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Effects of part-time grazing and feeding management on the fatty acid composition and antioxidant capacity in the milk of Turcana ewes

Daniel Mierlita
Department of Animal Science, University of Oradea, Oradea 410087, Magheru 26, Bihor, Romania.
e-mail: dadi.mierlita@yahoo.com

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Abstract

The effect of part-time grazing on milk production and quality, with three different feeding systems of indoor-supplied fodder, was studied in flock of Turcana sheep. The control group (group P) had permanent access to the pasture (full-grazing) without additional ration. For the other 3 groups (part-time grazing), the grazing period was limited to 6 hours/day, divided in two rounds meant to circumvent the high temperatures during the day. The diet of ewes in the part-time grazing groups was supplemented with 500 g dry matter (DM) mixture concentrate (group C); 1000 g DM grass hay (group H); or 500 g DM grass hay + 200 g DM concentrate + 180 g DM camelina oil calcium soaps (group S). Indoor-supplied diets ensured a contribution of 0.70 – 0.72 UFL and 97-104 g CP/day. Treatments had no effect on milk production or milk protein content. Camelina oil calcium soaps (Cs) supplementation caused an increase in milk fat and a higher quality of energy corrected milk (ECM) ($p < 0.01$). The full-grazing system proved beneficial for supplying milk rich in α-linolenic acid, *cis*-9, *trans*-11CLA and C18:1 *trans*-11, while also condoning a reduction in the oxidation process of fats ($p < 0.001$). Part-time grazing and supplementing the diet with concentrates or grass hay compromises nutritional characteristics of milk with increased proportion of hypercholesterolemic FA (HFA), a higher value of atherogenicity index (AI) and increased antioxidant capacity of milk ($p < 0.001$). Ewes fed via feeding systems, with a limited access to pasture and moderate amounts of concentrate, hay and Cs did not change milk fatty acid profile and oxidative stability of the milk with respect to full grazing group. The experiment confirmed that it is possible to improve the nutritional characteristics of milk production by implementing a feeding system that combines local resources and a reduced amount of indoors provided concentrate, hay and Cs.

Key words: Part-time grazing ewes, camelina oil calcium soaps, PUFA profile, TEAC assay.

Introduction

Raising dairy ewes on pasture is justified not only by the low cost of feeding, but mainly by the nutritional and sanogenetic quality of the milk obtained, as compared to that obtained from housed ewes fed preserved feed [1]. A low-input feeding system based on grazing increases the proportion of beneficial FAs; however, daily milk yields are lower [2]. Taking this into account, it must be said that during the highest temperatures of the day (over 33°C) during summer (July-August), the DM intake from grazing decreases. Supplementing the ration of grazing ewes with various preserved forage (concentrates, hay) is meant to increase the ingestion of DM (dry matter) and energy, and also to increase the milk production [3]. This feeding management of pasture-fed ewes has a negative influence on the sanogenetic quality of milk fat [4, 5]. To improve the nutritional characteristics and impact of milk fat on human health, fatty acids (FA) profiles must be modified for increasing the proportion of polyunsaturated fatty acids (PUFA), especially conjugated linoleic acid (CLA) and α-linolenic acid (C18: 3), at the expense of saturated fatty acids (SFA). Isomer C18: 2 *cis*-9, *trans*-11 (rumenic acid), which is the most important CLA isomer, is produced by rumenic biohydrogenation of linoleic acid (C18: 2) from feed and by desaturation (in the presence of Δ^9-desaturase enzyme) of *trans*-vaccenic acid (TVA; C18: 1 *trans*-11), in the mammary gland. *Trans*-vaccenic acid is produced by rumenic biohydrogenation of linoleic acid and linolenic acid from feed [6]. Enrichment of milk fat with PUFA and especially with t11-C18:1, c9, t11-CLA and α-linolenic acid (C18: 3) is achieved by increasing the intake of linoleic acid and linolenic acid via feeding [7].

AbuGhazaleh *et al.* [8] demonstrated that the greatest concentrations of *cis*-9, *trans*-11 CLA in milk fat can be obtained by adding fish oil along with plant oils high in linoleic acid (sunflower oil) or linolenic acid (linseed oil) to dairy cow diets. AbuGhazaleh and Jenkins [9] showed that the stimulatory effect of dietary fish oil and algae on milk *cis*-9, *trans*-11 CLA production is due to the inhibitory effect of docosahexaenoic acid (C22:6 n-3; DHA) on the ruminal reduction of FA to stearic acid.

Ruminant diet supplementation with oil (sunflower oil, soybean oil, flax oil, canola oil) has a more pronounced negative effect on intake, on fiber digestion, on rumen metabolism and on milk FA profile than by-pass type fats [10-12]. Using saponified fats in ruminant feed as a source of polyunsaturated fatty acids is a more convenient practice, because it is limited to rumenal biohydrogenation of FA [11].

The evaluation of an increase in unsaturated nutritionally beneficial FAs should take into consideration also the oxidative stability of altered milk fat. Different proportions of ALA (7% and 2% of total FA in milk fat from cows fed grass silage and maize

silage, respectively) were proposed to be important for the formation of lipid hydroperoxides. In a further study of Havemose *et al.*[13], differences in the oxidative stability of milk from cows fed grass-clover silage or hay were examined. A higher degree of lipid oxidation was found in milk of cows fed the silage. The higher content of natural antioxidants did not prevent oxidation and different contents of ALA (8% and 4% of total FAs in silage and hay, respectively) were thought to be the cause.

The traditional, and most frequently used sheep flock management system in the Romanian sub-mountainous area is based on the Turcana breed (well adapted to the mountainous terrain) and on part-time grazing during much of the summer period (July to August), because pastures are scarce and of poor quality, due to drought but also due to the scorching temperatures throughout the day, reaching well over 33°C, which limit grazing time [14]. Thus, it is important for farmers to supplement the ewes' diet so as to obtain milk of high nutritional quality at the lowest possible cost.

Camelina oil (CO) is rich in polyunsaturated fatty acids (FA) and rumen bypass of CO can contribute to increase polyunsaturated FA proportion in milk. However, supplementation with CO may result in both positive and adverse changes in the nutritional and dietetic properties of milk, as shown by increased unsaturated FA proportion and increased oxidation susceptibility[15]. The oxidative deterioration of milk fat containing a high PUFA proportion can increase the development of rancid odors and flavors in milk [16], which results in products of lower nutritional quality and safety due to the formation of secondary, potentially toxic compounds [15].

Although the effect of plant oils on milk FA composition is well documented under confinement feeding system, there is less information in the literature concerning the effect of camelina oil calcium soaps on milk PUFA content and oxidative stability of grazing dairy ewes. Studies have shown that milk PUFA levels are typically higher in ewes fed pasture-based diets than TMR (total mixed rations) [14]. Therefore, the objective of the present work was to evaluate the effects of the part-time grazing system and of supplementing ewes' indoor diets on the milk fatty acid composition with particular reference to *cis*-9, *trans*-11 CLA and FA n-3 and oxidative stability of the milk, compared to full-grazing.

Materials and Methods

The study was conducted at the University of Oradea (Romania) over a 10 week period, from mid-June to the end of August. The first 3 weeks were used as a covariate period (week 1) and adaptation to dietary treatments (weeks 2 and 3). After weaning the lambs (72 ± 14 days in milk), 40 multiparous Turcana breed ewes (live weight 44.9 ± 2.7 kg; parity 2.4 ± 0.16) were divided in four homogeneous groups (10 ewes/group). At the onset of the trial, ewes averaged 744.7 ± 148.5 g/day of milk yield, containing 66.8 ± 11.2 g/L of milk fat. Groups were randomly assigned to one of the four different feeding regimes described below.

The control group (group P) had permanent access to the pasture (ad libitum) during the entire experiment without additional ration. For the other 3 groups (part-time grazing), the grazing period was limited to 6 h/day, divided in two rounds (8:00 – 11:00 h and 19:00 – 22:00 h), in order to avoid the high temperatures during the day, which often exceed th 33°C limit, under the weather conditions in Romania. The diet of the ewes in the part-time grazing groups was supplemented with 500 g dry matter (DM) mixture concentrate for lactating sheep (group C); 1000 g DM grass hay (group H); or 500 g DM grass hay + 200 g DM mixture concentrate + 180 g DM calcium soaps (10% of the diet's DM [10]) (group Sc). Calcium soaps were prepared from camelina oil via the precipitation method as described by Alexander *et al.*[10] and introduced in concentrated mixture. Mixture concentrate consisted of corn (58%), triticale (20%), soybean meals (18%) and vitamin-mineral premix (5%). All three supplements provided an additional energy contribution of 0.70 – 0.72 UFL and 97-104 g crude protein (CP)/day. Animals in the part-time grazing groups (C, H and S) received mixture concentrate divided into 2 equal halves given during the morning and evening milkings. Grass hay was divided into 2 equal halves and given after each grazing period the to groups H and S.

All ewes grazed on the same field, although in separate plots, on a mountain pasture (1248 m altitude), consisting of a mixture of approximately 37% *Festuca rubra*, 18% *Phleum pratense*, 12% *Poa pratensis*, 14% *Dactylis glomerata* and 9% *Trifolium repens*. The pasture was divided into 20 paddocks of approximately 0.2 ha, using a combination of fixed and portable electric fences. The ewes were moved to a new paddock every 6 days, to allow a rest period of 30 days between grazing. Portable shade structures were placed in the paddocks to provide 1.5 m^2 of shade per ewe, moved in every paddock. Water and vitamin-mineral blocks were available in the paddock for the full grazing ewes (group P). For part-time grazing groups, drinking water and vitamin-mineral blocks were freely available to all animals when they were indoors. Ewes were milked twice daily (at 07:00 and 18:00 o'clock), individual milk production being recorded daily.

Samples of diets were collected in weeks 3, 5, 7 and 10 of the experiment period (n = 4), stored at -20°C, and then used for chemical composition analysis. They were analyzed for DM [17], NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber) on a Fibersac analyzer Ankom Technology, Fairport, NY [18], N (Kjeldahl technique), and ether extract [19].

Samples (n = 2) of diets were collected on weeks 6 and 10 for the determination of FA profile. These samples were immediately stored at -20°C, later lyophilized and kept until analysis.

Milk samples were collected during the weeks 6 and 10 (first 3 days of each week) and analyzed for chemical composition, fatty acid profile and antioxidant activity. Milk samples were refrigerated at 4–6°C and taken immediately to the laboratory for analyses of antioxidant capacity.

Milk samples were preserved with 2 tablets of Bronopol® (BroadSpectrum Micro-tabs II, D&F Control Systems Inc., USA). The samples were refrigerated at 4°C before being analysed for fat and protein content (N x 6.38) by infrared analysis (Milk Analyser System 4000, Foss Electric, Hillerod, Denmark). Monohydrate lactose content was measured on these samples using an enzymatic method [20]. Samples of milk collected on weeks 6 and 10 for FA analysis were frozen at -20°C without preservatives.

To determine FA in diets, FA methyl esters (FAME) were prepared by the one-step extraction-methylation method of Sukhija and Palmquist [21]. In order to determine the composition of fatty acids in milk, the fat was extracted according to the international standard ISO 14156/IDF 172:2001. Fatty acid methyl esters were prepared according to the method proposed by Christie [22] and Chouinard *et al.* [23]. FAME were determined by gas chromatography using a Varian GC 3600 equipped with FID and a fused silica capillary

column (SP 2560 Supelco), 100 m × 0.25 mm i.d., film thickness 0.20 µm. Helium was used as the carrier gas at a flow rate of 1 mL/min. The split ratio was 1:100. The oven temperature was programmed at 90°C and held for 1.50 min, then increased to 210°C at a rate of 9°C/min, held at this temperature for 25 min, then increased to 230°C at 15°C/min, and held for 7 min. The temperatures of the injector and the detector were set at 270°C. The fatty acid identification was based on external standards, and calculation of the distribution (in weight percentage) was based on the area of each fatty acid ester corrected for the response factors for the individual fatty acids. Internal standards were used to determine percentage of recovery. The CLA isomer reported is *cis*-9, *trans*-11 C18:2 and *trans*-10, *cis*-12 C18:2. The percentage of each fatty acid was calculated by dividing the area under the fatty acid peak by the sum of the areas under the total reported fatty acid peaks. Atherogenicity index (AI) was calculated according to Chilliard *et al.* [11] as follows: (C12:0 + 4 x C14:0 + C16:0)/(monounsaturated + polyunsaturated fatty acids).

Antioxidant activity of milk and experimental diets samples was estimated by TEAC method, according to the Renobales *et al.* [1] method which measures the ability of compounds to scavenge the 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical cation in relation to Trolox. A blank sample was used for the correction of the residual turbidity. The pH of milk samples was adjusted to 6.7, and samples were diluted ten times before any measurements. In the case of milk samples the results were expressed as µmol Trolox equivalent normalized according to the protein content of each milk sample (µmol Trolox equivalents/g protein), and for experimental diets as µmol Trolox equivalent/100 g.

All statistical analyses were performed using the Mixed statistical procedure of SAS [24]. Analysis of data concerning milk yield, milk compositions, FA in milk fat and TEAC were done using Proc Mixed in a repeated measures design. Treatment, block, week, and treatment × week were included in the model as fixed effects with week as the repeated measure on ewes. Covariance structure was autoregressive (1). The significance level was declared at $P<0.05$. Trends for significance were declared at $P = 0.05$ to 0.10.

Results and Discussion

Chemical composition and content in main fatty acids of fodder used (pasture, hay, mixture of concentrated and calcium soaps) are presented in Table 1. As expected, concentrated mixture had the highest CP content, while hay and pasture had the highest concentration of cell wall components (NDF and ADF). All fodders and, particularly, concentrated mixture were good sources of linoleic acid (C18: 2), but the pasture was richer in α-linolenic acid (C18: 3) (38.82% of total FAME), these results are consistent with those mentioned in the literature [2, 26, 27]. Linolenic acid was the major fatty acid in the camelina oil calcium soaps (Cs), accounting for approximately 39.8% of total fatty acid methyl esters (Table 1). The second major fatty acids in the Cs were linoleic acid and oleic acid, accounting for 20.1% and 12.7% respectively of total fatty acid methyl esters.

The effect of treatments on milk yield and composition is presented in Table 2. There was no effect ($P = 0.21$) of treatments (full grazing or part-time grazing or type of supplementation of diet) on milk yield. Grass hay supplementation of part-time grazing diet (group H) resulted in a increase in milk fat percentages and yield. Fodder fiber content is considered to be a key factor in achieving a higher fat content in milk [28]. Ewes having a diet supplemented with calcium soaps had a higher percentage of fat in milk and a higher daily production of fat and corrected milk production (ECM = energy corrected milk; Fat and Protein Corrected Milk = FPCM -1047 kcal/kg); these figures are

Table 1. Chemical composition and fatty acids content of fodder used in ewes' feeding[1].

Specification	Pasture	Grass hay	Concentrate mixture	Calcium soaps (camelina oil)
Chemical composition (g/kg DM)				
Dry matter (DM)	276	842	883	
Crude protein (CP)	149.4	103.7	193.8	
Crude fat	28.7	15.8	32.5	
NDF	497.4	512.4	106.1	
ADF	284.1	336.6	35.4	
Energy (UFL)	ND	0.71	1.38	
Content in fatty acids (% of FAME)				
C12:0	0.68	0.53	0.04	0.23
C14:0	0.89	0.68	0.21	0.18
C16:0	17.57	17.39	17.43	5.21
C16:1 n-9	0.38	0.93	0.64	0.12
C18:0	2.39	7.04	3.29	2.93
C18:1 n-9c	5.34	15.93	23.17	12.73
C18:1 c11	2.43	0.72	1.52	1.59
C18:2 n-6c	24.93	31.11	49.20	20.18
C18:3 n-6	3.76	0.46	0.27	1.54
C18:3 n-3	38.82	24.18	3.52	39.84
C20:1	0.67	1.03	0.71	12.33
C22:1	ND	ND	ND	3.12
SFA[2]	21.53	25.64	20.97	8.55
MUFA[3]	6.39	18.61	26.04	29.89
PUFA[4]	65.54	55.75	52.99	61.56
TEAC (µmol TE/100 g feed)	189.76	106.54	86.11	134.80

[1]Data presented are least square means (n = 4), except for FA (fatty acid) profile (n = 2 samples

[1]Data presented are least square means (n = 4), except for FA (fatty acid) profile (n = 2 samples per feeds). DM = dry matter; NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; FAME = fatty acid methyl esters; ND = not detected. TEAC: Trolox equivalent antioxidant capacity (µmol Trolox equivalents/100 g feed). [2]SFA = saturated FA (C12:0 + C14:0 + C16:0 + C18:0); [3]MUFA = monounsaturated FA (C16:1 + C18:1 + C20:1 + C22:1); [4]PUFA = polyunsaturated FA (C18:2 + C18:3).The UFL value was estimated in according INRA [25].

Table 2. Influence of ration structure and supplement of calcium soaps on milk yield and its composition[1].

Treatment[2]	P	C	H	Sc	SEM[3]	P[4]
Milk yield (g/day)	721.4	737.3	743.4	763.7	21.3	0.21
ECM[5] (kg/day)	0.714[b]	0.706[b]	0.756[ab]	0.802[a]	0.019	<0.01
FPCM[6]	0.783[b]	0.773[b]	0.831[ab]	0.884[a]	27.1	<0.01
Fat (g/L)	77.3[a]	73.8[b]	81.4[a]	85.6[a]	2.93	<0.01
Fat (g/day)	55.7[b]	54.4[b]	60.5[ab]	65.3[a]	2.01	<0.01
Proteins (g/L)	50.8	49.1	50.4	51.2	1.91	0.68
Proteins (g/day)	36.6	36.2	37.4	39.1	0.97	0.16
Lactose (g/L)	48.3	48.6	48.2	48.4	1.31	0.28
Lactose (g/day)	34.8	35.8	35.8	36.9	0.95	0.12
Milk urea (mg/dl)	32.9[a]	25.1[b]	29.3[ab]	24.2[b]	0.83	<0.05

[1]n = 10 ewes/group; [2]P: permanent access to the pasture (ad libitum) without additional ration; C: part-time grazing (6 h/d) + 500 g dry matter (DM) mixture concentrate for lactating sheep; H: part-time grazing (6 h/d) + 1000 g DM grass hay; Sc: part-time grazing (6 h/d) + 500 g DM grass hay + 220 g DM mixture concentrate + 180 g DM calcium soaps of camelina oil. [3]SEM: standard error of least square means. [4]Significance for treatment effect. [5]Energy Corrected Milk (ECM) = Milk yield (kg/day) x (0.071 x Fat (%) + 0.043 x Protein (%) + 0.2224). [6]Fat (6.5%) and Protein (5.8%) Corrected Milk (FPCM -1047 kcal/kg) = Milk Yield, kg x (0.25 + 0.085 x fat,% + 0.035 x protein,%).

significantly higher compared with ewes in other groups. Milk yield is directly attributed to energy intake by the higher energy content of the oil supplemented diet [29, 30]. However, milk urea (mg/dl) was higher for pasture than part-time grazing groups. The values of this parameter are close to the recommended levels for sheep milk [31]. Milk content and the daily output of protein and lactose were not affected by the feeding system based on grazing. Including fats rich in PUFA in dairy cattle feed resulted in a decrease in milk production and milk fat content. This effect is known as the Milk Fat Depression Syndrome (MFD). Exactly the opposite seems to occur in sheep and goats; for these, a supplementation of the diet with fat generates an increase of milk fat content and production [11, 28].

Thus, feeding management with a limited access to pasture and moderate amounts of concentrate, hay and camelina oil of calcium soaps (Cs) can improve milk yield and milk composition with respect to the group that did full grazing. Therefore, if access to pasture land is not available full-time, it is possible to improve the economic parameters of milk production by implementing a feeding system that combines local resources and a reduced amount of indoors provided concentrate, hay and Cs.

Results on the effect of experimental factors on the profile of milk fatty acids are presented in Table 3. The group that ingested the highest amount of grass (group P) had the highest concentration of unsaturated FA in the milk fat, both total unsaturated FA and PUFA. This value was similar to that reported by Renobales *et al.* [1] and Piredda *et al.* [32] in the milk of sheep allowed to graze on polyphyte pastures. Group P milk also had the highest concentration of α-linolenic acid (ALA), rumenic acid (CLA isomer c9t11), *trans*-vaccenic acid (TVA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Increased content of n-3 FA (ALA, EPA, DHA), CLA isomer c9t11 and TVA in fat milk is an advantage of pasture-based feed ration, in terms of dietary fat impact on human health [5]. The amount of the CLA isomer c9t11 in the milk of group P was 2.4-fold higher than that present in the milk of group C and over 30% higher compared with group H. The positive effect of pasture on milk fat content in CLA *cis*-9, *trans*-11, was found previously, in lactating ewes [5, 26]. Metabolic pathways that form the link between pasture feeding and increased c9,t11-CLA in milk are not well known. It is unknown how α-linolenic acid, which predominates in the fat of the pasture (55-65% of total FA[11]), participates as an intermediate in the formation of c9,t11-CLA, although pasture favours the formation of C18:1 *trans*-11 [33].

The concentration of vaccenic acid (TVA) in the milk of group P was 3.2-fold higher than that of group C and 2.0-fold higher than that of group H. Cows on pasture-based diets have been shown to have higher levels of TVA in their milk than those on preserved forages[34-36]. Couvreur *et al.* [35] reported a linear relationship between the proportion of fresh grass in dairy cow diets and milk TVA. Human tissues can directly convert vaccenic acid into the c9t11-CLA isomer [11].

Supplementation of ration based on pasture with concentrates (group C) and hay (group H), has led to increased content of saturated fatty acids in milk fat (SFA: C6:0, C8:0, C10:0, C14:0, C16:0) and a decreased amount of monounsaturated FA (C16:1, t11-C18: 1 and c9-C18:1) and polyunsaturated FA (c9,t11-C18:2, C18:3). The results suggest that the introduction of concentrates and hay in lactating ewes' diet led to important changes in the populations of rumen bacteria, which favoured biohydrogenation processes, thus a part of unsaturated FA from feed have been fully hydrogenated [2]. This argument is confirmed by significantly lower milk fat content of α-linolenic acid (ALA), rumenic acid (c9,t11-C18: 2) and vaccenic acid (t11-C18:1), indicating more

Table 3. Influence of ration structure and supplement of calcium soaps on fatty acids profile from milk fat [1] (% of total FAME[2]).

Treatment[3]	P	C	H	Sc	SEM[4]	P[5]
C4:0	2.52	2.13	2.21	2.80	0.340	0.10
C6:0	2.18	2.61	2.09	2.72	0.323	0.24
C8:0	2.14	2.92	2.12	2.46	0.286	0.12
C10:0	5.37^{c}	8.89^{a}	6.61^{b}	5.27^{c}	0.172	< 0.001
C12:0	3.51^{b}	5.04^{a}	4.70^{a}	2.09^{c}	0.081	<0.001
C14:0	7.83^{b}	10.99^{a}	9.37^{a}	6.75^{c}	0.440	<0.001
C14:1	0.07^{b}	0.14^{a}	0.12^{a}	0.11^{a}	0.012	<0.10
C15:0	0.86^{b}	0.83^{b}	1.07^{a}	0.65^{c}	0.054	<0.01
C16:0	16.62^{c}	24.81^{a}	23.68^{a}	20.93^{b}	1.202	<0.001
C16:1	0.59^{b}	0.62^{ab}	0.83^{a}	0.44^{bc}	0.027	<0.01
C17:0	0.50^{b}	0.49^{b}	0.71^{a}	0.50^{b}	0.084	<0.05
C18:0	16.05^{a}	10.37^{b}	12.23^{ab}	15.44^{a}	0.531	<0.001
C18:1 n9t	0.59^{ab}	0.32^{b}	0.27^{b}	0.75^{a}	0.067	<0.001
C18:1 *trans*-11 (TVA)	6.69^{a}	2.05^{c}	3.28^{b}	5.11^{a}	0.248	<0.001
C18:1 n9c	23.69^{a}	19.46^{b}	21.28^{ab}	22.76^{a}	0.603	<0.05
C18:1 *cis*-11	0.97	0.60	0.63	0.70	0.064	0.27
C18:2 n6t	0.36	0.26	0.39	0.31	0.036	0.63
C18:2 n6c	2.14	3.06	2.47	2.45	0.143	0.41
trans-10, *cis*-12 CLA	0.03	0.02	0.02	0.05	0.011	0.22
cis-9, *trans*-11 CLA (RA)	2.33^{a}	0.95^{c}	1.79^{b}	2.02^{a}	0.165	<0.001
C18:3 n-3 (ALA)	2.17^{b}	1.05^{c}	1.43^{c}	2.85^{a}	0.366	<0.001
C20:0	0.37	0.33	0.31	0.28	0.042	0.16
C20:4	0.12	0.20	0.22	0.20	0.029	0.08
C20:5 n-3 (EPA)	0.38^{a}	0.29^{b}	0.35^{ab}	0.41^{a}	0.024	<0.05
C22:6 n-3 (DHA)	0.44^{ab}	0.34^{b}	0.44^{ab}	0.50^{a}	0.017	<0.01
Others	1.48	1.22	1.36	1.45	0.278	0.12
Saturated FA	58.05^{c}	69.42^{a}	65.10^{b}	60.09^{c}	0.617	<0.001
Unsaturated FA	40.45^{a}	29.41^{c}	33.54^{b}	38.46^{a}	0.597	<0.001

[1]n = 10 ewes/group; [2]FAME = fatty acids methyl esters; [3]P: permanent access to the pasture (ad libitum) without additional ration; C: part-time grazing (6 h/d) + 500 g DM mixture concentrate for lactating sheep; H: part-time grazing (6 h/d) + 1000 g DM grass hay; Sc: part-time grazing (6 h/d) + 500 g DM grass hay + 220 g DM mixture concentrate + 180 g DM calcium soaps of camelina oil. [4]SEM: standard error of least square means. [5]Significance for treatment effect. [6]Atherogenic Index = (C12:0 + 4 × C14:0 + C16:0)/ (monounsaturated + polyunsaturated fatty acids); MUFA and PUFA: mono- and polyunsaturated fatty acids. RA: rumenic acid (*cis*-9, *trans*-11 CLA); TVA: trans-vaccenic acid (*trans*-11 C18:1); ALA: α -linolenic acid (C18:3 n-3) ; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; Total n-3 FA: C18:3 n-3, C20:5 n-3, C22:6 n-3. TEAC: Trolox equivalent antioxidant capacity (μmol Trolox equivalents/g protein).

Table 3. Continued.

Treatment[3]	P	C	H	Sc	SEM[4]	P[5]
Monounsaturated FA	32.60[a]	23.20[c]	26.42[b]	29.87[ab]	0.474	<0.001
Polyunsaturated FA	7.85[ab]	6.21[c]	7.12[b]	8.59[a]	0.315	<0.001
Total n-3 FA	2.99[b]	1.68[d]	2.22[c]	3.76[a]	0.248	<0.001
AI[6]	1.27[c]	2.51[a]	1.96[b]	1.30[c]	0.134	<0.001
Δ^9 – desaturase ratios:						
16:1/16:0 + 16:1	0.022[b]	0.025[b]	0.035[a]	0.020[b]	0.002	<0.05
18:1/18:0 + 18:1	0.66	0.68	0.68	0.62	0.031	0.60
RA/VA + RA	0.30[b]	0.32[b]	0.36[a]	0.27[b]	0.018	<0.05
TEAC values ((µmol Trolox equivalents/g protein)	3771.6[a]	2627.4[c]	2882.1[b]	3671.8[a]	189.6	<0.001

intense activity of the rumenal microflora responsible for PUFA biohydrogenation from the diet supplemented with mixed concentrate (group C) and hay (group H). Increases in the concentration of C18:0, C18:1 *cis*-9 and C18:1 *trans*-11 in milk fat and decreases in C16:0 were found in grazing sheep and cows compared with those fed with TMR [5, 37].

Ewes' diet supplementation with calcium soaps modified fatty acid composition in milk fat to a lower level of saturated fatty acids and a higher level of MUFA and PUFA, confirming that by adopting a strategy of adequate alimentation the nutritional and dietetic quality of ewes' milk can be improved. Calcium soaps in the diet have led, in particular, to a significant increase in the concentration of trans-vaccenic acid (t11-C18:1), rumenic acid (c9,t11-CLA) and α -linolenic acid (C18:3) in fat milk.

The increases in milk linolenic acid with the Cs treatments were relatively small compared with their intake. Similar low increases in n-3 fatty acids were reported by others when n-3 lipid supplements were used [36, 38]. This low transfer of n-3 fatty acids from feed to milk fat may be explained by extensive ruminal biohydrogenation of linolenic acid or by their partitioning toward other tissues within the body; n-3 fatty acids are almost totally confined to plasma cholesterol ester and phospholipids, which are poorly taken up by the mammary gland [36]. The increased C18:0 levels in ewe milk supplemented with Cs would be the result of total rumen biohydrogenation of part of the C18:2n-6 of the diet [27]. However, the fact that no great increase in the proportion of C18:0 was observed in the present case would most likely be due to the activity of the $\triangle^9$-desaturase enzyme which converts C18:0 derived from oil supplementation into C18:1 within the mammary gland [29].

Ewes maintained on pasture and whose rations were not supplemented with other fodder (group P), had the lowest concentration of FA C10:0, C14:0, C16:0 and C17:0 (P<0.10 to 0.05) in the milk fat, but they had the highest concentration of FA C18:0, t11-C18:1, c9-C18:1 c9, t11-C18:2 and C18:3 in milk fat (P <0.05 to 0.001). Decreased content in C12:0, C14:0 and C16:0 has led to lower atherogenic index (AI) of milk fat of ewes maintained on pasture (group P) and of those grazing part-time, whose diet was supplemented with Cs (group Sc), compared with ewes grazing part-time with concentrates and hay introduced to their diets (groups C and H). Consequently, the atherogenicity index of the milk of group P and Sc animals was the lowest of all groups, 1.27-1.30, whereas the highest value of AI was recorded in ewes from group C, 2.51 (Table 3). This value is comparable to that reported by Renobales *et al.*[1] for sheep milk (2.25 - 2.72) from animals which were taken out to a pasture for 4 h/d, and whose diet was supplemented with hay and commercial concentrate.

Product/substrate reports + product were estimated to assess the degree of desaturation specific to fatty acids during milk fat synthesis [12]. Higher levels of these reports have indicated that fatty acid desaturation, considered as a substrate, was more intense for part-time ewes with diets where concentrates or grass hay were introduced. The camelina oil calcium soaps supplement from feed had a reducing effect of the processes of desaturation. This conclusion was consistent with that reported by Mele *et al.* [12] and Bernard *et al.* [39] in studies on ewes and, respectively, on goats.

Increased content of unsaturated nutritionally beneficial FAs in milk fat, which is desirable due to its hypocholesterolemic effect, was recognized as an important factor for depressing the oxidative stability of milk fat causing deformation of the milk fat globule membrane, which affects the deterioration of the technological properties of sheep milk [40]. We found a variation in antioxidant activity between the milk from full-grazing group sheep (group P) and the part-time grazing groups (groups C and H) (Table 3). The TEAC (Trolox equivalent antioxidant capacity) value was significantly higher in the milk from P ewes group (P <0.001) compared with C and H group (3771.6 vs. 2627.4 and 2882.1 µmol Trolox equivalents/g protein) due to higher concentrations of *cis*-9, *trans*-11 CLA, which has been shown to have a high antioxidant activity [41]. An elevated concentration of unsaturated fatty acids, especially PUFA, in milk from the full-grazing production system, carries the risk of oxidative instability, which, however, may be controlled by a high concentration of natural antioxidants in the milk. This could be due to higher levels of α-tocopherol and β-carotene in pasture than in concentrate and grass hay [13, 42]. The

trend of decreasing oxidative stability of milk in the case of part-time grazing groups may be due to a higher degree of lipid oxidation caused by higher levels of C18:2 n-6, especially when the ewes diet was supplemented with concentrates (group C).

Camelina oil is obtained from camelina seed (*Camelina sativa* L), a traditional plant in Romanian agriculture, and is characterized by a high content of 18:2 n-6 and 18:3 n-3. Feeding a plant oil rich in PUFA is expected to increase the levels of beneficial PUFA in milk, which may decrease the oxidative stability of milk due to PUFA susceptibility to oxidation. In this context, it is important to evaluate milk oxidation status by measuring the antioxidant activity of milk by TEAC method. Part-time grazing diet supplementation with camelina oil calcium soaps (Cs) led to an increase in TEAC values in milk samples by 34.6-27.4% ($P<0.001$), compared to the part-time grazing diets supplemented with concentrate or grass hay (groups C and H). This increase in antioxidant capacity of milk was due to increased levels of natural antioxidants (tocopherols and phytosterols) of camelina oil [43, 44], which provide a better oxidative stability of polyunsaturated FA in camelina oil as compared to fish oil and other vegetable oils rich in PUFAs [45]. Even though milk PUFA concentrations were higher with S diet than with P diet in the present study, Cs supplementation had no influence on the oxidative stability of the milk.

Conclusions

Ewes maintained on pasture and whose ration was not supplemented with other fodder, had the lowest concentration of hypercholesterolemic fatty acids (HFA: C12:0, C14:0, C16:0) ($P<0.10$ to 0.05) in their milk fat, but instead had the highest concentration of TVA, c9, t11-CLA and α-linolenic acid in milk fat ($P<0.05$ to 0.001). Part-time grazing and supplementing the diet with concentrates or grass hay, compromises the milk FA profile, without any significant positive effect on milk yield. Feeding-system ewes with a limited access to pasture and moderate amounts of concentrate, hay and camelina oil calcium soaps (Cs) did not change milk fatty acid profile and oxidative stability of the milk with respect to the group that did full grazing. Thus, supplementary diet on Cs could be a valuable tool for farmers who wish to produce milk and dairy products enriched in n-3 FA and CLA and obtain a lower content of saturated fatty acids with high hypercholesterolemia potential.The experiment confirmed that manipulation in ewes part-time grazing diet may improve milk fatty acid profile and antioxidative capacity of milk, with beneficial effects on human health. Even though milk PUFA concentrations were higher with S diet than with P diet in the present study, Cs supplementation had no influence on the oxidative stability of the milk.

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