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REGULAR ARTICLES

Extensive analysis of milk fatty acids in two fat-tailed sheep breeds during lactation

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Abstract The profile of fatty acids (FA) in the milk fat of two Iranian fat-tailed sheep breeds, Sanjabi and Mehraban, was compared during lactation. Eight ewes of each breed, balanced in parity and carrying one foetus, were selected before parturition. Ewes were kept separated in individual pens during the experimental period, under the same management practices and fed the same diet, in order to eliminate any confounding effects on milk FA profile. Milk was sampled at biweekly intervals up to 10 weeks of lactation, starting 2 weeks after parturition. More than 100 FA were determined in milk fat by means of gas chromatography. The milk fat of Sanjabi ewes contained more *cis*-9 18:1, that of Mehraban ewes was richer in 10:0, 12:0 and 14:0, and no differences were found for 16:0 and 18:0. No breed differences were found for most branched-chain FA. Mehraban ewes showed a higher presence of vaccenic and rumenic acids in their milk fat. The milk fat of Sanjabi ewes had a lower atherogenicity index and n-6/n-3 FA ratio. The contents of several FA showed time-dependent changes, so breed differences were more apparent or disappeared as lactation progressed. The milk fat of Sanjabi ewes showed a better FA profile from the human health point of view.

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Introduction

Dairy fat is important in human diet. During recent decades, increasing emphasis has been placed on the contribution of dairy foods on the development or prevention of chronic human diseases. Some milk fatty acids (FA), including the saturated 12:0, 14:0 and 16:0 and some *trans* unsaturated FA, are thought to have negative effects on human health when consumed in high amounts, whereas butyric acid (4:0), some branched-chain fatty acids (BCFA), vaccenic acid (VA, *trans*-11 18:1) and rumenic acid (RA, *cis*-9, *trans*-11 18:2) may have potentially beneficial effects, the most relevant being the anticarcinogenic one (Shingfield et al. 2008).

It is well established that diet composition has a noticeable ability to modify the FA profile of sheep milk fat (Nudda et al. 2014). However, there are also physiological, environmental and management factors that affect milk FA contents (De la Fuente et al. 2009). Among those factors, breed has arisen interest in recent years because milk FA profile could be used as an indicator of breed typicity (Signorelli et al. 2008). A number of studies have compared FA contents in the milk fat of different local sheep breeds by using ewes that were kept under the same feeding and management conditions, in order to avoid any confounding effects on the obtained results (Tsiplakou et al. 2008; Talpur et al. 2009; Mierlita et al. 2011a, 2011b; Rozbicka-Wieczorek et al. 2015). Some of those studies have only focused on the concentration of conjugated linoleic acid (CLA) isomers (Tsiplakou et al. 2006; Rozbicka-Wieczorek et al. 2013). Moreover, only Tsiplakou et al. (2006) and Sinanoglou et al. (2015) took into account the effect of lactation stage, and most of the above-mentioned

studies did not supply detailed information about quantitatively minor FA.

Iran has the largest sheep population in the world after China, India and Australia (FAOSTAT 2015). With 31 recognized breeds, Iran has also one of the highest numbers of sheep breeds in the world according to the Domestic Animal Diversity Information System (www.fao.org/DAD-IS). Sanjabi and Mehraban are two fat-tailed sheep breeds that are widely kept in the provinces of Kermanshah and Hamadan, respectively, located in the western region of Iran. In this country, sheep milk, besides used for lamb rearing, is a valuable nutritional food that traditionally has enriched the nomadic shepherd's diet consumed fresh or as cheese, yoghurt and other dairy products (Izadifard and Zamiri 1997; Degen 2007). Moreover, Rüne Dân, an oil made from the Sanjabi sheep's butter, is famous throughout Iran because of its flavour, aroma and other characteristics, like its low melting point. However, to the best of our knowledge, there is no published research on the FA contents in the milk fat of those breeds.

The aim of the present work was to compare in detail the FA profile of the milk fat from Sanjabi and Mehraban ewes, which were kept indoors under the same feeding regime and management conditions, during lactation.

Materials and methods

The study was carried out in the Animal Research Station of Razi University Agriculture College (Kermanshah, Iran). Eight Sanjabi and eight Mehraban ewes, balanced in parity (2.5 ± 0.53 and 2.4 ± 0.9, respectively), were selected 1 month before lambing from a synchronized pure flock. Ewes were at their second and third lambing, carried one foetus, and were clinically healthy and free from internal and external parasites. Ewes' body weights at parturition were 59.5 ± 5.32 and 45.2 ± 7.57 kg, and lambs' birth weights were 4.11 ± 0.19 and 3.78 ± 0.17 kg in Sanjabi and Mehraban ewes, respectively. After parturition, the ewes were kept in individual straw-bedded pens, which were provided with individual water and feeding troughs, and fed the same diet, without grazing at all, in order to eliminate the confounding influences of diet and farm management on milk FA profile.

Diet was based on alfalfa hay (60 % dry matter, DM) and a concentrate mix (40 % DM) consisting of maize grain (26 % DM), soybean meal (9 % DM), wheat bran (3.5 % DM), and minerals and vitamins (1.5 % DM). Chemical composition of the diet was (dry matter basis) crude protein 13.9 %, neutral detergent fibre 36.1 %, acid detergent fibre 25.6 %, calcium 0.61 % and phosphorus 0.37 %. This diet supplied 0.6, 7.2 and 2.9 g/kg of oleic, linoleic and α-linolenic acid, respectively (calculated according to Sauvant et al. 2004). The ration was fed in two equal amounts at approximately 07:30 and 17:30 h. Daily feed amounts (as fed basis) offered until week six of lactation and from week seven onwards were 2.26 and 1.67 kg/day, respectively, in Sanjabi ewes, and 1.95 and 1.46 kg/day, respectively, in Mehraban ewes (National Research Council 1985). Visual observation of the troughs revealed that feed offered daily was completely eaten from day to day. Water was provided ad libitum to all ewes for the entire period. No cases of mastitis were observed during the experimental period.

Milk samples were collected individually at biweekly intervals up to 10 weeks of lactation, starting 2 weeks after parturition. Lambs were separated from their dams at 1630 h on the day before each test day and bottle-fed. On the test day, ewes were milked twice by hand at 0630 and 1630 h, and milk was weighed and sampled individually in each milking. The morning milk samples from each ewe were kept refrigerated at 4 °C in plastic containers, then they were mixed with the corresponding evening samples, and the resulting individual samples (60 ml each) were stored at −80 °C.

Fat of milk samples from each ewe was extracted as described by Luna et al. (2005), placed in amber vials, blanketed with a stream of nitrogen and stored at −20 °C until analysis. Few weeks later, they were transported frozen to Spain for analysis. Fatty acid composition in the 80 milk fat samples was determined by gas–liquid chromatography. Fatty acid methyl esters (FAME) were prepared according to ISO-IDF (2002). Analysis of FAME was performed on a gas–liquid chromatograph (Agilent 6890 N Network System) by injection onto a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm, Varian, Middelburg, The Netherlands) under similar conditions to those reported by Martínez Marín et al. (2012). Individual FAME quantification was performed using a milk fat with known composition (CRM 164; European Community Bureau of Reference, Brussels, Belgium). Some individual FA were identified by comparison with standards distributed by Nu-Chek (Elysian, MN, USA). When no commercial standards were available, FAME were analysed by electronic impact MS. Finally, when previous methodologies were not enough, identification of unknown compounds was based on chromatograms obtained under similar analytical conditions that were reported in previous published studies.

Data were analysed by repeated measurements analysis using the MIXED procedure of SAS 9.1 (SAS Institute Inc., Cary, NC, USA). The statistical model included the fixed effects of breed, week of lactation (WOL) and their interactions, and the random effects of each individual ewe within breed, assuming a compound symmetry structure on the basis of Schwarz's Bayesian information model fit criteria. Linear trends were investigated by polynomial contrasts. The CORR procedure was used when appropriate. Statistical significance was declared at $P < 0.05$.

Results and discussion

More than one hundred individual FA, including geometric and positional isomers, were quantified in milk fat, and the effects of breed, week of lactation (WOL) and their interaction on the FA profile were examined (Tables 1, 2 and 3). The ewes of the present study were homogeneous and maintained under the same feeding and management conditions. Hence, any observed differences of the FA contents in milk fat should be primarily related to breed effects. Although several FA and groups of FA presented significant breed and breed × WOL differences, we focused the discussion on those which are known to be relevant for the nutritional quality of milk fat, taking into account other published papers that compared local sheep breeds by using ewes kept under the same feeding and management conditions.

Overall, the sum of SFA, *cis* and *trans* monounsaturated FA (MUFA) and total CLA differed statistically between Mehraban and Sanjabi ewes, whereas no breed differences were found in the sum of polyunsaturated FA (PUFA) and BCFA (Table 1). Breed effects on the main groups of FA in the milk fat of autochthonous sheep breeds are controversial. Some authors have found no effects on SFA, MUFA, PUFA and total CLA contents (Tsiplakou et al. 2008; Rozbicka-Wieczorek et al. 2013, 2015; Sinanoglou et al. 2015), while other authors have observed differences in the contents of SFA, MUFA, PUFA and *trans* MUFA (Talpur et al. 2009; Mierlita et al. 2011a, 2011b). On the other hand, the values of the major FA and the FA sums observed in the present work are in good agreement with the values reported by the above-mentioned studies, with SFA comprising more than two thirds of total milk FA on average.

The content of 4:0 in milk fat did not differ between breeds (Table 1), in agreement with the observations of Talpur et al. (2009) in Kachi and Kooka ewes and Mierlita et al. (2011b) in Spanca and Turcana ewes, although it steadily decreased as lactation progressed in both breeds (Table 4), as reported by Sinanoglou et al. (2015) in Karagouniko and Chios ewes. The contents of 6:0, 8:0, 10:0, 12:0 and 14:0 in milk fat were higher ($P < 0.05$) in Mehraban than in Sanjabi ewes (Table 1), and all, but 6:0, showed an increasing linear trend in the former as lactation progressed (Table 4). Observed breed differences agreed with the results of Talpur et al. (2009), as well as those of Mierlita et al. (2011b) in Merino of Transylvania, Tsigay and Turcana ewes, but not with those of Tsiplakou et al. (2008) in Awassi, Lacaune, Friesland and Chios ewes, and Rozbicka-Wieczorek et al. (2015) in Wrzosówka and Polish Lowland ewes. Contrary to our results, Sinanoglou et al. (2015) did not find breed nor breed × lactation stage differences in 8:0, 10:0, 12:0 and 14:0 contents. Regarding 16:0, the most abundant fatty acid in milk fat, no breed effects were found (Table 1), but its content decreased in the milk fat of Sanjabi ewes as lactation progressed. It is worth mentioning that some studies did not find breed differences either (Tsiplakou et al. 2008; Talpur et al. 2009; Rozbicka-Wieczorek et al. 2015), while other do (Mierlita et al. 2011a, b; Sinanoglou et al. 2015). The increase of 8:0, 10:0, 12:0 and 14:0 contents in the milk fat of Mehraban ewes as lactation progressed, without changes in 16:0, and the simultaneous decrease of 18:0 and *cis*-9 18:1 (Table 4) would suggest a more pronounced change in the metabolic status of those animals (Loften et al. 2014), i.e. from negative to positive energy balance, compared with Sanjabi ewes. It might occur that more 18:0 and *cis*-9 18:1 were mobilized from adipose tissue at the beginning of lactation in Mehraban than in Sanjabi ewes, which in turn could inhibit the de novo synthesis of SFA in the mammary gland (Palmquist et al. 1993).

Eight odd-chain SFA were detected in milk fat, but only 15:0 and 17:0 were found in substantial amounts (Table 1). Both FA were more abundant in milk fat of Sanjabi ewes, but this difference was only significant for 17:0. These results were just opposed to those of Rozbicka-Wieczorek et al. (2015), but agreed with Mierlita et al. (2011a). The three most abundant BCFA were iso-17:0, iso-16:0 and anteiso-15:0, and their contents summed up to nearly 70 % of the group (Table 1). It is worth mentioning that non-terminal BCFA, as those reported in high concentrations in the fat tail adipose tissue and muscle of Damara sheep (Alves et al. 2013), were not detected in the present work. The contents of individual BCFA in milk fat were not different between Mehraban and Sanjabi ewes, except for iso-18:0, but WOL effect was significant in all of them. As a result, total BCFA content steadily increased as lactation progressed in both breeds, but the increment was more pronounced in Sanjabi than in Mehraban ewes, which showed a lower value than the former in the last WOL (Table 4). Except for the present work and that of Sinanoglou et al. (2015), who observed breed differences in several BCFA, to the best of our knowledge, no other published papers from studies carried out with ewes kept stalled under the same feeding and management conditions have reported the breed effects on BCFA. Odd- and branched-chain FA can serve as biomarkers of both rumen function in animals and dairy fat intake in humans (Vlaeminck et al. 2006).

The isomeric profile of 18:1 milk FA is detailed in Table 2. Oleic acid (*cis*-9 18:1) was the second most predominant FA in milk fat and quantitatively the most important MUFA, totalling more than 94 % of the total *cis* 18:1. Its content was higher in Sanjabi than in Mehraban ewes and decreased in the latter as lactation progressed (Table 4). With regard to the rest of the *cis* 18:1 isomers, *cis*-11 and *cis*-12 were the most abundant and differed between breeds. Most of the individual *trans* 18:1 FA, including the quantitatively main isomer VA, were higher in Mehraban ewes, so their sum was also higher in this breed (Table 1). No significant breed × WOL effects were observed in VA content, but it linearly decreased in the milk fat

Table 1 Milk fatty acid profile (g/100 g of fatty acid methyl esters) in two fat-tailed sheep breeds

				Probability		
Fatty acids	Mehraban	Sanjabi	SEM	Breed (*B*)	Week (*W*)	*B* × *W*
SFA						
4:0	3.58	3.77	0.056	0.34	<0.001	0.23
5:0	0.024	0.023	0.001	0.79	0.34	0.23
6:0	2.89	2.61	0.045	<0.05	0.12	<0.001
7:0	0.034	0.027	0.001	<0.05	0.20	<0.05
8:0	2.60	2.22	0.050	<0.05	0.13	<0.001
9:0	0.053	0.042	0.002	<0.05	0.09	<0.05
10:0	7.75	6.06	0.193	<0.001	<0.05	<0.001
12:0	4.14	3.27	0.099	<0.001	<0.001	<0.001
iso-13:0	0.023	0.027	0.001	0.24	<0.001	0.95
anteiso-13:0	0.011	0.013	0.001	0.14	<0.001	0.10
iso-14:0	0.158	0.176	0.006	0.30	<0.001	<0.05
14:0	9.96	8.64	0.179	<0.05	<0.001	<0.001
iso-15:0	0.265	0.297	0.007	0.11	<0.001	0.19
anteiso-15:0	0.436	0.472	0.014	0.37	<0.001	<0.05
15:0	0.901	0.952	0.018	0.32	<0.001	0.22
iso-16:0	0.342	0.373	0.009	0.22	<0.001	<0.01
16:0	23.54	23.85	0.204	0.59	0.57	<0.05
iso-17:0	0.441	0.456	0.008	0.57	<0.05	0.54
17:0	0.685	0.895	0.024	<0.001	<0.001	0.22
cyclo 17:0	0.012	0.011	0.001	0.36	<0.01	0.17
iso-18:0	0.093	0.122	0.004	<0.05	<0.01	<0.05
18:0	10.99	11.66	0.189	0.21	0.16	<0.05
keto-10 18:0	0.014	0.016	0.001	0.16	<0.01	0.18
19:0	0.098	0.111	0.002	0.12	0.05	<0.05
20:0	0.217	0.237	0.005	0.17	<0.001	0.16
21:0	0.049	0.053	0.001	0.48	<0.001	0.74
22:0	0.088	0.100	0.003	0.14	<0.001	0.23
23:0	0.042	0.053	0.002	0.08	<0.001	0.45
24:0	0.035	0.042	0.002	0.17	<0.001	0.57
MUFA						
cis-9 10:1 + 11:0	0.273	0.220	0.008	<0.01	<0.001	<0.01
cis-9 12:1	0.028	0.022	0.001	<0.05	<0.001	<0.001
cis-11 12:1 + 13:0	0.144	0.118	0.004	<0.05	<0.001	0.06
cis-9 14:1	0.134	0.106	0.005	<0.05	<0.001	<0.05
Other *trans* 15:1	0.072	0.077	0.002	0.19	<0.001	0.42
cis-7 + *trans*-10 + *trans*-11 + *trans*-12 16:1	0.315	0.336	0.005	0.16	<0.001	<0.001
cis-8 16:1	0.019	0.017	0.001	0.21	0.07	<0.001
cis-9 16:1 + anteiso-17:0	1.11	1.31	0.018	<0.001	0.07	0.14
cis-10 16:1	0.018	0.016	0.001	0.10	<0.05	<0.001
cis-11 16:1	0.010	0.010	0.000	0.68	<0.05	<0.05
cis-12 16:1	0.009	0.010	0.000	0.14	0.07	0.17
cis-13 16:1	0.074	0.050	0.004	<0.05	<0.001	<0.001
trans-4 16:1	0.022	0.039	0.003	<0.05	<0.05	0.22
trans-5 16:1	0.026	0.040	0.003	0.06	<0.001	0.44
trans-6 + *trans*-7 16:1	0.035	0.030	0.001	<0.05	<0.05	0.07
trans-8 16:1	0.017	0.012	0.001	<0.001	0.42	<0.05
trans-9 16:1	0.015	0.030	0.002	<0.05	0.06	0.12
trans-14 16:1	0.026	0.026	0.001	0.63	0.15	<0.001
Other *trans* 16:1	0.023	0.035	0.003	0.09	<0.05	0.31
cis-9 17:1	0.246	0.358	0.012	<0.001	0.07	0.17
Other 17:1	0.044	0.060	0.001	<0.001	<0.05	0.41
Total *cis* 18:1	20.25	23.59	0.425	<0.001	0.34	<0.001
Total *trans* 18:1	2.76	2.14	0.055	<0.001	<0.001	<0.05
cis-10 19:1	0.117	0.147	0.004	<0.05	0.08	0.06
Other 19:1	0.030	0.037	0.001	<0.05	<0.05	0.17
cis-9 20:1	0.005	0.004	0.000	<0.05	0.37	<0.001
cis-11 20:1	0.005	0.004	0.000	<0.05	<0.05	0.06
cis-15 24:1	0.015	0.019	0.001	0.06	<0.05	0.63
PUFA						
Total non-conjugated 18:2	2.88	2.78	0.046	0.45	0.22	<0.001
Total conjugated 18:2	0.644	0.436	0.020	<0.001	<0.05	<0.01
cis-6 *cis*-9 *cis*-12 18:3	0.047	0.057	0.002	0.20	<0.001	0.18
cis-9 *cis*-12 *cis*-15 18:3	0.400	0.482	0.014	<0.05	<0.05	0.09
trans-9 *cis*-12 *cis*-15 18:3	0.001	0.002	0.000	<0.001	<0.05	0.10
cis-9 *trans*-11 *cis*-15 18:3	0.052	0.063	0.001	<0.001	<0.05	<0.05
cis-9 *trans*-12 *cis*-15 18:3	0.012	0.015	0.000	<0.05	0.06	<0.001
Other 18:3 non-conjugated isomers	0.007	0.009	0.000	<0.001	0.11	0.06
20:2 n-6	0.009	0.012	0.000	<0.05	0.07	<0.001

Table 1 (continued)

			Probability			
20:3 n-6	0.023	0.024	0.001	0.52	<0.05	<0.001
20:3 n-3	0.015	0.018	0.001	<0.05	<0.001	<0.001
20:4 n-6	0.198	0.182	0.004	0.08	<0.001	<0.001
22:2 n-6	0.026	0.025	0.001	0.76	<0.001	<0.001
20:5 n-3	0.045	0.064	0.002	<0.001	0.06	0.46
22:4 n-6	0.018	0.017	0.001	0.55	<0.05	<0.001
22:5 n-6	0.008	0.008	0.000	0.67	0.15	<0.05
22:5 n-3	0.123	0.136	0.005	0.47	<0.05	0.30
22:6 n-3	0.038	0.061	0.003	<0.001	<0.001	0.06
ΣSFA	69.47	66.56	0.493	<0.05	0.30	<0.001
ΣMUFA	25.83	28.86	0.443	<0.05	0.35	<0.001
ΣPUFA	4.55	4.39	0.070	0.45	0.14	<0.001
ΣBCFA	1.77	1.94	0.035	0.13	<0.001	<0.01
Σ*cis* MUFA	22.83	26.42	0.440	<0.001	0.52	<0.001
Σ*trans* MUFA	3.00	2.45	0.054	<0.001	<0.001	<0.05
cis-9 14:1/(C14:0 + *cis*-9 14:1)	0.013	0.012	0.000	0.18	<0.001	0.72
cis-9 18:1/(C18:0 + *cis*-9 18:1)	0.638	0.660	0.004	0.09	0.66	0.23
RA/(VA + RA)	0.360	0.337	0.005	0.10	0.31	<0.05
AI	2.29	1.91	0.060	<0.05	<0.01	<0.001
n-6/n-3	4.22	3.50	0.099	<0.01	<0.001	<0.001

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *BCFA* branched-chain fatty acids, *RA* rumenic acid, (*cis*-9 *trans*-11 18:2), *VA* vaccenic acid (*trans*-11 18:1), *AI* atherogenicity index (12:0 + 4 × 14:0 + 16:0)/(MUFA + PUFA)

of Mehraban ewes as lactation progressed (Table 4). Vaccenic acid is the quantitatively main of several intermediate products in the rumen biohydrogenation pathways of linoleic and α-linolenic acids (Shingfield and Wallace 2014). Except for the present work, published data of breed effects on the sheep milk fat contents of *cis* and *trans* 18:1 and 16:1 isomers, other than oleic acid and VA, is scarce. Some of those FA may have potentially beneficial effects on human health (Shingfield et al. 2008; Mozaffarian et al. 2013).

Non-conjugated 18:2 were about fourfold and sixfold higher than conjugated 18:2 in Mehraban and Sanjabi ewes, respectively (Table 1). Linoleic acid (*cis*-9 *cis*-12 18:2) was by far the most abundant 18:2 and did not show breed differences (Table 3). However, its content linearly decreased in the milk fat of Mehraban ewes and linearly increased in that of Sanjabi ewes as lactation progressed (Table 4). Most previous studies that compared breed effects on sheep milk FA profile under the same conditions of feeding and management reported no

Table 2 Milk 18:1 fatty acid profile (g/100 g of fatty acid methyl esters) in two fat-tailed sheep breeds

				Probability		
Fatty acids	Mehraban	Sanjabi	SEM.	Breed (*B*)	Week (*W*)	*B* × *W*
cis-9	19.47	22.80	0.418	<0.001	0.33	<0.001
cis-11	0.409	0.501	0.012	<0.001	0.47	<0.05
cis-12	0.222	0.149	0.007	<0.001	<0.001	<0.001
cis-13	0.039	0.044	0.001	0.08	<0.001	0.62
cis-14	0.032	0.022	0.001	<0.001	0.68	<0.001
cis-15	0.025	0.031	0.001	<0.05	0.09	0.56
cis-16	0.055	0.044	0.001	<0.001	0.39	<0.001
trans-4	0.014	0.012	0.001	0.16	0.42	<0.05
trans-5	0.015	0.015	0.000	0.81	<0.001	<0.05
trans-6 + *trans*-7 + *trans*-8	0.231	0.213	0.004	<0.05	<0.001	<0.001
trans-9	0.208	0.185	0.004	<0.05	<0.001	<0.001
trans-10	0.303	0.239	0.007	<0.001	0.07	<0.001
trans-11	1.05	0.747	0.031	<0.001	<0.05	0.14
trans-12	0.264	0.199	0.007	<0.001	0.10	0.12
trans-15	0.400	0.294	0.014	<0.05	<0.05	0.85
trans-16	0.267	0.238	0.005	0.08	0.39	<0.05

Table 3 Milk non-conjugated and conjugated 18:2 fatty acid profile (g/100 g of fatty acid methyl esters) in two fat-tailed sheep breeds

				Probability		
Fatty acids	Mehraban	Sanjabi	SEM	Breed (*B*)	Week (*W*)	*B* × *W*
cis-9 *trans*-13 + *trans*-8 *cis*-12	0.342	0.299	0.006	<0.05	<0.01	<0.01
trans-8 *cis*-13 + *cis*-9 *trans*-12	0.164	0.148	0.003	0.14	0.11	<0.001
trans-9 *cis*-12	0.030	0.029	0.001	0.39	0.14	<0.001
trans-11 *cis*-15	0.069	0.069	0.003	1.00	<0.01	0.41
cis-9 *cis*-12	2.24	2.18	0.039	0.61	0.11	<0.001
cis-9 *cis*-15	0.018	0.028	0.001	<0.001	0.23	0.63
cis-12 *cis*-15	0.004	0.004	0.000	0.96	0.62	0.57
Other non-conjugated 18:2	0.016	0.023	0.001	<0.01	0.19	<0.01
cis-9 *trans*-11	0.596	0.379	0.020	<0.001	<0.05	<0.01
trans-9 *cis*-11	0.007	0.009	0.000	<0.01	0.93	0.65
trans-10 *cis*-12	0.007	0.006	0.000	0.18	0.52	<0.01
trans-11 *cis*-13	0.002	0.002	0.000	0.20	0.64	0.94
trans-12 *trans*-14	0.001	0.001	0.000	0.08	0.53	0.79
trans-11 *trans*-13	0.009	0.011	0.000	<0.01	0.09	0.49
trans-8 *trans*-10 + *trans*-9 *trans*-11 + *trans*-10 *trans*-12	0.022	0.029	0.001	<0.05	<0.01	0.06

Table 4 Contents of selected fatty acids (g/100 g of fatty acid methyl esters) in the milk fat of two fat-tailed sheep breeds during lactation

Fatty acids	Breed	Week of lactation					*P* linear	
		2	4	6	8	10		SEM
4:0	Mehraban	3.81	3.70	3.49	3.57	3.35	0.08	0.056
	Sanjabi	4.18	4.06	3.66	3.55	3.38	<0.001	
6:0	Mehraban	2.72	3.00	2.80	2.92	3.01a	0.11	0.045
	Sanjabi	2.75	2.79	2.63	2.63	2.23b	<0.05	
8:0	Mehraban	2.31	2.69	2.48	2.63	2.87a	<0.01	0.050
	Sanjabi	2.23	2.41	2.24	2.33	1.91b	0.20	
10:0	Mehraban	6.31	7.75a	7.32	8.01	9.36a	<0.001	0.193
	Sanjabi	5.88	6.26b	6.20	6.67	5.28b	0.68	
12:0	Mehraban	3.55	4.00	3.76	4.23	5.15a	<0.001	0.099
	Sanjabi	3.11	3.42	3.22	3.60	3.00b	0.97	
14:0	Mehraban	8.54	9.40	9.39	10.31a	12.15a	<0.001	0.179
	Sanjabi	8.50	8.55	8.77	9.04b	8.32b	0.94	
16:0	Mehraban	23.25b	23.48	22.85	23.69	24.42	0.21	0.204
	Sanjabi	25.23a	23.60	24.31	23.06	23.05	<0.05	
ΣBCFA	Mehraban	1.74	1.87	1.96	2.04	1.95b	0.09	0.040
	Sanjabi	1.62	1.75	1.95	2.11	2.25a	<0.001	
18:0	Mehraban	11.99	11.60	11.28	10.68	9.38b	<0.001	0.188
	Sanjabi	11.18	11.62	11.92	11.79	11.78a	0.52	
cis-9 18:1	Mehraban	21.78	19.23b	21.06	19.13	16.13b	<0.001	0.418
	Sanjabi	22.61	22.69a	22.01	21.93	24.73a	0.42	
trans-11 18:1	Mehraban	1.22a	1.10a	1.08a	1.00a	0.86	<0.01	0.031
	Sanjabi	0.88b	0.76b	0.63b	0.70b	0.76	0.13	
cis-9 *trans*-11 18:2	Mehraban	0.72a	0.60a	0.63a	0.58a	0.45	<0.01	0.020
	Sanjabi	0.42b	0.36b	0.32b	0.36b	0.44	0.68	
cis-9 *cis*-12 18:2	Mehraban	2.55a	2.32	2.35	2.13	1.83b	<0.001	0.039
	Sanjabi	2.05b	2.02	2.19	2.25	2.38a	<0.01	

Within each fatty acid at each week of lactation, least squares means followed by different letters are significantly different ($P < 0.05$) between breeds

BCFA branched-chain fatty acids

breed effects on milk fat linoleic acid content (Talpur et al. 2009; Rozbicka-Wieczorek et al. 2015; Sinanoglou et al. 2015). Most other non-conjugated 18:2 showed statistically significant breed × WOL differences although only *cis*-9 *trans*-13 18:2 was found in relatively large amounts, being higher ($P < 0.05$) in Mehraban than in Sanjabi ewes (Table 3).

Rumenic acid was the most abundant milk CLA isomer (Table 3). Its content was higher in Mehraban ewes, although it showed a decreasing linear trend as lactation progressed and no breed differences were observed in the last WOL (Table 4). Sinanoglou et al. (2015) reported a higher milk fat content of RA in late lactation in one of two sheep breeds, and Tsiplakou et al. (2006) found no effect of days in milk on milk fat CLA of any of four dairy sheep breeds. On the other hand, breed effects on RA content in ewe milk fat are controversial in the literature. Results of Talpur et al. (2009) and Mierlita et al. (2011a, 2011b) agreed with those in the present work, while Tsiplakou et al. (2006, 2008), Rozbicka-Wieczorek et al. (2013, 2015) and Sinanoglou et al. (2015) failed to show any differences. Mierlita et al. (2011b) pointed out that breed differences in the milk fat content of RA might be related to the availability of VA in the mammary gland. In agreement with that, the RA decrease in the milk fat of Mehraban ewes with increasing days in milk, observed in the present work, could be related to the lower availability of VA as lactation progressed (Table 3). It is well known that most RA in sheep milk fat derives from Δ-9 desaturation of VA in the mammary gland (Bichi et al. 2012). In the present work, RA and VA contents were highly correlated in both breeds ($r = 0.83$ and 0.67 in Mehraban and Sanjabi ewes, respectively; $P < 0.001$). From the whole data set, a strictly linear relationship between VA and RA was found: $RA\% = 0.558 * VA\% - 0.0147$; $r^2 = 0.74$; $P < 0.001$, which indicated that RA content in milk fat was a nearly constant proportion of VA content. In addition to RA, a multiplicity of minor geometrical and positional isomers was identified in the present work, though in very small amounts. Published information on the breed effects on those minor CLA isomers is scant.

In both breeds, the α-linolenic acid (*cis*-9 *cis*-12 *cis*-15 18:3) content in milk fat was about 77 % of total identified 18:3 (Table 1). The second most abundant 18:3 was *cis*-9 *trans*-11 *cis*-15 18:3, which is an isomer produced in the rumen biohydrogenation of α-linolenic acid (Bodas et al. 2010). The 20 and 22 PUFA found in the highest proportions in milk fat were 20:4 n-6, 20:5 n-3, 22:5 n-3 and 22:6 n-3 (Table 1). The milk fat contents of α-linolenic acid, its isomers and most of the long-chain n-3 PUFA were significantly higher in Sanjabi than in Mehraban ewes. No significant breed × WOL effects were observed in the n-3 FA contents, except for 20:3 n-3. The only PUFA n-6 that showed a breed difference was 20:2 n-6. Some authors have not found breed differences in the milk fat contents of α-linolenic acid, 20:5 n-3 and 22:6 n-3 (Talpur et al. 2009; Mierlita et al. 2011a, 2011b), while others observed differences in α-linolenic acid and 20:5 n-3 contents (Rozbicka-Wieczorek et al. 2015) or in 22:6 n-3 content (Sinanoglou et al. 2015).

In order to compare the milk FA profiles from a nutritional point of view, atherogenicity index (AI) and the ratio between n-6 and n-3 FA were the chosen indicators (Table 1). The AI value was lower in Sanjabi ewes, i.e. it had a better value, since fat with high AI is assumed to be more detrimental for human health (Ulbricht and Southgate 1991). The n-6 to n-3 FA ratio was higher in Mehraban than in Sanjabi ewes, but both values may be considered favourable from the human health point of view because they were around 4, which is the recommended value in dietary fat (Simopoulos 2008).

In conclusion, under the conditions of the present study, the average milk fat contents of three of the five quantitatively more important FA (10:0, 14:0 and *cis*-9 18:1), as well as other minor FA with nutritional importance (RA, VA, α-linolenic acid and most of the long-chain n-3) differed between Sanjabi and Mehraban breeds. The contents of several FA showed time-dependent changes, so breed differences were more apparent or disappeared as lactation progressed. Milk fat of Sanjabi ewes showed a better FA profile from the human health point of view.

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

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