COMPARATIVE STUDY REGARDING THE FATTY ACIDS PROFILE IN SHEEP MILK RELATED TO THE BREED AND PARITY

Mierlita D., Padeanu I., Maerescu Cristina, Chereji I, Halma Elena, Lup F.

University of Oradea, Department of Animal Science, Oradea City, Romania; e-mail: dadi.mierlita@yahoo.com

Abstract

Our objective was to analyse milk yield, milk components, and especially fatty acid profile of ewes' milk fat, in relation with breed, and parity. The sheep were used in a 3 x 2 factorial arrangement consisting of a combination of breeds (n = 3: Merinos of Transylvania, Tigaie and Turcana) and parity (n = 2: primipara and multiparous).

Turcana ewes yielded more milk, and milk fat had a lower content of saturated FA and a higher content of polyunsaturated FA, so that the atherogenic index recorded the best value for this breed. Turcana breed also had the highest concentration of cis-9, trans-11 CLA (conjugated linoleic acid) in milk, which was due not only to an increase of C18:1 trans-11 supply in the rumen, but also to an increase in Δ^9 – Desaturase enzyme activity. Milk n-3 FA content, mainly that in α – linolenic acid, was not influenced by breed, but it decreased as lactation number increased. Milk yield and its components increased with parity, while the proportion of n-3 FA and cis-9, trans-11 CLA in milk fat decreased, that is, the nutritional quality of milk decreased if we look at its influence on human health.

Keywords: sheep milk, n-3 FA and CLA, breeds and parity.

INTRODUCTION

Manipulation of milk fat fatty acid profile with the help of dietary and physiological factors can bring benefits to human health, from reduction in cholesterol, to inhibition of some forms of cancer (Simopoulos A., 1999). It is widely recognized that dietary factors have a supreme role in modulating fatty acid profile in milk from cows (Rego et. al., 2009), goats (Chilliard et. al., 2003; Andrade et. al., 2006) and ewes (Addis et. al., 2005; Gomez-Cortes et. al., 2008, 2009; De La Fuente et. al., 2009).

Nevertheless, the effects of physiological factors, such as breed and parity on milk fat FA profile were given little attention. Studies quoted in professional literature focus mainly on dairy cows (Kelsey et. al., 2003; Kay et. al., 2005; Ferlay et. al., 2006; Palladino et. al., 2010), while information about small size ruminants, such as ovine, are still scarce. Studies on the influence of some physiological factors linked to breed and parity on ovine milk FA content were carried out by: Barbosa et. al. (2003); Mihaylova et. al. (2004); Tsiplakou et. al. (2006); Federica et. al. (2008); Gerchev and Mihaylova (2009). In several of these studies there were no identical management and dietary practices provided to establish the effects of

physiological factors on various FA in milk, with a particular focus on CLA. The differences included pastures with different botanical compositions, located at different altitudes and being in different stages of vegetation or in different seasons. Carta et. al., (2002) noticed that sheep milk is much richer in n-3 FA and CLA than that of cow milk; a possible reason for that might be that sheep are pasture-fed, while dairy cows are usually fed on preserved and concentrate feed.

In these conditions, with our study we proposed to determine milk quality parameters and the fatty acid profile of milk fat for three breeds, regarded as local breeds on the verge of extinction due to their low productivity (Merino of Transylvania – MT, Tsigay – TG and Turcana - TU), in relation with parity. We gave a particular attention to milk fat n-3 FA and CLA content, not only because of their importance to human health, but also because they might help in the sustainable use of these local breeds' genetic resources. Due to its fat and protein content, TU and TG milk is used to produce some high quality traditional cheeses ('cas', 'telemea' and 'branza de burduf').

MATERIALS AND METHODS

Animals, experimental design and feeding

The study was conducted using 72 lactating ewes: Merino of Transylvania (MT) (n = 24; 12 primipara and 12 multiparous – third lactation); Tsigay (TG) (n = 24; 12 primipara and 12 multiparous – third lactation 3) and Turcana (TU) (n = 24; 12 primipara and 12 multiparous – third lactation). The ewes were selected from private herds, taking into account similar milk yields, weight and lambing dates, and divided into 6 groups of 12 each. We used a 3 x 2 factorial arrangement constituted by a combination of breeds (n = 3: MT, TG and TU) and parity (n = 2: primipara and multiparous). Each group was kept in sheepfolds within enclosed facilities, under identical environment practices.

The study was conducted after the weaning of lambs, from May to August 2010. The first two weeks of the experiment period were used to adapt rumen microflora to the new diet; no samples were taken in this period. The sheep were kept on a diet consisting of preserved fodder administered in the facilities, in order to eliminate the confusing results of grazing (changes in the pasture composition during grazing) and the influence of seasons. The diet of the sheep consisted of hay (0.5 kg alfalfa hay and 0,5 kg meadow hay), corn silage (ad libitum) and a concentrate mix (0,7 kg) of: corn – 41%; triticales – 30%; soybean meal – 10%; sunflower meal – 15% and vitamin-mineral premix – 4%. The diet met the nutritional needs (energy and protein

level) recommended for lactating ewes (Mierlita et. al., 2009) to eliminate any influence of nutritional factors on the results of the experiment. The ewes were fed every morning and evening at approximately the same hours, 9:00 a.m. and 7:00 p.m. respectively.

Sampling and chemical analysis

Individual milk yields were recorded once a week (on the same day of the week) for a two-month period, that is, 8 samples for each ewe. The ewes were milked manually twice a day, at approximately 7:00 a.m. and 5:00 p.m. Two milk samples for analysis were also collected from each ewe weekly: one was used to determine milk components (fats, proteins – N x 6.38 and lactose) and the other one to determine FA profile of milk fat. Milk samples for FA content were frozen at -20^0C as soon as they were obtained and kept like that until analysis, while milk samples for analysis of the chemical composition of milk were preserved with 2-bromo-2-nitropropane-1-2-diol and stored at 4^0C. The milk samples collected were used to determine fat content (Gerber method), total nitrogen (Kjeldahl method), lactose (with a phenol-HCl reducing solution using an Auto-analyzer: Technicon, France) and FA composition of milk fat.

Milk samples collected for FA analysis were defrosted in a water bath at 35^0C. Fatty acid methyl esters (FAME) were prepared by base-catalysed methanolysis of the glycerides according to ISO-IDF (2002). FAME was separated using a SHIMADZU GC-17A gas chromatograph fitted with a CHROMPACK, with a 100 m column length and 0.25 mm diameter; the stationary phase (a polyethylene glycol derivative) being a 0.2 μm thick coating film on the inner column. We used an FID detector, and 99.9 % purity helium as mobile phase.

The operating parameters of the gas chromatograph were the following: injector and detector temperature 260^0C; column temperature an oven with initial temperature of 150^0C for 5 minutes and a 4^0C temperature gradient per minute up to 235^0C; carrier gas flow rate 1.9 ml/minute and a split rate of 1:19. After the gas chromatograph reached the programmed operating parameters, 0.5 μl methyl esters solutions of fatty acids were injected manually using a Hamilton syringe. Individual FA were identified by comparisons with retention times of standards (Sigma-Aldrich, St. Louis, USA) and expressed in g /100 g of total fatty acid methyl esters.

Calculations and Statistical Analyses

Before analyses, the data on fatty acid composition were processed to compute the content of saturated fatty acids (STA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). In the case of PUFA, n-3 FA (C18:3 + C20:5 + C22:3 + C22:5 + C22:6), n-6 FA (C18:2 + C20:4) and the n-6 : n-3 ratio were calculated according to Ellis et. al.

(2006). Δ^9 – Desaturase ratios were also calculated according to Kelsey et. al., (2003) as product of Δ^9 – desaturase / (product of Δ^9 – desaturase + substrate of Δ^9 – desaturase); Δ^9 – Desaturase index based on Kay et.al., (2005) and atherogenic index according to Chilliard et. al. (2003), thus (C12:0 + 4 x C14:0 + C16:0) : (MUFA + PUFA).

Data obtained were analyzed using the MIXED procedure of SAS for repeated measurements. Differences caused by breed and parity were regarded significant for $p < 0.05$.

RESULTS AND DISCUSSION

Milk yield and chemical composition

Mean results for milk yield, percentages of fat, protein and lactose in milk for primipara and multiparous MT, TG and TU breeds are presented in Table 1.

Table 1

Milk production and milk composition in relation to breed and parity

Variables		Breed[1]			SEM[2]	p values effects[3]		
		Merino of Transylvania	Tsigay	Turcana		M vs. Tg	M vs. Tu	Tg vs. Tu
Milk yield, g/d	P	181^{a}	308^{A}	363^{A}	97.23	**	**	**
	M	228^{b}	430^{B}	554^{B}		**	**	**
Fat, %	P	4.89^{c}	6.34^{A}	6.65^{A}	0.877	**	*	*
	M	5.40^{d}	6.91^{B}	7.16^{B}		*	*	NS
Protein (N x 6,38), %	P	5.40^{a}	5.82	5.83^{A}	0.741	NS	NS	NS
	M	5.80^{b}	6.10	6.23^{B}		NS	NS	NS
Lactose, %	P	4.78	4.43^{c}	4.67^{a}	0.240	NS	NS	NS
	M	4.90	4.76^{d}	4.87^{b}		NS	NS	NS

P – primipara, M – multiparous (third lactation);
[1]Subcolumn means between rows with different superscript differ ($^{A, B}p < 0.01$; $^{a, b}p < 0.05$; $^{c, d}p < 0.10$);
[2] SEM = standard error of mean;
[3]M = Merinos of Transylvania; Tg = Tsigay; Tu = Turcana (** $p < 0.01$; * $p < 0.05$; NS = not significant $p > 0.10$).

Milk yield and its composition were characteristic to each breed; TU and TG ewes had higher milk yields, with higher fat and protein (N x 6,38) contents, than those of MT ewes, this latter one having both lower milk yield and milk components (TU > TG > MT). These results applied to both primipara (first lactation) and multiparous (third lactation) ewes, with the observation that both milk yield and milk components had lower values ($p<0.10$) in the case of primipara ewes. There were no significant differences in respect of milk lactose content in relation with breed and parity. The results of this study match those obtained by Mihaylova et. al.,

(2004), and Lujerdean et. al.(2009) both in respect of milk yield and of its components.

Fatty acid profile in milk fat

a) *Effect of breed*

Table 2 shows fatty acids profile of milk by breed (MT, TG and TU) and parity (primipara – first lactation and multiparous – third lactation). We found significant differences between breeds in C4:0, C6:0, C10:0, C12:0, C14:0, C17:0 and C18:0, belonging to SFA; in C16:1, C18:1 belonging to MUFA and in C18:2 and CLA belonging to PUFA. We found the highest concentration of SFA ($p<0.01$), especially capric acid (C10:0), lauric acid (C12:0), and myristic acid (C14:0), and the lowest concentration of MUFA ($p<0.01$), especially oleic acid (C18:1 *cis*-9), for MT ewes.

Table 2

Fatty acids content of milk fat in relation to sheep breed and parity

Fatty acid (g 100/g FAME)[1]	Merino of Transylvania		Tsigay		Turcana		SEM[2]	p – values effects	
	P	M	P	M	P	M		effect of breed	effect of parity
C4:0	4.65^{b}	4.76^{b}	4.62^{b}	4.93^{b}	3.54^{a}	3.11^{a}	0.17	*	NS
C6:0	2.11^{a}	2.01^{a}	3.03^{b}	2.78^{b}	1.87^{a}	2.31^{a}	0.22	*	NS
C8:0	2.70	2.56	1.97	2.05	1.81	2.40	0.13	NS	NS
C10:0	9.21^{b}	9.58^{b}	6.12^{a}	6.57^{a}	5.69^{a}	6.07^{a}	0.73	**	NS
C12:0	4.43^{c}	4.41^{c}	3.35^{ab}	3.57^{b}	2.60^{a}	2.62^{a}	0.17	***	NS
C14:0	12.16^{bc}	13.19^{c}	10.23^{a}	11.14^{b}	9.76^{a}	10.24^{a}	0.33	**	*
C15:0	1.40	1.45	1.42	1.56	1.29	1.44	0.07	NS	NS
C16:0	23.05^{a}	24.21^{b}	23.73^{a}	24.62^{b}	24.87^{b}	26.70^{c}	0.40	*	**
C17:0	0.49^{a}	0.61^{ab}	0.98^{b}	0.96^{b}	0.87^{b}	0.92^{b}	0.07	**	NS
C18:0	12.61^{b}	13.15^{b}	12.74^{b}	13.52^{b}	11.80^{a}	12.45^{ab}	0.24	*	NS
C14:1	0.43^{b}	0.28^{a}	0.23^{a}	0.22^{a}	0.19^{a}	0.17^{a}	0.03	NS	*
C16:1	0.97^{b}	0.72^{ab}	0.53^{a}	0.54^{a}	0.89^{b}	0.78^{ab}	0.02	*	NS
C18:1 *trans-9*	0.21^{a}	0.16^{a}	0.39^{a}	0.33^{a}	0.53^{b}	0.49^{b}	0.01	*	NS
C18:1 *trans-11* (VA)	9.02^{a}	6.77^{b}	7.52^{b}	6.15^{b}	10.57^{a}	8.88^{ab}	0.41	*	**
C18:1 *cis-9*	9.09^{a}	9.86^{a}	14.55^{b}	13.76^{b}	14.20^{c}	13.19^{b}	0.55	**	NS
C18:1 *cis-11*	0.50^{a}	0.35^{a}	0.49^{a}	0.42^{a}	0.71^{b}	0.49^{a}	0.07	NS	*
C18:2 *n-6 trans*	0.33^{a}	0.22^{a}	0.61^{b}	0.44^{a}	0.42^{ab}	0.34^{a}	0.06	*	*
C18:2 *n-6 cis*	1.70^{a}	1.73^{a}	2.53^{b}	2.20^{b}	2.81^{b}	2.48^{b}	0.07	*	NS
C18:2 *c-9, t-11* CLA	2.52^{b}	2.03^{a}	2.55^{b}	2.15^{a}	3.17^{b}	2.84^{b}	0.05	**	*
C18:3 n-6	0.61^{b}	0.55^{a}	0.68^{b}	0.60^{a}	0.64^{b}	0.57^{a}	0.03	NS	*
C18:3 n-3 (ALA)	0.99^{b}	0.86^{a}	1.10^{b}	0.92^{a}	1.03^{b}	0.88^{a}	0.04	NS	*
C20:4 n-6	0.17	0.13	0.18	0.17	0.17	0.14	0.01	NS	NS
C20:5 n-3, EPA	0.10^{a}	0.11^{a}	0.14^{b}	0.09^{a}	0.14^{b}	0.12^{ab}	0.01	NS	*
C22:3 n-3	0.09	0.08	0.07	0.07	0.10	0.08	0.00	NS	NS
C22:5 n-3, DPA	0.18	0.15	0.16	0.15	0.21	0.20	0.01	NS	NS
C:22:6 n-3,	0.09	0.07	0.08	0.09	0.12	0.09	0.00	NS	NS

DHA									
SFA	72,81^{c}	75.93^{c}	68.19^{b}	71.70^{b}	64.10^{a}	68.17^{b}	0.97	**	*
MUFA	20.22^{ab}	18.14^{a}	23.71^{bc}	21.42^{b}	27.09^{c}	24.09^{bc}	0.68	**	*
PUFA	6.98^{b}	5.92^{a}	8.10^{bc}	6.88^{ab}	8.81^{c}	7.74^{b}	0.11	*	**
n -3 FA	1.45^{a}	1.27^{b}	1.55^{a}	1.32^{b}	1.60^{a}	1.37^{b}	0.08	NS	*
n - 6 FA	2.81^{b}	2.63^{b}	4.00^{a}	3.41^{ab}	4.04^{a}	3.53^{ab}	0.07	*	NS
n - 6 : n - 3	1.94^{b}	2.07^{b}	2.58^{a}	2.58^{a}	2.52^{a}	2.58^{a}	0.03	*	NS
Atherogenic index[3]	2.80^{b}	3.38^{c}	2.14^{a}	2.57^{b}	1.85^{a}	2.20^{a}	0.14	***	*
Δ^9 – Desaturase ratios[4]									
C14	0.034^{b}	0.021^{a}	0.022^{a}	0.019^{a}	0.019^{a}	0.016^{a}	0.002	*	NS
C16	0.040^{c}	0.029^{b}	0.022^{a}	0.021^{a}	0.035^{bc}	0.028^{b}	0.002	*	*
C18	0.60^{a}	0.57^{a}	0.64^{ab}	0.60^{a}	0.68^{b}	0.64^{ab}	0.03	*	NS
CLA	0.22^{b}	0.23^{ab}	0.23^{ab}	0.24^{a}	0.25^{ab}	0.26^{a}	0.015	*	NS
Index Δ^9 – Desaturase[5]	0.30^{b}	0.27^{a}	0.33^{ab}	0.30^{a}	0.36^{b}	0.32^{ab}	0.018	**	*

P – primipara, M – multiparous (third lactation); [1]FAME - fatty acid methyl esters; [2] SEM = standard error of mean;

[3] Atherogenic index was calculated according to Chilliard et. al., (2003), as follows: (C12:0 + 4 x C14:0 + C16:0) : (MUFA + PUFA);

[4] Calculated for each pair of FA according to Kelsey et. al., (2003) as: (product of Δ^9 – desaturase) : (product of Δ^9 – desaturase + substrate of Δ^9 – desaturase); ie: C14 : C14:1 : (C14:1 + C14:0);

[5] Calculated according to Kay et. al., (2005), as follows: (C14:1 + C16:1 + C18:1 *cis-9* + CLA *cis-9, trans-11*) : (C14:0 + C16:0 + C18:0 + C18:1 *trans-11* + C14:1 + C16:1 + C18:1 *cis-9* + CLA *cis-9, trans-11*).

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids;

[a, b, c]: means with different superscripts differ significantly;

NS p>0.05, *p<0.05, **p<0.01, ***p<0.001.

Comparing TG and TU breeds, in the milk of the latter one there was a smaller content in butyric acid (C4:0), caproic acid (C6:0) lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0). The total of short chain fatty acids (SCFA) and of medium-chain fatty acids (MCFA) in milk (< 16 carbons) was higher (p<0,05) for MT ewes, while the lowest proportion was recorded for TU ewes (MT >TG >TU). Short and medium FA (C4:0 – C14:0 and approximately half of C16:0) are de novo synthesized in the mammary gland, from rumen fermentation of acetic acid and ß – hydroxybutyrate, being influenced both by the energy balance of diet and the specificity of rumen microflora (Chilliard et. al. 2003). Addis et. al. (2005) reported that ewes with higher milk yield also have a higher SCFA and MCFA in milk. This finding was not confirmed by our studies, as we found the highest levels of SCFA and MCFA in milk where the yield was lowest. Long chain fatty acids (LCFA) in milk fat showed a contrary tendency to that of SCFA and MCFA.

Global increase in total PUFA concentration in the milk of TU and TG ewes, by 28.37% and 16.12% respectively, compared to MT ewes is considered beneficial to human health.

The n-3 FA content in milk was not influenced by breed. These FA originate from feed, the concentration in milk depending on their amounts in rumen. Our experimental design avoided dietary confusions, as the ewes were fed the same diet and sampled on the same day. We assume that the minor differences between breeds ($p>0.05$) are due to rumen microflora particularities. The values obtained by us in this study for n-3 FA content of milk, and that of ALA (C18:3 n-3) in particular, are comparable to those reported previously by Dimitrov et. al., (2001) for Corriedale sheep; Mihaylova et. al., (2004) for Tsigay and Karakachan sheep; Mele et. al., (2006) and Federica et. al., (2008) for Sarda sheep; de Gerchev and Mihaylova, (2009) for Tsigay sheep; De La Fuente et al., (2009) and Sanchez et. al., (2010) for Spanish Churra sheep.

The n-6 : n-3 FA ratio was smaller in the milk fat of MT sheep (2.00 : 1) (MT< TU< TG), being closer to the optimum level suggested for human diet 1 : 1 (Simopoulos, 2008). Thus, our results confirmed conclusions drawn by several previous studies (Collomb et. al., 2006; Gerchev and Mihaylova, 2009), who demonstrated that sheep milk is an important n-3 FA source for humans. In contrast to our study, Bouattour et. al., (2007), found a much higher n-6/n-3 ratio, that is, 3.87 – 4.47 : 1, for Lacaune breed.

The *cis*-9, *trans*-11 CLA concentration in milk fat was significantly influenced by breed, the highest values being recorded for TU sheep ($p<0.05$). We recorded a strong correlation between milk fat VA (trans-11 C18:1) and *cis-9, trans-11* CLA, on the one hand, and *cis*-9, *trans*-11 CLA and the Δ^9 – Desaturase system, on the other hand; these correlations matched the findings reported by Kay et. al., (2005) in cow milk fat ($r = 0.16 – 0.63$). The Δ^9 – Desaturase enzyme acts at the level of mammary gland and of other tissues, and it introduces a double bond with a Δ^9 specificity and in this way converts myristic acid (14:0) into myristoleic acid (*cis-9* 14:1), palmitic acid (16:0) into palmitoleic acid (*cis-9* 16:1) stearic acid (18:0) into oleic acid (*cis-9* 18:1) and vaccenic acid (VA: *trans-11* 18:1) into a conjugated linoleic acid isomer, that is, rumenic acid (RA: *cis-9, trans-11* 18:2) (Bauman et. al., 2006). Garnsworthy et. al., (2010) argues that the Δ^9 – Desaturase enzyme activity has an important genetic component, which allows us to conclude that the higher level of *cis-9, trans-11* CLA in TU sheep milk fat is the result of an increased Δ^9 – Desaturase activity, shown by a higher index Δ^9 – Desaturase index, but also of a higher VA supply in rumen caused probably by rumen microflora differences. The lower level of *cis-9, trans-11* CLA in MT sheep milk fat might be caused by rumen bacteria that favors production of propionic acid and which are opposed to those that help VA production through biohydrogenation, that of linoleic acid in particular (Bauman et. al., 2006).

As MT sheep have a higher genetic potential for meat production than TG and TU sheep, their rumen bacteria favour the production of propionic acid, which is specific to tissue synthesis (Mierlita et. al., 2009).
Our findings about milk fatty acid profile for TG and TU breeds match those reported by Federica et. al. (2008) for local Italian breeds (Gentile di Puglia and Altamurana) and confirm the fact that when milk yield increased, myrisitc acid and the total of saturated FA (SFA) decreased, while polyunsaturated FA (PUFA) increased in milk fat. The differences recorded by us in comparison with previous studies (Addis et. al., 2005; Collomb et. al., 2006; Hervas et. al., 2009) resulted not only from differences in breeds, but mainly from the fact that the sheep were pasture-fed, which favored increase of n-3 FA and CLA , and decrease of SFA in milk fat.

The atherogenic index (Tabel 2), which defines food fats in respect of impact on human health, was lower ($p<0.001$) in the milk fat of TU sheep than in that of MT sheep (TU < TG < MT); there was a direct correlation between the saturated FA content and the value of this index (Addis et. al., 2005). Fats with a higher atherogenic index are more detrimental to human health.

b) *Effect of parity*

Parity influenced mainly the content of myrisitc acid (C14:0), palmitic acid (C16:0), myristoleic acid (C14:1), oleic acid (C18:1cis-9), vaccenic acid (VA: trans-11 C18:1), linolenic acid (ALA: C18:3 n-3) and rumenic acid (RA: cis-9, trans-11 C18:2) in milk fat (table 2). The saturated fatty acids (SFA) content was significantly higher ($p< 0,05$) in the fat of multiparous sheep (third lactation) than in that of primipara (first lactation) for all three breeds included in the study (75,93 vs. 72.81% for MT; 71.70 vs. 68.19% for TG and 68.17 vs. 64.10% for TU). Within SFA, palmitic acid had the highest proportion in milk fat for all three breeds, but also showed the highest difference ($p<0.01$) between multiparous and primipara sheep.
Higher parity led to an increase ($p<0.05$) of SCFA and MCFA in milk fat, and this increase was accompanied by a decrease ($p<0.05$) in the MUFA and PUFA content (Table 2).
Milk fat n-3 FA content, and especially that in α-linolenic (ALA), was influenced by parity; the values recorded for primipara sheep were higher ($p<0.05$) than those for the multiparous ones. As n-3 FA originates mainly from feed, we assume that the differences resulting from parity are due to rumen microflora particularities linked to age.
The FA family represented by C12:0, C14:0 and C16:0, which are synthesized de novo by sheep, increased ($p<0.05$) as lactation number increased, while the C18 FA family, such as oleic acid, VA, linoleic acid,

RA and ALA, decreased ($p<0.05$) with the increase in lactation number. These changes of fatty acid profile in milk fat as parity increased were also reported by Tsiplakou et. al., (2006) and De La Fuente et. al., (2009), as opposed to Kelsey et. al., (2003) and Cranix et. al., (2008), who did not find any changes that could be linked to cows' parity. Thus, a higher lactation number has a negative influence on the nutritious quality of milk, as the proportion of those FA in milk that are beneficial to human health, that is, n-3 FA and CLA, decreases, while that of saturated FA increases.

The mean value of *cis-9, trans-11* CLA was higher ($p<0.05$) in milk fat for primipara sheep than for the multiparous ones by 24.14% for MT, 18.6% for TG and 11.62% for TU. This increase of CLA for primipara sheep is due mainly to a greater amount of VA flowing from rumen to the mammary gland and not to changes in the Δ^9 – Desaturase system, which showed lower values than for multiparous sheep. These results match those obtained by Garnsworthy et. al., (2010) for cows, who mentions that Δ^9 – Desaturase activity is lower for first lactation than for the second and >2. Δ^9 – Desaturase activity for the other FA (C14, C16 and C18) and the Δ^9 – Desaturase index showed higher values ($p<0.05$) for primipara sheep, which probably explains the higher MUFA and PUFA concentrations in milk fat. In the case of primipara ewes, the trans 18:1 isomer content in milk fat is higher than that for multiparous ewes. This is due to an incomplete rumen biohydrogenation, caused probably by rumen microflora differences or by higher rumen feed passage rate. This aspect concurs with the lower concentration of stearic acid (C18:0) in milk fat of primipara sheep; stearic acid being the direct precursor of feed polyunsaturated fatty acids rumen biohydrogenation (Chilliard et. al., 2003). Reduction in the C14:0 and C16:0 content resulted in a decrease in primipara sheep milk fat atherogenic index, which means that this milk has a better impact on human health than that coming from multiparous animals.

CONCLUSIONS

Milk yield of ovine, its components, and especially polyunsaturated FA content in milk fat, with a particular focus on n-3 FA and CLA, which are beneficial to human health, are influenced by physiological ones, such as breed and parity. Results showed that Turcana (TU) breed sheep yielded more milk, with a higher component content (fat and protein) than the other two breeds (Turcana > Tsigay > Merino of Transylvania), and milk fat had a higher polyunsaturated FA content, especially in *cis-9, trans-11* CLA, and lower in saturated FA, which caused a lower atherogenic index (2.20 vs. 2.57 for Tsigay and 3.38 for Merino of Transylvania). Milk yield and its components increased with parity, while the proportion of n-3 FA and *cis-9,*

trans-11 CLA in milk fat decreased, that is, the nutritional quality of milk decreased if we look at its influence on human health. These changes meet new nutritional trends and consumers' expectations. The results obtained seem to show that increase in milk fat *cis*-9, *trans*-11 CLA in relation with breed and higher parity is due not only to an increased supply of *trans*-11 C18:1 in rumen, but also to an increased Δ^9 – Desaturase activity.

ACKNOWLEDGEMENTS: This work was supported by CNCSIS – UEFISCSU, project number PN II – IDEI 679/2008.

REFERENCES

1. Addis, M., Cabiddu, A., Pinna, G., Decandia, M., Piredda, G., Pirisi, A. and Molle G., 2005, Milk and cheese fatty acid composition in sheep fed Mediterranean forages with reference to conjugated linoleic acid *cis*-9, *trans*-11. J. Dairy Sci. 88: 3443-3454.
2. Andrade, P.V.D., Schmidely, Ph., 2006, Influence of procentage of concentrate in combination with rolled canola seed on performance, rumen fermentation and milk fatty acid composition in dairy goats. Livestock Science, 104 (1): 77-90.
3. Barbosa, E., Oliveira, C., Casal, S., Soares, L., Vale, A.P., Lopes, J.C., Brito, N.V., 2003, Quantification and variability of conjugated linoleic acids levels in sheep milk of two Portuguase breeds. Ejeafche 2(4), 493-497 http://ejeafche.
4. Bauman, D.E., Mather, I.H., Wall, R.J., Lock, A.L., 2006, Major advances associated with the biosynthesis of milk. J. Dairy Sci. 89:1235-1243.
5. Bouattour, M.A., Casals, R., Albanell, E., Such, X., Caja, G., 2007, Milk CLA and fatty acids profile in milk from Lacaune ewes fed whole sufflower grains. The 5th International Symposium on The Challenge to Sheep and Goats milk, Sardinia, Italia from 18-20 april, 2007; I-P014.
6. Carta, A., Piredda, G., Addis, M., Cabiddu, A., Fiori, M., Leroux, C., Barillet, F., 2002, Fatty acid composition of sheep milk from a backcross Sarda x Lacaune resource population: Preliminary QTL detection for CLA content. Ciheam-Options Mediteraneennes: 107-113; http://ressources.ciheam.org.
7. Chilliard, Y., Ferlay, A., Rouel, J., Lambere, G., 2003, A review of nutritional and physiological factors affecting goat milk synthesis and lipolysis. J. Dairy Sci. 86: 1751-1770.
8. Collomb, M., Butikofer, U., Maurer, J., Sieber, R., 2006, Composition en acides gras du lait de brebis produit a diverses altitudes. Revue suisse Agric. 38(6): 335-339.
9. Cranix, M., Steen, A., Van Laar, H., Van Nespen, T., Martin-Teroso, J., De Baets, B., Fievez, V., 2008, Effect of lactation stage on the odd- and branched-chain milk fatty acids of dairy cattle under grazing and indoor conditions. J. Dairy Sci. 91:2662-2677.
10. De La Fuente, L.F., Barbosa, E., Carriedo, J.A., Gonzalo, C., Arenas, R., Fresno, J.M., San Primitivo, F., 2009, Factors influencing variation of fatty acid content in ovine milk. J. Dairy Sci., 92 : 3791 – 3799.

11. Dimitrov, T., Stankov, I., Mihailova, G., Iliev, T., 2001, Fatty acid content of sheep milk from South-Bulgarian Corriedale and their crosses in production of cheese. Animal Sciences (Sofia), 2: 98-101 (BG).
12. Ellis, K.A., Innocent, G., Grove-White, D., Cripps, P., McLean, G.W., Hovard, C.V., Mihm, M., 2006, Comparing the fatty acid composition of organic and conventional milk. J. Dairy Sci. 89: 1938-1950.
13. Federica, S., Contarini, G., Annicchiarico, G., Napolitano, F., Orru, L., Catillo, G., 2008, Breed differences in sheep milk fatty acid profiles: Opportunities for sustainable use of animal genetic resources. Small Ruminant Research 78 : 24-31.
14. Ferlay, A., Martin, B., Pradel, Ph., Coulon, J.B., Chilliard, Y., 2006, Fatty acid composition and milk lipolytic system in Tarentaise and Montbeliarde cow breeds. J. Dairy Sci. 89: 4026-4041.
15. Garnsworthy, P.C., Feng, S., Lock, A.L., Royal, M.D., 2010, Short communication: Heritability of milk fatty acid composition and stearoyl-CaA desaturase indices in dairy cows. J. Dairy Sci. 93(4): 1743-1748.
16. Gerchev, G. and Mihaylova, G., 2009, Variation in fatty acid composition of milk from Tsigai sheep in the region of Central Balkan Mountains. Biotechnology in Animal Husbandry 25 (5-6): 945-952.
17. Gomez-Cortes, P., Frutos, P., Mantecon, A.R., Juarez, M., De La Fuente, M.A., Hervas, G., 2008, Addition of olive oil to dairy ewe diets: Effect on milk fatty acid profile and animal performance. J. Dairy Sci. 91: 3119-3127.
18. Gomez-Cortes, P., Frutos, P., Mantecon, A.R., Juarez, M., De La Fuente, M.A., Hervas G., 2009, Effect of supplementation of grazing dairy ewes with a cereal concentrate on animal performance and milk fatty acid profile. J. Dairy Sci. 92: 3964-3972.
19. Guler, Z., Keskin, M., Masatcioglu, T., Gul, S., Bicer, O., 2007, Effects of breed and lactation period on some characteristics and free fatty acid composition of raw milk from Damascus goats and German Fawn x Hair goat B1 crossbreds. Turk. J. Vet. Anim. Sci. 31(5): 347-354.
20. Hervas, G., Gomez-Cortes, P., De La Fuente, M.A. Mantencon, A.R., Juarez, M., Giraldez, F.J., Frutos, P., 2009, Effect of supplementation of grazing dairy ewes with a cereal concentrate on milk fatty acid profile. Options Mediterraneennes, 85: 439-444.
21. ISO-IDF., 2002, Milk fat preparation of fatty acid methyl esters. International Standard ISO 15884-IDF 182:2002.
22. Kay, J.K., Weber, W.J., Moore, C.E., Bauman, D.E., Hansen, L.E., Hansen, L.B., Chester-Jones, H., Crooker, B.A., Baumgard, L.H., 2005, Effects of week of lactation and genetic selection for milk yield on milk fatty acid composition in Holstein cows. J. Dairy Sci. 88:3886-3893.
23. Kelsey, J.A., Corl, B.A., Collier, R.A., Bauman, D.M., 2003, The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. J. Dairy Sci. 86:2588-2597.
24. Kuchtik, J., Sustova, K., Urban, T., Zapletal, D., 2008, Effect of the stage of lactation on milk composition, its properties and the quality of rennet curdling in East Friesian ewes. Czech J. Anim. Sci., 53(2) : 55-63.
25. Lujerdean, A., Bunea, A., Pica, E.M., 2009, Characterization of Turcana, Tsigay and Merino of Cluj sheep milk in early and late lactation period. Bulletin USAMV-CN, Anim, Sci. and Biotechnologies, 66(1-2): 26-31.
26. Luna, P., Juarez, M., De La Fuente, M.A., 2005, Validation of a rapid milk fat separation method to determine the fatty acid profile by gas chromatography. J. Dairy Sci. 88: 3377-3381.

27. Mele, M., Buccioni, A., Petacchi, F., Serra, A., Banni, S., Antongivanni, M., Secchiari, P., 2006, Effect of forage/concentrate ratio and soybean oil supplementation on milk yield, and composition from Sarda ewes. Anim. Res. 55: 273-285.
28. Mele, M., Serra, A., Conte, G., Pollicardo, A., Del Viva, M., Secchiari, P., 2007, Whole extruded linseed in the diet of dairy ewes during early lactation: effect on the fatty acid composition of milk and cheese. Ital. J. Anim. Sci. 6(suppl. 1): 560-562.
29. Mierlita, D., Maerescu, C.M., Daraban, St., Lup, F., 2009, Effects of energy and protein content in the diet on milk yield and milk fatty acid profile in dairy ewes. Bulletin USAMV-CN, Anim, Sci. and Biotechnologies, 66(1-2): 67-73.
30. Mihaylova, G., Gerchev, G., Moeckel, P., Jahreis, G., 2004, Comparative study on fatty acid content in milk of Tsigay and Karakachan sheep. Bulg. J. Vet. Med. 7(3), 181 – 187.
31. Palladino, R.A., Buckley, F., Prendiville, R., Murphy, J.J., Callan, J., Kenny, D.A., 2010, A comparasion between Holstein-Friesian and Jersey dairy cows and their F1 hybrid on milk fatty acid composition under grazing conditions. J. Dairy Sci. 93(5): 2176-2184.
32. Peterson, D.G., Kelsey, J.A., Bauman, D.E., 2002, Analysis of variation in *cis*-9, *trans*-11 conjugated linoleic acid (CLA) in milk fat dairy cows. J. Dairy Sci. 85:2164-2172.
33. Rego, O.A., Alves, S.P., Antunes, L.M.S., Rosa, H.J.D., Alfaia, C.F.M., Prates, J.A.M., Carita, A.R.J., Fonseca, A.J.M., Bessa, R.J.B., 2009, Rumen biohydrogenation-derived fatty acids in milk fat from grazing dairy cows supplemented with rapeseed, sunflower, or linseed oils. J. Dairy Sci. 92: 4530-4540.
34. Sanchez, J.P., San Primitivo, F., Barbosa, E., Varona, L., De La Fuente, L.F., 2010, Genetic determination of fatty acid composition in Spanish Churra sheep milk. J. Dairy Sci. 93(1): 330-339.
35. SAS., 1999, SAS OnlineDoc®, Version 8, SAS Institute Inc., Cary. NC.
36. Simopoulos, A.P., 1999, Essential fatty acids in health and chronic disease. Am. J. Clin. Nutr. 70: 560-569.
37. Simopoulos, A.P., 2008, The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp. Biol. Med. 233:674-688.
38. Thorsdottir, I., Hill, J., Ramel, A., 2004, Seasonal variation in *cis*-9, *trans*-11 conjugated linoleic acid (CLA) content in milk fat from the Nordic Countries. J. Dairy Sci. 87:2800-2802.
39. Tsiplakou, E., Mountzouris, K.C., Zervas, G., 2006, The effect of breed, stage of lactation and parity on sheep milk fat CLA content under the same feeding practices. Livestock Science 105: 162 – 167.