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Effect of Diet Composition on Milk Composition and Fatty Acid Profile of Najdi Ewes

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ABSTRACT

The aim of our work was to investigate the effect of dietary supplementation on the milk composition, fatty acid profile, physical properties, and tocopherol contents of milk fat from dairy ewes. Sixty-four multiparous Najdi ewes were selected, randomly distributed into four groups (n = 16), and fed four different diets. The diets were composed of traditional feed (TF, barley and alfalfa hay) and three complete feeds with different compositions (CF_1, CF_2, and CF_3). Twenty-four Najdi ewes were randomly divided into four groups (n = 6) after lambing and fed different diets. Milk fat percentage was higher in the milk of ewes fed TF (5.47%), CF_1 (4.95%), or CF_2 (6.40%); however, milk protein percentage was higher in the milk of ewes fed CF_1 (4.29%) and CF_3 (4.64%) compared to TF (3.78%) and CF_2 (3.57%). Total (C12:0 + C14:0 + C16:0) saturated fatty acids were significantly lower in milk fat from ewes fed TF (45.67%) and CF_1 (42.13%) compared to CF_3 (53.65%) and CF_2 (49.67%). Linoleic acid (C18:2Δ9c,12c; n-6) was significantly higher in milk fat from ewes fed CF1 (4.17%). While, no significant difference was detected for α-linolenic acid (C18:3Δ9c,12c,15c; n-3). The percentage of trans-vaccenic acid (C18:1Δ11t) was significantly greater in milk fat from ewes fed CF_2 (3.54%) followed by CF_1 (2.00%), CF_3 (1.24%) and TF (0.37%). The conjugated linoleic acid (C18:2Δ9c,11t) content was significantly higher in milk fat ewes fed CF_1 (0.97%). These results indicate that the FA profile and total tocopherols are significantly affected by the diet type.

INTRODUCTION

Milk from most mammalian species is considered to fulfill all nutritional requirements for human infants and neonates (Abdelrahman *et al*., 2017a). It is a principal source of lipids, proteins, carbohydrates, minerals, enzymes, vitamins, and trace elements necessary for human growth and development (Paksory *et al*., 2018). The Fatty Acid (FA) content and quality of food have received much attention over the last decade (Abdelrahman *et al*., 2017b). Studies have revealed that FA profiles and physicochemical properties of an animal product, are not the same within or amongst a breed and they can be significantly altered by different factors, such as, change of feed and lipid supplementation (Nudda *et al*., 2014; Caredda *et al*., 2017; Kholif *et al*. 2018). Most studies have tended to focus on increasing the content of valuable fatty acids such as short-chain FA (SCFA), poly unsaturated FA (PUFA), and conjugated linolenic acid (CLA) (Kholif *et al*., 2018). Yurchenko *et al*. (2018) also reported that the FA profile and contents in milk influence the quality, texture, aroma, and flavor of milk and milk products. Palmquist (2006) has reported that the content of FAs with 18-carbon chain (C18) in milk fat is directly related to the level of C18 acids in the animal's diet. Moreover, the incorporation of high levels of unsaturated FAs (UFAs) in the diet has little effect on the level of UFA in milk fat (Palmquist, 2006). In addition, Moreover, milk from ewes, compared to the milk of goats and cows, is characterized by higher protein, minerals and fat content; greater opalescence and whiteness; and higher and higher digestibility (Pulina and Bencini, 2004; Yurchenko *et al*., 2018; Teng *et al*., 2018). The aim of our research was to find the effect of diet composition on milk quality, FA profile, physical properties, and tocopherol contents of milk fat.

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MATERIALS AND METHODS

A total of 64 multiparous Najdi ewes, with healthy udders, of approximately three years of age and body weights, ranging from 60 to 65 kg, were used in this study. All ewes were in the second lactation stage and lambed singles. Ewes were allocated into four semi-open shed (groups) to ensure close observation and managed similarly without any discernible variations in management throughout the study. Water was freely available. Complete feeds were formulated with different nutrient contents such as traditional feeding system, normal protein and high energy (CF_1); high protein and normal energy (CF_2), and normal protein with medium energy level (CF_3) as shown in Table I. The first 8 weeks of lactation, all newborn lambs were breastfed normally. Then, lambs were weaned, and the ewes were hand milked once a day. The experimental protocol for handling ewes had been approved by the Ethics Committee of the King Saud University, Riyadh, Saudi Arabia.

Immediately after lambing, colostrum was collected. Milk samples were collected from each ewe till two months after lambing, and analyzed for chemical components. The major milk composition was measured using a Milk analyzer (Minor Type 78100, FOSS Electric, Denmark). Udder health status was evaluated throughout the experiment by Somatic Cell Counts (SCC). The SCC was determined as count/Ml using Fossomatic Minor somatic cell counter (Fossomatic 90, FOSS Electric, Denmark).

Milk fat was obtained from ewe milk using the method explained by Luna *et al*. (2005) and fatty acid methyl esters (FAMEs) were prepared using the procedure of Sbihi *et al*. (2015). The calculated molecular weight was determined by multiplying the percentage of each fatty acid by the molecular weight of its triacyglycerol. The iodine value was calculated using the method described by Ham *et al*. (1998) using the percentage of UFAs. The calculated saponification value was obtained from the mean molecular weight. The instruments employed for determining kinematic viscosity, refractive index, and specific gravity were Ubbelohde-type size 2 Viscometer (Koehler, Bohemia, New York, USA), an Abbe refractometer (Bellingham and Stanley, Ltd., Kent, England), and a density meter DM40 (Mettler-Toledo, Columbus, USA), respectively. The tocopherol isomers were determined according to the ISO (2006) standard. The instrument used for analysis was HPLC (Shimadzu, Kyoto, Japan) with a fluorescence detector.

Statistical analysis

Statistical analyses were performed using GraphPad prism (version 5). ANOVA (Bonferroni test: compare all pairs of columns) at a 95% confidence level with a pair-wise comparison was used to compare measures of chemical composition, fatty acids, and tocopherol content of ewes' milk and milk fat. Values with a significant difference of $P < 0.05$ were indicated as significant.

Table I. Chemical composition and fatty acid profile of traditional feed (barley and alfalfa hay)and complete feeds (CF_1, CF_2 and CF_2) on dry matter basis).

Nutrition's	TF		Complete feed		
	Barley	Alfalfa hay	CF_1	CF_2	CF_3
Chemical composition					
Dry matter, %	88.5	93.44	92.96	93.60	92.92
Crud protein, %	11.45	17.90	12.2	13.7	12.2
ME, Mcal /kg	2.93	2.82	2.87	2.17	2.39
NDF, %	31.15	43.30	38.3	41.8	42.2
ADF, %	5.71	34.58	26.1	26.9	26.4
Ash, %	2.65	9.46	11.04	11.27	8.75
Fatty acids profile					
C6:0	0.02	-	0.08	-	-
C8:0	0.21	-	0.48	0.26	0.29
C10:0	-	-	0.79	0.29	0.42
C12:0	-	2.39	14.26	4.81	8.26
C14:0	0.1	2.88	5.69	2	3.53
C16:0	19.5	20.83	17.08	15.96	12.7
C16:1Δ9*c*	-	-	0.22	0.31	0.32
C17:0	-	0.43	-	0.31	-
C18:0	1.48	4.72	4.33	5.53	2.52
C18:0 epoxy	-	2.37	0.95	0.67	-
C18:1Δ9*t*	-	-	-	0.21	-
C18:1Δ9*c*	16.46	7.67	34.51	37.63	29.74
C18:2Δ9*c*,12*c*	55.65	25.38	19.29	28.09	37.40
C18:3Δ9*c*,12*c*,15*c*	6.11	28.03	0.98	2.03	3.80
C20:0	0.19	2.43	0.52	0.74	0.51
SFA	21.5	36.05	44.18	30.57	28.23
MUFA	16.46	7.67	34.73	38.15	30.06
PUFA	61.76	53.41	20.27	30.12	41.2

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; NDF, neutral detergent fibre; ADF, acid detergent fibre; -, not detected.
CF_1, normal protrin and high energy; CF_2, high protrin and normal energy; CF_1, normal protrin and medium energy.

Table II. Colostrum composition (least square means) for different feeding treatments.

Composition	TF	CF_1	CF_2	CF_3
Fat %	10.35 ± 0.74	9.42 ± 0.98	9.06 ± 1.01	9.20 ± 1.09
Protein %	11.98 ± 0.32	12.51 ± 0.42	12.47 ± 0.44	12.07 ± 0.47
Lactose %	2.29 ± 0.15	2.56 ± 0.22	2.57 ± 0.22	2.95 ± 0.26
Total solids %	26.44 ± 0.87	26.13 ± 1.15	25.76 ± 1.19	25.44 ± 1.28

CF, complete feed; TF, traditional feed. For other abbreviations, see Table I.

Table III. Effect of feeding treatments on milk composition and somatic cell counts of Najdi ewes.

Composition	TF	CF_1	CF_2	CF_3
Fat %	5.47 ± 0.46^a	4.95 ±0.69^a	6.40 ± 0.69^a	4.46 ± 0.60^b
Protein %	3.78 ± 0.18^b	4.29 ±0.25^a	3.57 ± 0.22^b	4.64 ± 0.24^a
Lactose %	4.54 ± 0.19^b	5.03 ±0.29^a	4.05 ± 0.26^c	4.40 ± 0.27^{ab}
Total solids %	14.2 8± 0.64	13.77 ±0.90	12.49 ± 0.80	13.66 ± 0.85
Somatic cell count	642.30 ± 199	465.39 ±317	838.07 ± 240	704.06 ± 253

Values having different superscripts in a row are significantly different at $p \leq 0.05$.
For abbreviations, see Table I and II.

RESULTS AND DISCUSSION

Data on colostrum composition of Najdi ewes for different feeding regimes are presented in Table II. Diets were not observed to affect colostrum composition significantly (P>0.05). The mean values for fat, protein, lactose, and total solid contents were: 9.51, 12.26, 2.59, and 25.94, respectively. These values of colostrum composition observed in our study were found to be in the same range with those previously reported by Banchero *et al*. (2004) in sheep.

The mean percentage of fats were statistically similar (*P*>0.05) in milk ewes fed TF (5.47%), CF_1 (4.95%), and CF_2 (6.40%), but significantly (P<0.05) lower for ewes fed CF_3 (4.46%) as explained in Table III. The average milk fat content was lower than the values reported for Lacuane (Castillo *et al*., 2009) and Awassi ewes (Nudda *et al*., 2002) but in the same range as those reported for Najdi ewes by Ayadi *et al*. (2014). Therefore, breed differences and nutrition programs can explain the discrepancies between our results and previous studies. The content of protein was significantly higher (P<0.05) in the milk of the ewes fed CF_1 (4.29%) and CF_3 (4.64%) than those in the ewes fed TF (3.78%) and CF_2 (3.57%). The level of lactose in the milk of ewes fed TF, CF_1, and CF_2 were significantly (P<0.05) affected by the treatments. High content was observed in the milk of ewes fed CF_1 (5.03%), while a low level was observed in the ewes fed CF_2 (4.05%). As shown in Table III, the highest value of SCC was found in the milk ewes fed CF_2 (2.79) while the lowest value was found in milk ewes fed CF_1 (2.26). Nudda *et al*. (2004) showed that the SCC in milk is affected by nutrition. Furthermore, Sharma *et al*. (2011) reported that an elevated SCC in milk has a negative influence on the quality of raw milk. However, the SCC of the Najdi ewe's milk was much lower than that of other ewes' milk as well as cow's milk (Sharma *et al*., 2011).

The FA profiles of milk fat from ewes which were fed TF, CF_1, CF_2, and CF_3 are summarized in Table IV which reveals that the FA profile was significantly affected by the fat content and the type of FA in the ewes' diet. The mean values of SCFAs (C6:0, C8:0 and C10:0) in the TF fed ewe-milk fat were not significantly affected (*P* > 0.05) by the type of feed. However, they were significantly higher (P< 0.05) than the mean value of SCFAs in the milk fat of ewes fed CF_3 (1.31%, 1.65%, and 6.42%, respectively). Moreover, CF_1 fed ewe-milk fat (8.75%) had the highest content of lauric acid (C12:0), followed by milk fat from ewes fed CF_2 (7.56%), CF_3 (6.24%) and TF (4.38%), respectively (Table II). The highest content of myristic acid (C14:0) was found in milk fat of ewes fed CF_2 (14.58%) and CF_3 (14.28%) as shown in Table II compared to its content in the milk fat of ewes fed CF_1 (12.26%) and TF (11.14%). The low levels of lauric and myristic acids in the milk fat of milk from the ewes fed TF may be explained by the low content of these fatty acids in TF (Table I). Tridecylic acid (C13:0) was detected only in milk fat from ewes fed TF.

Dietary treatment affected the content of palmitic acid (C16:0) in milk fat (*P*>0.05) from ewes fed TF (30.16%) and

Table IV. Fatty acid profiles (%) of milk fats of control and treated ewes. The values are Mean± SD.

Fatty acid	TF	CF_1	CF_2	CF_3
C6:0	$1.52^{a} \pm 0.06$	$1.32^{b} \pm 0.11$	$1.28^{b} \pm 0.04$	$1.31^{b} \pm 0.06$
C8:0	$1.88^{ab} \pm 0.21$	$1.89^{b} \pm 0.10$	$1.73^{ab} \pm 0.04$	$1.65^{a} \pm 0.08$
C10:0	$7.38^{a} \pm 0.27$	$7.36^{a} \pm 0.35$	$7.10^{a} \pm 0.12$	$6.30^{b} \pm 0.54$
C11:0	$0.20^{b} \pm 0.02$	$0.31^{a} \pm 0.02$	$0.14^{c} \pm 0.02$	-
C12:0	$4.38^{d} \pm 0.22$	$8.75^{a} \pm 0.45$	$7.56^{b} \pm 0.12$	$6.24^{c} \pm 0.10$
C13:0	0.14 ± 0.02	00	--	--
C14:0	$11.14^{c} \pm 0.70$	$12.36^{b} \pm 0.43$	$14.58^{a} \pm 0.20$	$14.28^{a} \pm 0.23$
C14:1Δ9*c*	$0.19^{b} \pm 0.02$	$0.17^{b} \pm 0.02$	$0.26^{a} \pm 0.02$	-
C15:0	$0.89^{b} \pm 0.10$	$0.63^{c} \pm 0.08$	$1.05^{a} \pm 0.03$	$0.57^{c} \pm 0.03$
isoC15:0	$0.38^{b} \pm 0.06$	$0.15^{c} \pm 0.05$	$0.54^{a} \pm 0.06$	$0.35^{b} \pm 0.04$
anteisoC15:0	--	0.14 ± 0.02	--	--
C16:0	$30.16^{b} \pm 0.39$	$21.12^{c} \pm 0.44$	$31.51^{a} \pm 0.98$	$29.15^{b} \pm 0.23$
isoC16:0	$0.33^{a} \pm 0.02$	$0.18^{b} \pm 0.02$	$0.10^{c} \pm 0.01$	--
C16:1Δ9*c*	$1.56^{ab} \pm 0.17$	$1.57^{ab} \pm 0.15$	$1.83^{a} \pm 0.21$	$1.47^{b} \pm 0.05$
C16:1Δ11*c*	$0.28^{a} \pm 0.02$	$0.24^{a} \pm 0.05$	$0.23^{a} \pm 0.02$	-
C17:0	$0.80^{b} \pm 0.06$	$0.93^{a} \pm 0.07$	$0.37^{c} \pm 0.05$	$0.40^{c} \pm 0.03$
antisoC17:0	$0.37^{a} \pm 0.03$	$0.25^{bc} \pm 0.04$	$0.21^{c} \pm 0.02$	$0.31^{ab} \pm 0.03$
isoC17:0	0.32 ± 0.02	--	--	--
C17:1Δ10*c*	$0.57^{a} \pm 0.10$	$0.48^{a} \pm 0.06$	$0.23^{b} \pm 0.03$	$0.22^{b} \pm 0.02$
C18:0	$9.47^{a} \pm 0.45$	$9.94^{a} \pm 0.75$	$5.47^{c} \pm 0.27$	$8.58^{b} \pm 0.12$
C18:1Δ9*c*	$20.79^{ab} \pm 1.10$	$19.95^{b} \pm 0.57$	$15.46^{c} \pm 0.90$	$21.58^{a} \pm 0.58$
C18:1Δ11*c*	$0.43^{d} \pm 0.11$	$0.97^{a} \pm 0.14$	$0.74^{bc} \pm 0.04$	$0.79^{b} \pm 0.05$
C18:1Δ13*c*	$0.17^{d} \pm 0.03$	$0.45^{a} \pm 0.05$	$0.29^{c} \pm 0.03$	$0.24^{bc} \pm 0.03$
C18:1Δ14*c*	$0.32^{a} \pm 0.11$	$0.14^{b} \pm 0.01$	--	--
C18:1Δ15*c*	0.26 ± 0.02	--	--	--
C18:1Δ8*t*	--	$0.27^{b} \pm 0.02$	$0.44^{a} \pm 0.09$	--
C18:1Δ9*t*	--	0.41 ± 0.08	--	--
C18:1Δ11*t*	$0.37^{d} \pm 0.03$	$2.00^{b} \pm 0.12$	$3.54^{a} \pm 0.34$	$1.24^{c} \pm 0.17$
C18:1Δ13*t*	$0.26^{a} \pm 0.06$	$0.25^{a} \pm 0.02$	$0.28^{a} \pm 0.02$	$0.27^{a} \pm 0.02$
C18:1Δ16*t*	$0.75^{a} \pm 0.09$	$0.66^{ab} \pm 0.16$	$0.56^{b} \pm 0.03$	$0.50^{cb} \pm 0.04$
C18:2Δ9*c*,12*c*	$2.56^{b} \pm 0.16$	$4.17^{a} \pm 0.24$	$2.50^{b} \pm 0.19$	$2.67^{b} \pm 0.06$
C18:2Δ9*c*,12*t*	$0.25^{a} \pm 0.06$	$0.27^{a} \pm 0.08$	$0.18^{a} \pm 0.02$	--
C18:2Δ9*c*,11*t*	$0.34^{b} \pm 0.06$	$0.97^{a} \pm 0.10$	$0.23^{c} \pm 0.03$	$0.31^{bc} \pm 0.02$
C18:3Δ9*c*,12*c*, 15*c*	$1.16^{a} \pm 0.09$	$1.27^{a} \pm 0.10$	$1.20^{a} \pm 0.08$	$1.13^{a} \pm 0.08$
C20:0	$0.24^{a} \pm 0.02$	$0.24^{a} \pm 0.03$	$0.14^{b} \pm 0.02$	$0.20^{ab} \pm 0.05$
C20:4Δ5*c*,8*c*, 11*c*,14*c*	$0.20^{b} \pm 0.03$	$0.32^{a} \pm 0.04$	$0.26^{ab} \pm 0.04$	$0.23^{b} \pm 0.03$

SD, standard deviation; -, not detected. Values having different superscripts in a row are significantly different at $p \leq 0.05$.
For other abbreviations, see Table I and II.

CF_3 (29.15%) which are significantly higher ($P < 0.05$) than that from the CF_1 fed ewe-milk fat (21.12%) and significantly lower ($p < 0.05$) than CF_2 fed ewe-milk fat (31.51%). On the other hand, the percentage of palmitoleic (C16:1Δ9*c*) and *cis*-11-hexandecenoinc (C16:1Δ11*c*) acids were not affected ($P > 0.05$) by dietary treatment, except

that the pamitoleic acid content was statistically higher in milk fat from ewes fed CF_2 (1.83%) than in CF_3 (1.47%), and the average mean value was approximately 1.60% and 0.25%, respectively. Branched-chain FAs (BCFA) present in ewe's milk fat are 13-methylmyristic acid (isoC15:0), 12-methylmyristic acid (anteisoC15:0), isopalmitic acid (isoC16:0), 15-methylpalmitic acid (isoC17:0), and 14-methylpalmitic acid (anteisoC17:0). 12-anteisoC15:0 and isoC17:0 were detected only in milk fat from ewes fed CF_1 and TF, respectively. IsoC15:0 was significantly higher (P<0.05) in milk fat of ewes fed CF_2 (0.54%) than that in milk fat of ewes fed TF (0.38%), CF_3 (0.35%), and CF_1 (0.15%). While isoC16:0 was statistically higher (P< 0.05) in milk fat from ewes fed TF (0.33%) than that in milk fat from ewes fed CF_1 (0.18%) and CF_2 (0.10%), anteisoC17:0 was statistically higher ($p < 0.05$) in milk fat of ewes fed TF (0.37%) compared to milk fat of ewes fed CF_1 (0.25%) and CF_2 (0.21%). As indicated in Table III, the highest content of BCFAs is found in the TF fed ewe-milk fat (1.39%). Our results are in accordance with a previous report (Teng *et al*., 2018) which stated that diets affected the level of BCFAs in sheep's milk. A higher content of BCFAs was found in milk from sheep fed Pasteur diets compared to those fed feeding grains. Moreover, Teng *et al*. (2018) mentioned that the liberation of free BCFAs via the lipolysis of corresponding triglycerides is responsible for the unique flavors of sheep and goat milk products. The level of these flavor compounds can differ significantly in sheep and goat milk according to the geographic location of the breed (Teng *et al*., 2018).

The average values of stearic acid (C18:0) were 9.47, 9.94, 5.47, and 8.58% of the milk fat in ewes fed TF, CF_1, CF_2, and CF_3, respectively. The concentration of stearic acid was significantly lower (P<0.05) in milk fat of ewes fed CF_2 compared with TF, CF_1, and CF_3. The contents of oleic acid (C18:1Δ9*c*) in milk fat of ewes fed TF (20.79%), CF_1 (19.95%), and CF_3 (21.58%) were significantly higher than in milk fat of ewes fed CF_2 (15.46%). There was no correlation between the content of oleic acid in the diets and milk fat of ewes fed CF_1, CF, and CF_3. Chilliard *et al*. (2000) reported that 40% of C18:0 extracted from the blood into the mammary glands is converted to oleic acid to preserve milk fluidity. Furthermore, Chilliard *et al*. (2001) explained that the decrease in the stearic acid concentration may be due to the decrease of bio-hydrogenation of unsaturated fatty acids or increase in Delta-9 desaturase activity in the mammary glands.

TVA, which is produced in the rumen during the biohydrogenation of UFAs, is the *trans*-FA that is found in the greatest proportion in ruminant fat (Prieto-Manrique *et al*., 2018). TVA is the major *trans*-C18:1 present in the milk fat of ewes. TVA was significantly higher in CF_2 fed ewe-milk fat (3.54%) compared to CF_1 fed ewe-milk fat (2.00%). The CF_1 fed ewe-milk fat had the highest content (4.17%) of LA (C18:2Δ9*c*,12*c*; ω6), while, there is no statistically significant difference between the LA content in milk fat of ewes fed TF (2.56%), CF_2 (2.50%), and CF_3 (2.67%). Moreover, the content of ALA (ω-3) was not significantly affected by the type of diet ($P < 0.05$) as revealed by its content in the milk fat from ewes fed TF (1.16%), CF_1 (1.27%), CF_2 (1.20%), and CF_3 (1.13%). Table I shows that the CF_3 fed ewes had a high LA content (37.40%), while TF fed had high ALA (6.11% (barley) and 28.03% (alfalfa hay). Buccioni *et al*. (2015) reported that the concentration of ALA and LA were greater when the ewes' diets supplemented with high contents of ALA and LA. Increased intakes of PUFAs have also been associated with health benefits such as improved brain function and reduced risk of dementia (Kholif *et al*., 2018; Yurchenko *et al*., 2018). CLA (C18:2Δ9*c*,11*t*) was not affected ($p > 0.05$) by dietary treatment with TF (0.34%), CF_2 (0.23%), and CF_3 (0.31%). While, CLA content was significantly greater ($P < 0.05$) in CF_1 fed ewe-milk fat (0.97%) than with other diets. Similarly, Prieto-Manrique *et al*. (2018) noted that pasture-based diets increased the content of CLA in cow milk fat. TVA is the precursor of CLA, which has many health benefits for humans, such as preventive action against cancer and obesity (Kholif *et al*., 2018; Prieto-Manrique *et al*., 2018).

The average SFAs were 68.88%, 65.21%, 71.57%, and 69.03% for milk fat of ewes fed TF, CF_1, CF_2, and CF_3, respectively (Table V). SFAs were significantly higher (P< 0.05) in CF_2 fed ewe-milk fat than in milk fat from ewes fed TF, CF_1, and CF_3. The lowest content of SFAs was observed in milk fat of ewes fed CF1. Total SFA contents detected in milk fat of ewes fed TF, CF_1, and CF_3 (<70%) are in line with results reported by Matar *et al*. (2017). While, total SFA (>70%) in CF_2 fed ewe-milk fat is similar to that reported by Matar *et al*. (2017). Furthermore, hyperchloesterolermic FAs (HFA) were significantly higher ($P < 0.05$) in milk fat from ewes fed CF_2 (53.65%) followed by CF_3 (49.67%) compared to milk fat from ewes fed TF (45.67%) and CF_1 (42.13%), which has the lowest HFA content. Mierlita and Vicas (2015) reported that high content of HFAs (C12:0 + C14:0 + C16:0) has a negative effect on human health. Total PUFA was significantly greater ($P < 0.05$) in CF_1 fed ewe-milk fat (7.24%) compared to milk fat from ewes fed TF (5.19%), CF_2 (4.56%), and CF_3 (4.65%). Furthermore, no significant difference ($P > 0.05$) was found in total PUFA content between milk fat of ewes fed CF_2, and CF_3. Similarly, the n-6/n-3 ratio was significantly higher in CF_1 fed ewe-milk fat (3.59) compared to the

Table V. Fatty acid classes and indices (%) of control and treated ewes. The values are Mean± SD.

	TF	CF1	CF2	CF3
SFA	68.88^{b} ± 0.90	65.21^{c} ± 0.40	71.57^{a} ± 1.00	69.03^{b} ± 0.49
USFA	31.12^{b} ± 0.90	34.79^{a} ± 0.40	28.43^{c} ± 1.00	30.97^{b} ± 0.49
MUFA	25.93^{c} ± 1.04	27.55^{b} ± 0.56	28.87^{a} ± 0.70	26.32^{bc} ± 0.48
PUFA	5.19^{b} ± 0.16	7.24^{a} ± 0.32	4.56^{c} ± 0.33	4.65^{c} ± 0.20
n-6	2.76^{b} ± 0.17	4.49^{a} ± 0.24	2.75^{b} ± 0.22	2.91^{b} ± 0.15
n-3	1.16^{a} ± 0.09	1.26^{a} ± 0.10	1.20^{a} ± 0.08	1.13^{a} ± 0.08
SFA/UFA	2.22^{b} ± 0.09	1.88^{c} ± 0.04	2.52^{a} ± 0.12	2.23^{b} ± 0.05
n-6/n-3	2.41^{b} ± 0.32	3.59^{a} ± 0.33	2.30^{b} ± 0.13	2.58^{b} ± 0.25
BCFA	1.39^{a} ± 0.04	0.71^{c} ± 0.05	0.84^{b} ± 0.07	0.66^{c} ± 0.06
OCFA	2.02^{a} ± 0.13	1.87^{a} ± 0.12	1.56^{b} ± 0.05	0.97^{c} ± 0.06
SCFA	10.78^{a} ± 0.18	10.57^{a} ± 0.51	10.11^{a} ± 0.13	9.25^{b} ± 0.56
MCFA	49.62^{c} ± 1.03	45.53^{d} ± 0.81	57.79^{a} ± 1.35	52.06^{b} ± 0.40
LCFA	39.60^{b} ± 1.13	43.90^{a} ± 0.50	32.09^{c} ± 1.30	38.69^{b} ± 0.60
Total C18:1	23.34^{b} ± 1.16	25.08^{a} ± 0.49	21.32^{c} ± 0.87	24.62^{ab} ± 0.48
Total C18:2	2.81^{b} ± 0.16	4.44^{a} ± 0.30	2.67^{b} ± 0.20	2.67^{b} ± 0.06
Total C18:1 *cis*	21.97^{a} ± 1.16	21.50^{a} ± 0.48	16.49^{b} ± 0.92	22.60^{a} ± 0.59
Total C18:1 *trans*	1.37^{d} ± 0.04	3.58^{b} ± 0.07	4.82^{a} ± 0.43	2.03^{c} ± 0.21
AI	2.54^{c} ± 0.13	2.27^{d} ± 0.05	3.43^{a} ± 0.17	2.99^{b} ± 0.06
HFA	45.67^{c} ± 0.74	42.13^{d} ± 0.90	53.65^{a} ± 1.20	49.67 ^{b}± 0.19

SFA, saturated fatty acid; USFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; BCFA, branched-chain fatty acid; OCFA, odd-chain fatty acid; SCFA, short-chain fatty acid; MCFA, medium-chain fatty acid; LCFA, long-chain fatty acid; SD, standard deviation. Values having different superscripts in a row are significantly different at $p \leq 0.05$.
For other abbreviations, see Table I and II.

ratios in TF (2.41), CF_2 (2.30), and CF_3 (2.58), which are not significantly affected by dietary treatment. The n-6/n-3 ratios found in milk fat of ewes fed TF, CF_2, and CF_3 were similar to that found by Matar *et al*. (2017) for Najdi ewes' milk fat during milking periods (2.03) and was lower than the milk fat of ewes fed CF_1. Mierlita (2016) reported that a ratio of n-6/n-3 below 4 is required in the diet to combat life diseases such as coronary heart disease and cancers. The level of SCFA was not significantly affected by the type of diet in milk fat of ewes fed TF (10.78%), CF_1 (10.57%), and CF_2 (10.11%). The content of SCFAs was significantly lower in CF_3 fed ewe-milk fat (9.25%) compared to other diets. As indicated by Cabiddu *et al*. (2017), the increase in SCFA content is due to a high intake of PUFA. On the other hand, the content of medium-chain fatty acid (MCFA) was significantly affected by the diet. It was significantly higher (P<0.05) in milk fat from ewes fed CF_2 (57.79%) than that in TF (49.62%) and CF_3 (52.06%), which was significantly lower in milk fat from ewes fed CF_1 (45.53%). Zhang *et al*. (2006) summarized that the inclusion of oil seeds having different fatty acid composition did not influence the level of total MCFAs. Furthermore, Buccioni *et al*. (2015) showed that the incorporation of unsaturated vegetable oils in the ruminant diet decreases the neo-synthesis of SCFA and MCFA owing to the inhibitory effect of large amounts of transmission long chain fatty acids on the expression of genes involved in fatty acid synthesis.

The main effect of treatment by long-chain fatty acid (LCFA) was detected in CF_2 fed ewe-milk fat, which had the highest content (43.90%) of LCFAs. Oleic acid was the main LCFA detected. Similar results were obtained by Mierlita (2016) in ewes that were fed concentrated mixture. The difference in the mean values of LCFAs was not statistically significant (P>0.05) and differed between milk fat from ewes fed TF and CF_3 diets. Feeding did not give rise to significant differences in BCFA content with regard to CF_1 (0.71%) and CF_3 (0.66%); while, it was significantly greater in TF fed ewe-milk fat (1.39%) followed by CF_2 fed ewe-milk fat (0.84%). TF supplementation increased remarkably (P<0.05) odd-chain fatty acids in milk fat from ewes fed TF (2.02%) and CF_1 (1.87%) compared with milk

Table VI. Physical properties and tocopherol contents of milk fat. The values are Mean± SD.

	TF	CF_1	CF_2	CF_3
PP				
MW	788.6 [a] ± 2.12	784.7 [b] ± 8.26	776.9 [c] ± 1.06	787.8 [a] ± 1.84
IV	31.64 [b] ± 0.67	37.58 [a] ± 0.41	29.74 [c] ± 1.17	31.66 [b] ± 0.42
KV (40°C)	26.83 [b] ± 0.19	27.92 [a] ± 0.20	26.15 [c] ± 0.26	26.82 [b] ± 0.13
SV	213.40 [c] ± 0.60	214.5 [b] ± 0.30	216.60 [a] ± 0.30	213.6 [c] ± 0.50
SG (25°)	0.9140 [c] ± 0.0002	0.9151 [a] ± 0.0001	0.9147 [b] ± 0.0001	0.9140 [c] ± 0.0001
RI (25°C)	1.4540 [b] ± 0.0001	1.4530 [c] ± 0.0001	1.4540 [a] ± 0.0002	1.4540 [b] ± 0.0001
Toc (µg/g)				
α-Toc	18.79 [a] ± 0.76	14.09 [b] ± 0.60	14.39 [b] ± 0.60	15.54 [b] ± 0.36
γ-Toc	1.24 [a] ± 0.09	0.92 [b] ± 0.05	0.95 [b] ± 0.04	1.07 [b] ± 0.07
δ-Toc	0.26 [a] ± 0.02	0.21 [b] ± 0.02	0.17 [b] ± 0.02	0.20 [b] ± 0.02
Total	20.29 [a] ± 0.85	15.22 [b] ± 0.66	15.52 [b] ± 0.65	16.81 [b] ± 0.41

PP, physical properties; MW, molecular weight (g/mol); IV, iodine value (gI_2/100 g fat); KV, kinematic viscosity (mm^2/s); SV, saponification value (mg KOH/g); SG, specific gravity; RI, refractive index; Toc, tocopherol; SD, standard deviation. Values having different superscripts in a row are significantly different at $p \leq 0.05$.
For other abbreviations, see Table I and II.

fat from ewes fed CF_3 (0.97%). As shown in Table III, the content of total C18:1 and C18:2 were significantly higher in CF_1 fed ewe-milk fat. On the other hand, total C18:1 *cis* was significantly higher ($P < 0.05$) in milk fat from ewes fed TF (21.97%), CF_1 (21.50%), and CF_3 (22.60%). The content of total C18:1 *trans* was significantly higher in milk fat in ewes fed CF_2 (4.82%) followed by CF_1 (3.58%). Observations related to the physical properties and tocopherol contents of different extracted milk fat samples are summarized in Table VI. Only α-, β, γ-tocopherol were present in the milk fat of ewes. α-Tocopherol was the main isomer of total tocopherols (≈93%). These results correlate favorably with Revilla *et al*. (2017) who stated that α-tocopherol is the major isomer present in ewes' milk along with the other three isomers. Total tocopherols and α-tocopherol concentrations were significantly higher ($p < 0.05$) in TF fed ewe-milk fat (20.29 and 18.79 µg/g) compared with CF_1 fed ewe-milk fat (15.22 and 14.09 µg/g), CF_2 (15.52 and 14.39 µg/g) and CF_3 (16.81 and 15.54 µg/g). The tocopherol contents, in the milk of ewes fed TF, were slightly higher than that found in conventional ewes' milk (20.05 g/g) (Revilla *et al*., 2014) and were remarkably higher than that found in organic ewes' milk (14.89 µg/g) (Revilla *et al*., 2014). Furthermore, Revilla *et al*. (2014) detected only two isomers in conventional and organic ewes' milk collected from Spain. In addition, dietary treatments did not affect ($p > 0.05$) the total tocopherol and α-tocopherol content in milk fat from ewes fed CF_1, CF_2, and CF_3. Revilla *et al*. (2017) reported that α-tocopherol content is related to the content of PUFAs due to the antioxidant activity of vitamin E. It was found that milk fat from ewes fed CF_1 (7.24%) had the highest PUFA content and not milk fat from TF (5.19%). This result can be explained by the reflection of vitamin E levels of the TF diet in the milk produced by ewes fed with this diet, as indicated by Revilla *et al*. (2014).

The current work established that the diverse diets used, improved the quality of the milk fat in ewes. As stated before, the four supplement diets used in this study contain different metabolizable energy and crude protein content (Table I): TF [high-energy (HE) and recommended protein (RP)], CF_1 [HE and high-protein (HP)], CF_2 [low-energy (LE) and HP], and CF_3 (LE and RP). We found in this study that the content of total SFAs and C18:1 *trans* were lower in the milk fat of ewes fed HE diets. Consumption of large amounts of SFAs and C18:1 *trans* is a negative health behavior. A high intake of SFA contributes to the development of coronary heart disease (Dias *et al*., 2015); while, high intake of TFA has been associated with increased risk of coronary heart disease, sudden death, diabetes mellitus and increased markers of systematic inflammation (Dias *et al*., 2015). Furthermore, the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) have recommended a minimum consumption level for both TFA and SFA. This study revealed that, the content of n-3, n-6, PUFA, and CLA were high in the milk fat of ewes fed HE diets as compared to those fed LE diets; while, TVA was high in the milk fat of ewes fed LE compared to milk fat of ewes fed HE. Gómez-Cortés *et al*. (2018) stated that

no relationship was found between TVA and coronary heart disease, and on the contrary, a number of positive health effects have been attributed to TVA. Moreover, TVA is a precursor of the most relevant bioactive compound, such as rumenic acid, present in milk fat. Higher concentration of conjugated linolenic acids, notably rumenic acid, has many positive health effects such as, anti-tumor, anti-atherosclerosis, anti-diabetic as well as anti-obesity effects, and also modulates the immune system (Gómez-Cortés *et al*., 2018). Although, the content of TVA in TF fed ewe-milk fat (0.37%) and CF_1 (2.00%) is low as compared to CF_2 fed ewe-milk fat (3.54%) and CF_3 (1.24%), the content of CLA is high in milk fat of ewes fed HE (TF and CF_1). The conversion rate of TVA to CLA is a lot higher in TF (47.89%) and CF_1 (32.66%) fed ewes as compared to CF_2 (6.10%) and CF_3 (20.00%) fed ones. Efforts to increase the CLA concentration has been the aim of many researches to better the quality of cheese, yogurt, biscuits, and cream dairy products.

Revilla *et al*. (2017) concluded that high levels of CLA increases the quality of cheese obtained from sheep's milk. Moreover, Zhang *et al*. (2006) reported that cheese with high levels of CLA and ALA can be made from milk fat that contains a high concentration of these fatty acids. Comparison of the two HE diets, TF and CF_1, revealed that the CF_1 diet resulted in a high content of valuable fatty acids. As shown in Tables II and III, PUFA, n-3, n-6, CLA, and TVA were significantly higher in CF_1 fed ewe-milk fat compared to TF fed ewe-milk fat. Furthermore, the content of SFAs is significantly lower in milk fat of ewes fed CF_1. These results highlighted that the high content of crude protein in supplement diets has a negative effect on the quality of fatty acids in milk fat of ewes, while, no significant difference was detected between milk fat of ewes fed CF_2 and CF_3, in terms of PUFA, n-3, n-6, and CLA contents. Several food and diet experts have highlighted that dietary recommendations for a fatty diet are based on values of n-6/n-3 and PUFA/SFA ratios. The values found in our experiments for ewe milk fat were below the recommended values (below 4 and 0.45, respectively) (Aguilar *et al*., 2014).

CONCLUSION

The diet that included higher energy and normal protein (CF_1) remarkably improved the quantity of valuable fatty acids. The total content of saturated fatty acids was lowest in milk fat of ewes fed CF_1, which also has the highest content of PUFA. Our results will be beneficial for dairy producers who wish to use diverse sources of milk for cheese production.

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Conflict of interest declaration

The authors have declared that there is no conflict of interests

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