Acta Alimentaria, Vol. 46 (4), pp. 457–463 (2017)
DOI: 10.1556/066.2017.46.4.8

FATTY ACID PROFILE AND CHOLESTEROL CONTENT OF GHEZEL SHEEP MILK DURING LACTATION PERIOD

A. Alizadeh[a]*, M.R. Ehsani[b] and L. Rofehgarinejad[a]

[a]Department of Food Science and Technology, Tabriz branch, Islamic Azad University, Tehran Rd., 5157944533, Tabriz. Iran
[b]Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Daneshgah Blvd, Simon Bulivar Blvd, 1477893855, Tehran. Iran

(received: 3 December 2016; accepted: 23 January 2017)

The chemical composition, fatty acid profile, and cholesterol content of milk fat were analysed during the lactation period of thirty Iranian Ghezel sheep. They were fed dry hay for the first three months and then grazed on fresh grass to the end of lactation, along with barley and wheat middling during the whole period. Fatty acid profile analysis showed palmitic acid to be the dominant fatty acid (45.24±1.88%). During lactation C6:0, C8:0, C10:0, C12:0, and C14:0 contents decreased, while C18:0, C18:1, C18:2, and CLA increased significantly, which can be associated with the change of nutrition from hay to fresh grazing. The cholesterol content of the sheep milk reached 14.88 mg/100 ml milk or 283.43 mg/100 g fat as an average for the whole period of milking. Regression analysis showed a significant increase in cholesterol from 5.42 to 32.87 mg/100 g milk during the lactation period.

Keywords: chemical composition, cholesterol, fatty acid, milk, sheep

Milk has an important role in human nutrition due to its complex nutrients that meet the metabolic and growth needs. Although milking cows produce a significant proportion of worldwide milk production, small ruminants, such as goats and sheep, have a special place regarding the specific composition of their milk and its impact on health (Strzalkowska et al., 2009). Considering that dairy goat and sheep farming are a vital sector in some Mediterranean and Middle Eastern economies, they require development towards becoming more industrial. Information on the composition of sheep milk and related products would be essential for this to occur (Park & Haenlein, 2006). Milk composition is affected by several factors such as breed, stage of lactation, milking system, health, environment, and feeding (Bocquier & Caja, 1999; Kuchtik et al., 2008). The effect of lactation period on milk composition in different sheep breeds have been studied by several authors (Kuchtik et al., 2008), but the results do not support each other probably because of different factors that may change sheep milk composition (Nudda et al., 2002)

According to the FAO (2014) reports, Iran ranks eighth in the world for sheep milk production, which is about 5% of the total milk production by country. The sheep in the region are mostly, more than 95%, of the Ghezel breed, which has been noted as one of the two distinctive milk type breeds in Iran (Valizadeh, 2010). The population of this breed is about 1.8 million in Iran, and classified as fat-tail, heavy weight ones with mean lactation yield of 100–220 kg (Izadifard & Zamiri, 1997; Baneh et al., 2013). Sheep milk in the region is mostly processed as cheese. Lighvan is a village in a mountainous area in the province of

* To whom correspondence should be addressed.
Phone: +98 912 3309182; e-mails: a.alizadeh@iaut.ac.ir, ainaz_alizadeh@hotmail.com

Azerbaijan in Iran, and is famous for its specific semi-hard sheep-milk cheese. Although Lighvan cheese's composition has been analysed in several papers, there is no study that investigates this breed's milk composition. Meanwhile, studies on the cholesterol content of sheep's milk is limited to just a few studies and needs to be distinguished more with details of different breeds.

Therefore, the aim of the present study is to evaluate the major chemical components, fatty acid profile, and cholesterol content of sheep milk throughout the lactation period in order to report an average for the content and needed changes in the procedure during the lactation period to help dairy technologists, cheese producers, nutritionists, and physicians.

1. Materials and methods

1.1. Animals feeding and sampling

The study was carried out on thirty Iranian Ghezel sheep, maintained in the most experienced and biggest traditional farm of the region, Eidi. The manuscript contains experimental animals, the study was conducted in accordance with the internationally accepted principles for laboratory animal use and care, and our ethical committee on animal care approved the protocol. Research has been done in the veterinary Faculty, Tabriz branch, Islamic Azad University that is under supervision of the Science minister of Iran. The sheep were selected with the help of a veterinarian in charge of milking the region's animals. All of the selected animals were in good condition and clinically healthy. They were numbered and fed the same diet as all cattle, a prevalent way of feeding in the region. For the first three months of lactation (February–April), feeding was with dry hay in caves, but throughout the second three months (May–July), hay was substituted with free fresh grazing. Barley and wheat middling were also added to their diet during the period of lactation. This method of feeding was similar to that of the other sheep keepers in the region, without any changes made in order to attain the actual milk composition in the area. Milking was carried out by a combined lamb-suckling and hand-milking procedure during the suckling period and by twice daily hand-milking from weaning to the end of lactation. Lambs were weaned about 100 days after lambing. Milk samples were gathered from each encoded sheep every month from two weeks of parturition after the colostrum stage to the end of lactation lasting about six months. Milk samples were kept in a freezer at –45 °C for analysis.

1.2. Chemical composition analysis

Milk samples were analysed for fat, protein, lactose, and total solids (TS) contents using MilkoScan (Minor 78100, FOSS, Denmark), which was calibrated each month before the tests. Soluble nitrogen content was detected by the standard Kjeldahl method according to the AOAC 16.041 (1990).

1.3. Fatty acid profile

Fatty acid composition of the frozen milk samples was determined with the direct transesterification method as proposed by Lepage and Roy (1986). A 100 ml milk sample was mixed with 2 ml methanol:benzene (4:1) and then 200 ml acetyl chloride was added. The methanolised sample was kept at 45 °C for 1 h, 5 ml K_2CO_3 was added to cooled vials, and finally it was centrifuged at 2000 *g*. The upper phase was injected to GC.

1.3.1. Gas chromatography (GC). GC (Model 610, Buck Scientific, USA) was equipped with a flame ionization detector and capillary column (TR-CN100, fused silica, 60 m × 0.25 mm × 0.2 μm film thickness, Teknokroma, Italy). The injection volume was 1 μl and the split ratio was set at 1:100. Injection and detection temperatures were 240 °C and 260 °C, respectively. The initial temperature of the oven was set at 190 °C for eight min, and then increased with a rate of 1 °C min^{-1} to reach 260 °C. Helium was used as the carrier gas with a flow rate of 2 ml min^{-1}. Fatty acid methyl esters were identified by comparison of their retention times with authentic standards analysed under the same conditions. Results were expressed as % w/w of total fatty acid.

1.4. Cholesterol analysis

1.4.1. Sample preparation. Five ml KOH was added to 0.5 g milk and mixed for 15 s. The lower phase was heated at 80 °C for 15 min and vortexed every five minutes for 10 s. Then, 1 ml of water and 5 ml hexane were added to the sample and shaken vigorously for 1 min. After centrifugation at 2000 *g*, the upper phase was separated for injection to GC (Fletouris et al., 1998).

1.4.2. GC condition for cholesterol analysis. The column was capillary TRB sterol (30 m × 0.22 mm × 0.22 μm) (Teknokroma, Italy). The detector was FID adjusted at 300 °C. Oven temperature was set at 285 °C, while injection temperature was 300 °C. Carrier gas was helium with a flow rate of 3 ml min^{-1}. Injection volume was 1 μl.

1.4.3. Quantitative analysis. Equal concentrations of standard cholesterol and alpha-cholestane (as internal standard) were prepared and injected to GC. The resulted peak areas were divided to reach a factor (F).

An identified quantity of internal standard (50 μg) was added to the sample and injected to GC. The final concentration of cholesterol was quantified by multiplying 50 F and cholesterol peak area, which accordingly divided by internal standard peak area.

1.5. Statistical analysis

One-way analysis of variance and subsequent comparison of means by the least significant difference (LSD) method at 5% probability level was carried out. Chemical composition was assessed by regression model. Software including SAS and SPSS were used for statistical calculations.

2. Results and discussion

2.1. Milk composition

Chemical composition of milk samples from thirty sheep (Ghezel breed) gathered during the six months of the lactation period is shown in Table 1. Total average chemical composition was obtained as: fat=5.41±1.48, protein=6.58±0.78, lactose=4.79±0.80, and total solids 17.52±2.03 (% w/w) (mean ± SD). Comparing these data with literature revealed an unexpected result related to protein content, which was more than fat content, while most studies have reported the opposite (Park et al., 2007). In order to analyse this result, soluble nitrogen and, accordingly, the casein content of the samples were detected. Casein content

was 79±3% (mean ±SD) of the total protein, which was quite normal. This means that the higher proportion of protein content in the sheep milk did not correlate with whey proteins that might originate from diseases or other factors. Higher protein content could be related to nutrition, in that a high level of nutrition and energy balance will decrease fat content and increase protein content in sheep milk (Bocquier & Caja, 1999). Furthermore, cereals in the diet reduce acetate synthesis in the rumen, which is a precursor of fat (Pulina et al., 2006). Breed could also be an important factor that identifies sheep milk composition (Bocquier & Caja, 1999). In the first month, the average fat content of the samples in this study was very low. Samples were aimed to be gathered after second weeks of parturition, where there was a sharp increase in milk yield as reported by Izadifard and Zamiri (1997) in the second week from lambing for Ghezel sheep.

Table 1. Sheep milk chemical composition across the 6 months of lactation

Lactation months	Fat (%)		Protein (%)		Lactose (%)		TS (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1st	2.61	0.83	5.46	0.65	5.57	0.15	14.42	0.83
2nd	4.91	1.43	5.78	0.54	5.31	0.24	16.64	1.23
3rd	5.16	1.15	6.65	0.66	5.07	0.22	17.46	1.06
4th	5.60	1.08	7.09	0.70	4.65	0.29	18.04	1.40
5th	5.27	1.01	7.25	0.56	4.82	0.15	17.89	1.06
6th	7.93	1.24	9.03	2.46	3.29	1.44	20.67	2.21
LSD (5%)	1.02		1.03		0.55		1.23	

Data are mean±SD (n=30). LSD (5%): Least Significant Difference at $P<0.05$

Milk composition changes during lactation were significant ($P<0.05$), the results of which are shown in Table 1. It can be concluded that fat, protein, and TS contents increased, while lactose content decreased during the lactation period. In milk of sheep and other cattle, at the peak of lactation with maximum lactose synthesis, protein and fat synthesis cannot keep up with the lactose increase (Nudda et al., 2002). It was obvious in our study that at the first half of lactation lactose synthesis was high, but it decreased towards the end, because of the lower milk yield. Sharp changes in sheep milk composition at the sixth month were related to the very low daily milk yield that decreased from 1600 g at first month to about 200 g at sixth month. It would therefore be advisable to study a five-month period of lactation in future studies. Fat content did not change significantly between the second and fifth month, while the maximum protein content was reported for the fourth and fifth months ($P<0.05$) excluding the sixth month. The lowest and highest lactose contents occurred at the fourth and first months, respectively, and finally, the lowest total solids content was recorded for the first month ($P<0.05$).

2.2. Fatty acid profile

Fatty acid profiles of the sixty milk samples gathered during the six months of lactation are shown in Table 2. Palmitic acid was the dominant fatty acid. Although, high palmitic acid is

a feature of the majority of mammals, in this study the content was more than twice the oleic acid content (45.24 vs. 21.08%), while most studies have reported approximately equal percentages of the two fatty acids (Jandal, 1996; Park et al., 2007; Carloni et al., 2009). Medium-chain fatty acids (MCFAs) of C10:0, C12:0, C14:0, and C16:0 contributed to 62% of the total in this study, while the short-chain fatty acid (SCFA) content of C4:0, C6:0, and C8:0 was 4.05%. Obviously, C4:0 and C10:0 percentages were less and palmitic acid contents were higher than previously reported by Park and co-workers (2007). Such results could relate to nutrition and the cereals rich in palmitic acid that were added to the sheep's diet. Other significant factors such as race, climate, and some other minor elements could also be important.

Table 2. Sheep milk fatty acids profile during six months of lactation period

Fatty acid (%)	Months of lactation												
	1st Mean ±SD		2nd Mean ± SD		3rd Mean ±SD		4th Mean ±SD		5th Mean ± SD		6th Mean ± SD		LSD 5%
C4:0	0.97	0.14	0.86	0.28	0.68	0.21	0.83	0.22	0.81	0.29	0.81	0.36	0.13
C6:0	2.73	0.66	2.37	1.00	1.89	0.56	1.12	0.36	1.24	0.24	0.95	0.46	0.18
C8:0	2.59	0.48	2.13	0.87	1.75	0.37	0.97	0.26	1.12	0.24	0.60	0.32	0.16
C10:0	4.85	1.17	4.17	1.46	3.78	1.12	1.69	0.52	2.02	0.47	0.99	0.55	0.23
C12:0	3.58	0.96	3.11	0.84	3.24	1.04	1.52	0.40	1.81	0.30	1.08	0.39	0.19
C14:0	13.30	1.19	14.42	1.26	14.87	2.13	8.83	1.84	11.42	0.72	9.14	3.08	0.25
C15:0	0.85	0.38	0.90	0.35	0.79	0.13	0.84	0.17	1.10	0.17	0.84	0.20	0.11
C16:0	45.64	2.99	46.36	4.14	45.74	2.50	41.85	3.67	47.26	1.56	44.60	2.98	0.21
C16:1	1.34	0.24	1.24	0.23	1.45	0.29	2.23	0.68	1.45	0.46	1.82	0.31	0.14
C18:0	6.90	1.14	7.29	1.05	7.19	1.11	9.24	2.32	9.10	0.77	9.06	2.51	0.24
C18:1	15.85	2.39	15.76	1.97	17.17	2.60	28.84	3.78	20.64	1.85	28.26	5.09	0.30
C18:2	1.33	0.33	1.20	0.21	1.27	0.23	1.75	0.29	1.62	0.32	1.60	0.30	0.11
CLA	0.09	0.05	0.20	0.12	0.18	0.08	0.29	0.17	0.42	0.15	0.28	0.10	0.10

Data are mean±SD (n=30). LSD (5%): Least Significant Difference at $P<0.05$

Changes in fatty acid profile during the lactation period were significant except for C4:0, C15:0, C16:0, and C16:1 ($P<0.05$). Regression analysis for the fatty acids indicated that during lactation, C6:0, C8:0, C10:0, C12:0, and C14:0 contents decreased, while C18:0, C18:1, C18:2, and CLA contents increased significantly ($P<0.05$). This should be in accordance with the change of nutrition from hay to fresh grazing.

Fatty acids of 4–14 carbon atoms are synthesized de novo in the mammary gland, while C18 acids are take in from the circulating blood. C16:0 originates from both, and is less altered (Huppertz et al., 2009).

Polyunsaturated fatty acid (PUFA) content is very important, as these fatty acids have an impact on health. In particular, CLA has such biological activity (Strzalkowska et al., 2009). Milk is an important source of CLA that could respond to more than 75% of human

nutritional demands. Grazing has an important effect on the CLA content of the milk, and the height of the region might also increase CLA content (Carloni et al., 2009). Ligvan is located at a height of 2300 m from sea level, but CLA content was not high compared to previous studies by Park and co-workers (2007).

2.3. Cholesterol content

The cholesterol content of the sheep milk was obtained as 14.88 mg/100 ml milk as an average for the whole period. Considering the average fat content of the milk (5.25 g/100 g milk), the cholesterol content could be reported as 283.43 mg/100 g fat. Although there are not enough precise reports on the cholesterol content of sheep milk, Goudjil and co-workers (2003) have reported a range between 15–30 mg/100 ml milk and 288 mg/100 g fat. Wojtowski and co-workers (2001) reported 26.11 mg/100 ml milk for sheep milk, as well.

Our results showed lower cholesterol content for the Ghezel breed of sheep. However, many factors, such as nutrition, can affect the cholesterol content of sheep milk.

Regression analysis of cholesterol content during the lactation period showed a significant increase ($P<0.05$). Increase in fat content during this period could be a reason for the cholesterol content increase as discussed by Wojtowski and co-workers (2001) for an increase from 23.5 to 30 mg/100 ml milk. Calculations of cholesterol content on fat basis, as shown in Table 3, represent its increase without depending on fat content.

Table 3. Sheep milk cholesterol content during lactation period

	Lactation months					
	1st	2nd	3rd	4th	5th	6th
Cholesterol (mg/100 ml milk)	5.42±2.95 [a]	7.90±1.39 [b]	7.97±5.66 [b]	15.26±2.90 [c]	19.87±7.66 [d]	32.87±7.40 [e]
Cholesterol (mg/100 mg fat)	207.66	160.89	154.45	272.50	377.04	414.50

Data are mean±SD (n=30). [a,b]: Different letters show significant difference between the samples ($P<0.05$)

3. Conclusions

It can be concluded that with the progress of lactation, the fat, protein, and total solids content of sheep milk increased, while lactose content decreased significantly. Furthermore, the sheep milk of the region had a high protein and low fat content compared to other reports. Fatty acid profile analysis showed palmitic acid to be dominant, with permanent content during lactation period. Oleic acid and other UFAs increased with lactation because of fresh grazing during the second half of lactation. The cholesterol content of the sheep milk increased towards the end of lactation independent of fat content. Investigations of different breeds around the world could elucidate sheep milk composition's vague points to define a precise range for its composition and also to select the breeds with superior nutritional benefits in terms of milk composition.

*

We kindly acknowledge supports from the Tabriz Branch, Islamic Azad University, Tabriz, Iran.

References

AOAC (1990): *Official Methods of Analysis of the Association of Official Analytical Chemists* 15th edition. Association of official analytical chemists AOAC, Arlington, VA. Method no. AOAC 16.041

Baneh, H., Rokouei, M., Ghafouri-Kesbi, F., Veysi, A. & Niknafs, S. (2013): Multivariate genetic analysis on body weight traits in Ghezel sheep. *Songklanakarin J. Sci. Technol*., *35*, 131–135.

Bocquier, F. & Caja, G. (1999): *Effect of nutrition on the composition of sheep's milk.* Proceedings of 5th Great Lakes. Dairy Sheep Symposium; Vermont, USA. Available at http://www.dsana.org/Resources/Proceedings/5Proceedings1999.pdf (last accessed 2 December 2016)

Carloni, M., Fedeli, D., Roscioni, T., Gabbianelli, R. & Falconi, G. (2009): Seasonal variation of fat composition in sheep's milk from areas of central Italy. *Mediterr. J. Nutr. Metab., 2*, 1–6.

FAO (2014): *FAOSTAT Database collections.* Food and Agriculture Organization of the United Nations. Rome, Italy. Available at http://www.faostat.fao.org. (last accessed: 3 December 2016)

Fletouris, D.J., Botsoglou, A. & Psomas, I.E. (1998): Rapid determination of cholesterol in milk and milk products by direct saponification and capillary gas chromatography. *J. Dairy Sci., 81*, 2833–2840.

Goudjil H., Torrado, S. & Fontecha, J. (2003): Composition of cholesterol and its precursors in ovine milk. *Dairy Sci. Technol., 83*, 153–160.

Huppertz, T., Kelly, A.L. & Fox, P.F. (2009): Milk lipids-composition, origin and properties. -in: Tamime, A.Y. (Ed.) *Dairy fats and related products.* Blackwell, UK. pp. 1–27.

Izadifard, J. & Zamiri. M.J. (1997): Lactation performance of two Iranian fat-tailed sheep breeds. *Small Ruminant Res., 24*, 69–76.

Jandal, J.M. (1996): Comparative aspects of goat and sheep milk. *Small Ruminant Res., 22*, 177–185.

Kuchtik, K.J., Sustova, K., Urban, T. & Zapletal, D. (2008): Effect of the stage of lactation on milk composition, its properties and the quality of rennet curding in East Friesian ewes. *Czech J. Anim. Sci., 2*, 55–63.

Lepage, G. & Roy, C.C. (1986): Direct trans-esterification of all classes of lipids in a one-step reaction. *J. Lipid Res., 27*, 114–120.

Nudda, A., Bencini, R., Mijatovic, S. & Pulina, G. (2002): The yield and composition of milk in Sarda, Awassi and Merino sheep milked unilaterally at different frequencies. *J. Dairy Sci., 85*, 2879–2884.

Park, Y.W., Juarez, M., Ramos, M. & Haenlein, G.F.W. (2007): Physicochemical characteristics of goat and sheep milk. *Small Ruminant Res., 68*, 88–113.

Park, Y.W. & Haenlein, G.F.W. (2006): *Handbook of milk and non-bovine mammals.* Wiley-Blackwell Ltd, UK, pages 472.

Pulina, G., Nudda, A., Battacone, G. & Cannas, A. (2006): Effects of nutrition on the contents of fat, protein, somatic cells, aromatic compounds, and undesirable substances in sheep milk. *Anim. Feed Sci. Tech., 131*, 255–291.

Strzalkowska, N., Jozwik, A., Bagnicka, E., Krzyzewski, J., Horbanczuk, K., Pyzel, B. & Horbanczuk, J.O. (2009): Chemical composition, physical traits and fatty acid profile of goat milk as related to the stage of lactation. *Anim. Sci. Pap. Rep., 27*, 311–320.

Valizadeh, R. (2010): Iranian sheep and goat industry at a glance. -in: Karim, S.A. & Joshi, A. (Eds) *Climate changes and stress management*: *Sheep and goat production*. SSPH Pub., India. pp. 431–440.

Wojtowski, J., Dankow, A., Gut, A. & Pikul, J. (2001): Fatty acid composition and cholesterol content of sheep and goat milk fat during lactation. *Arch. Tierzucht., 44,* 299–308.