats were selected randomly within the herds, were genetically different from each other, and reared intensively. A commercial concentrated feed (2500 kcal/kg) and dried clover were used ad libitum for consumption by the Saanen does. The goats (n = 125) were investigated for genetic parameters such as the *CSN3* and *AGPAT6* gene. Moreover, some of them were screened for milk traits during the lactation period. For all procedures, ethical approval was received (2012-04/03).

Milk traits. Saanen does were milked by machine twice a day. The milk data, including the yields and components [protein, fat, total solid (TS), solid not fat (SNF), casein, lactose, and total MY], were recorded throughout lactation from Saanen goats from Herd 1 (in 2012). During lactation, milk samples were collected in 100 mL sterile sample containers twice a month. Samples were transported to the laboratory via a cold chain (+4 °C) within two hours. The total MY of lactation per animal was evaluated by the Fleischmann method (Trapez II), according to the International Committee for Animal Recording (ICAR) Guidelines 2014 (BERGER and THOMAS, 2005). The samples were analyzed for milk contents, such as total protein, fat, TS, SNF, total casein, and lactose by Fourier transform infrared (FTIR) spectroscopy (MilkoScan™ FT1, Foss Electric, Hillerød, Denmark) (BERGE et al., 2010).

***CSN3* and *AGPAT6* gene analysis.** Blood samples were collected aseptically from the jugular vein in EDTA. All samples were transported to the laboratory at low temperature, and were kept at -20 °C until DNA extraction at the genetic laboratory

of the Faculty of Veterinary Medicine, Uludag University. The genomic DNA was extracted by the phenol-chloroform method according to POWELL and GANNON (2002). The characteristics of the DNA, the purity and amount, were determined by spectrophotometer according to the ratio of absorbance at 260 and 280 nm (260/280) (Thermo/NanoDrop 2000C). The extracted DNA samples were stored at -80 °C until the PCR-RFLP was

performed. The polymorphisms of *CSN3* (550 C→T) and *AGPAT6* (g.9263C>G) were determined by the PCR-RFLP method, according to CHESSA et al. (2003), PRINZENBERG et al. (2005), and HE et al. (2011) respectively. The 407 bp PCR product for *CNS3* and 241 bp PCR products for the *AGPAT6* gene were amplified using the established primer sets and conditions given in Table 1.

Table 1. Primer sets and PCR conditions of *CSN3* and *AGPAT6* gene

Gene	Primer sets	Product size	PCR conditions	References
<i>CSN3</i> - 550 C→T	KCNI-F: GGTATCCTAGTTATGGACT-CAAT KCN I-R: GTTGAAGTAACTTGGGCTGTGT	407 bp	94°C 1,5 m [94°C 45 s 56,5°C 45 s 72°C '45 s] x 35cycle 72°C 5 m	Chessa et al. 2003, Prinzenberg et al., 2005
<i>AGPAT6</i> - g.9263C>G	F: ATCTGGCATTTCACACATT R: CTGACTCCATCTAAGAGCCT	241 bp	95°C 5 m [94°C 30 s 53°C 30 s 72°C 35 s]x 34 cycle 72°C 10 m	He et al., 2011

PCR products were evaluated by electrophoresis on 2-2.5% agarose gel. Differentiation between the M and F alleles of the *CSN3* gene and the G and C alleles of the *AGPAT6* gene was performed by RFLP analysis. A 5 µL of each PCR product was digested with PstI and NcoI-HF restriction enzyme for the *CSN3* and *AGPAT6* genes, respectively, at 37 °C for about 16 hours, according to the manuals. The enzyme products were estimated by 3% agarose gel electrophoresis (COSENZA et al., 2008). The bands of PCR and enzyme products were visualized using the DNr Minilumi imaging system.

Statistical analyses. Popgene v1.32 software was used for calculation of the genotype frequencies of *CSN3* and *AGPAT6* gene. Data were processed by the software to estimate the genotype frequencies and possible deviation from the Hardy-Weinberg equilibrium of Saanen goats (YEH et al., 2000). Statistical analysis of milk yield/composition was carried out by the Minitab 15 statistical software (Minitab Inc., 2000) using the general linear model procedure (GLM) with the following model for milk composition:

$$Y_{ijk} = \mu + C_i + D_j + E_k + e_{ijk}$$

Y_{ijk} = the dependent variable (LL, protein, fat, TS, SNF, casein and lactose rate);
 μ = the overall mean;
 C_i = the fixed effect of *AGPAT6* gene (g.9263C>G) (i = CC, GC, GG);
 D_j = the fixed effect of does age (k = 1, 2, 3, 4≥ age);
 E_k = the fixed effect of birth type (j = single, twin, triplet);
 e_{ijkl} = the random error.

The model for protein, fat, TS, SNF, casein, lactose and lactation MY is as follows:

$$Y_{ijkl} = \mu + C_i + D_j + E_k + F_l + e_{ijkl}$$

Y_{ijkl} = the dependent variable (protein, fat, TS, SNF, casein, lactose and lactation MY);
 μ = the overall mean;
 C_i = the fixed effect of *AGPAT6* gene (g.9263C>G) (i = CC, GC, GG);
 D_j = the fixed effect of does age (j = 1, 2, 3, 4≥ age);
 E_k = the fixed effect of birth type (k = single, twin, triplet);
 F_l = the fixed effect of lactation length period (l = 1:180-210 day, 2:211-230 day, 3:231-250 day, 4:251-270 day, 5:271-288 day);
 e_{ijkl} = the random error.

Table 2. The milk composition (%) of Saanen goats according to some genetic and environmental factors

Factor	n	Protein%	Fat%	TS**%	SNF*%	Casein%	Lactose%	LL (day) *
<i>AGPAT6</i> -g.9263C>G	GG 6	3.12 ± 0.12	3.41 ± 0.15	11.16 ± 0.29	8.20 ± 0.15	2.43 ± 0.10	4.29 ± 0.05	242.9 ± 5.68
	GC 20	3.39 ± 0.07	3.63 ± 0.09	11.81 ± 0.18	8.59 ± 0.09	2.65 ± 0.06	4.31 ± 0.03	244.6 ± 8.21
	CC 35	3.35 ± 0.05	3.71 ± 0.06	11.83 ± 0.12	8.52 ± 0.06	2.62 ± 0.04	4.29 ± 0.02	242.3 ± 13.28
		NS	NS	NS	NS	NS	NS	NS
Age	1 18	3.15 ± 0.08 ^c	3.30 ± 0.11 ^b	11.10 ± 0.21 ^b	8.27 ± 0.11 ^c	2.44 ± 0.07 ^c	4.32 ± 0.04	218.7 ± 9.87 ^b
	2 11	3.26 ± 0.09 ^{bc}	3.64 ± 0.13 ^a	11.66 ± 0.24 ^a	8.40 ± 0.12 ^b	2.54 ± 0.08 ^{bc}	4.37 ± 0.04	252.3 ± 10.99 ^a
	3 17	3.46 ± 0.08 ^a	3.74 ± 0.10 ^a	12.01 ± 0.20 ^a	8.65 ± 0.10 ^a	2.71 ± 0.06 ^a	4.30 ± 0.03	261.2 ± 9.04 ^a
	4 ≤ 15	3.29 ± 0.08 ^{ab}	3.65 ± 0.11 ^a	11.62 ± 0.20 ^a	8.32 ± 0.10 ^{bc}	2.57 ± 0.07 ^b	4.22 ± 0.04	241.0 ± 9.42 ^a
		*	*	**	*	*	NS	**
Birth type	1 19	3.25 ± 0.07	3.66 ± 0.09	11.66 ± 0.18	8.37 ± 0.09	2.53 ± 0.06	4.31 ± 0.03	241.1 ± 8.22
	2 33	3.34 ± 0.05	3.63 ± 0.07	11.76 ± 0.14	8.53 ± 0.07	2.60 ± 0.04	4.33 ± 0.02	240.8 ± 6.56
	3 9	3.29 ± 0.12	3.46 ± 0.16	11.37 ± 0.29	8.38 ± 0.15	2.56 ± 0.10	4.27 ± 0.05	248.0 ± 13.34
		NS	NS	NS	NS	NS	NS	NS
Mean		3.29 ± 0.05	3.59 ± 0.07	11.60 ± 0.13	8.43 ± 0.06	2.57 ± 0.04	4.31 ± 0.03	243 ± 5.81

TS*: total solid, SNF*: solid not fat, LL*: lactation length; *** - P<0.001, ** - P<0.01, * - P<0.05, NS - not significant, ^{abc} - Different superscripts within a column indicate significant differences.

Table 3. The milk yields (kg) of Saanen goats according to some genetic and environmental factors.

Factor	n	Milk yield (kg)	Protein (kg)	Fat (kg)	TS* (kg)	SNF* (kg)	Casein (kg)	Lactose (kg)
<i>AGPAT6</i> -g. <i>9263C>G</i>	GG	416.9 ± 38.60	11.58 ± 0.47	13.01 ± 1.41	41.28 ± 4.04	29.87 ± 2.90	8.66 ± 0.83	16.16 ± 1.65
	GC	365.0 ± 24.38	11.04 ± 0.66	12.12 ± 0.89	39.44 ± 2.55	28.68 ± 1.83	8.69 ± 0.52	14.67 ± 1.04
	CC	384.4 ± 17.44	11.00 ± 1.05	13.23 ± 0.63	42.12 ± 1.82	30.05 ± 1.31	9.08 ± 0.37	15.39 ± 0.74
Age	1	257.3 ± 31.12 ^d	8.06 ± 0.85 ^c	9.36 ± 1.14 ^d	29.60 ± 3.25 ^e	21.65 ± 2.34 ^c	6.32 ± 0.67 ^c	11.48 ± 1.33 ^c
	2	331.4 ± 32.76 ^c	8.82 ± 0.89 ^c	10.13 ± 1.20 ^c	33.26 ± 3.42 ^c	24.30 ± 2.46 ^c	7.04 ± 0.70 ^c	12.91 ± 1.40 ^c
	3	433.7 ± 28.31 ^b	12.78 ± 0.77 ^b	14.12 ± 1.03 ^b	45.39 ± 2.96 ^b	32.78 ± 2.13 ^b	10.03 ± 0.60 ^b	16.57 ± 1.21 ^b
	4≤	532.7 ± 29.59 ^a	15.12 ± 0.80 ^a	17.52 ± 1.08 ^a	55.54 ± 3.09 ^a	39.41 ± 2.23 ^a	11.85 ± 0.63 ^a	20.66 ± 1.26 ^a
Birth type	1	398.6 ± 24.50	11.36 ± 0.67	13.13 ± 0.89	41.85 ± 2.56	29.98 ± 1.86	8.90 ± 0.52	15.70 ± 1.04
	2	396.6 ± 21.62	11.58 ± 0.59	13.53 ± 0.79	42.82 ± 2.26	30.57 ± 1.62	9.13 ± 0.46	15.92 ± 0.92
	3	371.2 ± 39.80	10.69 ± 1.08	11.69 ± 1.45	38.18 ± 4.16	28.05 ± 2.99	8.40 ± 0.85	14.59 ± 1.70
LL (day)	180-210	301.5 ± 44.45 ^e	7.60 ± 1.21 ^c	8.70 ± 1.62 ^c	28.39 ± 4.65 ^c	20.60 ± 3.34 ^{bc}	5.97 ± 0.95 ^c	10.95 ± 1.90 ^c
	211-230	338.5 ± 28.54 ^d	9.00 ± 0.78 ^b	9.40 ± 1.04 ^c	32.02 ± 2.98 ^c	23.75 ± 2.15 ^b	7.05 ± 0.61 ^c	12.43 ± 1.22 ^{bc}
	231-250	362.8 ± 27.69 ^c	10.77 ± 0.75 ^b	12.25 ± 1.01 ^b	38.93 ± 2.89 ^b	27.86 ± 2.08 ^b	8.50 ± 0.59 ^b	14.30 ± 1.18 ^b
	251-270	449.8 ± 27.75 ^b	14.29 ± 0.75 ^a	16.11 ± 1.01 ^a	51.38 ± 2.90 ^a	37.26 ± 2.09 ^a	11.25 ± 0.59 ^a	19.10 ± 1.18 ^a
Mean	271-288	491.3 ± 31.12 ^a	14.38 ± 0.85 ^a	17.46 ± 1.14 ^a	54.00 ± 3.25 ^a	38.20 ± 2.34 ^a	11.29 ± 0.67 ^a	20.24 ± 1.33 ^a
		388.79 ± 17.35	11.21 ± 0.47	12.79 ± 0.64	40.95 ± 1.82	29.53 ± 1.31	8.82 ± 0.37	15.40 ± 0.74

TS*: total solid, SNF*: solid not fat, LL*: lactation length; *** - P<0.001, ** - P<0.01, * - P<0.05, NS - not significant, ^{a,b,c,d,e} - Different superscripts within a column indicate significant differences.

Table 4. The allele and genotype frequencies of *CSN3* and *AGPAT6* gene in Saanen goats

Genotype	Herd 1		Herd 2		Total		Allele	Allele frequency	Herd 1 Allele frequency	Herd 2 Allele frequency	Total Allele frequency
	n	Genotype frequency (%)	n	Genotype frequency (%)	n	Genotype frequency (%)					
<i>CSN3</i>	FF	68	98.55	56	100	124	F	0.992	0.992	1	0.996
	MF	1	1.45	0	0	1	M	0.008	0.008	0	0.004
	MM	0	0	0	0	0					
<i>AGPAT6</i> *	GG	7	10.15	8	14.29	15	G	0.268	0.268	0.420	0.336
	GC	23	33.33	31	55.36	54	C	0.732	0.732	0.580	0.664
	CC	39	56.52	17	30.35	56					
Total	69		56		125						100

* - *AGPAT6* (HWE): χ^2 - 0.1267; P - 0.7218

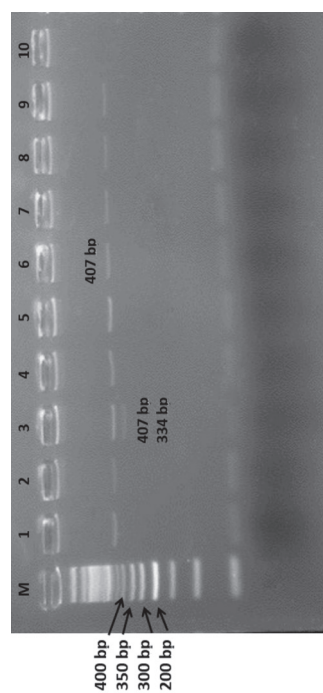


Fig. 1. The RFLP products of the *CSN3* gene. The PCR products (407 bp) obtained from DNA samples of Saanen goats were digested with the *PstI* restriction enzyme. Samples were visualized by 3% agarose gel electrophoresis. (Line M: 100 bp DNA ladder, Line 1-2: MF genotype with 407 and 334 bp products, Line 3: MF genotype with 407 and 334 bp product, Lane 10: Negative control)

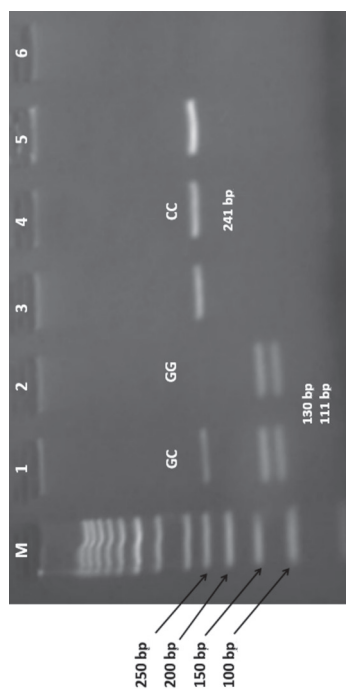


Fig. 2. The RFLP products of the *AGPAT6* gene. The PCR products (241 bp) obtained from DNA samples of Saanen goats were digested with the *NcoI* restriction enzyme. Samples were visualized by 3% agarose gel electrophoresis. (Line M: 100 bp DNA ladder, Line 1: GC genotype with 241, 130 and 111 bp products, Line 2: GG genotype with 130 and 111 bp products, Line 3-5: CC genotype with 241 bp product, Line 6: Negative control.)

Results

Milk traits. According to the results; LL, protein, fat, TS, SNF, casein, lactose rates were; 243 ± 5.81 days, $3.29 \pm 0.05\%$, $3.59 \pm 0.07\%$, $11.60 \pm 0.13\%$, $8.43 \pm 0.06\%$, $2.57 \pm 0.04\%$, $4.31 \pm 0.03\%$ (Table 2) respectively. Moreover, the mean yield of total milk, protein, fat, TS, SNF, casein and lactose were determined as; 388.9 ± 17.5 kg, 11.21 ± 0.47 kg, 12.79 ± 0.64 kg, 40.95 ± 1.82 kg, 29.53 ± 1.31 kg, 8.82 ± 0.37 kg, 15.40 ± 0.74 kg, respectively (Table 3). The impact of age on all MY parameters (protein, fat, total solid, solid not fat, casein, and lactose yield) ($P < 0.001$) was found to be statistically significant. Furthermore, the percentages of milk components, such as protein ($P < 0.05$), fat ($P < 0.05$), TS ($P < 0.01$), SNF ($P < 0.05$), casein ($P < 0.05$), and lactation length ($P < 0.01$) were significantly affected by age. It was observed that the fat, TS, and LL increased with age, and the highest MY were determined in four year old does.

***CSN3* and *AGPAT6* gene.** The genotype and allele frequencies of *CSN3* and *AGPAT6* gene were determined in Saanen goats. The PCR-RFLP results revealed that two different genotypes (FF and MF) were detected for the *CNS3* gene (Fig. 1); the frequencies of the FF and MF genotype for 550 C→C SNP were 99.20% and 0.80%, respectively (Table 4). Also, the allele frequencies of F and M were 0.996 and 0.004. Since only one individual had a heterozygote genotype (MF), the herds were identified as monomorphic (FF) for the *CSN3* gene. Therefore the effect of the *CSN3* gene on milk yield and composition was not estimated. For the *AGPAT6* gene, three different genotypes (GG, GC, CC) were observed, as shown in Fig. 2. The following genotype frequencies of *AGPAT6* (g.9263C>G) were determined respectively: GG (12.00%), GC (43.20%), CC (44.80%) with the allele frequencies G (0.336) and C (0.664). The most frequent genotype was CC for the *AGPAT6* gene in the investigated Saanen flock. The effect of the *AGPAT6* gene on the investigated milk traits was determined not to be statistically significant in Saanen goats in the current study.

Discussion

Milk traits. Accordingly to the literature, there is high variability in the recorded lactation MY of Saanen goats. The result regarding the MY obtained in this study was lower than those reported by BOICHARD et al. (1989), BELICHON et al. (1998), TORRES-VÁZQUEZ et al. (2009), TOLU et al. (2010), and VLAD et al. (2014), but higher than those determined by ULUTAS et al. (2010), BOLACALI and KUCUK, 2012, AKTAS et al. (2012) and ISHAG et al. (2012). In common with MY, the LL also differs throughout the literature. the LL in the present study (243 ± 5.81) was detected to be lower than that reported by TOLU et al. (2010), BOLACALI and KUCUK (2012). However, this parameter was found to be higher than that reported by BOICHARD et al. (1989), ULUTAS et al. (2010), AKTAS et al. (2012), ISHAG et al. (2012), and VLAD et al. (2014). The difference between LL values might originate from the distinction of milk yields depending on nutritional levels affected by the climatic conditions of the breeding area or differences in genotypic structure.

Contrary to ZOA-MBOÉ et al. (1997), the findings of the present study were in agreement with those reported by TORRES-VÁZQUEZ et al., (2009), ZOA-MBOÉ et al. (1997), and BOLACALI and KUCUK (2012). ZOA-MBOÉ et al. (1997) indicating that the factor of age influenced all milk, fat, and protein yield/composition traits, except the fat percentage. The impact of age on lactation traits might be explained by the developing udder structure due to the increase in body size with age. Hence milk production capacity improves with advanced age. In close agreement with ISHAG et al. (2012), the effect of lactation length on all MY parameters (lactation milk, protein, fat, total solid, solid not fat, casein, and lactose yield) ($P < 0.001$) were defined as statistically significant. The peak MY's were identified in does with 251-270 and 271-288 days of LL. In this study, no significant differences were observed due to the effects of birth type on milk production traits ($P > 0.05$). These observations differ from the work of BOLACALI and KUCUK (2012), ISHAG et al. (2012), who determined that the effect of birth type on total milk yield was significant.

CSN3 and *AGPAT6* genes. The A and B alleles of the *CSN3* gene have been studied in various goat breeds, including Saanen goats (YAHYAUI et al., 2001/2003; PRINZENBERG et al., 2005; KUMAR et al., 2009; CHIATTI et al., 2007; CARAVACA et al. 2009; SZTANKÓOVÁ et al., 2009; GUPTA et al., 2009; CAROLI et al., 2001 and STRZELEC and NIŻNIKOWSKI, 2011; CHESSA et al., 2003). The frequencies of the M and F alleles of the *CSN3* gene were investigated in the current study, and the results showed that the genotype frequencies were 99.20% for FF and 0.80% for MF in the Saanen breed. These findings were partially compatible with the work of PRINZENBERG et al. (2005), who reported that the frequencies of M allele were null. Only one individual was identified as a heterozygote (MF), as shown in Fig. 1. In another study, performed for the same SNP by STRZELEC and NIŻNIKOWSKI (2011), the “a” and “g” alleles were identified (the “a” allele accepted to B variant of *CSN3* gene) and “aa” and “gg” genotype frequencies were determined as 78.13% and 21.88% in the Saanen breed. However, these results were not similar to our data because the frequency of the B variant (“aa”), which is known to contain the M allele, was found to be dominant in that study in contrast to the present study. The observed variation in genotype frequencies could be explained by the differences in genetic structure. Since the herd was found to be monomorphic for *CSN3* gene polymorphism (550 C→T), this effect on milk traits was not evaluated.

The genotypic frequencies of the *AGPAT6* gene were similar to data recorded by MIKAILAITÉ (2017), who reported that the frequencies were 42.9%, 42.9%, and 14.2% for CC, GC, and GG genotypes, respectively. These findings are in agreement with those reported by HE et al. (2011). The genotype frequencies were established to be 10.8% for CC, 19.8% for GC, and 69.4% for GG. Also, the allele frequencies of the *AGPAT6* gene for g.9263C>G polymorphism were 0.793 and 0.207 for G and C. Therefore, our results of allele frequencies (G:0.336, C:0.664) conflict with the work of HE et al. (2011). This might be due to the variation in the genetic structure in the investigated goats that

had different origins. HE et al. (2011) emphasized that individuals with GG and GC genotypes had higher milk fat and protein percentages, as well as milk yield, compared to those of the CC genotype, in addition to the genotype/allele frequencies of the gene. Unlike the findings of HE et al. (2011), the effect of the *AGPAT6* gene on milk traits was determined as insignificant in this study. Moreover, these results were not similar to data indicated by MIKAILAITÉ (2017), who claimed that goats with the GC genotype had higher protein content than the others. The reason for the differences in the effects on milk traits compared to previous studies could be explained by flock differences or the sample size of the study. The significant effects of the *AGPAT6* gene for g.9263C>G on milk traits, especially milk fat content, could be the result of the larger amount of phenotypic or genotypic data.

Conclusions

In conclusion, the effect of some environmental factors on milk yield and composition and the effect of *CSN3* and *AGPAT6* gene polymorphisms on milk production traits in Saanen goats were established. Parameters such as SNF, lactose, or casein yield of Saanen milk, which are important factors for the dairy industry, were first reported in this study. Environmental factors, such as maternal age and lactation length, were found to be significant for milk production traits in Saanen goats. So, consideration of these environmental factors, which were found to be statistically significant in the present study, could be useful as selection criteria in dairy goat breeding for higher milk yield and better composition. Furthermore, screening of the other regions of the *CSN3*, *AGPAT6* genes, and also the investigation of the phenotypic association between them could be useful in order to clarify the efficiency of target genes.

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SAŽETAK

Istraživanje je provedeno kako bi se odredili učinci čimbenika okoliša te učinci genskih polimorfizama *CSN3* i *AGPAT6* na prinos i sastav mlijeka u sanske koze. Tijekom laktacije koza provedena je genotipizacija te prikupljeni uzorci za utvrđivanje prinosa i sastava mlijeka. Genotipovi gena *CSN3* i *AGPAT6* određeni su PCR-RFLP-om. Prinos mlijeka (MY), duljina laktacije (LL), udio proteina, udio masnoće, udio suhe tvari (TS), udio nemasne suhe tvari (SNF), udio kazeina, udio laktoze bili su kako slijedi: 388,9 ± 17,5 kg, 243 ± 5,81 dana, 3,29 ± 0,05 %, 3,59 ± 0,07 %, 11,60 ± 0,13 %, 8,43 ± 0,06 %, 2,57 ± 0,04 %, 4,31 ± 0,03 %. Prinos u kg za protein, masnoću, suhu tvar, nemasnu suhu tvar, kazein i laktozu bili su kako slijedi: 11,21 ± 0,47 kg, 12,79 ± 0,64 kg, 40,95 ± 1,82 kg, 29,53 ± 1,31 kg, 8,82 ± 0,37 kg, 15,40 ± 0,74 kg. Utvrđeni genotipovi i učestalost alela gena *CSN3* i *AGPAT6* bili su: FF (99,20 %), MF (0,80 %), F (0,996), M (0,004), GG (12 %), GC (43,20 %), CC (44,80 %), G (0,336), C (0,664). Statistički znakoviti utjecaji utvrđeni su za: duljinu laktacije na pokazatelje prinosa mlijeka (proteine, masnoću, TS, SNF, kazein, laktozu, MY) ($P < 0,001$); dobi na pokazatelje prinosa mlijeka ($P < 0,001$) zatim na proteine, masnoću, SNF, udio kazeina ($P < 0,05$), te na udio suhe tvari i duljinu laktacije ($P < 0,01$). Tip legla nije znakovito utjecao na pokazatelje prinosa mlijeka. Istražene sanske koze pokazale su varijacije gena *AGPAT6* (g.9263C>G), dok je stado za gen *CSN3* bilo monomorfno (FF). Učinak gena *AGPAT6* na svojstva mlijeka nije bio statistički znakovit. Čimbenici kao što su dob majke i duljina laktacije znakovito su utjecali na svojstva mlijeka sanske koze, stoga bi oni mogli biti korisni za unapređenje uzgojnih strategija mliječnih pasmina koza.

Ključne riječi: prinos i sastav mlijeka; PCR-RFLP; sanska koza; *CSN3*; *AGPAT6*
