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AWASSI SHEEP MILK FATTY ACID PROFILE DURING LACTATION UNDER A TRADITIONAL GRAZING SYSTEM IN SEMI-ARID REGIONS

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ABSTRACT

The effects of lactation stage, farm and the stage × farm interaction on the main components, physicochemical characteristics and fatty acid profile of Awassi ewes' milk were studied (n = 16) over all lactation stages starting from day 10 to avoid colostral milk which is very rich in nitrogen due to globulins. Milk was sampled on the following control days: D10, D30, D70, D100 and D130. Ewes grazed on available natural pasture and supplemented with maize, soybean meal, concentrate according to their needs. Milk samples were analyzed for milk composition using Milkoscan FT120 instrument and milk fatty acids profile was performed with a gas chromatography equipped with flame ionization detection (GC-FID). Monounsaturated fatty acid and polyunsaturated fatty acid (PUFA) contents were negatively correlated with the advance of lactation stage. The PUFAs as C18:2t isomers and conjugated linoleic acid (CLA) had their highest contents at D30 (0.87±0.05%, 1.13±0.05%; respectively). Omega 3 fatty acids had their highest value, 1.41±0.05%, at D70 and the omega 6: omega 3 ratio had its lowest value, 1.52±0.19%, during D30–D70, which was optimal. Thus, the increase in healthy milk fatty acids from grazing ewes will provide healthier dairy products for later human consumption especially from earlier periods in lactation.

Keywords: Milk composition, fatty acids, lactation stage, Awassi, sheep.

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INTRODUCTION

In the Middle East, sheep production plays an important role in supporting the livelihoods of resource-poor farmers in the low rainfall regions and dry areas in many geographical regions such as Central Asia, Middle East, North Africa etc. These regions suffer from feed scarcity due to drought, increasing effects of climate as well as declines in productivity (Abi Saab *et al.*, 2004; Ben Salem and Smith, 2008). Awassi is the dominant sheep breed in the Middle east and attracts importance in different regions like Spain, Australia, CA etc. (Galal *et al*., 2008), it is well adapted to the prevailing conditions and its major concentration is in the Bekaa Valley (800–1000 m above sea level, 528 mm year^{-1} rainfall). The majority of Awassi sheep are reared under semi-extensive or extensive management systems in which seasonal pastures contribute to the majority of the animals' diet, and concentrate supplementation is given during winter when accessibility to grazing diminishes (Khazaal, 2005; Abi Saab *et al*., 2011). These management systems amplify the seasonality of milk production (Khazaal, 2005), so that the lactation period generally starts in February and ends in early summer. And changes in dietary composition can affect the milk fat content and composition (Bauman and Griinari, 2003; Vasta *et al.*, 2008). In addition to dietary factors, several other influences affect the chemical composition of milk such as breed, parity, lactation period as well as environmental factors (Pulina *et al.*, 2007; De La Fuente *et al*., 2009; Nudda *et al.*, 2014).

Nowadays, the repercussions of milk fat on human health are of interest to consumers who are now seeking healthier versions of their diet since cardiovascular disease and hyperlipidemia contribute to more than 33.4% of the disease-related mortality in Lebanon (Hospital-based Mortality System (HMS) - Statistics Department, 2019). In this sense, some studies are giving specific attention to enhance fatty acid (FA) composition of the milk in order to improve human health (Lock and Bauman, 2004). Many authors have studied the dietary effect of food by-products on FA profiles of milk from Awassi ewes such as olive cake, tomato pomace and sugar beet pulp (Abbeddou *et al.*, 2008; Hilali *et al.*, 2010; Abbeddou *et al.*, 2015; Hilali *et al.*, 2018) as well as the effect of supplementation of some lipids (Husvéth *et al.*, 2010; Titi and Al-Fataftah, 2013). Similar effects were studied by Tsiplakou *et al.*, (2006) but were restricted to examining conjugated linoleic acid (CLA) content in milk of the sheep breeds Awassi, Lacaune, Friesland and Chios, and did not consider the effect of days in milk. Many studies have investigated lactation stage effect on milk FA profile for other sheep breeds, including Karagouniko and Chios

(Sinanoglou *et al.*, 2015) and Sanjabi and Mehraban ewes *(*Payandeh *et al.*, 2016), whereas others studied seasonal effects on the FA profile in milk from Lacha (Perea *et al.,* 2000; Barron *et al.,* 2001*)*, Churra (De La Fuente *et al.*, 2009) and East Friesian breeds (Mayer and Fiechter, 2012). In general, proportions of monounsaturated FAs (MUFAs) and unsaturated FAs were the highest at late lactation but the opposite was observed for saturated FAs (De La Fuente *et al.*, 2009; Sinanoglou *et al*., 2015). However, De La Fuente *et al*., (2009) described an increase of CLA throughout progression of the lactation period; seasonal variation led to an increment of 44% of CLA from winter until spring–summer. Similar seasonal changes were mentioned by Perea *et al*., (2000), with higher proportions of unsaturated and long-chain FAs in milk taken in June and higher proportions of saturated and short-chain FAs (SCFAs) in milk taken in April and February. These observations were due to the fact that ewes were grazing in June with high grassland abundance (Atti *et al*., 2006; Mierlita, 2012; Nudda *et al.*, 2014).

Given the above observations, along with the little available information concerning FA profile variation throughout the lactation period in milk from Awassi sheep, this study aimed to establish a detailed milk FA profile of Awassi ewes from lambing covering the different lactation periods under a traditional grazing system in semi-arid areas.

MATERIALS AND METHODS

Animals and diets: The experiment was carried out in two large farms in Bekaa valley, Lebanon. In each farm, eight lactating Awassi ewes with average weight of 56.8 kg were enrolled from the day of lambing. Animals were aged 2–6 years and at least in their second parity. In the first farm, ewes were reared at Anjar (33°44′34.3″N, 35°56′57.4″E, average rainfall 540 mm year^{-1}) and were grazed on cultivated ryegrass during March–April and then on available pasture; i.e. Medicago s., trifolium p., Avena s., Vicia s.; in spring until the end of lactation. The second farm was at Mrayjat village (33°48′28.5″N, 35°49′04.4″E, average rainfall 6*90* mm year^{-1}) and ewes were grazed on available pasture in the village as Avena s., Hordeum b., Medicago s., Vicia s. Ewes in both farms received 0.5 kg concentrate supplementation consisting of barley, wheat bran and vitamin premix (crude protein 16%) and had continuous access to clean water (Table 1).

Table 1. Proximate analysis of pasture and feed.

	ANJAR					MRAYJAT				
	Pasture				Feed	Pasture				Feed
Month	**Mar**	**May**	**Jun**	**Jul**		**Mar**	**May**	**Jun**	**Jul**	
Days in milk	**D30**	**D70**	**D100**	**D130**		**D30**	**D70**	**D100**	**D130**	
Dry Matter%	13.66	15.57	17.08	25.87	89.26	15.89	24.21	39.73	19.82	89.77
Ash%	12.68	12.97	16.12	12.04	12.51	13.19	9.64	9.77	16.39	6.93
CP%	30.01	23.38	19.59	16.6	19.15	23.34	14.81	9.41	18.42	15.17

Milk sampling: Animals were monitored throughout lactation during February–August to cover the entire period of lactation at the following times: D10, D30, D70, D100 and D130. On the date of sampling, ewes were hand milked twice a day, at 08:30 and 16:30, and samples were mixed and stored at −20 °C in two 50-mL tubes for milk composition and FA analysis respectively, at the Lebanese Agricultural Research Institute – Fanar Station.

Milk composition analysis: An infrared spectroscopy instrument MilkoScan FT120 (FOSS, Hillerød, Denmark) was used to determine main milk components: fat, protein, total solids (TS) and lactose (all %); as well as physicochemical characteristics of density [specific gravity (SG) × 1000], freezing point depression (FPD, °C) and total acidity (°SH).

For FA analysis, frozen milk samples were thawed at 40 °C using a water bath with moderate mixing in order to homogenize the fat. The FA extraction was according to Molkentin and Precht (2000) with slight modifications: 1 mL of milk was added to a glass tube and mixed with 2 mL of methanol followed by 1 mL of chloroform and then vortexed for 1 min. Then 1 mL of chloroform was added and followed by 1 mL of distilled water – this step produced different layers, and the lower phase was transferred to another glass tube and evaporated under a nitrogen stream (Reacti-Vap; Thermo Fisher, Waltham, MA, USA). The extracted lipids were methylated by adding 1 mL of hexane followed by 20 µL of sodium methylate solution (2 M in methanol), vortexed for 3 min and then centrifuged for 1 min at 480×g (Z383K, Hermle Labortechnik GmbH, Wehingen, Germany). Afterwards, 100 mg of sodium hydrogen sulfate monohydrate was added and vortexed again for 2 min, then centrifuged at 480×g for 1 min. Then 1 µL of the clear supernatant was automatically injected into gas chromatograph (GC). The GC parameters for FA analysis included a highly polar (90% biscyanopropyl/10% phenylcyanopropyl polysiloxane) capillary column Rtx-2330 (105 m × 0.25 mm ID, 0.20 µm df) installed on a

Shimadzu GC (GC 2010; Shimadzu Corp., Kyoto, Japan) with a flame-ionization detector (FID) and a split-injection port. The temperatures of the injector and detector were maintained at 250 °C. Helium was used as a carrier gas with a flow rate of 1.28 mL min^{-1} on linear velocity control. The initial oven temperature was set to 60 °C for 3 min and then the following thermal program was used: increase by 8 °C min^{-1} up to 150 °C; isotherm for 3 min; increase by 3 °C min^{-1} up to 170 °C; isotherm for 5 min; increase by 10 °C min^{-1} up to 220 °C; isotherm for 5 min; increase by 5 °C min^{-1} up to 225 °C; and isotherm for 25 min. The identification and quantification of FAs were performed with the GC Solution program version 2.3 (Shimadzu Corp.). The response factor of the fatty acid methyl ester (FAME) standard (Supelco 37 component FAME Mix varied conc. in dichloromethane, North Harrison road, Bellefonte, Pennsylvania, USA) was used to correct FAME proportions. Their quantity in the milk fat was calculated based on the fat content of milk.

Statistical analysis: The mixed procedure of SAS software v. 9.2 (SAS Institute Inc., Cary, NC, USA) was used to analyze the repeated measured variables. The statistical model included stage of lactation through the different days of sampling as the source of variation, farms and their interactions, and animals as the random effect. Least squares means were calculated considering all milk components and FAs. The SAS correlation procedure was used to estimate Pearson's correlations between parameters.

RESULTS

Milk composition and physicochemical characteristics: Milk components were significantly influenced by the progression of lactation on the farms (Table 2). Lactation advanced with steady protein and TS contents showing an overall increment of 26 and 32%, respectively, during D10–D130. As expected, fat content increased sharply by 93% ($P < 0.01$), particularly at the end of the study period coinciding with the approach of the end of the lactation season; whereas lactose content decreased by 15% within the same period ($P < 0.01$).

Milk collected from Anjar showed significantly lower protein content and higher fat and TS contents compared to Mrayjat ($P < 0.01$). However, no significant differences were observed in lactose content between the farms. Interaction between lactation stage and farms (Stage × Farm) showed high significance in the different milk main components during lactation ($P < 0.01$).

Differences in the physicochemical characteristics of milk were clearly observed throughout lactation progression (Table 3). Density remained steady from D10–D100 ($P < 0.01$). For FPD and total acidity, a smooth increment was observed during D30–D70 and remained steady afterwards. Differences between farms were significant for density, FPD (both $P < 0.01$) and acidity ($P < 0.05$).

FA profile: The progression of milking season significantly affected the milk FA profile (Table 4). The highest proportions of FAs in milk were for palmitic (C16:0, 26%), oleic (C18:1c9, 20%), myristic (C14:0, 10%), stearic (C18:0, 9%) and capric acids (C10:0, 6%). In general, SCFAs (FAs of C4:0–C10:0) increased slightly during D10–D30 and then showed a smooth drop at D70 followed by another pronounced drop of 13, 21, 23 and 22%, for C4:0, C6:0, C8;0 and C10:0 respectively, at D100 before increasing again ($P < 0.05$). Butyric (C4:0) and caproic acids (C6:0) showed no significant differences between the farms. However, Anjar farm showed 15 and 16% higher amounts of caprylic (C8:0) and capric (C10:0) acids, respectively, compared to Mrayjat ($P < 0.05$). The medium-chain FAs (MCFAs), like C12:0 and C14:0, increased with the advance of lactation at different magnitudes (15 and 36% at D70, respectively) until D100 when a drop was observed, followed by a marked increment toward the end of lactation. This drop was observed earlier (D10) with another MCFA, C16:0, followed by an increase of 37% at the end of the study period. A positive correlation was found between MCFA and days of lactation ($r = 0.71$; $P < 0.0001$). Comparing the two farms, Anjar had 14% higher lauric acid (C12:0) and 4% lower palmitic acid (C16:0; $P < 0.05$), whereas there was no significant difference in myristic acid (C14:0). In addition, C18:0 showed its maximum contents at the beginning of lactation followed by a drop of 18% at D30 then a plateau was observed until declining again by 40% at D130 ($P < 0.05$). C18:0 showed negative correlation with days of lactation ($r = -0.53$; $P < 0.01$). Focusing on MUFAs, a decrease of 23% was noted from beginning till the end of lactation and confirmed by the negative correlation between MUFAs and days of lactation ($r = -0.62$; $P < 0.01$). This was also the case for C18:1c isomers but not for C14:1, which increased with advance of the milking season ($P < 0.05$). Other MUFAs, like C10:1 and C16:1 isomers, showed a drop of 38% at D30 and 23% at D70, respectively, in comparison to D10 then a significant increase later until the end of lactation. However, the C18:1 t isomer showed a sharp increment of 60% at D30 followed by a severe drop until D130. No significant differences were noted between farms in MUFAs, C16:1 isomers and C18:1 c isomers. Significant variations between farms were observed in C10:1, C14:1 and C18:1 t isomers ($P < 0.05$). In regard to polyunsaturated FAs (PUFAs) in general and C18:2 t isomers particularly, an increment occurred at D30 (14% and 37%, respectively), then a drop was observed until the end of lactation. This last decline was also shown in the negative correlation between PUFAs and days in milk ($r = -0.50$; $P < 0.01$). Likewise, CLA c9 t11 showed a similar trend throughout

lactation with the highest point at D30 with an increment of 47% compared to the beginning of lactation. The CLA c9 t11 was positively correlated with PUFAs (r = 0.62, $P < 0.01$). However, C18:2 c isomers showed a drop of 37% at D30 followed by an increase for the rest of lactation. However, C18:3 isomers showed a different pattern with 47% higher amount at D70 compared with D10. Differences between farms were significant for all the PUFAs ($P < 0.05$). Moreover, omega 3 FAs had the highest amount at D70 ($P < 0.05$). The omega 3 FAs were negatively correlated with C18:1 c11 (r = −0.52; $P < 0.01$). However, omega 6 FAs showed its lowest amount at D30 ($P < 0.05$). Meanwhile, omega 9 showed a decreased pattern toward the end of lactation. The omega 9 was negatively correlated with SCFAs and MCFAs (r = −0.69 and −0.72, respectively; both $P < 0.01$).

The stage × farm interaction had a significant effect ($P < 0.05$) on the majority of the FAs particularly on MUFAs (C18:1n9c, C18:1c isomers and C18:1 t isomers), PUFAs (C18:2 n6c, CLA c9t11 and C18:2c isomers), as well as on omega 3, 6 and 9.

Table 2. Milk main components throughout lactation for the different farms (%).

	Protein	Fat	TS	Lactose
Stage[1]				
D10	4.63 ± 0.30^{c}	4.36 ± 0.30^{d}	14.95 ± 0.40^{d}	4.73 ± 0.12^{a}
D30	4.80 ± 0.31^{c}	5.30 ± 0.31^{c}	15.81 ± 0.42^{cd}	4.42 ± 0.12^{ab}
D70	5.17 ± 0.31^{bc}	5.23 ± 0.30^{c}	16.27 ± 0.41^{c}	4.58 ± 0.12^{ab}
D100	5.26 ± 0.33^{abc}	7.12 ± 0.32^{b}	18.02 ± 0.43^{b}	4.28 ± 0.13^{bc}
D130	5.86 ± 0.42^{ab}	8.42 ± 0.41^{a}	19.71 ± 0.56^{a}	4.00 ± 0.17^{c}
Farms				
Anjar	5.44 ± 0.18^{a}	6.96 ± 0.18^{b}	17.88 ± 0.24^{b}	4.10 ± 0.07
Mrayjat	6.12 ± 0.21^{b}	5.60 ± 0.21^{a}	17.40 ± 0.28^{a}	4.25 ± 0.09
P-values				
Stage	**	**	**	**
Farm	**	**	**	ns
Stage × Farm	**	**	**	**

$^{a–d}$ Least squares means within a column within a category with different superscripts differ ($P < 0.05$).
[1]D10-D130; corresponds to Day 10 to Day 130.
$^{**}P < 0.01$; ns = not significant

Table 3. Physicochemical characteristics of milk throughout lactation in the two farms.

	Density (SG ×1000)	FPD[1] (°C)	Total acidity (°SH)
Stage[2]			
D10	1036.41 ± 0.85^{a}	0.59 ± 0.02^{b}	10.59 ± 1.02^{b}
D30	1034.29 ± 0.88^{ab}	0.58 ± 0.02^{b}	11.25 ± 1.06^{b}
D70	1035.50 ± 0.86^{a}	0.65 ± 0.02^{a}	12.66 ± 1.04^{ab}
D100	1031.93 ± 0.92^{b}	0.65 ± 0.02^{a}	12.97 ± 1.11^{ab}
D130	1031.67 ± 1.17^{b}	0.66 ± 0.02^{a}	15.02 ± 1.41^{a}
Farms			
Anjar	1032.69 ± 0.51^{a}	0.61 ± 0.01^{a}	13.48 ± 0.62
Mrayjat	1035.96 ± 0.60^{b}	0.68 ± 0.01^{b}	15.30 ± 0.72
P-values			
Stage	**	**	**
Farm	**	**	ns
Stage × Farm	**	ns	**

$^{a–d}$ Least squares means within a column within a category with different superscripts differ ($P < 0.05$).
[1] FPD = freezing point depression.
[2]D10-D130; corresponds to Day 10 to Day 130.
$^{**}P < 0.01$; ns = not significant

Table 4. Milk fatty acid composition during lactation (% of total fatty acids).

Fatty acids	Stage[1]					Farm		*P*-values		
	D10	D30	D70	D100	D130	Anjar	Mrayjat	S	F	S × F
SFA										
C4:0	4.25 ± 0.16^{ab}	4.35 ± 0.16^{a}	4.07 ± 0.15^{ab}	3.54 ± 0.17^{c}	4.11 ± 0.22^{ab}	4.05 ± 0.09	3.90 ± 0.11	*	ns	ns
C6:0	2.86 ± 0.13^{ab}	3.00 ± 0.13^{a}	2.73 ± 0.13^{ab}	2.17 ± 0.14^{c}	2.43 ± 0.19^{bc}	2.63 ± 0.08	2.35 ± 0.09	*	ns	*
C8:0	2.45 ± 0.14^{a}	2.62 ± 0.14^{a}	2.35 ± 0.13^{ab}	1.80 ± 0.15^{c}	1.90 ± 0.19^{bc}	2.27 ± 0.08^{b}	1.94 ± 0.10^{a}	*	*	*
C10:0	6.17 ± 0.41^{bc}	7.10 ± 0.41^{ab}	6.84 ± 0.4^{bc}	5.36 ± 0.43^{d}	5.64 ± 0.56^{cd}	6.58 ± 0.24^{b}	5.53 ± 0.29^{a}	*	*	*
C12:0	3.19 ± 0.21^{bc}	3.57 ± 0.21^{bc}	3.65 ± 0.21^{b}	3.00 ± 0.23^{c}	3.10 ± 0.3^{bc}	3.64 ± 0.13^{b}	3.14 ± 0.15^{a}	*	*	*
C14:0	7.54 ± 0.45^{e}	8.94 ± 0.45^{d}	10.25 ± 0.44^{bc}	9.50 ± 0.47^{cd}	11.20 ± 0.62^{b}	10.03 ± 0.27	9.69 ± 0.32	*	ns	*
C16:0	21.95 ± 0.65^{d}	22.76 ± 0.65^{cd}	24.8 ± 0.63^{b}	24.63 ± 0.68^{bc}	31.17 ± 0.89^{a}	25.18 ± 0.38^{a}	26.21 ± 0.46^{b}	*	*	*
C18:0	11.48 ± 0.49^{a}	9.40 ± 0.49^{b}	10.4 ± 0.47^{ab}	10.71 ± 0.51^{ab}	7.15 ± 0.67^{c}	9.57 ± 0.29	9.1 ± 0.34	*	ns	ns
C20:0	0.17 ± 0.03^{c}	0.15 ± 0.03^{c}	0.32 ± 0.03^{b}	0.55 ± 0.03^{a}	0.52 ± 0.04^{a}	0.25 ± 0.02^{a}	0.33 ± 0.02^{b}	*	*	*
Unsaturated fatty acids										
C10:1	0.16 ± 0.02^{c}	0.10 ± 0.02^{d}	0.16 ± 0.02^{c}	0.15 ± 0.02^{cd}	0.26 ± 0.03^{b}	0.20 ± 0.01^{b}	0.17 ± 0.01^{a}	*	*	ns
C12:1	0.01 ± 0.01^{bc}	0.004 ± 0.01^{c}	0.01 ± 0.01^{bc}	0.01 ± 0.01^{bc}	0.02 ± 0.01^{b}	0.034 ± 0.004^{b}	0.011 ± 0.004^{a}	*	*	ns
C14:1	0.17 ± 0.04^{e}	0.33 ± 0.04^{d}	0.45 ± 0.04^{c}	0.58 ± 0.04^{b}	0.85 ± 0.05^{a}	0.36 ± 0.02^{a}	0.59 ± 0.03^{b}	*	*	*
C16:1n9	0.51 ± 0.06^{ac}	0.45 ± 0.06^{abc}	0.30 ± 0.05^{bcd}	0.20 ± 0.06^{d}	0.38 ± 0.08^{abcd}	0.48 ± 0.03^{b}	0.22 ± 0.04^{a}	*	*	*
C16:1 isomers	1.19 ± 0.08^{b}	1.04 ± 0.08^{bc}	0.91 ± 0.08^{c}	0.94 ± 0.09^{c}	1.62 ± 0.12^{a}	1.28 ± 0.05	1.24 ± 0.06	*	ns	*
C18:1n9c	23.98 ± 0.8^{a}	18.96 ± 0.8^{b}	19.01 ± 0.78^{b}	20.23 ± 0.84^{b}	17.15 ± 1.1^{b}	19.56 ± 0.47	20.4 ± 0.56	*	ns	*
C18:1 c isomer	1.32 ± 0.09^{a}	1.18 ± 0.09^{abd}	0.79 ± 0.08^{e}	0.90 ± 0.09^{cde}	0.90 ± 0.12^{bcd}	1.11 ± 0.05	1.02 ± 0.06	*	ns	*
C18:1n9t	0.05 ± 0.02	0.02 ± 0.02	0.00 ± 0.02	0.05 ± 0.02	0.00 ± 0.03	0.05 ± 0.01	0.03 ± 0.01	ns	ns	ns
C18:1t isomers	1.96 ± 0.18^{d}	4.84 ± 0.18^{a}	2.64 ± 0.17^{bc}	2.14 ± 0.19^{cd}	1.69 ± 0.25^{d}	2.24 ± 0.11^{a}	3.07 ± 0.13^{b}	*	*	*
C18:2 n6c	2.19 ± 0.09^{a}	1.82 ± 0.09^{b}	1.89 ± 0.09^{b}	1.94 ± 0.09^{ab}	2.03 ± 0.12^{ab}	1.75 ± 0.05^{a}	2.44 ± 0.06^{b}	*	*	*
CLA c9t11	0.60 ± 0.05^{cd}	1.13 ± 0.05^{a}	0.81 ± 0.05^{b}	0.72 ± 0.05^{bcd}	0.70 ± 0.07^{bcd}	0.68 ± 0.03^{a}	0.85 ± 0.04^{b}	*	*	*
C18:2 c isomers	2.32 ± 0.09^{a}	1.88 ± 0.09^{b}	1.93 ± 0.09^{b}	1.97 ± 0.09^{b}	2.09 ± 0.12^{ab}	1.89 ± 0.05^{a}	2.48 ± 0.06^{b}	*	*	*
C18:2 n6t	0.01 ± 0.01^{b}	0.11 ± 0.01^{a}	0.02 ± 0.01^{b}	0.03 ± 0.01^{b}	0.01 ± 0.02^{b}	0.03 ± 0.01	0.03 ± 0.01	*	ns	*
C18:2 t isomers	0.55 ± 0.05^{b}	0.87 ± 0.05^{a}	0.51 ± 0.04^{b}	0.43 ± 0.05^{b}	0.44 ± 0.06^{b}	0.68 ± 0.03^{b}	0.48 ± 0.03^{a}	*	*	*
C18:3 n3	0.70 ± 0.05^{e}	1.18 ± 0.05^{b}	1.32 ± 0.05^{a}	1.01 ± 0.05^{c}	0.63 ± 0.06^{ef}	0.86 ± 0.03^{a}	0.89 ± 0.03^{b}	*	*	*
C18:3 isomers	0.70 ± 0.05^{d}	1.19 ± 0.05^{b}	1.32 ± 0.05^{a}	1.01 ± 0.05^{c}	0.63 ± 0.06^{de}	0.87 ± 0.03^{a}	0.89 ± 0.03^{b}	*	*	*
C20:4 n6	0.21 ± 0.01^{a}	0.10 ± 0.01^{c}	0.12 ± 0.01^{bc}	0.12 ± 0.02^{bc}	0.16 ± 0.02^{ab}	0.14 ± 0.01	0.14 ± 0.01	*	ns	*
C20:5 n3	0.04 ± 0.01^{cd}	0.06 ± 0.01^{bcd}	0.10 ± 0.01^{a}	0.09 ± 0.01^{ab}	0.09 ± 0.01^{ab}	0.07 ± 0.01	0.06 ± 0.01	*	ns	*
C22:6 n3	0.03 ± 0.01^{a}	0.01 ± 0.01^{ab}	0.00 ± 0.01^{b}	0.02 ± 0.01^{ab}	0.02 ± 0.01^{ab}	0.03 ± 0.00^{b}	0.014 ± 0.04^{a}	*	*	ns
Calculated fatty acids										
Omega 3	0.74 ± 0.05^{c}	1.24 ± 0.05^{b}	1.41 ± 0.05^{a}	1.10 ± 0.05^{b}	0.72 ± 0.07^{c}	0.93 ± 0.03	0.95 ± 0.04	*	ns	*
Omega 6	2.41 ± 0.09^{a}	2.04 ± 0.09^{c}	2.12 ± 0.09^{bc}	2.24 ± 0.1^{abc}	2.40 ± 0.13^{ab}	1.97 ± 0.05 ^{a}	2.72 ± 0.07^{b}	*	*	*
Omega 9	24.49 ± 0.81^{a}	19.46 ± 0.81^{bc}	19.32 ± 0.78^{bc}	20.43 ± 0.85^{b}	17.56 ± 1.11^{c}	20.05 ± 0.48	20.64 ± 0.57	*	ns	*
MUFA	29.3 ± 0.92^{a}	26.81 ± 0.92^{ab}	24.18 ± 0.89^{c}	25.07 ± 0.97^{bc}	22.71 ± 1.26^{cd}	25.09 ± 0.54	26.77 ± 0.65	*	ns	*
PUFA	4.53 ± 0.15^{bc}	5.28 ± 0.15^{a}	4.83 ± 0.15^{b}	4.42 ± 0.16^{bc}	4.23 ± 0.21^{cd}	4.43 ± 0.09^{a}	4.94 ± 0.11^{b}	*	*	*
SCFA	15.77 ± 0.75^{ab}	17.11 ± 0.75^{a}	16.01 ± 0.73^{ab}	12.90 ± 0.79^{c}	14.09 ± 1.03^{bc}	15.57 ± 0.44^{b}	13.75 ± 0.53^{a}	*	*	*
MCFA	33.05 ± 1.01^{e}	35.67 ± 1.01^{de}	39.07 ± 0.99^{c}	37.16 ± 1.07^{cd}	45.6 ± 1.4^{b}	39.38 ± 0.6	39.16 ± 0.71	*	ns	*
Omega 6: Omega 3	3.76 ± 0.2^{b}	1.68 ± 0.2^{c}	1.52 ± 0.19^{c}	2.04 ± 0.21^{c}	3.35 ± 0.28^{b}	2.26 ± 0.12^{a}	4.76 ± 0.14^{b}	*	*	*

$^{a–d}$ Least squares means within a line within a category with different superscripts differ ($P < 0.05$).

[1]D10-D130; corresponds to Day 10 to Day 130.

Omega 3: C18:3c9,12,15; C20:3c11,14,17; C20:5c5,8,11,14,17; Omega 6: C18:2c9,12; C18:2t9,12; C18:3c6,9,12; C20:3c8,11,14; C20:4c5,8,11,14; Omega 9: C16:1n9, C18:1c9; C22: 1c13; MUFA = monounsaturated fatty acids: C10:1; C12:1; C14:1; C16:1c7; C16:1c9; C18:1t11; C18:1c9; C18:1 minor isomer (C18:1 t 8–13; C18:1c9-12); C20:1; C22:1c13; PUFA = polyunsaturated fatty acids: C18:2t9,12; C18:2c9,12; C18:2c9t11; C18:3c6,9,12; C18:3c9,12,15; C20:2c11,14; C20:3c8,11,14; C20:3c11,14,17; SCFA = short-chain fatty acids: C4:0–C11:0; MCFA = medium chain fatty acids: C12:0–C16:0; S = Stage, F = Farm

* $P < 0.05$; ns = not significant

DISCUSSION

Variations among milk main components and physical characteristics in the course of lactation have generally been very well studied in different sheep breeds (Antunovic *et al*., 2001; Ochoa-Cordero, 2002; Oravcová *et al*., 2006; Kuchtík *et al.*, 2017) and in Awassi particularly (Nudda *et al.*, 2002; Kridli *et al.*, 2007; Iñiguez and Hilali, 2009; Titi and Al-Fataftah, 2013). Kuchtík *et al.*, (2017) described the increased trend for milk components like fat, protein and TS with the progression of lactation. Their findings are in accordance with our study except that their experiment started at day 33 and in our trial an ascending phase was observed from D10 onwards. The studied farms showed significant differences in most milk components, with 20% higher fat and 13% lower protein content for Anjar compared to Mrayjat farm ($P < 0.05$). This may be due to the variability in pasture available in each region because during spring the ewes on Anjar farm were fed mainly on ryegrass, which is rich in fiber compared to other forage species (Cabiddu *et al*., 2003). Similar findings were reported by Pirisi *et al.*, (2001) on Sarda ewes fed on ryegrass pasture and a fat content was noted of 6.98% and protein content of 5.66%. Regarding lactose content, no significant differences between farms were observed. It is well known that lactose content in milk is more stable during the lactation season and is little affected by feed (Morand-Fehr *et al.*, 2007).

In regard to the physicochemical characteristics of milk, the total acidity followed the trend of protein content with the progression of lactation; total acidity tended to increase after D10 as mentioned by Brito *et al*., (2006), this was related to the content of casein in milk protein which can alter its acidity. The FPD was 12% lower at the end lactation compared with D10 ($P < 0.05$). These results were in accordance with those of Pavić *et al.,* (2002). In addition, the values of density in our study were within the ranges found by Park *et al*., (2007).

The FAs show remarkable changes with the advance of lactation (Strzałkowska *et al*., 2009; Sinanoglou *et al*., 2015). The percentages of the highest FA shares of C10:0, C14:0, C16:0, C18:0 and C18:1c9 were in accordance with Park *et al*., (2007). The decline in SCFAs (C4:0–C10:0) from D30 toward the end of lactation was similarly reported by Sinanoglou *et al.,* (2015) for which early lactation started at day 42. Tsiplakou *et al.*, (2008) concluded that grazing animals had decreases in SCFAs because pasture was rich in long-chain FAs thus inhibiting *de novo* synthesis of SCFAs in the mammary gland. However, in our study, lauric (C12:0), myristic (C14:0) and palmitic acids (C16:0) had their highest amounts at the end of lactation (3.10 ± 0.3, 11.20 ± 0.62 and 31.17 ± 0.89%, respectively). Similar observations were described by Mayer and Fiechter (2012) and Payandeh *et al*., (2016) for lauric and myristic acids, but no trend was reported for palmitic acid. Another study reported a similar profile of palmitic acid (Sinanoglou *et al*., 2015). In addition, the constant profile observed after D30 for stearic acid (C18:0) and the drop toward the end of lactation were similar to that described by Mayer and Fiechter (2012). Among the MUFAs, oleic acid (C18:1n9c) was the major FA and decreased 29% from the beginning of lactation at D10 until its end – this decline was confirmed by the negative correlation between C18:1n9c and days in milk ($r = -0.61$, $P < 0.0001$). In this sense, Payandeh *et al.*, (2016) described the decline of C18:1n9c as a reflection of the metabolic status of the animal, emphasizing that the animal mobilizes long-chain FAs from its adipose tissue in order to cope with the negative energy balance at the onset of lactation (Palmquist *et al.*, 1993). The amounts of C18:1n9c in both Anjar and Mrayjat farms were higher than those reported by Hilali *et al.,* (2018) for Awassi ewes in the control group fed a conventional diet (19.56 ± 0.47, 20.4 ± 0.56 and 18.32 ± 0.293%, respectively) in comparison to our study, for which animals were grazed on natural rangelands and so led to these results (Atti *et al*., 2006). Furthermore, C18:1t isomers, C18:2t isomers and CLA (C18:2 c9t11) showed their highest contents at D30 at the end of March (4.84 ± 0.18, 0.59 ± 0.03 and 1.13% ± 0.05%, respectively) which coincided with the start of spring in Lebanon when ewes grazed fresh pasture during most of the day. These findings were in accordance with De La Fuente *et al*., (2009), who found that spring resulted in significantly higher levels of PUFAs. In general, biohydrogenation of PUFAs in the rumen results in trans FAs that promote the synthesis of CLA (Lock and Garnsworthy, 2002). This was confirmed by the positive correlation between CLA (C18:2 c9t11) and C18:1t13 ($r = 0.75$, $P < 0.0001$). Nevertheless, a decrease in CLA amount was observed after D30, coinciding with the start of spring, thus supporting the conclusion of Cabiddu *et al*., (2003) that a decrease in CLA percentage in milk occurred in spring when pasture changed its phases from vegetative to reproductive growth. Moreover, the amounts of CLA (C18:2 c9t11) found in both Anjar and Mrayjat farms were higher than those reported by Hilali *et al*., (2018) using conventional rations (0.68 ± 0.03, 0.85 ± 0.04 and 0.335 ± 0.015%, respectively). In addition, omega 3 FAs had their highest values at mid lactation (D70), and there were no significant differences between farms. However, the average amounts for Anjar and Mrayjat farms were higher than those of Hilali *et al.*, (2018) using conventional diets (0.93 ± 0.03, 0.95 ± 0.04 and 0.35 ± 0.248%, respectively). Interestingly, the omega 6:omega 3 ratio was 60% lower at D70 compared to D10 ($P < 0.05$), and was almost 2:1. Simopoulos (2002) stated that the lower this ratio is the lower is the risk of chronic disease, and noted that western diets had very high values for this ratio of 15:1–16.7:1. The omega 6:omega 3 ratio

result is an interesting point, emphasizing the richness of the natural pasture in the Bekaa region (both farms) in PUFAs. Natural pasture in the Bekaa region are now sought to enrich animal diets and consequently milk fat, which is considered the main source of CLA in consumers' diets (Strzałkowska *et al.*, 2009), and are known very widely to have beneficial effects on human health by reducing chronic disease (Shingfield, 2008).

Conclusion: The advance in lactation season had a significant effect on the FA profile in milk of Awassi sheep. The stage × farm interaction had a significant effect on some of the FAs particularly on MUFAs, PUFAs and omega 3, 6 and 9. Grazing on natural rangelands in the Bekaa Valley resulted in milk rich in CLA, C18:1n9c. From a nutritional point of view, this may be regarded as positively for the quality of ghee and butter that are consumed widely in the Middle East. Moreover, FA-profile of these products made in the spring will differ from the FA-profile of the products made from milk from other parts of the lactation period.

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