The levels of milk fatty acids and alterations of correlations between them in weaning process in damascus goats

HÜSEYİN ÖZKAN[1]*, İREM KARAASLAN[2], UFUK KAYA[3], SEVDA DALKIRAN[4], MURAT YÜKSEL[5], AKIN YAKAN[1,2]

[1] Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Genetics, 31060 Hatay, Turkey
[2] Hatay Mustafa Kemal University, Technology and Research & Development Center (MARGEM), 31060 Hatay, Turkey
[3] Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Biostatistics, 31060 Hatay, Turkey
[4] Hatay Mustafa Kemal University, Institute of Health Sciences, Department of Molecular Biochemistry and Genetics, 31060 Hatay, Turkey
[5] Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, 31060 Hatay, Turkey

SUMMARY

Weaning in goat breeding is applied with varying periods and practices. During lactation stages, there are notable changes in the fatty acid profile of goat milk. Weaning is one of the potential breeding practices that may affects the milk fatty acid profile in goats. The aim of this study was to determine the changes in fatty acid profile of milk during weaning process in Damascus goats. Possible changes in the relationships between milk fatty acids and Somatic Cell Score (SCS) were also investigated in the study. Milk samples were collected during morning milking from 24 healthy Damascus goats in the weaned day (WD) and one week after the weaning (Post Weaned Day, PWD). SCS and fatty acid profile of collected milk samples in both sampled days were determined. Nutritive value, odour and atherogenic indexes of milk samples were also evaluated. While SCS was dramatically decreased after weaning, significant changes at varying levels were determined in milk fatty acids. The levels of C10:0, C12:0, C16:0, and C20:5 n3 fatty acids were lower in the PWD samples than the WD samples, while C11:0, C16:1 n7, C17:0, C17:1 n8, C18:1 n9, C20:0, C20:3 n6, C21:0, C23:0, and C24:0 fatty acids were found at higher levels. Compared to WD samples, Long-chained Fatty Acids (LCFA), Unsaturated Fatty Acids (UFA), Mono-Unsaturated Fatty Acids (MUFA), and Nutritive value parameters were higher in PWD samples. On the other hand, Medium-Chained Fatty Acids (MCFA), Saturated-Fatty Acids (SFA), and atherogenic index were found lower in PWD samples than WD samples. Furthermore, it was determined that the correlations between the fatty acids in milk were changed after weaning application. It was determined that the weaning process had considerable effects on the milk fatty acid profile, and it was thought that the milk quality of Damascus goats increased significantly after weaning application.

KEY WORDS

Milk fatty acids; Goat milk; Weaning in goats; Relationship in milk fatty acids.

INTRODUCTION

Goat is the first domesticated farm animals and population of goat in the world is growing day by day[1]. In general, goat breeding is mostly done for milk and dairy products. In addition to being precious dairy resource, goats are considerable farm animals for meat production. Therefore, the growth and development of goat kids are particularly important in goat breeding[2].

In mammals, lactation is essential to provide immune-related molecules to the offspring in addition to requirements of milk ingredients. As other mammals, goat kids are allowed to have access to milk in a certain period of lactation following parturition. During this period, ingredients of milk have tremendous effect on the health and growth characteristics of kids[3]. Fatty acids are remarkable ingredients of the milk. Particularly, goat milk has richer short- and medium-chain fatty acids[1]. Fatty acids originate by two sources in goat milk. While the first fatty acid source is exogenous resources in ration, another one is de novo synthesis in the organism[1,4]. Fatty acid synthesis is strongly related with stage of post-partum period, and it is thought that the milk fatty acid profile may be related to the weaning period[5,6,7]. In goat breeding, weaning is applied with varying periods and practices depending on the breeding strategies. However, some researchers report that weaning period has crucial effects to the parameters related to the development and meat quality of offspring[4]. Strong relation has been reported between compositions of fatty acids in milk of goats and mus-

Corresponding Author:
Özkan Hüseyin (hozkan@mku.edu.tr).

Table 1 - Formulas used in the calculation of fatty acid parameters

Fatty Acid Parameters (%)*	Formulas of Parameters
Short Chained Fatty Acids (SCFA)	C4:0 + C6:0 + C8:0 + C10:0
Medium Chained Fatty Acids (MCFA)	C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C15:1 n5 + C16:0 + C16:1 n7
Long Chained Fatty Acids (LCFA)	C17:0 + C17:1 n8 + C18:0 + C18:1 n9 + C18:2 n6 trans + C18:2 n6 cis + C20:0 + C18:3 n6 + C20:1 + C18:3 n3 + C21:0 + C20:2 n6 + C22:0 + C20:3 n6 + C22:1 n9 + C20:3 n3 + C20:4 n6 + C23:0 + C22:2 n6 + C24:0 + C20:5 n3 + C24:1 n9 + C22:6 n3
Saturated Fatty Acids (SFA)	C4:0 + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0
n6	C18:2 n6 trans + C18:2 n6 cis + C18:3 n6 + C20:2 n6 + C20:4 n6 + C22:2 n6
n3	C18:3 n3 + C20:3 n3 + C20:5 n3 + C22:6 n3
Polyunsaturated Fatty Acids (PUFA)	n6 + n3
Monounsaturated Fatty Acids (MUFA)	C14:1 n5 + C15:1 n5 + C16:1 n7 + C17:1 n8 + C18:1 n9 + C20:1 n7 + C22:1 n9 + C24:1 n9
Unsaturated Fatty Acids (UFA)	MUFA + PUFA
Odour Index (OI)	(C4:0+C6:0+C8:0+C10:0)
Atherogenic Index (AI)	(C12:0+(4*C14:0)+C16:0)/(MUFA + PUFA)
Nutritive Value (NV)	(C18:0+C18:1 n9)/C16:0

*: [2-10]

cle of kids[3,8].

Depending on the breeding strategies, it is known that changes occur in milk compositional parameters with the weaning process. On the other hand, there is limited knowledge about the changes on milk fatty acids and relations each other after weaning application in goats. In this study, in addition to determination of the alterations of milk fatty acids after weaning, the possible changes on the relationship between fatty acids in milk has been investigated in Damascus goats.

MATERIALS AND METHODS

Animals and Collection of Samples

The study approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (Approval no: 2021/02-08), carried out on in a private farm in Hatay Province in Turkey. Samples were collected from healthy 24 Damascus goats in their 3rd-4th lactation. The animals gave singleton birth and were at the weaning process. Goats were fed with 1 kg/goat dry alfalfa and 1 kg/goat concentrate feed (88.91% dry matter, 16.51% crude protein and 2650 kcal/kg total Metabolic Energy).

Animals were weaned at postpartum 105th day (105±4 days). The milk samples were collected at morning milking on two different days: Weaned day (WD, postpartum 105th day) and one week later (Post-Weaned day, PWD, postpartum 112th day). Milk samples were collected under sterile conditions. Udders of goats were washed with alcohol-based disinfectant and cleaned with water and sterile cotton gauze swabs before collecting of milk samples. Approximately 50 mL collected milk samples from each animal were transferred to the laboratory in cold chain about 30 min.

Determination of Somatic Cell Count and Cream Layer Collection from Milk Samples

Somatic Cell Count (SCC) of the samples were determined by somatic cell counter (Lactoscan SCC 6010, Bulgaria) and obtained SCC data were normalized and transformed into Somatic Cell Score by the method (SCS=log2(SCC/100.000) + 3) reported by Ali et al[9]. Approximately 50 mL milk from each sample was centrifuged (NF800R, NUVE, TURKEY) at + 4 °C at 1800 xg for 15 min. Thereafter, the samples were kept at – 20 °C for 15 min. Obtained cream layers were collected into 1.5 mL volume sterile tubes and stored at – 80 °C until fatty acid analysis.

Extraction and Analysis of Fatty Acids

For fatty acid analysis, 2 mL 2N KOH in methanol was used for 500 µL cream layer and incubated 4 min at room temperature. After incubation, 4 mL n-Heptane was added to samples and kept for 2 min at room temperature. The samples were centrifuged at 200 xg for 5 min. The upper phases containing methyl esters were transferred to 1.5 mL vials. Fatty acids of samples were identified using gas chromatograph (GC-2025, Shimadzu, JAPAN) equipped with flame ionization detector. Separation was carried out with Restek Rt-2560 column (100 m length, 0.25 mm internal diameter x 0.20 µm film thickness). The injection was performed at 200 °C (held for 2 min) and then the oven temperature was raised to 250 °C at a rate 4 °C/min and finally held constant for 15 min. The temperatures of the flame ionization and injector were adjusted at 250 °C with the following gas flow: Carrier gas hydrogen: 40 mL/min, instrument air: 400 mL/min. The hydrogen flow rate was 1.2 mL/min, the split ratio was 1:50 and the injection volume was set to 1 L. The peaks of FAME were identified by comparing the retention times with those of FAME Mix Standard (Restek, USA). Fatty acids analysis was performed in an accredited laboratory (Technology and Research and Development Center, Hatay Mustafa Kemal University, Hatay/Turkey). The detailed information about the formulation of fatty acid parameters were presented in Table 1.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics software Version 23.0 and R (version 4.1.2). Firstly, the variables were checked for normality by using Shapiro-Wilk test.

The relationship between SCS and fatty acid parameters in each sampling time (WD and PWD) was established using Pearson correlation analysis. The Pearson correlation coefficients between variables were calculated and visualized as a heatmap correlogram. The correlograms that was consisted of correlation coefficient matrices were visualized using the R corrplot package[11]. SCS and fatty acid parameters were analyzed using a linear mixed model for repeated measures. The model included the fixed effect of sampling time and the random effect of goat.

$$Y_{ij} = \mu + S_i + G_j + e_{ijC}$$

Where, Y_{ij}, dependent variable; μ, overall mean; S_i, the fixed effect of sampling time (i = Weaned day and Post-Weaned day); G_j, random effect of goat and e_{ijC}, residual error. Pairwise comparisons were done using a Bonferroni adjustment. Data were calculated as "Mean ± Standard Error of Mean". $P<0.05$ was considered as significant in all analyses.

RESULTS

The SCS was dramatically decreased from 5.78±0.08 to

Table 2 - Fatty acid profile of Weaned and Post-Weaned milk samples (Mean ± SEM)

Variables (%)	Name	Sampling Time		P
		Weaned Day	Post-Weaned Day	
C4:0	Butyric acid	0.122 ± 0.006	0.123 ± 0.005	0.801
C6:0	Caproic acid	1.058 ± 0.056	1.052 ± 0.064	0.942
C8:0	Caprylic acid	2.593 ± 0.151	2.584 ± 0.122	0.962
C10:0	Capric acid	13.514 ± 0.373	12.133 ± 0.454	0.021
C11:0	Undecanoic acid	0.117 ± 0.007	0.184 ± 0.012	<0.001
C12:0	Lauric acid	5.764 ± 0.166	5.130 ± 0.168	0.009
C13:0	Tridecylic acid	0.087 ± 0.003	0.093 ± 0.005	0.285
C14:0	Myristic acid	14.236 ± 0.416	13.486 ± 0.366	0.173
C14:1 n5	Myristoleic acid	0.440 ± 0.034	0.440 ± 0.030	0.99
C15:0	Pentadecylic acid	1.156 ± 0.077	1.239 ± 0.080	0.449
C15:1 n5	Pentadecenoic acid	0.258 ± 0.026	0.301 ± 0.023	0.208
C16:0	Palmitic acid	29.509 ± 0.492	28.039 ± 0.501	0.038
C16:1 n7	Palmitoleic acid	1.267 ± 0.034	1.595 ± 0.125	0.015
C17:0	Margaric acid	0.671 ± 0.038	0.808 ± 0.036	0.011
C17:1 n8	Heptadecenoic acid	0.200 ± 0.013	0.274 ± 0.018	0.001
C18:0	Stearic acid	11.856 ± 0.628	12.413 ± 0.654	0.534
C18:1 n9	Oleic acid	13.659 ± 0.456	16.432 ± 0.478	<0.001
18:2 n6 trans	Linoleic acid	0.150 ± 0.019	0.184 ± 0.009	0.103
18:2 n6 cis	Linolelaidic acid	1.828 ± 0.107	1.659 ± 0.134	0.32
C18:3 n3	-linolenic acid	0.919 ± 0.049	0.938 ± 0.056	0.798
C18:3 n6	-linoleic acid	0.016 ± 0.002	0.018 ± 0.001	0.273
C20:0	Arachidic acid	0.056 ± 0.011	0.114 ± 0.012	0.001
C20:1 n7	Gondoic acid	0.007 ± 0.001	0.008 ± 0.001	0.115
C20:2 n6	Eicosadineoic acid	0.014 ± 0.001	0.016 ± 0.001	0.259
C20:3 n6	Dihomo-g-linolenic acid	0.021 ± 0.002	0.028 ± 0.002	0.016
C20:4 n6	Arachidonic acid	0.052 ± 0.004	0.053 ± 0.006	0.869
C20:3 n3	Eicosatrienoic acid	0.014 ± 0.003	0.020 ± 0.004	0.29
C20:5 n3	Eicosapentaenoic acid	0.006 ± 0.001	0.003 ± 0.0005	0.032
C21:0	Heneicosylic acid	0.331 ± 0.013	0.545 ± 0.028	<0.001
C22:0	Behenic acid	0.015 ± 0.002	0.015 ± 0.002	0.868
C22:1 n9	Erucic acid	0.009 ± 0.001	0.009 ± 0.002	0.743
C22:2 n6	Docosadienoic acid	0.003 ± 0.001	0.003 ± 0.0002	0.868
C22:6 n3	Docosahexaenoic acid	0.010 ± 0.001	0.014 ± 0.001	0.001
C23:0	Tricosylic acid	0.009 ± 0.001	0.012 ± 0.001	0.038
C24:0	Lignoceric acid	0.026 ± 0.002	0.031 ± 0.002	0.022
C24:1 n9	Nervonic acid	0.008 ± 0.003	0.003 ± 0.0003	0.096

5.08±0.10 in PWD samples compared to WD samples (P<0.001). On the other hand, significant differences in fatty acid profile were determined between WD and PWD samples. While C10:0, C12:0, C16:0, and C20:5 n3 fatty acids decreased, C11:0, C16:1 n7, C17:0, C17:1 n8, C18:1 n9, C20:0, C21:0, C20:3 n6, C23:0, and C24:0 fatty acids were increased with the 7 days after of weaning (Table 2).

MCFA contents were significantly decreased, while LCFA contents were increased in PWD (P<0.001). While UFA and MUFA parameters were increased, the contents of SFA were decreased in PWD (P<0.001). In addition, Nutritive value of the milk was increased (P<0.01) whereas atherogenic index were decreased (P<0.001) (Figure 1).

In addition to comparing the results between sampled days, the correlations between studied parameters were also investigated in both WD and PWD samples. According to the analysis, negative and positive correlations were determined between parameters in both sampled days with varying strengths. The correlations were presented in correlograms in Figure 2 and Figure 3.

DISCUSSION

The primary function of milk is to provide nutritional requirements as well as the requirements of the immune system for newborn mammals for a certain period. Following the parturition, synthesis of milk begins with colostrum and continuous varying periods depending on the breeding strategies[2]. In general, SCS is related with the mammary health status in farm animals. Although high SCS are usually interpreted as mastitis, this is not always the case due to the apocrine secretion

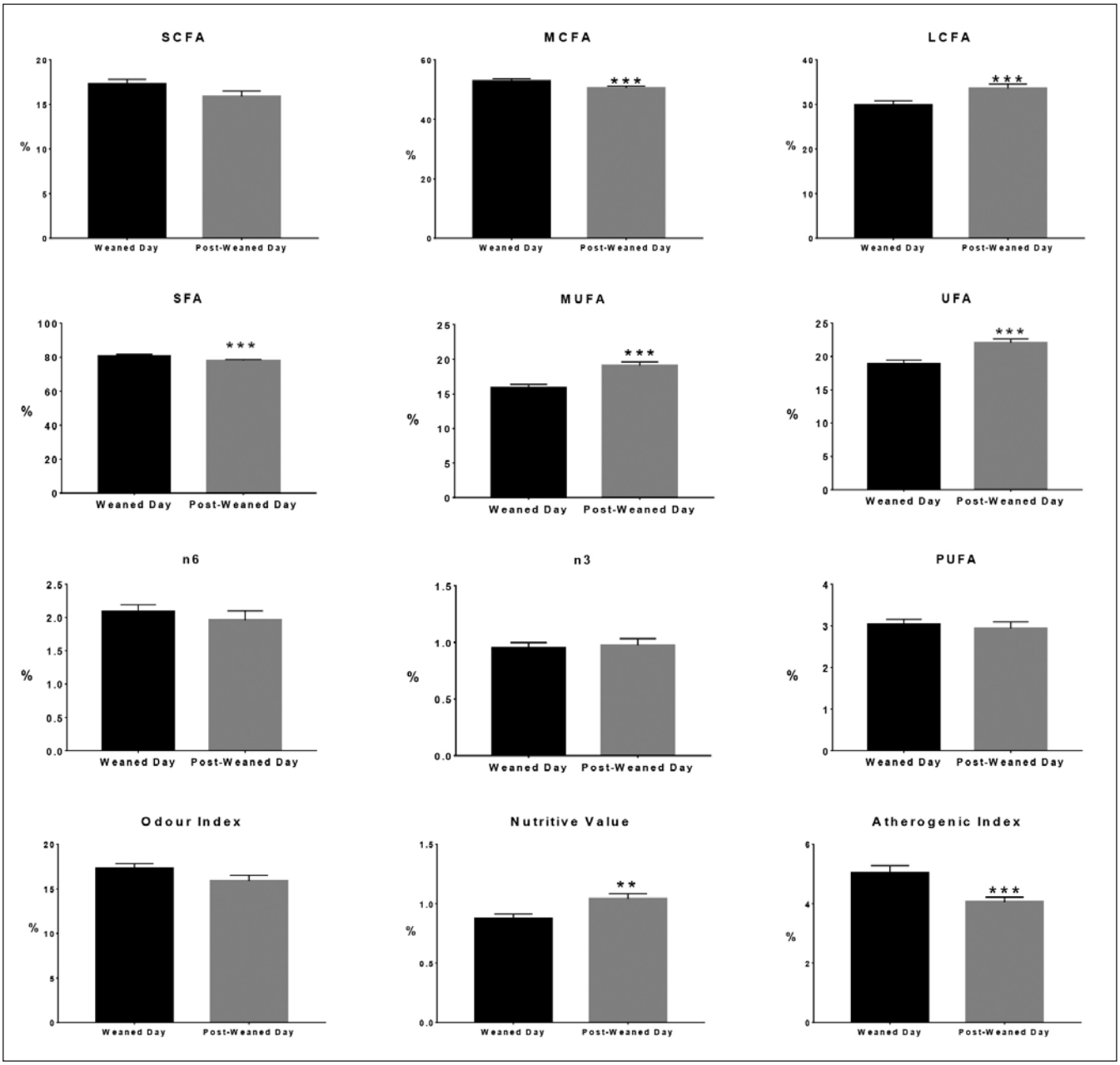

Figure 1 - Fatty acid related milk quality parameters in sampled days.

SCFA: Short-chained fatty acids; **MCFA**: Medium-chained fatty acids; **LCFA**: Long-chained fatty acids; **SFA**: Saturated fatty acids; **MUFA**: Mono-unsaturated fatty acids; **UFA**: Unsaturated fatty acids; **n6**: Omega 6; **n3**: Omega 3; **PUFA**: Poly-unsaturated fatty acids; ****:** P<0.01; *****:** P<0.001

mechanism in goats[7,12]. The SCS can fluctuate widely throughout the lactation period, even in healthy goats[7]. In this study carried out in Damascus goats, SCS levels in milk has been investigated in both weaning and post weaning period. SCS levels in milk of WD samples have been found to be similar in different breed goats in suckling and early periods reported by Fernández et al.[13] and Zamuner et al.[14]. On the other hand, it has been determined that there has a decrease in the SCS in PWD samples. It has been thought that it might be related to possible physiological changes in mammary gland together with other organs due to the completion of suckling[15,16].

Although SCS is associated with udder health, it has been reported that there is a relationship between SCS and milk quality[7,17]. Furthermore, milk fatty acid profile has also related with mammary health and milk quality. It has been known that milk derived fatty acids have immunomodulatory and anti-inflammatory properties[2]. While positive correlation has been detected between SCS and C22:6 n3 in WD samples, negative correlation has been found between SCS with C15:1 n5, C21:0, and C22:2 n6 fatty acids in PWD samples. Also, it has been reported that C22:6 n3 fatty acid has potentially important role in response to inflammation[18]. This might explain the positive correlation between SCS and C22:6 n3[19,20]. SCS correlated fatty acids are synthesized both mammary gland and other metabolic organs such as liver[21]. On the other hand, it has been reported that de novo fatty acid synthesis in mammary gland is strongly related with SCS in ruminants[21,22]. In addition, longer fatty acids come to mammary gland with circulating blood from other organs[21,23]. It has been thought that the reason of the determined correlation might be related to systemic activities of the leukocytes in the circulating blood. Even if the SCS has been determined in physiological limits in both sampled days, the variable correlations have been thought to related with the physiological changes occurred depending on the weaning process[5,6].

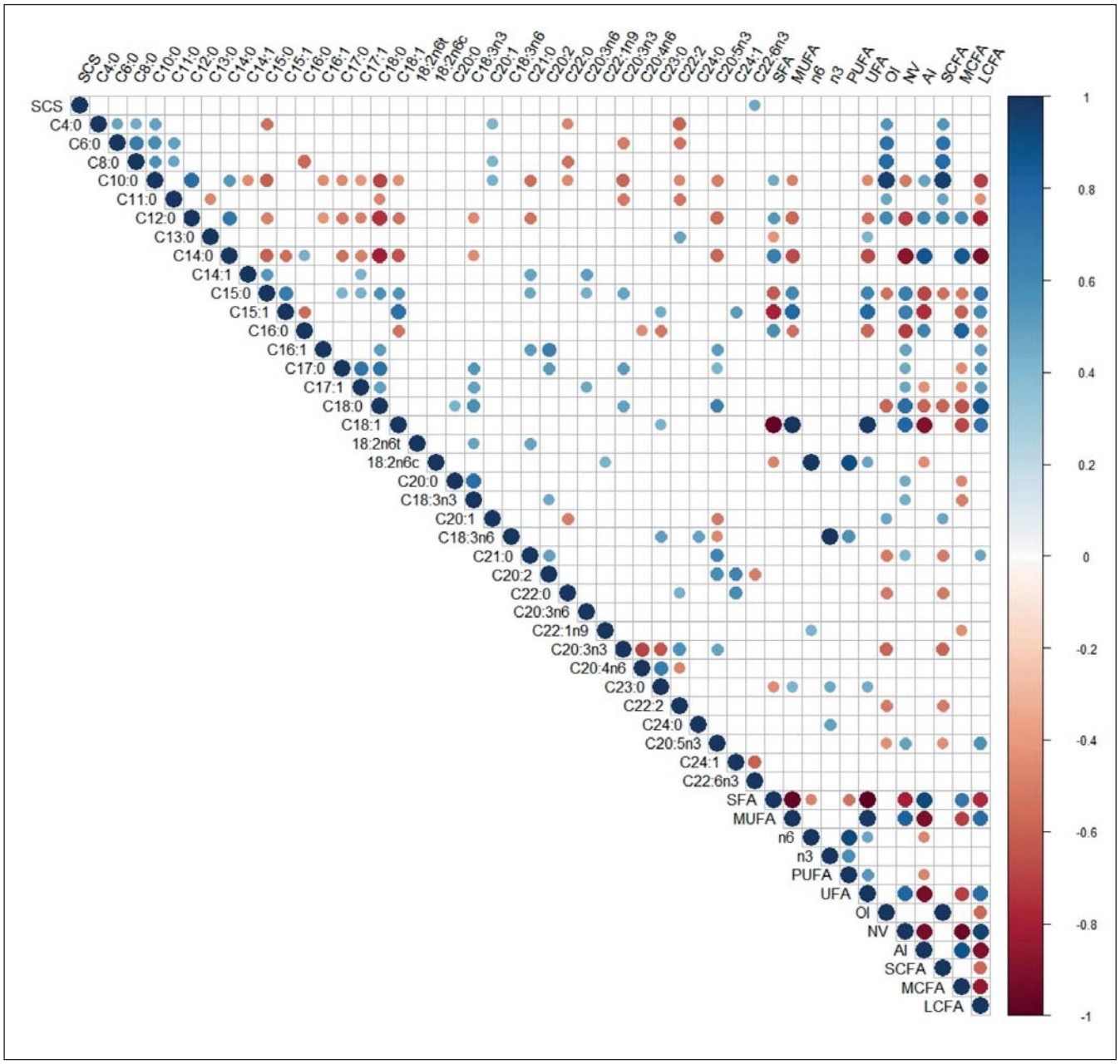

Figure 2 - Correlations between studied parameters in correlogram in Weaned Day. The correlation coefficients in the correlogram were shown in the upper right angle. Larger circles and higher colour density were represented stronger correlations. In addition, the blanks in the correlograms were indicated that there were no significant correlations.

Fatty acid profile of milk is essential for the quality of milk. In general, milk fatty acid profile is affected by numerous genetic and environmental factors. In addition to ration and season, stage of lactation and suckling are also important environmental variables[24,25]. While it has been determined that C10:0, C12:0, and C16:0 fatty acids decreased, C11:0 and most of the long-chained fatty acids increased with the weaning application. In addition, C12:0 fatty acid, which has atherogenic effect in human diets, was significantly decreased in PWD samples. C12:0 fatty acid has also reported to have antibacterial and antiviral properties[25]. On the other hand, it has been reported that SCFA and MCFA primarily preferred for the supplying energy requirements[20,26,27]. It has been thought that the diminishes of C10:0, C12:0, and C16:0 fatty acids in milk might be explained with the weaning process[28].

In goats, most of the fatty acids (60%) in milk is de novo synthesized in mammary epithelial cells[24]. While SCFA and MCFA are the major products in mammary gland, circulating blood contribute to LCFA and MUFA contents of the milk[26,29]. It has been determined that most of the long-chained fatty acids increased after the weaning process. In addition to MUFA and UFA contents of the milk, NV has increased. Moreover, atherogenic index has decreased. In contrast to our findings, it has been reported in a study conducted on sheep that the atherogenic index of milk increased after weaning[6]. Even if related parameters might be affected by different factors, it has been thought that the main factors might be the differences between breed type and weaning period. On the other hand, it has been inferred that the quality of milk increased in Damascus goats after weaning and milk of weaned goat is more appropriate for the human health[21].

According to Pearson correlation results, positive correlations have been found between most of the short- and medium-chained fatty acids in both sampled days, as expected[30]. After

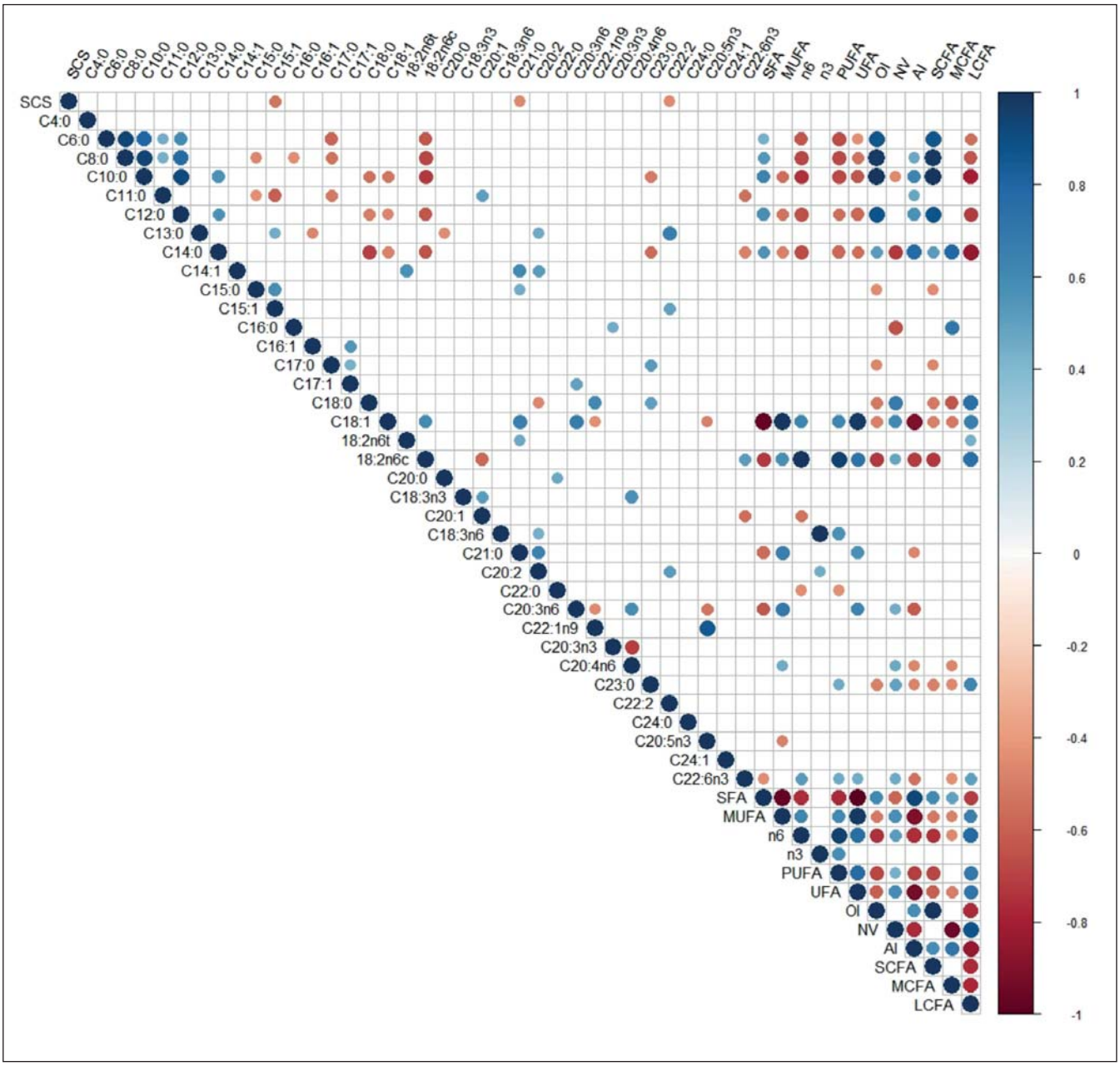

Figure 3 - Correlations between studied parameters in correlogram in Post-Weaned Day. The correlation coefficients in the correlogram were shown in the upper right angle. Larger circles and higher colour density were represented stronger correlations. In addition, the blanks in the correlograms were indicated that there were no significant correlations.

weaning, the correlation of C4:0 with other short chain fatty acids disappeared in PWD samples. While most of the correlations have been determined similar in both sampled days, correlations between some of the fatty acids have changed after weaning. It has been known that the fatty acid metabolism is under the control of complex molecular mechanisms[2,30]. In addition to systemic compensators, it has been reported that the major regulators of fatty acid metabolism in milk are attendant proteins such as SCD, FASN, and ACACA enzymes[27,30,31,32]. Furthermore, one of the main reasons of the changes on the correlations between fatty acids in milk might be the replenish of mammary gland after weaning process. In addition, stage of lactation is probably what influenced fatty acid relations[32]. It has been reported that the major reason of the correlations among de novo fatty acids (C4:0-C14:0) in both sampled days might be their similar source of origin[32]. On the other hand, the mobilization of lipids should be considered reason for the changes in correlations between long-chained fatty acids in WD and PWD samples.

CONCLUSION

In conclusion, the results of our study have revealed it is evident that weaning has an important influence in milk SCS. In line with the results of this study, it might be considered that weaning application has significant effects on milk fatty acid profile. It is thought that the milk quality of goats significantly increased after weaning of kids. To elucidate the underlying mechanisms of fatty acid profile of goat milk during weaning process, more studies are needed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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