Determination the effect of *CSN1S1, CSN3* and *AGPAT6* genes and lactation rank on physicochemical properties of goat milk

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SUMMARY

The physiochemical properties of milk are important factors in terms of the process of dairy production, quality, and profitability. However, the knowledge about the effect of genetic factors such as *CSN1S1, CSN3,* and *AGPAT6* genes on these traits is insufficient. The objective of this study was to determine the effect of these genes and lactation rank (LR) on total acidity, citric acid, density (density), free fatty acid (FFA), freezing point degree (FPD) and urea parameters. A total of fifty (n=50) Saanen goats, which is known as the highest milk-producing breed within the goats were genotyped using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) methods. For genetic analysis, the blood samples were taken from the jugular vein by aseptic conditions. The DNA isolations were performed by the phenol-chloroform method from blood samples. Milk samples were collected during the lactation and evaluated by Fourier transform infrared (FTIR) spectroscopy for the physicochemical properties of Saanen goat milk. The statistical analysis was carried out using the least-squares of the GLM procedures. The results indicated that no significant differences were confirmed between the investigated genes with the physicochemical properties of goat milk. However, the effect of the *AGPAT6* gene on density and the effect of LR on citric acid composition was found tended to be significant. Moreover, we determined the significant correlations between the physicochemical properties in the present study. According to the results, acidity was correlated with the citric acid (-0.304), density (0.641), FFA (-0.332) significantly. Other significant correlations were observed between the citric acid and FPD (0.275), LR (-0.313); the density and FFA (-0.315), FPD (0.436); the urea and LR (-0.369). These results and novel correlations may be useful for future studies on evaluating the potential impact of *AGPAT6* gene on these traits to achieve breeding and commercial goals in the goat production and dairy industry.

KEY WORDS

Goat milk, physicochemical properties, *CSN1S1, CSN3, AGPAT6.*

INTRODUCTION

Goat milk prevails from the others due to the composition and nutritional properties such as organic materials and minerals. Besides these properties, the physicochemical compounds such as acidity, citric acid, density (density), free fatty acid (FFA), freezing point degree (FPD) and urea amounts are important parameters to define the effect of various dairy production methods on a quality of fresh milk or milk products[1]. As a result of that, these parameters obtain product quality and profitability and processability in terms of the technological properties of milk for the dairy industry[2]. It was known that the aging time and temperature significantly associated with the FFA level also changes the storage time[1]. On the other hand, the acidity of milk is used to identify the growth of the bacteria during fermentation on cheese or yogurt making process[3]. The organic acids, such as citric acid, play a crucial role in the flavor of dairy products; thus, they subscribe to the quality of cheese[4]. The impact on the technological process makes the physicochemical properties of milk as an important criterion for the dairy industry. The composition of milk is impressed by lots of environmental and genetic factors such as health problems, stage of lactation, breeding conditions, feed intake, or genetic polymorphisms[2].The development of molecular technics will provide researchers a better knowledge of the candidate markers (such as SNPs-single nucleotide polymorphisms) that are associated with milk yield and quality, also the accuracy of genomic breeding values rather than classic selection methods[5].

The four casein loci consist of αS1-casein *(CSN1S1)*, β-casein *(CSN2)*, αS2-casein *(CSN1S2)*, and κ-casein *(CSN3)* located on 250-300 kb region of chromosome 6 in goats[6]. The polymorphisms of these genes have significant impacts on various dairy phenotypes[6]. The *CSN1S1* gene, which's eighteen alleles have been identified up to now, is associated with different levels of alpha S1-casein (α*S1-CN*) protein of milk[7]. It was indicated that the polymorphisms of that gene effect not only the casein content but also its structural and nutritional characteristics and technological properties of goat milk[7]. Zhang et al.[8] indicated that an 11-bp indel mutation was significantly associated with the FPD and acidity of Guanzhong dairy goat milk.

The *CSN3* gene is another member of a casein gene family that most of the coding sequences of the mature protein (162 aa) are presented in exon 4 (Gene ID:100861231)[9]. Similarly, the *CSN3* gene was described considerably polymorphic, has 21 alleles (A, B, B′, B″, C, C′, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, and R) have been defined so far [9-10]. The genes encoding caseins such as *CSN1S1* or *CSN3* are strong candidates to clarify the variation between the protein contents of milk, moreover the rheological parameters and cheese yield in general[10].

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The 1-acylglycerol-3-phosphate O-acyltransferases 6-*AGPAT6* gene (also known as *GPAT4*) located in caprine chromosome 27 (have 14 exons) is involved in triacylglycerol and glycerophospholipid biosynthesis pathways[11]. Because of the role in lipid metabolism, it was claimed that the *AGPAT6* gene could be effective on milk fat yield and composition. The polymorphisms of *AGPAT6* gene have been studied in Holstein cattle[12], Brown Swiss cattle[13] and Guanzhong goats[11] up to now; according to the results, significant effects on milk fat and protein composition were reported. Moreover, the genotypic frequencies of investigated genes exhibited variation between the breeds and is thought that these differences might alter the physical and chemical properties of milk on investigated breeds [10,11].

Nevertheless, the studies about the effect of the polymorphisms of candidate genes (*CSN1S1, CSN3,* and *AGPAT6*) on physicochemical properties (acidity, density, FPD, FFA or urea) of Saanen goat milk have been rather limited in the literature, to the best of our knowledge. Therefore, determination of the effect of *CSN1S1, CSN3,* and *AGPAT6* genes on the physicochemical properties of goat milk was aimed in the present study.

MATERIALS AND METHODS

Animal sources and milk analysis

The study was performed in Saanen goats (n=50) ranged from one to six years old. The goats were breeding intensively at the farm located in a district of Bursa province in the Marmara Region of Turkey. The animals were fed with the same diets according to standard commercial implementation. During the lactation, Saanen does were milked by machine twice a day, and milk samples were collected in sterile sample containers twice a month. Samples were transported to the laboratory via the cold chain (+4°C). The samples were analyzed for physicochemical properties such as urea, acidity, density, citric acid, FPD, and FFA with Fourier transform infrared (FTIR) spectroscopy (MilkoScan™ FT1, Foss Electric, Hillerød, Denmark). For all processing was carried out in compliance with the ethical requirements, and ethical approval was received from the local Ethics Committee for Animal Research with the number of 2019-07/05.

Genomic analysis

The genomic DNA was extracted from the blood samples using phenol-chloroform, according to Green and Sambrook[14]. The investigated animals were genotyped for *CSN1S1*(B1, B3 and E allele), *CSN3* (550 C T) and *AGPAT6* (g.9263C>G) polymorphisms by PCR-RFLP method according to the methodology described by Cosenza et al.[15], Chessa et al.[16], Prinzenberg et al.[10] and He et al.[11], respectively. The PCR products of *CSN1S1, CSN3,* and *AGPAT6* genes were digested with *MnlI* (for *CSN1S1*-B1), *DdeI* (for *CSN1S1*-B3), *MspI* (for *CSN1S1*-E), *PstI* and *NcoI-HF* restriction enzyme (NEB) at 37 for 16 hours. After then, the RFLP products were separated on the 2-3% agarose gel containing ethidium bromide at 100 - 120 V for 45 to 60 minutes. The bands were visualized with the DNr Minilumi imaging system, and the animals were genotyped according to the agarose gel results.

Statistical analysis

The genotype frequencies of tested polymorphisms were calculated by direct counting. The chi-square test ($\chi 2$) for deviation from Hardy-Weinberg equilibrium (HWE) has been estimated by Pop-gen (Ver. 1.31) software[17]. The indexes of genetic variety, including expected heterozygosity (He), effective allele numbers (Ne), and the polymorphism information content (PIC) were calculated according to Nei & Roychoudhury[18]. The phenotypic correlation coefficients were estimated using Pearson's correlation coefficient (PCC) option of correlation procedures. The PCC was categorized into three groups by the levels of PCC ranges: 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 correlation[19].

In order to detect the differences between groups for investigated parameters, the general linear model (GLM) was carried out using the Minitab software (MINITAB®, USA, v17.1.0) program. The following mixed model was chosen by considering the adjusted R^2 to adjust possible significant differences between the genotypes: $Y_{ijklmn} = \mu + C_i + A_j + B1_k + B3_l + E_m + L_n + e_{ijklmn}$, where *Yijk* symbolized the observed value; μ is the overall mean for each trait; *Ci is* the fixed effect of *CSN3* gene *(i= FF, MF); Aj is* the fixed effect of *AGPAT6* gene *(i= CC, GC, GG);* $B1_k$ is the fixed effect of B1 genotype (m = B1, B1/NB1, NB1); $B3_l$ is the fixed effect of B3 genotype (o = B3, B3/NB3, NB3); E_m is the fixed effect of B1 genotype (p = E, E/NE, NE); L_n is the fixed effect of lactation rank; e_{ijklmn} is the random error. For all statistical analyses, a probability level of P<0.05 was accepted as statistically significant; the P value less than 0.10 (P < 0.1) was considered as a tendency in the current study.

RESULTS

Genotypic distributions of the population

The genotype frequencies and the indexes of He, Ne, and PIC values, including X_2 and *P* significance of HWE, are given in Table 1. According to the results, the most frequent genotypes were observed B1-B1 for *CSN1S1-B1* (0.96); B3-NB3 for *CSN1S1-B3 (0.48), NE-NE for CSN1S1-E (0.59)*; FF for *CSN3* (0.98) and CC genotype for *AGPAT6* gene (0.58). The NB1-NB1 and MM genotypes were not found in the investigated flock. Although only one goat with MF and two goats with B1-NB1 genotypes were observed in the population, the breed was adjusted polymorphic for *CSN1S1, CSN3,* and *AGPAT6* gene. In the case of the *CSN1S1*gene, the X_2 values for the genotypes of B1, B3, and E allele were 0.0208 and 0.2416 and 0.0088 with the probability values of 0.885 (P>0.05) and 0.623 (P>0.05) and 0.925 (P>0.05) respectively. For *CSN3* (550C T) and *AGPAT6* (g.9263C>G) polymorphisms the X_2 and P values were determined 0.0051 and 0.4356 with 0.943 (P>0.05) and 0.509 (P>0.05) respectively. These indicated that the investigated goat population had not deviated from the HWE (Table 1) for *CSN1S1, CSN3,* and *AGPAT6* genes.

Association of genetic and environmental factors with investigated traits

The results about the effect of genotype *(CSN1S1, CSN3,* and *AGPAT6* gene polymorphisms) and LR on acidity, citric acid, density, FFA, FPD, and urea are presented in the related table (Table 2). In respect of the studied parameters, the effect of *CSN1S1* (B1, B3, E) and *CSN3* gene on the physicochemical properties of Saanen goat milk were determined statistically insignificant. However, the effect of the *AGPAT6* gene on goat milk density (g/L) was considered as a tendency (P=0.086) in the present study. According to the results, the milk belongs to GC genotyped animals could be denser than others. Similarly, the LR was determined tended to be significant on the citric acid composition

Table 1 - The genotype frequencies of *CSN1S1, CSN3* and *AGPAT6* genes, population genetic indices and accordance with HWE.

Gene	*Genotype*	*n*	*%*	*He*	*Ne*	*PIC*	*2(HWE)*	*P(HWE)*
CSN1S1- B1	B1-B1	48	96	0,0392	1,0408	0,0384	0,0208	0,885
	B1-NB1[1]	2	4					
	NB1-NB1	-	-					
CSN1S1- B3	B3-B3	5	10	0,4488	1,8142	0,3481	0,2416	0,623
	B3-NB3	24	48					
	NB3-NB3	21	42					
CSN1S1- E	E-E	3	6	0,3750	1,6000	0,3047	0,0088	0,925
	E-NE	19	38					
	NE-NE	28	56					
CSN3	FF	49	98	0,0198	1,0202	0,0196	0,0051	0,943
	MF	1	2					
	MM	-	-					
AGPAT6	CC	29	58	0,3750	1,6000	0,3047	0,4356	0,509
	GC	17	34					
	GG	4	8					

[1] NB1: non-B1, [3] NB3: non-B3, [4] NE: non-E, $\chi2$(HWE) - Hardy-Weinberg equilibrium $\chi2$ value, n: number of goats,
He: gene heterozygosity; Ne: effective allele number, PIC: polymorphism information content, The consistent with HWE.

of Saanen milk (p=0.090). The citric acid ratio seemed to be at the highest value on the second lactation. However, LR had no significant effect on the other investigated parameters.

Correlations between the physicochemical properties of goat milk

The summary of the descriptive values of Pearson's correlation coefficients is shown in table (Table 3). As a result, two types of correlations, intermediate or high, were observed in the current study. The acidity was intermediately correlated with the citric acid (-0.304) and FFA composition (-0.332), but also highly correlated with the density of goat milk (0.641) with, the significance levels of P<0.05 and P<0.001 respectively. Although the citric acid was correlated only with FPD value (0.275) and LR (-0.313), we observed a tendency for the correlation between density and citric acid herein (P=0.077). Another intermediate level of correlation was adjusted between the density with FFA and FPD parameters. The FFA and FPD did not significantly correlate with the investigated physicochemical properties in the current study (P>0.05). Results indicated that urea only correlated with the LR (P<0.01).

DISCUSSION

The effect of genetic and environmental factors on investigated traits

The physiochemical properties of milk are important factors in terms of the process of dairy production, quality, and profitability. The milk acidity is a parameter that is associated with hygienic conditions of animals and an indirect sign of microbiological quality or might be an illusion by water or ammonia[20]. Zhang et al.[8] indicated that an 11-bp indel mutation was significantly associated with the acidity of Guanzhong dairy goat milk; according to study the II (homozygote insertion type-insertion/insertion) genotype exhibited higher values (7.49 ± 0.51) for physicochemical properties of goat milk. Differing from Zhang et al.[8], our results show that no significant differences were observed between the acidity and *CSN1S1* gene polymorphism (B1, B3, or E allele) (P>0.05). This alteration might be depended on the breed differences or variation of investigated polymorphisms. Similar to *CSN1S1* gene polymorphisms, the effect of the *CSN3* gene on that trait was found statistically not significant in our study (P>0.05). These findings are in agreement with those reported by Kyselová et al.[2] who determined the influence of *CSN3* genotype on the Czech Fleckvieh breed.

Lipolysis causes the emission of FFA into the milk that leads to affect the sensory quality of the milk and dairy products negatively[20]. Despite the source of energy is another factor that may affect milk composition especially milk fatty acid profile[20], Chilliard et al.[21], Dagnachew and Ådnøy[6] indicated that the polymorphisms of *CSN1S1* gene had significant effect on FFA concentration of Norwegian and Alpin dairy goats, respectively. Contrary to Chilliard et al.[21] and Dagnachew and Ådnøy[6], the effect of the *CSN1S1* gene on the FFA value of Saanen milk was found statistically insignificant. The variation of the results could be related to flock differences.

The freezing point is known to be preferred as a reliable method to confirm the adulteration of milk with water and being an indicator to determine the amount of water into the milk. This parameter based on many factors such as milk compound, the milking number, the month of milking, the breed, the feeding or weather conditions, and the health of the mammary gland[22,23]. In recent years, the effect of some genetic polymorphisms on FPD was noted by the scientists[8,24], Zhang et al.[8] pointed out that the II (homozygote insertion type-insertion/insertion) and ID (heterozygote type-insertion/deletion) genotypes exhibited higher values for FPD in Guanzhong dairy goat than DD (homozygote deletion type-deletion/deletion). On the other hand, Balia et al.[24] reported that the freezing point was not affected by *CSN1S1* gene but significantly influenced by the stage of lactation. Although the *CSN1S1* gene affected the casein content of milk, it was similar to data recorded by Balia et al.[24], the impact of this gene on FPD was found not significant in the present study.

Table 2 - Levels of significance, least squares means and standard errors for the effect of *CSN1S1, CSN3* and *AGPAT6* gene polymorphisms on physico-chemical properties of Saanen milk.

	Genotype	n	Acidity (°SH)	Citric acid (%)	Density (g/L)	FFA (mevk/L)	FPD (°C)	Urea (mg/100ml)
CSN1S1- B1	B1-B1	48	7.289 ± 0.59	0.052 ± 0.014	1028.36 ± 1.15	0.574 ± 0.08	0.510 ± 0.01	0.041 ± 0.003
	B1-NB1[1]	2	7.263 ± 0.796	0.063 ± 0.019	1027.51 ± 1.55	0.582 ± 0.10	0.508 ± 0.02	0.038 ± 0.007
	NB1-NB1	-	-	-	-	-	-	-
	P		0.963	0.426	0.413	0.901	0.929	0.450
CSN1S1- B3	B3-B3	5	7.323 ± 0.548	0.053 ± 0.013	1027.64 ± 1.07	0.574 ± 0.07	0.508 ± 0.01	0.047 ± 0.004
	B3-NB3[3]	24	7.455 ± 0.829	0.061 ± 0.020	1028.06 ± 1.61	0.570 ± 0.10	0.505 ± 0.02	0.035 ± 0.006
	NB3-NB3	21	7.050 ± 0.850	0.058 ± 0.021	1028.12 ± 1.66	0.589 ± 0.11	0.513 ± 0.02	0.037 ± 0.006
	P		0.517	0.899	0.949	0.911	0.500	0.157
CSN1S1- E	E-E	3	7.240 ± 1.110	0.058 ± 0.027	1028.03 ± 2.17	0.552 ± 0.14	0.509 ± 0.02	0.033 ± 0.008
	E-NE	19	7.075 ± 0.599	0.057 ± 0.015	1027.92 ± 1.17	0.583 ± 0.08	0.512 ± 0.01	0.045 ± 0.005
	NE-NE	28	7.517 ± 0.488	0.058 ± 0.012	1027.85 ± 0.95	0.599 ± 0.06	0.505 ± 0.01	0.041 ± 0.004
	P		0.479	0.998	0.990	0.855	0.602	0.246
CSN3	FF	49	6.943 ± 0.331	0.0624 ± 0.008	1027.06 ± 0.65	0.630 ± 0.04	0.505 ± 0.01	0.044 ± 0.003
	MF	1	7.61 ± 1.15	0.0527 ± 0.028	1028.82 ± 2.23	0.525 ± 0.15	0.512 ± 0.02	0.035 ± 0.009
	MM	-	-	-	-	-	-	-
	P		0.546	0.715	0.411	0.457	0.737	0.256
AGPAT6	CC	29	7.250 ± 0.606	0.056 ± 0.015	1027.78 ± 1.18	0.613 ± 0.08	0.510 ± 0.01	0.042 ± 0.005
	GC	17	7.505 ± 0.695	0.057 ± 0.017	1028.75 ± 1.35	0.567 ± 0.09	0.515 ± 0.01	0.039 ± 0.005
	GG	4	7.073 ± 0.722	0.060 ± 0.018	1027.28 ± 1.41	0.554 ± 0.09	0.501 ± 0.01	0.038 ± 0.005
	P		0.475	0.909	**0.086~**	0.217	0.236	0.183
LR	***1***	12	7.178 ± 0.619	0.064 ± 0.015	1027.59 ± 1.21	0.582 ± 0.08	0.505 ± 0.01	0.043 ± 0.005
	2	10	7.119 ± 0.713	0.065 ± 0.017	1028.08 ± 1.39	0.601 ± 0.09	0.512 ± 0.01	0.039 ± 0.005
	3	15	7.727 ± 0.669	0.051 ± 0.016	1028.39 ± 1.30	0.562 ± 0.09	0.515 ± 0.01	0.039 ± 0.005
	≥4	13	7.080 ± 0.680	0.050 ± 0.017	1027.69 ± 1.33	0.567 ± 0.08	0.503 ± 0.01	0.037 ± 0.005
	P		0.104	**0.090~**	0.533	0.774	0.173	0.159

LR: Lactation rank/number, ~: tended to be significant

The *AGPAT6* gene, which has an important role in glycerolipid biosynthesis catalysis, also known as a candidate gene for milk fat content[11]. Unfortunately, little knowledge exists on literature about the effect of that gene on goat milk. He et al.[11] indicated that the GG and CG genotypes for 9263C>G locus showed highly better milk performance than genotype CC individuals in Chinese dairy goat breeds for milk yield and fat performance. In another research, Cecchinato et al.[13] investigated the impact of 96 SNPs from 54 candidate genes, including the *AGPAT6* gene, on milk yield/quality and urea nitrogen content, according to result no significant effect observed on milk yield or urea concentration in Brown Swiss cattle. The present results indicated the non-significant effect of *AGPAT6* gene on urea concentration in Saanen milk. This conclusion is consistent with data found in the literature Cecchinato et al.[13]. In other respects, the *AGPAT6* gene might be tended to significant (P=0.086) on density in current research. Effect of lactation stage, length, or milking frequency on milk yield[25,26] and cheese-making properties such as quality of rennet curdling were well-documented in previous studies[27]. Moreover, the lactation number or LR was investigated, and Vijayakumar et al.[26] declared that a greater milk yield during the early stage with 4× milking frequencies per day was detected on the 3rd lactation[33]. Bhosale et al.[25] and Novotna et al.[28] emphasized that the effect of lactation number was significantly associated with the titrable acidity (°SH) in goat and sheep breed, respectively (P<0.05). Unlike the findings of Bhosale et al.[25] and Novotna et al.[28], the LR was not found effective on the physicochemical properties of goat milk except citric acid (P=0.090-tended to be significant) in the present study. Moreover, it was known that the citric acid concentration decreased during the lactation. The reason for different citric acid concentration levels during the lactation or different lactations might be nutritionally based, such as insufficient mineral absorption.

Correlations between the physicochemical properties of goat milk

The correlation between acidity and density values found -0.304 (P<0.05) in the present study, accordance with Chornobai et al.[29] and Kaw cka et al.[23] who indicated coefficients 0.2115 and 0.49 respectively. On the other hand, an important correlation between the acidity and urea was determined by Todaro et al.[30], Kuchtík et al.[27] (P≤0.001) and Kaw cka et al.[23] (P<0.05) in Saanen goats, East Friesian ewes and Mountain sheep of Poland respectively. Unlike these findings, the urea of milk that provides to control the protein level of forages was significantly correlated only with the LR in our study. The organic acids of milk, such as citric, pyruvic, malic, or lactic acids, play an important role in the flavor and storage time of dairy products; thus the correlations between the physicochemical parameters of milk is so critical for the consumer[4]. Consistent with our results, Kaw cka et al.[23] claimed that the citric acid positively correlated with FPD of the milk. The

Table 3 - Pearson correlations among some values of physico-chemical properties of goat milk.

Variables	Acidity	Citric Acid	Density	FFA	FPD	Urea
Citric acid	-0.304 *					
Density	0.641 ***	-0.252~				
FFA	-0.332 *	0.118	-0.315 *			
FPD	0.229	0.275 *	0.436 **	0.050		
Urea	-0.145	-0.180	0.052	0.110	-0.016	
Lactation rank	0.005	-0.313*	-0.068	-0.132	-0.203	-0.369 **

* $P<0.05$; ** $P<0.01$; *** $P<0.001$, ~: tended to be significant (P:0.077).

significant correlations between the citric acid-FPD, citric acid-acidity, FPD-density, and FFA-density were observed in the present investigation, in close agreement with Kaw cka et al.[23]. However, the correlation between FPD and acidity was found insignificant, differing from that research.

CONCLUSIONS

The physicochemical properties of milk are an important issue for the milk industry in case of an impact on the technical process of milk products. Thus the determination of the factors influenced that properties have an important role. Although the effect of *CSN1S1* (B1, B3, E) and *CSN3* gene on studied parameters were not found significant, the effect of the *AGPAT6* gene on density and the LR on citric acid ratio were established tended to be significant in the current study. Moreover, the identification of these characteristics in Saanen goats also contributed to the limited literature knowledge. Further studies that will be done to enlarged the investigated population or the true effects on these traits might be useful for the goat breeding and dairy industry. Moreover, significant correlations were observed between the investigated physicochemical parameters. So take these correlations into consideration while performing the selection program according to milk yield or quality might be beneficial for breeders.

References

1. Park YW, Jeanjulien C, Siddique A (2017) Factors Affecting Sensory Quality of Goat Milk Cheeses: A Review. J Adv Dairy Res 5: 185.
2. Kyselová J., Ječmínková K., Matějíčková J., Hanuš O., Kott T., Štípková M., Krejčová M. (20110). Physiochemical characteristics and fermentation ability of milk from Czech Fleckvieh cows are related to genetic polymorphisms of β-casein, κ-casein, and β-lactoglobulin. Asian-Australas J Anim Sci, 32(1):14-22.
3. Abay B.T., Kebede T.B. (2018). Physicochemical Properties and Comparisons of Goat and Cow Milk. Review. IJERD, 6(3): 416-419.
4. Buffa M., Guamis B., Saldo J., Trujillo A.J. (2004). Changes in organic acids during ripening of cheeses made from raw, pasteurized or high-pressure-treated goats' milk. Lebensm.-Wiss. u.-Technol, 37: 247-253.
5. Spelman R, Garrick D (1997) Utilisation of marker assisted selection in a commercial dairy cow population. Liv Prod Sci, 47: 139-147.
6. Dagnachew BS., Ådnøy T. (2014) Additive and dominance effects of casein haplotypes on milk composition and quality in Norwegian dairy goats.
7. Mastrangelo S., Sardina M.T., Tolone M., Portolano B. (2013). Genetic polymorphism at the *CSN1S1* gene in Girgentana dairy goat breed. Anim. Prod. Sci. 53, 403-406.
8. Zhang Y, Wang K, Liu J, , Zhu H, , Qu L, , Chen H, Lan X, Pan C, Song X. (2019). An 11-bp Indel Polymorphism within the *CSN1S1* Gene Is Associated with Milk Performance and Body Measurement Traits in Chinese Goats. Anim, 9(12). pii: E1114.
9. Yahyaoui M.H., Coll A., Sanchez A., Folch J.M. (2001). Genetic polymorphism of the caprine kappa casein gene. J Dairy Res, 68: 209-216.
10. Prinzenberg E.M., Gutscher K, Chessa S, Caroli A, Erhardt G. (2005). Caprine κ-Casein (*CSN3*) Polymorphism: new developments in molecular knowledge. J. Dairy Sci, 98: 1490-1498.
11. He C., Wang C., Chang Z.H., Guo B.L., Li R., Yue X.P., Lan X.Y, Chen H., Lei C.Z. (2011). *AGPAT6* polymorphism and its association with milk traits of dairy goats. Genet Mol Res, 10(4): 2747-2756.
12. Bionaz M., Loor J.J. (2008). *ACSL1, AGPAT6, FABP3, LPIN1,* and *SLC27A6* Are the most abundant isoforms in bovine mammary tissue and their expression is affected by stage of lactation. J Nutr, 138: 1019-1024.
13. Cecchinato A, Ribeca C, Chessa S, Cipolat-Gotet C, Maretto F, Casellas J, Bittante G. (2014). Candidate gene association analysis for milk yield, composition, urea nitrogen and somatic cell scores in Brown Swiss cows. Anim, 8(7):1062-70.
14. 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.
15. Cosenza G., Pauciullo A., Gallo D., Coiımoro L., D'avino A., Mancusi A., Ramunno, L. (2008). Genotyping at the *CSN1S1* locus by PCR-RFLP and AS-PCR in a Neapolitan goat population. Small Rumin. Res, 74,:4-90.
16. Chessa S., Ceriotti G., Dario C., Erhardt G., Caroli A. (2003). Genetic polymorphisms of αS1-, αS2- and k-casein in Maltese goat breed. Ital. J. Anim. Sci, 1:58-60.
17. Yeh F.C., Yang R.C., Boyle T.B.J., Ye Z.H., Mao J.X. (2000). Popgene (v.1.32) the user-friendly shareware for population genetic analysis University of Alberta, Canada: (http://www.ualberta.ca/~fyeh/, Accessed January 01, 2015.)
18. Nei M, Roychoudhury A (1974) Sampling variances of heterozygosity and genetic distance. Genetics 76: 379-390.
19. 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, Türkiye.
20. Nascimento T.V.C., Gonçalves de Almeida Júnior W.L., E.S., Júnior, Menezes D.R., Dias F.S., Matiuzzi da Costa M. (2017). Physical and chemical characteristics of milk from goats supplemented with different levels of total digestible nutrients in the dry period. Acta Sci. Anim. Sci, 39(4):429-435.
21. Chilliard Y., Rouel J., Leroux C. (2006). Goat's alpha-s1 casein genotype influences its milk fatty acid composition and delta-9 desaturation ratios. Anim Feed Sci Tech, 131: 474-487.
22. Janštová B., Dra ková M., Navrátilova P., Hadra L., Vorlová L. (2007). Freezing point of raw and heat-treated goat milk. Czech J. Anim. Sci, 52: 394-398.
23. Kawęcka A., Pasternak M., Słoniewska D., Miksza-Cybulska A., Bagnicka E.(2020) Quality of mountain sheep milk used for the production of traditional cheeses. Ann. Anim. Sci, 20(1): 299-314.
24. Balia F., Pazzola M., Dettori M.L., Mura M.C., Luridiana S., Carcangiu V., Piras G. Vacca G.M. (2013). Effect of *CSN1S1* gene polymorphism and stage of lactation on milk yield and composition of extensively reared goats. J. Dairy Res, 80: 129-137.
25. Bhosale S.S, Kahate P.A., Kamble K., Thakare V.M., Gubbawar S.G. (2009). Effect of Lactation on Physico-Chemical Properties of Local Goat Milk. Vet. World J., 2:17-19.
26. Vijayakumar M., Park J.H., Ki K.S., Lim D.H., Kim S.B., Park S.M., Jeong H.Y., Park B.Y., Kim T.I. (2017). The effect of lactation number, stage, length, and milking frequency on milk yield in Korean Holstein dairy cows using automatic milking system. Asian-Australas J Anim Sci, 30(8):1093-1098.
27. Kuchtík J., Šustová K., Urban T., Zapletal D. (2008). Effect of the stage of lactation on milk composition, its properties and the quality of rennet curdling in East Friesian ewes. Czech J. Anim. Sci., 53(2): 55-63.
28. Novotná L., Kuchtík J., Sustová K., Zapletal D., Filipík R. (2009). Effects of Lactation Stage and Parity on Milk Yield, Composition and Properties of Organic Sheep Milk. J Appl Anim Res, 36(1): 71-76.
29. Chornobai C.A., Damasceno J.C., Visentainer J.V., de Souza N.E., Matsushita M.(1999). Physical-chemical composition of in natura goat milk from cross Saanen throughout lactation period. Arch Latinoam Nutr. 1999 Sep;49(3):283-6.
30. Todaro M., Scatassa M.L., Giaccone P.R. (2005). Multivariate factor analysis of Girgentana goat milk composition. Ital. J.Anim. Sci., 4: 403-410.