

## The Relationship Between Milk Fatty Acid Profile and Expression Levels of *SCD*, *FASN* and *SREBPF1* Genes in Damascus Dairy Goats

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### ABSTRACT

In this study, the relationship between the expression levels of *SCD*, *FASN*, *SREBPF1* genes and milk fatty acid profiles in goat milks with low (LSFA) and high (HSFA) saturated fatty acid content was investigated and correlated. In HSFA group, *SCD*, *FASN* and *SREBPF1* genes were approximately 7, 9 and 4 folds more expressed than LSFA in milk somatic cells, respectively (P<0.01). Also, positive correlations were determined between *SCD* and *FASN* (0.907; P<0.001), *SCD* and *SREBPF1* (0.628; P<0.001), *FASN* and *SREBPF1* (0.720; P<0.001) genes. Positive and important correlation was found between C6:0 (Caproic acid) and *SCD* (0.468; P<0.05) and *SREBPF1* (0.388; P<0.05) genes. On the other hand, positive correlations were found between all of these three genes and C8:0 (Capric acid) and C10:0 (Caprylic acid). In milk samples, C14:0 (Myristic acid) and *SREBPF1* genes were correlated positively (0.469; P<0.05). Moreover, positive correlation was found between the odour index and *SCD* (0.553; P<0.01), *FASN* (0.444; P<0.05), *SREBPF1* (0.499, P<0.05) genes. The results showed that *SCD*, *FASN* and *SREBPF1* genes have important effects on the milk fatty acid profile and milk quality of goats and these genes may be candidate in selection applications in goats.

**Keywords:** Gene expression, Goat, Milk fatty acids, Somatic cells

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### Damascus Keçilerinde Süt Yağ Asidi Profili ile *SCD*, *FASN* ve *SREBPF1* Genlerinin Ekspresyon Seviyeleri Arasındaki İlişki

#### ÖZ

Bu çalışmada, doymuş yağ asidi içeriği düşük (LSFA) ve yüksek (HSFA) olan keçi sütlerinde *SCD*, *FASN*, *SREBPF1* genlerinin ekspresyon düzeyleri ile süt yağ asidi profilleri ve aralarındaki ilişki araştırılmıştır. HSFA grubunda süt somatik hücrelerinde *SCD*, *FASN* ve *SREBPF1* genleri, LSFA grubuna göre yaklaşık sırasıyla 7, 9 ve 4 kat daha fazla ifade edilmiştir (P<0,01). Ayrıca, *SCD* ve *FASN* (0,907; P<0,001), *SCD* ve *SREBPF1* (0,628; P<0,001), *FASN* ve *SREBPF1* (0,720; P <0,001) genleri arasında pozitif korelasyonlar tespit edilmiştir. C6:0 (Kaproik asit) ile *SCD* (0,468; P<0,05) ve *SREBPF1* (0,388; P<0,05) genleri arasında pozitif ve önemli korelasyonlar bulunmuştur. Bununla birlikte, bu üç genin tamamı ile C8:0 (Kaprik asit) ve C10:0 (Kaprilik asit) yağ asitleri arasında pozitif korelasyonlar belirlenmiştir. Süt örneklerinde C14:0 (Miristik asit) ve *SREBPF1* geni arasında pozitif korelasyon gözlenmiştir (0,469; P<0,05). Ayrıca, koku indeksi ile *SCD* (0,553; P<0,01), *FASN* (0,444; P<0,05), *SREBPF1* (0,499, P<0,05) genleri arasında pozitif korelasyon tespit edilmiştir. Sonuçlar, *SCD*, *FASN* ve *SREBPF1* genlerinin keçilerin süt yağ asidi profili ve süt kalitesi üzerinde önemli etkileri olduğunu ve bu genlerin keçilerde seleksiyon uygulamalarında aday olabileceğini göstermiştir.

**Anahtar Kelimeler:** Gen ekspresyonu, Keçi, Süt yağ asitleri, Somatik hücre

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## INTRODUCTION

The basic principle of animal breeding is to obtain healthy populations with superior characteristics in terms of yield and quality. With this principle, sustainable breeding studies continue in animal husbandry. Researchers have focused on characters such as meat yield, milk yield and milk quality in ruminants, which are important farm animals (Akcapinar and Ozbeyaz 1999, Ozkan and Yakan 2017). Increasing awareness of nutrition raises the demand for healthy and quality food. The world population is increasing day by day. Currently, the population of around 8 billion people is expected to be around 10 billion by 2050 (Anonymous 2020a). Increasing population and consumption awareness requires higher quality production in animal foods such as eggs, meat, milk and dairy products (Haile et al. 2016, Balthazar et al. 2017).

The World Health Organization (WHO) states that the saturated/unsaturated fatty acids and the n6/n3 ratios should be less than 1.0 and 4.0, respectively (WHO, 2013). Studies show that goat milk has more positive values in terms of these values than cow and sheep milk (Ceballos et al. 2009, Silanikove et al. 2010, Yakan et al. 2019). On the other hand, goat milk is more digestible than cow's milk. The goat milk's fat globules are smaller and also milk is richer in terms of short and medium chain fatty acids (Kompan and Komprij 2012, Yurchenko et al. 2018). Goat breeding is an increasingly important form of livestock for some dairy products such as cheese and ice cream (Yurchenko et al. 2018). It is estimated that there are more than 1000 goat breeds in the world and the total number of goats is around 1 billion today (Crisa et al. 2016, Anonymous 2020b). Today, the number of goats increased approximately 2-folds compared to 10 years ago and it is reported that more than 10 million goats exist in Turkey (Anonymous 2020a). In addition to pure and native breeds such as the Hairy goat, Angora goat, cultural breeds such as the Saanen, German Alaca Noble goat, Toggenburg and Damascus goat, and hybrid breeds such as the Kilis, Akkeçi, Bornova goat constitute the Turkish goat population. Damascus goat, due to its high milk yield and fertility properties, is an important animal breeding in countries such as Turkey, Syria, Lebanon, Israel and Cyprus (Keskin 2002, Yakan et al. 2019).

While quality in animal products has been associated with hygiene and cheating formerly, today it is evaluated in terms of parameters such as composition, processing and technology of the product (Raynal-Ljutovac et al. 2005, Yakan et al. 2019). Milk fatty acid composition is one of the most important factors affecting the quality of milk and dairy products. The amounts and rates of saturated and unsaturated fatty acids in milk composition considerably affect milk quality (Yakan et al. 2019). It has been reported that the consumption of saturated

fatty acids triggers cardiovascular diseases; however polyunsaturated fatty acids (PUFA) are protective against cardiovascular diseases (Simopoulos et al. 2008). On the other hand, it has been stated that subacute ruminal acidosis may be estimated in ruminants with the fatty acid profile in milk, and milk fatty acids may be used as biomarkers for detecting subacute ruminal acidosis (Colman et al. 2015, Yurchenko et al. 2018).

In goats, milk secretion type is apocrine. Therefore, the number and content of somatic cells in milk are different. The somatic cells that provide the natural defense mechanism of the mammary gland are composed of leukocytes, polymorphous nuclear cells, and largely epithelial cells. In ruminants, somatic cell count is one of the most important parameter that gives information about mammary health and animal physiology. Molecular activity in milk somatic cells is reported to be an important marker for understanding about biological activity in the mammary gland (Murrieta et al. 2006, Feng et al. 2007). In molecular studies related to mammary health and activity, it is possible to study from milk somatic cells without the need invasive methods such as biopsy in tissues (Feng et al. 2007, Jacobs et al. 2013). In addition to giving ideas about mammary health, somatic cells reflect the biological activity in molecular pathways such as lipogenesis in mammary tissue (Murrieta et al. 2006).

Although there are a lot of researches about the molecular activities of genes in the lipogenesis pathway in non-ruminant species, what is known about the relationship between these genes and milk fat synthesis in ruminants, especially goats, is quite limited (Yao et al. 2017). The mammary gland in lactation is one of the major organs where lipid synthesis takes place (Rudolph et al. 2010). It has been reported that *SCD* (Stearoyl-CoA Desaturase), along with many factors in goats, is particularly effective in the formation of milk fatty acid composition (Kompan and Komprij 2012). *SCD* is highly responsible for fatty acid elongation in milk (Kompan and Komprij 2012). *FASN* (Fatty Acid Synthase) and *SREBPF1* (Sterol Regulatory Element-Binding Transcription Factor 1) are another important regulators of lipogenic pathway. In this study, it is aimed to investigate the expression levels of *SCD*, *FASN* and *SREBPF1* genes in the goat milk with low and high saturated fatty acid content and to reveal the relationship between the expression levels of these genes and the milk fatty acid profile in goat milk.

## MATERIALS and METHODS

### Animal Materials

In the study, 20 milk samples were taken from 2-3 years old Damascus goats, which are in the 4th month of lactation. Goats were under semi-intensive

feeding. Approximately 100 ml milk collected to nuclease free and sterile tubes from each goat from the morning milking (50 ml volume falcons were used for sample collection). Samples were CMT (California Mastitis Test) negative and animals were healthy. Then, milk samples were quickly transferred to the laboratory for analyzes under cold chain.

#### **Cream and Somatic Cell Collection**

The samples were centrifuged at 1800 xg for 15 minutes at + 4 °C. After centrifugation, samples were kept at – 20 °C for cream layer for 10 min. The cream layer of samples was collected to new tubes with the help of a spatula. Cream samples were kept at – 20 °C until fatty acid analyzes.

After cream layer was removed, the supernatants in falcon tubes were discarded and the pellets at the bottom of falcons were transferred to 15 ml, sterile and nuclease-free falcon tubes. Cell suspensions in the falcon tubes were completed with PBS to 15 ml. Samples were centrifuged at 1800 xg for 15 minutes at +4 °C. Then, supernatants of samples were poured out; 1 ml TRI Reagent (Sigma-Aldrich, USA) solution was added to the cell pellets of samples. Total RNA isolation was made from the cell pellets homogenized in TRI Reagent.

#### **Fatty Acid Analysis**

Approximately 500 µl cream from each sample was used for fatty acid analysis. The creams were homogenized with 2 ml of 2N methanolic KOH for 4 minutes at room temperature. Thereafter, 4 ml of n-Heptane (Merck, USA) was added to the homogenate. After homogenization for 2 minutes at room temperature, samples were centrifuged at 200 xg for 5 minutes to phase separation. Methyl esters collected in the upper phase were transferred to vials with a volume of 1.5 ml and fatty acids were determined by HP Innovax column (60 m length, 0.25 mm i.d. x 0.25 µm film) in Gas Chromatography (HP Agilent 6890, USA). Injector temperature was set at 250 °C and detector temperature at 270 °C. Helium was used as the carrier gas. Injection was splitless with a total injection volume of 1 µl and injector was washed three times with n-Heptan. Oven temperature was programmed initially at 90 °C for 3 min and was increased to 250 °C with a 3 °C/min ramp rate. The determined peaks on chromatogram were verified by comparison of retention times of internal standards (FAME Mix, Supelco, USA)

According to the results of fatty acid contents of milks, the samples were divided into two groups as Low Saturated Fatty Acid Content (LSFA) and High

Saturated Fatty Acid Content (HSFA). The groups were created by ordering the samples from large to small according to the SFA contents. The first 10 samples constitute LSFA group, while the last 10 samples formed HSFA group.

#### **Total RNA Isolation and cDNA Synthesis**

Total RNA isolation was performed from milk somatic cells according to the TRI-Reagent (Sigma-Aldrich, USA) kit protocol (Rio et al. 2010). After chloroform-isopropyl alcohol-ethyl alcohol steps, the RNA pellets were obtained from the samples. Depending on their size, the RNA pellets were diluted with 30-100 µl of nuclease-free water and checked for quality.

The purity (A260/280) and concentration of the RNA isolated from the samples were checked in the nucleic acid meter (Merinton, SMA-1000 UV Spectrophotometer). Also, 28S and 18S rRNA subunits were evaluated in 1% agarose gel for RNA quality controls (100 V and 25 min).

The DNase treatment was performed to samples for possible genomic DNA contamination (DNase I, RNase free, Thermo Scientific, USA, Cat no: EN0525). According to the Revertaid First Strand cDNA Synthesis kit (Thermo-Scientific, USA) protocol, cDNA synthesis was performed. In the thermal cycler (Bio-Rad T100, USA), the reaction was carried out for 10 min at 25 °C, 120 min at 37 °C, and 5 min at 85 °C. After reaction, the final volume of the samples were completed to 200 µL with nuclease free water and kept at – 80 °C until gene expression analyses were performed.

#### **Quantitative Real-Time PCR Analysis**

Amplification of genes were performed using 10 µl of each cDNA sample with the kit containing SYBR Green I dye (Power SYBR® Green PCR Master, ThermoFisher Scientific, USA, Cat no: 4367659). The reaction was arranged 10 minutes at 95 °C, followed by 15 seconds at 95 °C, 60 seconds at 60 °C, and 40 cycles in qPCR (Bio-Rad CFX-96 Touch Real time PCR, USA). Each cDNA sample was studied as duplicate and *ACTB* gene was used as reference gene. The forward and reverse sequences of the primers used in the amplification of genes are shown in Table 1.

**Table 1.** Forward and reverse sequences of primers of studied genes

GENES	FORWARD AND REVERSE PRIMER SEQUENCES*	PRODUCT LENGTH
<i>FASN</i>	F: 5'-GCACACAATATGGACCCCA-3' R: 5'-CATGCTGTAGCCTACGAGGG-3'	183
<i>SCD</i>	F: 5'-ATCGCCCTTACGACAAGACC-3' R: 5'-CATAAGCCAGACCGATGGCA-3'	186
<i>SREBPF1</i>	F: 5'-AACATCTGTTGGAGCGAGCA-3' R: 5'-TCCAGCCATATCCGAACAGC-3'	134
<i>ACTB</i>	F: 5'-TGGATCGAGCATCCCCAAAG-3' R: 5'-ACTGGCCCTTCTCCTTAGA-3'	169

\*: All primer sequences were designed in this study by the authors via Primer-BLAST (NCBI).

### Statistical Analysis

SPSS package program (Version 23.0) was used to calculate the obtained data. Student-t test was used to measure the difference between the groups. Spearman correlation coefficient was used to calculate the correlation between parameters. Odour (Yakan et al. 2019), thrombogenic and atherogenic indexes were calculated using fatty acid parameters (Ulbricht and Southgate 1991). On the other hand, the  $2^{-\Delta\Delta Ct}$  method was used for gene expression calculations,

and results were given as fold changes (Livak and Schmitgen 2001).  $P < 0.05$  was accepted as significant.

### RESULTS

The saturated fatty acid content was  $64.92 \pm 0.90$ (%) in the LSFA group, while HSFA group saturated fatty acid content was  $71.27 \pm 0.45$ (%), ( $P < 0.001$ ). Between groups, variable levels of significance in terms of fatty acids were determined (Table 2).

**Table 2.** Milk fatty acid profiles of LSFA and HSFA groups (Mean $\pm$ SEM)

Fatty acids (%)	LSFA	HSFA	P
C4:0 (Butyric Acid)	1.62 $\pm$ 0.07	1.47 $\pm$ 0.15	-
C6:0 (Caproic Acid)	2.06 $\pm$ 0.08	2.16 $\pm$ 0.12	-
C8:0 (Caprylic Acid)	2.42 $\pm$ 0.11	2.68 $\pm$ 0.14	-
C10:0 (Capric Acid)	7.60 $\pm$ 0.38	9.28 $\pm$ 0.33	**
C11:0 (Undecanoic Acid)	0.23 $\pm$ 0.02	0.19 $\pm$ 0.02	-
C12:0 (Lauric Acid)	2.95 $\pm$ 0.17	3.68 $\pm$ 0.17	-
C14:0 (Myristic Acid)	8.63 $\pm$ 0.30	10.63 $\pm$ 0.47	**
C14:1 (Miristoleic Acid)	0.44 $\pm$ 0.04	0.64 $\pm$ 0.31	-
C15:0 (Pentadecylic Acid)	1.52 $\pm$ 0.09	1.37 $\pm$ 0.04	-
C15:1 (Pentadecenoic acid)	0.38 $\pm$ 0.02	0.31 $\pm$ 0.01	*
C16:0 (Palmitic Acid)	24.43 $\pm$ 0.69	26.7 $\pm$ 0.56	*
C16:1 (Palmitoleic acid)	0.97 $\pm$ 0.11	0.82 $\pm$ 0.10	-
C17:0 (Margaric acid)	1.02 $\pm$ 0.04	0.87 $\pm$ 0.05	**
C17:1 (Heptadecenoic Acid)	0.49 $\pm$ 0.03	0.38 $\pm$ 0.01	*
C18:0 (Stearic Acid)	11.27 $\pm$ 0.47	10.87 $\pm$ 0.48	-
C18:1 (Oleic Acid)	26.48 $\pm$ 1.13	21.18 $\pm$ 0.50	***
C18:2 n6 (Linoleic Acid)	1.46 $\pm$ 0.25	1.29 $\pm$ 0.20	-
C18:3 n3 (Alphalinolenic Acid)	1.57 $\pm$ 0.17	1.68 $\pm$ 0.17	-
C20:0 (Arachidic Acid)	0.71 $\pm$ 0.08	0.89 $\pm$ 0.20	-
C20:1 (Gondoic Acid)	1.42 $\pm$ 0.08	0.98 $\pm$ 0.04	***
C20:2 n6 (Eicosadienoic Acid)	0.48 $\pm$ 0.08	0.41 $\pm$ 0.09	-
C20:3 n3 (Eicosatrienoic Acid)	0.18 $\pm$ 0.02	0.18 $\pm$ 0.03	-
C20:3 n6 (Dihomo-g-linolenic Acid)	0.13 $\pm$ 0.02	0.14 $\pm$ 0.02	-
C20:4 n6 (Arachidonic Acid)	0.18 $\pm$ 0.01	0.16 $\pm$ 0.01	-
C22:0 (Behenic Acid)	0.38 $\pm$ 0.01	0.33 $\pm$ 0.01	**
C22:1 (Erucic Acid)	0.22 $\pm$ 0.03	0.16 $\pm$ 0.01	-
C24:0 (Lignoceric Acid)	0.15 $\pm$ 0.02	0.19 $\pm$ 0.04	-
C24:1 (Nervonic Acid)	0.82 $\pm$ 0.12	0.64 $\pm$ 0.04	-

\*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ; -:  $P > 0.05$

Differences were found in some parameters determined in samples grouped according to saturated fatty acid content (Table 3). While the

nutritional value was lower in LSFA than HSFA group ( $P<0.01$ ), atherogenic and thrombogenic index values were higher ( $P<0.001$ ) (Table 3).

**Table 3.** Some parameters were determined according to the fatty acid compositions in the groups (Mean $\pm$ SEM)

PARAMETERS	LSFA	HSFA	P
$\Sigma$ SFA	64.92 $\pm$ 0.90	71.27 $\pm$ 0.45	***
$\Sigma$ MUFA	29.79 $\pm$ 1.07	24.03 $\pm$ 0.56	***
$\Sigma$ PUFA	3.87 $\pm$ 0.35	3.71 $\pm$ 0.28	-
$\Sigma$ UFA	33.66 $\pm$ 0.85	27.74 $\pm$ 0.44	***
$\Sigma$ n6	2.18 $\pm$ 0.25	1.87 $\pm$ 0.22	-
$\Sigma$ n3	1.70 $\pm$ 0.19	1.84 $\pm$ 0.17	-
$\Sigma$ n6/n3	1.38 $\pm$ 0.17	1.12 $\pm$ 0.19	-
OI	13.70 $\pm$ 0.60	15.59 $\pm$ 0.69	-
NV	1.56 $\pm$ 0.08	1.21 $\pm$ 0.05	**
AI	1.46 $\pm$ 0.07	2.07 $\pm$ 0.09	***
TI	1.27 $\pm$ 0.04	1.60 $\pm$ 0.03	***

**SFA:** Saturated fatty acids; **MUFA:** Monounsaturated fatty acids; **PUFA:** Polyunsaturated Fatty acids; **UFA:** Unsaturated fatty acids; **OI:** Odour index; **NV:** Nutritional Value; **AI:** Atherogenic index; **TI:** Thrombogenic index

\*:  $P<0.05$ ; \*\*:  $P<0.01$ ; \*\*\*:  $P<0.001$ ; -:  $P>0.05$

**OI** = (C4:0+C6:0+C8:0+C10:0); **NV** = (C18:0+C18:1)/C16:0;

**AI** = (C12:0+(4\*C14:0)+C18:0)/ $\Sigma$ UFA

**TI** = (C14:0+C16:0+C18:0)/((0.5\*C18:1)+(0.5\* $\Sigma$ MUFA)+(0.5\* $\Sigma$ n6)+(3\* $\Sigma$ n3)+( $\Sigma$ n3/ $\Sigma$ n6))

Correlation values between milk fatty acid parameters such as SFA, MUFA, PUFA, and UFA in milk are given in Table 4.

**Table 4.** Correlations between some parameters in milk

PARAMETERS	$\Sigma$ MUFA	$\Sigma$ PUFA	$\Sigma$ UFA	$\Sigma$ n6	$\Sigma$ n3	$\Sigma$ n6/n3	OI	NV	AI	TI
$\Sigma$ SFA	-0.937***	0.023	-0.997***	-0.119	0.332	-0.364	0.623**	-0.820***	0.929***	0.889***
$\Sigma$ MUFA		-0.244	0.932***	-0.014	-	0.383*	-0.657**	0.815***	-0.892***	-0.789***
$\Sigma$ PUFA				0.811***	0.582**	0.212	0.183	-0.194	-0.018	-0.053
$\Sigma$ UFA				0.156	-0.341	0.398*	-0.591**	0.826***	-0.929***	-0.886***
$\Sigma$ n6					0.056	0.683***	0.147	0.003	-0.198	-0.005
$\Sigma$ n6/n3							-0.023	0.341	-0.442*	-0.114
OI								-0.361	0.480*	0.411*
NV									-0.756***	0.808***
AI										0.847***

\*:  $P<0.05$ ; \*:  $P<0.01$ ; \*\*\*:  $P<0.001$

RNA samples had appropriate purity (A260/280=1.87 $\pm$ 0.03) and concentration (283.07 $\pm$ 42.37 ng/ $\mu$ L). *SCD*, *FASN* and *SREBPF1* genes were expressed approximately 6 (6.35;  $P<0.05$ ),

9 (9.17;  $P<0.05$ ) and 4 (4.26;  $P<0.05$ ) folds more in the HSFA group compared to the LSFA group, respectively (Figure 1).

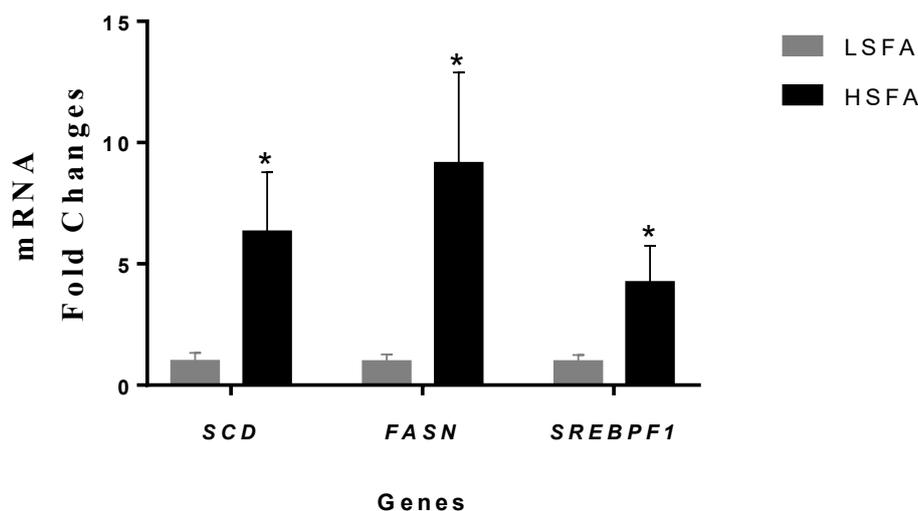


Figure 1. mRNA fold changes of genes in LSFA and HSFA groups (\*: P<0.05)

Some correlations were determined between *SCD*, *FASN*, *SREBPF1* genes and fatty acid parameters. The correlation results showed that there were important, positive and high correlations between genes (Between *SCD* and *FASN*: 0.907; P<0.001; between *SCD* and *SREBPF1*: 0.628; P<0.001; between *FASN* and *SREBPF1*: 0.720; P<0.001). Also, with variable levels of significance, positive and negative correlations were found between genes and fatty acids (Table 5).

While there was a positive correlation between saturated fatty acid ratio and *SCD* (0.444; P<0.05), *FASN* (0.344; P>0.05) and *SREBPF1* (0.405; P<0.05), a negative correlation was found between

the monounsaturated fatty acid ratio and the expression levels of *SCD* (-0.540; P<0.01), *FASN* (-0.439; P<0.05) and *SREBPF1* (-0.471; P<0.05). In addition, there was a positive correlation between *SCD* gene and polyunsaturated fatty acid ratio (0.426; P<0.05) and negative correlation between the unsaturated fatty acid ratio and *SCD* (-0.433; P<0.01) and *SREBPF1* (-0.414; P<0.05) were found. But then, a positive correlation was found between the *SCD* and n3 ratio. Odour index was found correlated with *SCD*, *FASN* and *SREBPF1* genes (0.553; 0.444; 0.499, respectively, P<0.05). A positive correlation was found between *SREBPF1*, a lipogenic transcription factor, and the atherogenic index (0.398; P<0.05).

Table 5. Correlations between genes and fatty acid parameters

PARAMETERS*	<i>SCD</i>	<i>FASN</i>	<i>SREBPF1</i>
C6:0 (Caproic Acid)	0.468*	0.370	0.388*
C8:0 (Caprylic Acid)	0.573**	0.457*	0.466*
C10:0 (Capric Acid)	0.492*	0.400*	0.508*
C14:0 (Myristic Acid)	0.197	0.241	0.469*
C17:1 (Heptadecenoic Acid)	-0.325	-0.254	-0.432*
C18:1 (Oleic Acid)	-0.466*	-0.377*	-0.478*
C18:3 n3 (Alphalinolenic Acid)	0.460*	0.420*	0.232
C20:1 (Gondoic Acid)	-0.385*	-0.284	-0.490*
C20:2 n6 (Eicosadienoic Acid)	0.519*	0.339	0.253
ΣSFA	0.444*	0.344	0.405*

ΣMUFA	-0.540**	-0.439*	-0.471*
ΣPUFA	0.426*	0.259	0.211
ΣUFA	-0.433*	-0.355	-0.414*
Σn6	0.301	0.099	0.113
Σn3	0.391*	0.356	0.211
Σn6/n3	-0.083	-0.217	-0.087
OI	0.553**	0.444*	0.499*
NV	-0.277	-0.140	-0.202
AI	0.287	0.247	0.398*
TI	0.229	0.090	0.223

‡: Statistically significant correlations were showed in table

\*: P<0.05; \*\*: P<0.01

## DISCUSSION

In recent years, with the increasing demand for goat milk, goat husbandry has gained importance and the level of breeding has increased (Novotna et al. 2019). In this study, *SCD*, *FASN* and *SREBPF1* genes expression levels and fatty acid profiles of goats that divided into two groups according to milk SFA contents were investigated.

Saturated fatty acids in the diets are associated with cardiovascular diseases (Siri-Tarino et al. 2010). Investigation of the factors that play roles in the formation of variation in dietary fatty acids has become an important goal. An important part of milk fatty acids in ruminants are saturated fatty acids (Haile et al. 2016). One of the most important parameters in the formation of milk quality is fatty acid profile (Chilliard et al. 2003). Numerous candidate genes and transcription factors in lipogenic pathways regulate the formation of milk fatty acid qualities and quantities via production and secretion of fatty acids (Moioli et al. 2007, Bionaz and Looor 2008).

In HSFA group, while C10:0, C14:0 and C16:0 fatty acids ratios were more than LSFA group (P<0.01), MUFAs as C15:1, C17:1, C18:1 and C20:1 and C22:0 fatty acids ratio were significantly lower (P<0.05). According to milk fatty acid composition, no significant difference was found between the groups in terms of other unsaturated fatty acids (Table 2). In accordance with the literature, while the atherogenic and thrombogenic index values were found to be high in the HSFA group, the unsaturated fatty acid ratio and thus the nutritional value were found lower (Haile et al. 2016, Idamokoro et al. 2019) (Table 3). As expected, a negative correlation was found between the monounsaturated fatty acid ratio and the odour, atherogenic and thrombogenic indexes. In addition, it was determined that there was a positive correlation between the nutritional value and the

unsaturated fatty acid ratio and it was understood that this correlation probably occurred due to MUFAs.

In HSFA group *SCD*, *FASN* and *SREBPF1*, primary lipogenic genes, were expressed more than 4 folds compared to the LSFA group and these results showed that these genes played an important role in the synthesis of saturated fatty acids in goat mammary gland (Figure 1).

*SCD* has a role as the key regulator in de novo fatty acid synthesis in mammary gland together with *ACC* and *FASN* (Bernard et al. 2008). It is reported that the main function of *SCD* in the mammary gland plays a role in the conversion of long chain fatty acids, especially C16:0 and C18:0, to C16:1 and C18:1, respectively. *SCD* has a significant effect on the fluidity of milk as well (Feng and et al. 2007). In a study it is reported that the *SCD* gene is more expressed during the lactation period in goat mammary tissue compared to the dry period, and increased *SCD* expression led to increase of the MUFA content and fat storage in the cell (Yao et al. 2017). In the same study, it is stated that concentration of oleic acid and synthesis of triacylglycerol are decreased with silencing of *SCD* gene. On the other hand, it is reported in some studies that that animals with high *SCD* activity in milk may be selected for high quality milk production that have high UFA content such as oleic acid (Feng et al. 2007, Jacobs et al. 2013). However, in this study, while HSFA groups' *SCD* gene expression was approximately 6 folds more than LSFA groups (Figure 1), UFA contents of HSFA groups' were lower than LSFA groups (Table 5). In addition, there was no difference in oleic acid levels between the groups. It is thought that lactation period and animal race may be caused these results. Since, mammary gland molecular activity and composition of milk change in different periods of lactation. On the other hand, it is reported that the milk fatty acid composition of three different goat breeds were

different under similar environmental conditions (Idamokoro et al. 2019).

Abnormal *SCD* expression in humans is known to be associated with obesity and its complications (Huang et al. 2015, Southam et al. 2015). In cattle and goats, it is reported that the polymorphism in *SCD* gene has led to change of milk fatty acid composition and therefore milk quality characteristics have changed (Conte et al. 2010, Alim et al. 2012). It is reported in a study, the over-expression of the *SCD* gene causes significant upregulation of some lipogenic genes especially as *FASN* and *PPARG* in lactating goats (Yao et al. 2017). In this study, it is determined that there is a very high correlation between *SCD* and *FASN* gene (0.907;  $P < 0.001$ ). *SREBPF1*, which is responsible for the activity of a large number of lipogenic genes such as *SCD* and *FASN* in mammals, shows a high level of positive correlation with both *SCD* (0.628;  $P < 0.001$ ) and *FASN* (0.720;  $P < 0.001$ ). *SREBPF1*, a lipogenic transcription factor, is a trigger of *SCD* and *FASN* genes and it regulates milk fatty acid profiles with activation in mammary epithelial cells in lactating ruminants. *SREBPF1* is required to trigger *SCD* expression (Huang et al. 2015, Souhtam et al. 2015). Disorders in milk fatty acid synthesis have been reported as a result of abnormal *SREBPF1* activity (Xu et al. 2016). It is reported that *SREBPF1* gene expression increased approximately 2 times in cattle, rats and humans, while it increased up to 30 times in goats due to mammary gland physiology in the period from pregnancy to lactation (Xu et al. 2016). *SREBPF1* activity is well defined in humans, rodents and cattle, but what is known about *SREBPF1* in goats is limited.

In a study investigating the lipogenic activity in goat breast epithelial cells, *SREBPF1* is found to trigger *FASN* gene expression (Xu et al. 2016). In another study, it is reported that *FASN* is a key metabolic regulator that has a primary role in long chain fatty acid synthesis (Han et al. 2018). *FASN* gene which is expressed in large numbers in tissues in mammals has shown that it is one of the major genes in goat mammary gland (Haile et al. 2016). In this study on Damascus goats, the positive correlation between the genes of which the expression levels were investigated compatible with the other studies (Xu et al. 2016, Haile et al. 2016, Han et al. 2018). In addition, a significant positive correlation was determined between *FASN* and C18:3 n3. In selection studies, *FASN* gene expression levels should be taken into consideration to obtain high quality milk according to milk fatty acid profile, especially in terms of C18:3 n3. Short and medium chain fatty acids are fatty acids between C4:0-C14:0. In goats, it is reported that approximately half of C16:0 with these fatty acids are de novo synthesized in mammary epithelial cells (Haile et al. 2016). In this study, no correlation is found between C16:0 and *SCD*, *FASN* and *SREBPF1* genes. However, a positive correlation is found between C14:0 used in the calculation of

atherogenic and thrombogenic indexes and *SREBPF1* (0.469;  $P < 0.05$ ). With these results, it is thought that there is a relationship between milk with high atherogenic and thrombogenic indexes and *SREBPF1*.

Significant positive correlations were found between target genes of study (*SCD*, *FASN*, *SREBPF1*) and C6:0, C8:0, and C10:0 fatty acids (Table 5). *SCD*, *FASN* and *SREBPF1* genes have important effects on the synthesis of these fatty acids used in the calculation of the odour index. Capric and caprylic acids have been used in medical therapy for metabolic-related digestive disorders such as hyperlipoproteinemia, premature infant feeding, epilepsy, cystic fibrosis, and coronary heart diseases (Kompan and Komprej 2012). Considering this situation, it is thought that the expression levels of these genes in the mammary epithelial cells may give important ideas in selection applications on milk quality in goats.

## CONCLUSION

In conclusion, the expression levels of *SCD*, *FASN* and *SREBPF1* genes, which are important in the regulation of energy metabolism, are investigated in milk with low and high saturated fatty acid content and their relationship with fatty acid profile in this study. It is thought that successful results may be obtained by investigating these genes in the selection applications on milk quality in ruminants. Also, more studies at the molecular levels are needed to investigate the importance of fatty acid profile in the formation of milk quality.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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