ERCİYES ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ
Journal of Faculty of Veterinary Medicine, Erciyes University

Araştırma Makalesi / Research Article
17(3), 325-333, 2020
DOI: 10.32707/ercivet.828862

Milk Traits of Damascus Goats at Different Lactation Stages: 2. Fatty Acid Profiles and Related Lipogenic Genes Expression Levels*

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How to cite: Özkan H, Yakan A, Karaslan İ,Çamdeviren B. Milk traits of Damascus goats at different lactation stages: 2. Fatty acid profiles and related lipogenic genes expression levels. Erciyes Univ Vet Fak Derg 2020; 17(3): 325-333.

Summary: In the present study, goat milk fatty acid profile and some of the lipogenic genes activities in milk somatic cells were evaluated during the lactation periods in Damascus goats. Relationship between fatty acid profile and lipogenic genes during the lactation were also investigated. The lactation periods of goats were divided to three stages as early, mid and late lactation. Beside fatty acid profile, *FASN, SCD, ACACA, SREBPF1,* and *PPARγ* genes expression levels were determined in milk. It was determined that fatty acid contents of milk samples changed during the lactation (P<0.05). *FASN* and *SCD* gene expression levels were downregulated in mid and late lactation stages (P<0.05). While *ACACA* was almost 3 folds upregulated in mid lactation stage (P<0.05), *SREBPF1* was approximately 9 folds downregulated in late lactation stage (P<0.01). Also, significant correlations were found between fatty acids and lipogenic genes in different lactation stages (P<0.05). In conclusion, it was determined that Damascus goats showed different metabolic activities in different stages of lactation in terms of both fatty acid profile and some lipogenic genes. It was thought that more studies at the molecular level is needed for understanding the complex milk fatty acid profile mechanism of goats.
Key words: Damascus goat, lactation period, lipogenic gene expression, milk fatty acids

Farklı Laktasyon Dönemlerindeki Damascus Keçilerinde Süt Özellikleri: 2. Yağ Asidi Profili ve Onunla İlişkili Bazı Lipogenik Genlerin Ekspresyon Seviyeleri

Özet: Bu çalışmada, laktasyon periyodundaki Damascus keçilerinde süt yağ asidi profili ve süt somatik hücrelerinde bazı lipogenik genlerin aktiviteleri değerlendirilmiştir. Ayrıca, laktasyon boyunca yağ asidi profili ile lipogenik genler arasındaki ilişki araştırılmıştır. Keçilerin laktasyon dönemleri erken, orta ve geç laktasyon dönemi olarak üçe ayrılmıştır. Yağ asidi profilinin yanı sıra sütte *FASN*, *SCD*, *ACACA*, *SREBPF1* ve *PPARγ* genlerinin ekspresyon düzeyleri belirlenmiştir. Süt örneklerinde yağ asidi içeriğinin laktasyon boyunca değiştiği tespit edilmiştir (P<0.05). *FASN* ve *SCD* gen ekspresyon seviyeleri erken ve geç laktasyon dönemlerinde az ifade edilmiştir (P<0.05). *ACACA* orta laktasyon döneminde yaklaşık 3 kat fazla ifade edilirken (P<0.05), *SREBPF1* geç laktasyon döneminde yaklaşık 9 kat az ifade edilmiştir (P<0.01). Ayrıca farklı laktasyon dönemlerinde yağ asitleri ve lipogenik genler arasında anlamlı korelasyonlar bulunmuştur (P<0.05). Sonuç olarak Damascus keçilerinin laktasyonun farklı dönemlerinde hem yağ asidi profili hem de bazı lipogenik genler bakımından değişik metabolik etkinlik gösterdiği belirlenmiştir. Keçilerin karmaşık olan süt yağ asidi profili mekanizmasını anlamak için moleküler düzeyde daha fazla çalışmaya ihtiyaç olduğu düşünülmektedir.
Anahtar kelimeler: Damascus keçisi, laktasyon periyodu, lipogenik gen ekspresyonu, süt yağ asitleri

Introduction

Major advances in animal breeding have been achieved over the past century with effective breeding techniques. Goat is one of the most important farm animals and goat milk and dairy products are valuable dietary resources for human nutrition (Albenzio et al., 2012). Fatty acid profile is a significant parameter for nutritional quality of milk and goat milk has substantial differences in terms of milk fatty acid profile (Bernard et al., 2012).

Fatty acids are essential components of mammals (Suburu et al., 2014). The proportions of dietary fatty acids affect sustainable health of human. While overfeeding with a diet rich in saturated fatty acids such as C12:0, C14:0 and C16:0 have negative effects on human health, it is reported that some of the short fatty acids such as C4:0 has positive effects (Bernard et al., 2012). Goat milk has higher levels of fatty acids compared to cow's milk (Lin et al., 2013). Also, goat milk is richer than cow's milk in C6:0, C8:0 and C10:0 fatty acids than cow's milk (Chillard, 1997; Marounek et al., 2012).

In ruminants, a number of fatty acids in milk are *de novo* synthesized in the mammary gland. It is reported that those fatty acids shorter fatty acids than

Geliş Tarihi/Submission Date : 04.08.2020
Kabul Tarihi/Accepted Date : 16.10.2020

C16:0 are derived mammary epithelial cells (Zhang et al., 2015). On the other hand, arterial blood is the other source of milk fatty acids and long chain fatty acids are derived from triacylglycerides or non-esterified fatty acids (NEFA) in arterial blood (Bernard et al., 2012; Zhang et al., 2015). Sources of C16:0 are either mammary gland or arterial blood (Zhang et al., 2015).

As well as nutrition, lactation stage has an important effect on milk fatty acid profile in ruminants. A series of physiological changes occur in the ruminants during the lactation period. In lactation the molecular activity of the mammary glands along with lipogenic organs changes. Therefore, a number of alterations occur in the milk fatty acid profile directly and indirectly depending on the complex molecular mechanisms of mammary gland (Suburu et al., 2014;Zhang et al., 2015).

Thanks to the development of technology in the last 20 years, the functioning of molecular mechanisms has become more comprehensible. On the other hand, molecular mechanism of fatty acids synthesis in mammary gland has not completely understood, yet (Ataşoğlu et al., 2009; Sun et al., 2016). In this study, goat milk fatty acid profile and some of the lipogenic genes activities in milk somatic cells are evaluated during the lactation periods in Damascus goats. Relationship between fatty acid profile and lipogenic genes during the lactation has also been investigated.

Materials and Methods

Animals and milk sampling

In the present study, milk samples of healthy 20 goats randomly selected from 200 animals were used. The breed type of goats was Damascus. On the other hand, goats were in 3rd-4^{th}lactation periods. A week after births, goats were on the pasture for grazing (06:00 to 18:00 a day). Goats consumed concentrate feed (300 g/head/day) in addition to grazing. The lactation periods of goats were divided to into three stages: Early (10-20 days after parturition, Early Lactation Stage (ELS)), mid (100-110 days after parturition, Mid Lactation Stage (MLS)) and late (200-210 days after parturition, Late Lactation Stage (LLS)). Milk samples were collected in these three stages of lactation.

Collection of cream layer and somatic cells

Approximately 50 mL volume milk samples were centrifuged at + 4 °C at 1800 xg for 15 min. Following the centrifugation, samples were kept at -20 °C for about 15 min**.** Cream layers of samples were collected in new sterile tubes with a volume of 1.5 mL when they were ready to be collected for fatty acid analyzes. Cream samples were kept at –20 °C until fatty acid analyzes.

After removing the cream layers, the supernatants were removed and the cell pellets were taken to new 15 ml volume sterile and RNase-DNase free falcon tubes with the help of PBS (Phosphate Buffered Saline). Cell suspensions in the falcon tubes were completed with PBS to 15 mL and centrifuged at + 4 °C at 1800 xg for 15 min. After centrifugation, supernatants of samples were discarded and approximately 1 mL TRIzol Reagent (Sigma-Aldrich, USA) was added to pellets for total RNA isolation. Pellets were homogenized with TRIzol Reagent and stored -80 °C until molecular analyzes.

Fatty acid analyzes

Fatty acid profile of samples was determined with using approximately 500 µL cream from each sample. After homogenization the creams with 2 mL of 2N methanolic KOH for 4 min at room temperature, 4 mL n-Heptane (Merck, USA) was added to the samples. After waiting at room temperature for 2 min, samples were centrifuged at 200 xg for 5 min. Aqua phase containing Methyl esters was transferred to vials with a volume 1.5 mL. Fatty acids of milk samples were analyzed by Gas Chromatography equipped with flame ionization detector (Shimadzu GC-2025, Japan), auto-injector (Shimadzu AOC-20i, Japan) and Restek Rt-2560 column (100 m length, 0.25 mm ID x 0.20 µm). Temperatures of injector and detector were both kept at 250 °C. Hydrogen was used as carrier gas and the gas flow was 1.20 ml/min. Injection mode was split mode with split ratio of 1:50 and total injection volume was 1 µL. Injector was rinsed with n-heptan, three times pre-run and six times post-run. Temperature gradient program was used. The initial oven temperature was 100 °C (hold for 2 min) and it was then increased by 4 °C/min until 250 °C (hold for 15 min). The run was 54.50 min. For the verification of fatty acids, the determined sample peaks retention times were compared with that of internal standard (FAME Mix, Restek, USA).

Total RNA isolation and cDNA synthesis

Total RNA's of samples were isolated according to the TRIzol Reagent kit protocol (Rio et al., 2010). Protocol of kit was as follows: After waiting approximately 15 min at room temperature, 0.25 mL chloroform was added to each sample and the samples were gently homogenized. Samples were centrifuged with chloroform at + 4 °C at 12000 xg for 15 min after waiting 10 min at room temperature. Following the separation of RNA with centrifugation, 400 µL aqua phases were transferred to new 1.5 mL nuclease free -sterile eppendorf tubes immediately afterwards. Isopropyl alcohol was added (half of using TRIzol) for precipitation of RNA and then centrifuged at + 4 °C at 12000 xg for 10 min. Thereafter, supernatant of sam-

ples were discarded and 70% ethyl alcohol was added to the each sample. Samples were centrifuged at + 4 °C at 7500 xg for 5 min and supernatants were discarded. This step was repeated twice. Finally, RNA pellets were centrifuged for washing with 99% ethyl alcohol at + 4 °C at 7500 xg for 5 min. Supernatants was discarded from samples, and RNA pellets were dried at room temperature for about 10-15 min.

RNA pellets were dissolved and homogenized with 30-100 µL of nuclease free water and checked for quality. Samples purity and concentration were controlled in the nucleic acid meter (Merinton, SMA-1000 UV Spectrophotometer). In addition to this step, rRNA subunits (28S and 18S rRNAs) were evaluated in 1% agarose gel electrophoresis (100V and 25 min).

After DNA digestion was performed for possible genomic DNA contamination with DNase kit (DNase I, RNase free, Thermo Scientific, USA, Cat no: EN0525), RNA samples were converted to cDNA via RevertAid first Strand cDNA synthesis kit (Thermo-Scientific, USA). Thermal cycler (Bio-Rad T100, USA) protocol was as follows: 10 min at 25 °C, 120 min at 37 °C, and 5 min at 85 °C. Following the reaction, samples volumes were completed to 200 µL with nuclease-free water and stored – 80 °C until qPCR analyzes.

Quantitative Real-Time PCR application

After converting total RNA samples to cDNA, *FASN* (Fatty Acid Synthase), *SCD* (Sterol CoA Desaturase), *ACACA* (Acetyl-CoA Carboxylase Alpha), *SREBPF1* (Sterol Regulatory Element Binding Transcription Factor 1), and *PPARγ* (Peroxisome proliferator-activated receptor gamma) genes expression levels were determined. Amplification of target genes was performed using 10 µL of each cDNA of samples. SYBR Green dye containing kit (Power SYBR® Green PCR Master, ThermoFisher Scientific, USA, Cat no: 4367659) was used for qPCR analyses. The reaction was arranged 10 min at 95 °C, followed by 15 sec at 95 °C, 60 sec 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 *G6PD* gene was used as reference gene. Primers forward and reverse sequences were shown in Table 1.

Table 1. Forward and reverse sequences of primers amplified genes

Genes	Forward and Reverse Sequences of Primers	Product Length
FASN*	F: 5’-GCACACAATATGGACCCCCA-3’ R: 5’-CATGCTGTAGCCTACGAGGG-3’	183
SCD*	F: 5’-ATCGCCCTTACGACAAGACC-3’ R: 5’-CATAAGCCAGACCGATGGCA-3’	186
ACACA*	F: 5’- GCCTGCCCGAGTTTTGAGTG-3’ R: 5’-CGCACTCTGGAGCGGATAAA-3’	105
PPARγ*	F: 5’-GTTCAACGCGCTGGAATTAG-3’ R: 5’-GGGCTTCACATTCAGCAAAC-3’	97
SREBPF1*	F: 5’-AACATCTGTTGGAGCGAGCA-3’ R: 5’-TCCAGCCATATCCGAACAGC-3’	134
G6PD#	F: 5’-TGACCTATGGCAACCGATACAA-3’ R: 5’-CCGCAAAAGACATCCAGGAT-3’	76

*: Sequences of Primers were designed by authors, **#:**Sequences of primer were designed by Garcia-Crespo et al. (2005)

Statistical analysis

Descriptive statistics were described as “Mean±Standard error of mean”. Pearson correlation coefficient was performed to determine the relationships between the fatty acids and genes of goat milk. MIXED procedure of SPSS (V22.0; SPSS Inc., Chicago, IL, USA) was used to analyses the effect of the period of lactation as early, mid and late term on the fatty acid parameters of goat milk by using the following model with repeated measures:

$Y_{ijk} = \mu + P_i + e_{ijk}$

Where, Yijk, dependent variable; µ, overall mean; Pi, effect of period of lactation (i = early, mid and late term); and eijk, residual error.

Animals within group were assessed as a random effect, while period of assessed as a fixed effect. $P<0.05$ was considered as significant in all analyses. 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 Schimitgen 2001).

Results

Table 2. Fatty acids profile of milk samples in different stages of lactation (Means±SE)

Fatty acids	ELS	MLS	LLS	P
C4:0 (%)	1.52±0.12^{ab}	1.47±0.10^{b}	1.87±0.15^{a}	<0.05
C6:0 (%)	1.92±0.15	2.25±0.14	2.19±0.15	-
C8:0 (%)	2.14±0.23^{b}	3.25±0.20^{a}	2.58±0.17^{b}	<0.01
C10:0 (%)	5.98±0.72^{c}	10.94±0.67^{a}	8.65±0.44^{b}	<0.001
C12:0 (%)	2.22±0.23^{c}	4.56±0.33^{a}	3.28±0.16^{b}	<0.001
C14:0 (%)	6.41±0.46^{b}	9.68±0.64^{a}	9.94±0.23^{a}	<0.001
C14:1 (%)	0.06±0.01^{c}	0.14±0.03^{b}	0.42±0.02^{a}	<0.001
C15:0 (%)	0.46±0.03^{b}	1.02±0.05^{a}	1.04±0.03^{a}	<0.001
C15:1 (%)	0.28±0.01^{a}	0.24±0.01^{b}	0.29±0.01^{a}	<0.01
C16:0 (%)	22.69±0.75^{b}	24.71±1.11^{b}	28.64±0.69^{a}	<0.001
C16:1 (%)	1.01±0.03^{a}	0.99±0.09^{a}	0.76±0.02^{b}	<0.01
C17:0 (%)	1.28±0.18^{a}	1.11±0.11^{a}	0.74±0.01^{b}	<0.01
C17:1 (%)	0.74±0.08^{a}	0.78±0.09^{a}	0.24±0.03^{b}	<0.001
C18:0 (%)	18.63±0.96^{a}	12.57±0.91^{b}	13.61±0.52^{b}	<0.001
C18:1 (%)	20.17±0.93	19.10±1.06	21.30±0.79	-
C18:2 n6 *trans* (%)	1.16±0.20^{a}	0.98±0.08^{a}	0.28±0.02^{b}	<0.001
C18:2 n6 *cis* (%)	5.39±0.55^{a}	2.74±0.17^{b}	2.03±0.06^{b}	<0.001
C20:0 (%)	0.64±0.07^{a}	0.34±0.06^{b}	0.04±0.01^{c}	<0.001
C18:3 n6 (%)	0.56±0.07^{b}	0.90±0.08^{a}	0.46±0.04^{b}	<0.001
C18:3 n3 (%)	2.74±0.53^{a}	1.09±0.16^{b}	0.72±0.03^{b}	<0.001
C20:1 (%)	2.39±0.32^{a}	0.20±0.05^{b}	0.06±0.01^{b}	<0.001
C20:2 n6 (%)	0.82±0.14^{a}	0.14±0.05^{b}	0.11±0.01^{b}	<0.001
C22:0 (%)	0.16±0.03	0.14±0.06	0.11±0.01	-
C20:3 n3 (%)	0.12±0.03^{b}	0.11±0.03^{b}	0.20±0.02^{a}	<0.05
C20:4 n6 (%)	0.28±0.05	0.33±0.07	0.17±0.02	-
C22:2 n6 (%)	0.09±0.01	0.13±0.02	0.09±0.01	-
C20:5 n3 (%)	0.09±0.01^{ab}	0.06±0.01^{b}	0.10±0.01^{a}	<0.05
C22:6 n3 (%)	0.04±0.01^{b}	0.04±0.02^{b}	0.10±0.01^{a}	<0.001
∑SFA	64.05±1.62^{b}	72.05±1.24^{a}	72.68±0.84^{a}	<0.001
∑MUFA	24.66±0.90^{a}	21.45±1.04^{b}	23.07±0.80^{ab}	<0.05
∑PUFA	11.29±1.34^{a}	6.51±0.53^{b}	4.25±0.10^{c}	<0.001
∑UFA	35.95±1.62^{a}	27.95±1.24^{b}	27.32±0.84^{b}	<0.001
∑n-6	8.42±0.88^{a}	5.32±0.39^{b}	3.33±0.09^{c}	<0.001
∑n-3	2.99±0.56^{a}	1.30±0.19^{b}	1.12±0.04^{b}	<0.001
∑n6/n3	3.54±0.40^{b}	4.68±0.32^{a}	3.02±0.11^{b}	<0.001
OI	11.57±1.12^{b}	17.91±0.02^{a}	15.28±0.83^{a}	<0.001
NV	1.73±0.09^{a}	1.34±0.09^{b}	1.24±0.06^{b}	<0.001
AI	1.35±0.10^{b}	2.13±0.19^{a}	2.12±0.10^{a}	<0.001
TI	1.27±0.07^{b}	1.56±0.11^{a}	1.75±0.06^{a}	<0.001

SFA: Saturated Fatty acids; **MUFA:** Monounsaturated fatty acids; **PUFA:** Polyunsaturated Fatty acids; **UFA:** Unsaturated fatty acids; **OI**: Odour index; **NV:** Nutritive Value; **AI:** Atherogenic index;
TI: Thrombogenic index
OI= (C4:0+C6:0+C8:0+C10:0);
NV=(C18:0+C18:1)/C16:0;
AI= (C12:0+(4*C14:0)+C18:0)/ΣUFA;
TI= (C14:0+C16:0+C18:0)/((0.5*C18:1)+(0.5*ΣMUFA)+(0.5*Σn6)+(3*Σn3)+(Σn3/Σn6));
$^{a, b, c, ab}$:Means with different letters in rows differ significantly

It was determined that most of the fatty acids levels of milk samples changed during the lactation period. In ELS, besides C16:1, C17:0, C18:0, C18:2 n6 *trans*, C18:2 n6 *cis*, C20:0, C18:3 n3, C20:1 fatty acids, polyunsaturated fatty acid (PUFA), unsaturated fatty acid (UFA), monounsaturated fatty acid (MUFA), n6, n3, and nutritive value levels were highest, while C10:0, C12:0, C14:0, C14:1, C15:0, C16:0, SFA (saturated fatty acid), odour index, atherogenic index and thrombogenic index levels were lowest. On the other hand, C4:0, C15:1, C18:0, C20:5 n3 fatty acids, and MUFA levels were the lowest, meanwhile C8:0, C10:0, C12:0 fatty acids, n6/n3 ratio, and odour index levels were highest in the MLS. In addition, the levels of C4:0, C20:3 n3, C20:5 n3, C22:6 n3 fatty acids were highest, while C16:1, C17:1, C18:2 n6 *trans*, C18:2 n6 *cis*, C20:0, C18:3 n6, C18:3 n3, C20:1, PUFA, n6, n3, n6/n3, nutritive value were lowest in LLS (Table 2).

Significant changes were also determined in gene expression levels. Compared to ELS, *FASN* and *SCD* genes expression levels were downregulated almost 10 folds in MLS and LLS (P<0.05). On the other hand, *ACACA* gene expression levels were almost 3 folds upregulated in MLS. In addition to this, *SREBPF1* were approximately 9 folds downregulated in LLS (Figure 1).

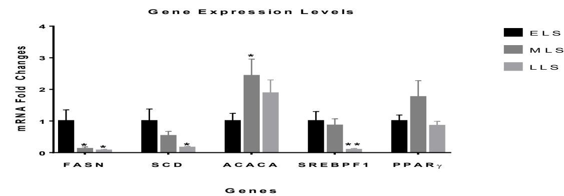

Figure 1. Expression levels of lipogenic genes in somatic cells during early, mid and late lactation
*: P<0.05; **: P<0.01

Table 3. Correlations between lipogenic genes and fatty acid parameters in different lactation stages

Parameters	ELS					MLS				LLS			
	FASN	*SCD*	*ACACA*	*SREBPF1*	*PPARγ*	*FASN*	*SCD*	*SREBPF1*	*PPARγ*	*FASN*	*SCD*	*SREBPF1*	*PPARγ*
FASN		0.969***					0.940***	0.815***			0.723**	0.661**	
SCD					-0.564*			0.894***				0.932***	0.534*
ACACA				-0.600*				-0.595*	0.706**				0.699**
SREBPF1									-0.531*				
C4:0 (%)	0.522*	0.513*		0.501*	-0.729**	0.496*	0.577*	0.566*					
C6:0 (%)	0.668**	0.624*											
C8:0 (%)	0.587*	0.549*											
C15:0 (%)			0.628*		0.554*								
C15:1 (%)									0.609*				
C16:1 (%)			0.647*							-0.552*	-0.564*		
C18:2 *trans* (%)									0.574*				
C20:2 n6 (%)													-0.477*
C22:0 (%)											-0.473*	-0.474*	-0.688**
C20:4 n6 (%)											-0.534*	-0.469*	-0.496*
C22:2 n6 (%)									0.488*				
C20:5 n3 (%)							-0.583*						
∑PUFA													-0.471*
OI	0.584*	0.557*											
AI		0.507*											

PUFA: Polyunsaturated Fatty acids; **OI**: Odour index; **NV:**Nutritional Value; **AI:** Atherogenic index
OI= (C4:0+C6:0+C8:0+C10:0);
NV= (C18:0+C18:1)/C16:0;
AI = (C12:0+(4*C14:0)+C18:0)/ΣUFA*****: P<0.05; ******:P<0.01; *******:P<0.001; **-**:P>0.05
*:P<0.05; **: P<0.01
Only statistically significant differences were showed in Table 3.

In addition to determining fatty acid profile and lipogenic genes expression levels in milk during lactation periods, correlations between these parameters were also investigated. Regulation of C4:0 fatty acid syntheses were found significantly correlated with *FASN* and *SCD* genes in ELS and MLS. However, no correlation was found between C4:0 fatty acids and the gene studied in LLS. Also, positive and significant correlations were found between other short-chain fatty acids with *FASN* and *SCD* in ELS. In the meantime, *FASN* (r=0.584; P<0.05) and *SCD* (r=0.557; P<0.05) were found significantly correlated with odour index in this stage of lactation. Atherogenic index of ELS samples were also correlated with expression levels of *SCD* (r=0.507; P<0.05).

C15:0 fatty acid was found positively correlated with *ACACA* (r=0.628; P<0.05) and *PPARγ* (r=0.554; P<0.05) genes in ELS. On the other hand, there was also positive correlation between C15:1 fatty acid and *PPARγ* in MLS (r=0.609; P<0.05). While C16:1 fatty acid was correlated with *ACACA* (r=0.647; P<0.05) in ELS, negative correlations were determined between this fatty acid with *FASN* (r=-0.552; P<0.05) and *SCD* (r=-0.564; P<0.05) in LLS. C18:2 n6 *trans*, one of the longer fatty acid, positively correlated with *PPARγ* in MLS (r=0.574; P<0.05).

Some of the fatty acids longer than C16:0 were found strongly correlated especially in MLS and LLS (P<0.05). While *SCD* gene expression levels were found negatively correlated with C20:5 n3 (r=-0.583; P<0.05), positive correlation was found between *PPARγ and* C22:2 n6 fatty acid (r=0.488; P<0.05). Once again, negative correlations were found between C22:0 fatty acids with *SCD* (r=-0.473; P<0.05), *SREBPF1* (r=-0.474; P<0.05), and *PPARγ* (r=-0.688; P<0.01) in LLS. Similar correlations were also found C20:4 n6 and *SCD* (r=-0.534; P<0.05), *SREBPF1* (r=-0.469; P<0.05), and *PPARγ* (r=-0.496; P<0.01) in this stage of lactation. On the other hand, PUFA and *PPARγ* were found negatively correlated in LLS (r=-0.471; P<0.05) (Table 3).

Correlations between studied genes were also investigated (Table 3). *FASN* and *SCD* were found significantly and highly correlated in all lactation stages (P<0.01). Also, *FASN* and *SCD* gene expression levels were significantly correlated with *SREBPF1* gene expression levels in MLS and LLS samples (P<0.01). While *ACACA*, the major regulator of fatty acid biosynthesis, were positively correlated with *PPARγ* in MLS (r=0.706; P<0.01) and LLS (r=0.699; P<0.01), negative correlations were found between *ACACA* and *SREBPF1* genes in ELS (r=-0.600; P<0.05) and MLS (r=-0.595; P<0.05). But in this study, *SREBPF1*, major lipogenic transcription factor, were negatively correlated with *PPARγ* in MLS (r=-0.531; P<0.05) (Table 3).

Discussion and Conclusion

It was reported that the levels of C4:0, C6:0, C8:0 and C10:0 fatty acids were higher in goat milk than cow milk and all these fatty acids were more responsible for milk odour index (Kompan and Komprej, 2012; Sun et al., 2016). C6:0, C8:0 and C10:0 fatty acids were relatively abundant in goat milk, so they were named as caproic, caprylic, and capric acid, respectively (Kompan and Komprej, 2012). In a study conducted on semi-intensive feeding Damascus goats, a similar breed of this study, it was reported that the ratios of C4:0-C8:0 fatty acids were similar levels in all lactation stages (Güler et al., 2007). Unlike the results of Güler et al. (2007), the highest level of C4:0, also known as butyric acid, was found in LLS. Besides, the highest levels of C6:0, C8:0, and C10:0 fatty acids were in MLS as expected. The main reason for this difference was thought to be the factors such as the age and season of lactation. It was reported that the amount of C6:0-C10:0 fatty acids increased during the peak period of lactation in goats (Strzałkowska et al., 2009).

While positive correlations were determined between C4:0 fatty acids and most of the targeted genes expression levels in ELS and MLS, no correlation was found between C4:0 and any other gene in LLS. Mammary gland was reported to be the primary organ where the synthesis of short-chain fatty acids in milk occurs (Zhang et al., 2015). Therefore, physiological changes at the molecular levels in the mammary gland were thought to be the main reason for the reduction of C4:0 fatty acids in MLS.

FASN, a vital enzyme expressed in many tissues at different levels, was reported to perform majority of the enzymatic stages of fatty acid synthesis (Badaoui et al., 2007; Suburu et al., 2014). In most of the studies, researchers were focused on the relation between *FASN* gene and long chain fatty acid (Kadegowda et al., 2009; Suburu et al., 2014; Zhu et al., 2014). *FASN* is a multi-functional polypeptide enzyme that produces saturated fatty acids and is a priority for the synthesis of C16:0 fatty acid (Suburu et al., 2014). It is reported to be responsible for the production of C14:0 and C18:0 as well as C16:0 fatty acids (Jayakumar et al., 1995). On the other hand, a limited number of studies are found to investigate the relationship between *FASN* and short chain fatty acids in goat milk (Izadi et al., 2016; Zhu et al., 2014). Although *FASN* and *SCD* genes were reported to be crucial in fatty acid metabolism, it was found that these genes do not have a direct relationship to the short-chain fatty acid ratios causing high odour index in Damascus goat's milk. It is because *FASN* and *SCD* gene expression levels decreased significantly in MLS and LLS while odour index was high. It was reported in a study conducted on goats, *de novo* lipogenesis mediated by *SREBPF1* in mammary gland

(Zhang et al., 2018). Also, *SREBPF1* was reported to be involved in the desaturation of fatty acids (Sun et al., 2016). This transcription factor was reported to be particularly effective on *FASN* and partially *ACACA* gene (Yao et al., 2017; Xu et al., 2016).It was thought that lower level of C15:0 might be caused by the association of C15:0 with *ACACA* and *PPARγ* in ELS (Table 3). There was also positive correlation between C15:1 and *PPARγ* in MLS (P<0.05). It was reported that *PPARγ*, one of the leading regulators of lipogenesis, had a close relationship with lipogenic genes such as *FASN* and *ACACA* (Zhu et al., 2015). It was reported that SCD was directly regulated in the mammary gland by *SREBPF1* and *PPARγ* (Shi et al., 2013). In an another study, it was reported that *FASN* and *SCD* genes positively affect fatty acid composition of ruminant milk in terms of human consumption (Izadi et al., 2016).

While C16:1 fatty acid was positively correlated to *ACACA* in ELS (P<0.05), negative correlation was found between this fatty acid and *FASN* and *SCD* in LLS (P<0.05). Also some of the fatty acids longer than C16:0 were found strongly correlated in MLS and LLS (P<0.05). Although arterial blood was showed as the source of long chain fatty acids (Zhang et al., 2015), it was understood with this study that the mammary gland and milk somatic cells were also responsible for the long fatty acids in goat milk.

It was reported in a study that *ACACA* is one of the most important target genes of *SREBPF1* in goat mammary epithelial cells (Xu et al., 2018). *PPARγ* was also reported to regulate genes associated with fatty acid metabolism throughout lactation by triggering *SREBPF1* (Bionaz and Loor, 2008; Kast-Woelbern et al., 2004; Shi et al., 2013). Likewise, in a study conducted on bovine mammary epithelial cells, *PPARγ* and long chain fatty acids were reported to affect the regulation of lipogenic genes (Kadegowda et al., 2009). But in this study, *SREBPF1*, a major lipogenic transcription factor, was negatively correlated with *PPARγ* in MLS (r=-0.531; P<0.05). It was understood that the correlation between these two genes in mammary gland might change in the different lactation stages in goats and lipogenic pathway in mammary gland were re-regulated with the metabolism-responsible tissues of the organism in different lactation stages. Izadi et al. (2016) and Yao et al. (2017) were reported that *SCD* has a key role in synthesis of MUFA and long chain fatty acids in general. In this study, however, it was determined that there was no direct relationship between *SCD* gene expression levels and MUFA in milk. Although *SCD* known as an enzyme responsible for the synthesis of MUFA and long-chain fatty acids, it was understood from this study that *SCD* did not have the same effect on somatic cells at every stage of lactation (Yao et al., 2017). MUFA's, relatively longer fatty acids, were reported to have largely originated from arterial blood (Bernard et al., 2012; Zhang et al., 2015). Therefore, it was thought that tissue specific activity of *SCD* gene might led to these results.

Although it was reported that the synthesis of long chain fatty acids mostly depends on diet (Zidi et al., 2010), positive correlations were found between longer fatty acids and expression levels of lipogenic genes in milk somatic cells.

Synthesis of fatty acids in goat mammary gland has not fully been understood at the molecular level. However, the main cause of differences between breed in terms of fatty acid profile is reported to be genetic (Zidi et al., 2010). Genetic factors at the DNA levels are responsible for the formation of quantitative characters but more studies are needed for understanding the environmental effects to molecular activity. Studies about fatty acid synthesis in goat mammary gland are mostly conducted through cell culture (Shi et al., 2013; Sun et al., 2016). This is one of the factors that limit the understanding of the physiological process of the milk fatty acid profile of goats. On the other hand, the role of lipogenic genes in mammary gland of goats remains unclear. While obtained results, such as nutritive value and odour index, show that milk is more preferable in the first period of lactation (ELS), it is thought that other lipogenic genes should be investigated in goat milk with new studies on the regulation of milk fatty acid composition.

In conclusion, the relationship between molecular activity in milk somatic cells and milk fatty acid profile was investigated in different lactation stages with this study. In this way, substantial data was obtained from Damascus goat milk. Researches about molecular mechanism of mammary gland of goats were mostly focused on cell culture studies. On the other hand, it is generally known that long chain fatty acids in milk originate from arterial blood. But in this study, significant correlations were found between long chain fatty acids in milk and lipogenic genes expressed in milk somatic cells. In this context, it was understood that more studies are needed for understanding the complex mechanism of regulation the milk fatty acid profile of goats.

Acknowledgement

This study was supported by Scientific Research Council of Hatay Mustafa Kemal University with 19M057 project number.

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