doi: 10.17221/8241-CJAS

Time-dependent changes in milk fatty acid composition of ewes fed a winter ration supplemented with linseed or sunflower oils

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ABSTRACT: The effects of adding sunflower and linseed oils to a standard winter ration with a lower concentrate content on the milk fatty acid composition in lactating ewes were investigated. Eighteen dairy ewes randomly chosen from the ewe flock were divided into three groups: the first group was fed a winter ration, the second one a winter ration supplemented with sunflower oil, and the third a winter ration supplemented with linseed oil for a period of 12 days. In the treatment groups, the concentrate was partially replaced by 3.0 g/100 g sunflower or linseed oils. Milk samples were taken daily from morning milking for the analysis of fatty acid composition to determine their temporal daily variations. The responses to sunflower oil compared with linseed oil addition after the end of experiment were slightly higher for conjugated linoleic acid (CLA) ($P < 0.05$), linoleic acid ($P < 0.001$), *trans*-10 18:1 ($P < 0.001$), 6:0 to 16:0 ($P \approx 0.05 - P < 0.001$), whereas a higher content of α-linolenic acid (ALA) (3-fold) ($P < 0.001$), oleic acid ($P < 0.001$), and 18:0 ($P < 0.001$) was found in milk after linseed oil addition. The responses to both oil additions were relatively sustainable with regard to CLA, *trans*-11 18:1 (vaccenic acid, VA), and ALA content after the last 6 days of supplementation. The winter ration supplementation with sunflower or linseed oil led to a 3-fold increase in CLA milk fat content (0.6–2.0 or 1.8 g/100 g fatty acid methyl esters (FAME), $P < 0.001$) and a 3-fold increase in VA milk fat content (1.2–3.8 or 4.1 g/100 g FAME, $P < 0.001$), however the content of *trans*-10 18:1 was 5–6-fold higher, compared with unsupplemented winter ration. Plant oil supplementation enhanced the total content of CLA, VA, ALA by 5.0 and 3.9 g/100 g FAME ($P < 0.001$) for linseed and sunflower oil supplementation, and decreased the total content of 12:0, 14:0, and 16:0 by 9.3 and 5.8 g/100 g FAME ($P < 0.001$) compared to winter diet, respectively.

Keywords: ewes' standard winter diet; supplementation of plant lipids; daily changes of milk fatty acid composition; CLA content

INTRODUCTION

The specific profile of ewe milk fatty acids (FA) is particularly valuable for human nutrition and health. The biological effects and nutritional value of fat and FA must be considered on the basis of individual FA (Lock and Bauman 2004). Several *in vitro* cell cultures and *in vivo* animal models suggested that *cis*-9,*trans*-11 18:2 (conjugated linoleic acid, CLA) show anti-carcinogenic, anti-

Supported by the Slovak Research and Development Agency (Projects No. APVV-0458-10 and No. APVV-0416-10) and Research and Development Operational Programme funded by ERDF (Project Comenius University in Bratislava Science Park, ITMS 26240220086).

doi: 10.17221/8241-CJAS

atherogenic, anti-diabetic, and other advantageous physiological effects (Benjamin and Spener 2009). The *trans*-11 18:1 (vaccenic acid, VA) should also be considered as a FA with particular benefits for human health. The anti-carcinogenic effect of VA is associated with its conversion to CLA, humans convert on average 19% of dietary VA to CLA via Δ9-desaturase (Turpeinen et al. 2002; Field et al. 2009). Nevertheless, a ruminant milk fat contains higher levels of saturated FA (SFA), particularly lauric acid (LAA, 12:0), myristic acid (MA, 14:0), and palmitic acid (PA, 16:0) showing hypercholesterolemic properties, and lower contents of some *trans* FA (TFA) representing a dietary risk (Shingfield et al. 2013). In contrast to SFA 12:0 to 16:0, the 18:0 is non-atherogenic (Chilliard et al. 2007). It is suggested that CLA has similar beneficial effects in humans. It is therefore desirable to increase especially the content of CLA and VA and at the same time to lower that of LAA, MA, and PA in milk and dairy products.

Ration is the most important factor determining the CLA and VA content in milk fat. Addition of lipids to dairy ruminant ration can be used to increase ration energy density, milk fat content, and to modify milk FA composition. Supplementation with plant lipids such as linseed oil (rich in *cis*-9,*cis-12*,*cis-15* 18:3 (α-linolenic acid, ALA)) or sunflower oil (rich in *cis*-9,*cis*-12 18:2 (linoleic acid, LA)) enhances the content of CLA, VA, and ALA in milk (Bauman et al. 2000; Bauman and Griinari 2003). CLA can be a product of incomplete biohydrogenation of LA in the rumen and primarily of Δ9-desaturation of VA in the mammary gland (Griinari et al. 2000). Dietary ALA and LA are metabolized and biohydrogenated in the rumen leading to the formation of 18:0 and various isomers of monounsaturated FA (MUFA) and polyunsaturated FA (PUFA). Some of these intermediates are absorbed in the gut and secreted into milk while others are metabolized in body tissues, especially in the mammary gland, where a *cis* double bond is being added to the FA structures by the action of Δ9-desaturase enzyme. Rumen-escaped dietary PUFA and 18:0 formed in the rumen are residual precursors and the final products of biohydrogenation. Biohydrogenation affects the interaction between the ration components and the mammary fatty acid synthesis.

The milk fat CLA responses after lipid supplementation were sometimes transient and declined over time (Bauman et al. 2000; Dhiman et al. 2000). Roy et al. (2006) found that the content of CLA in cow milk from starch-rich based diets supplemented with sunflower oil were transient and declined after one to two weeks. Nevertheless, cows feeding a high forage hay based diet supplemented with linseed oil resulted in a gradual enrichment with CLA and VA in milk fat and the increase was maintained until the end of experiment. Transient decreases in *cis*-9,*trans*-11 CLA content were found to be associated with high proportions of corn silage and/or concentrates, and subsequent increases in the milk fat content of unhealthy *trans* FA (TFA), especially *trans*-10 18:1 (Ferlay et al. 2003, Chilliard et al. 2007).

Despite the fact that the effects of lipid oil supplementation on milk FA synthesis bear some similarities across ruminants, ewes often respond differently than cows or goats in many aspects of mammary lipid metabolism (Chilliard et al. 2003). The effects of plant oil supplementation on milk FA synthesis of ewes were reported by Antongiovanni et al. (2004), Zhang et al. (2006), Luna et al. (2008), Toral et al. (2010), Gomez-Cortes et al. (2011). The CLA milk fat content was in a relatively broad range of 0.8–2.5 g/100 g fatty acid methyl esters (FAME). The changes in milk FA composition after plant oil supplementation were dependent on the amount of oil included in the rations, the FA profile of lipid supplement, the form of lipid supplement, the composition of basal ration (forage type, starch and concentrate content) and their interaction with dietary lipids.

Based on the fact that milk SFA and TFA are established dietary risk factors, and unsaturated FA (UFA) decrease health risks, one might suggest reduced consumption of dairy products to decrease the intake of SFA and TFA. Nevertheless, dairy products are key sources of high-quality proteins, vitamins, minerals, and bioactive lipids. Altering the FA composition of milk represents one means to lower SFA and increase *cis* MUFA and PUFA intake with the human diet (Shingfield et al. 2013). The objective of this study was to investigate the daily changes in milk FA composition during a 12-day period when lactating ewes were fed a winter diet without oil supplementation, or with linseed/sunflower oil supplementation. Standard winter ration with a relatively lower concentrate content was used to determine the effects of addition of these plant oils to a winter basal ration (WBR) in order to increase the winter fat milk content of FA that

doi: 10.17221/8241-CJAS

Table 1. Ingredients of experimental diets (dry matter basis) (g/kg)

	WBR	LO	SO
Corn silage	430	430	430
Meadow hay	348	348	348
Concentrate	222	194	193
Linseed oil	–	28.2	–
Sunflower oil	–	–	29.2
WBR basic nutrients contents			
NEL (MJ)	4.2		
PDI	68.4		
NS	114		
Fibre	130		
Ca	6.1		
P	3.6		
Na	1.4		
K	14.8		

WBR = winter basal diet, LO = linseed oil supplemented ration, SO = sunflower oil supplemented ration, concentrate = commercial concentrated feed mixture, NEL = net energy of lactation, PDI = protein digestible in intestine, NS = nitrogen-containing substances

are potentially important for human nutrition and health (CLA, VA, ALA) and to decrease that of FA with negative effects on human health (LA, MA, PA).

MATERIAL AND METHODS

The study was carried out at the experimental ewe farm in Trenčianska Teplá, Animal Production Research Centre Nitra, Slovak Republic. The farm keeps 350 dairy ewes belonging to three pure breeds and crossbreeds based on Tsigai (n = 160), Improved Valachian (n = 130), and Lacaune (n = 60) breeds in the first to eighth parity. The ewes consumed the unsupplemented winter basal ration (uWBR) containing corn silage (1190 g), meadow hay (961 g), commercial concentrated feed mixture (614 g), and mineral supplement, in total 2765 g per ewe per day (Table 1). Sunflower oil and linseed oil were mixed manually with concentrate and other components of WBR immediately before feeding. The effect of partial supplementation of concentrate fed mixture of WBR with 3.0% sunflower oil or 3.0% linseed oil (g/100 g dry matter (DM)) on the temporal changes of fatty acid composition of ewes' milk fat was investigated. The FA composition of components of experimental rations is presented in Table 2. Eighteen dairy ewes randomly chosen from the ewe flock fed WBR were divided into three groups, each containing 6 lactating ewes with initial lactation days of 64 ± 3 and an average body weight of 68 kg. The first group was fed WBR, the second one was fed WBR supplemented with sunflower oil, and the third one was fed WBR supplemented with linseed oil. In the course of the experimental period, the ewes were kept in group-pens and the ration was offered once per day at 8:00 h. Machine milking was carried out twice daily (at 7:00 and 19:00 h) and daily temporal variations in FA composition were determined from the morning milk samples. On the first day of experiment ewes were milked before feeding the plant oil supplemented WBR.

Table 2. Fatty acid composition of experimental diet (g/kg dry matter)

Fatty acid	Corn silage	Meadow hay	Concentrate	Concentrate + linseed oil	Concentrate + sunflower oil
10:0	0.0	0.1	0.0	0.0	0.0
12:0	0.4	0.7	0.0	0.0	0.0
14:0	0.6	1.1	0.2	0.1	0.2
15:0	0.1	0.5	0.1	0.1	0.1
16:0	15.1	20.7	16.0	6.3	7.4
17:0	0.3	0.4	0.1	0.1	0.1
18:0	3.0	3.2	2.4	3.5	3.6
OA	21.1	7.3	21.4	20.4	23.7
LA	46.4	22.1	55.6	22.3	60.9
ALA	6.6	32.7	5.1	46.2	1.0
20:0	0.5	2.2	0.3	0.2	0.3
22:0	0.8	2.4	0.3	0.2	0.5
24:0	1.4	1.5	0.3	0.1	0.2

OA = oleic acid, LA = linoleic acid, ALA = α-linolenic acid

doi: 10.17221/8241-CJAS

The contents of FA were determined in bulk milk samples from each group.

The content of about seventy FA in the ewes' milk fat as well as in the experimental rations was analyzed using capillary gas chromatography (GC). Lipids from milk samples were extracted using chloroform-methanol mixtures (2 : 1). The extracts were filtered through anhydrous sodium sulfate, and then dried and stored under nitrogen at –18°C. For the FAME preparation, the base-catalyzed methylation procedure with a solution of sodium methoxide in methanol was used. GC analyses of milk and experimental rations extracts were performed on a 6890N Gas Chromatograph (Agilent Technologies, Waldbronn, Germany) with Flame Ionization Detector and 5973 Network Mass Selective Detector. FAME were separated in a capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness) of HP-88 stationary phase (Agilent Technologies, Santa Clara, USA). The initial column temperature of the programmed run was set to 45°C and was held for 2 min, then followed by a step up ramp of 15°C/min to 145°C, and then of 5°C/min to 240°C and held for 5 min. Helium was used as the carrier gas with a linear velocity set at 20 cm/s. 2 μl samples representing approximately 10 mg/ml FAME were injected using a split 50 : 1 at injection temperature of 300°C. Separated FA were identified by reference materials, published retention data, and mass spectrometric measurements. The chromatograms were evaluated quantitatively using a method of internal normalization and published response factors of flame ionization detector for FAME (Ackman 2002). The FA composition of milk fat was expressed in grams of each individual FAME per 100 g of sum detected FAME. The average relative standard deviation of the analyzed FAME with the content > 0.5 g/100 g was 1.1%, for the whole analytical procedure and 5 replicate samples. The CLA content in analyzed milk samples represents the total content of gas chromatographic unseparated triplet of *cis*-9,*trans*-11 + *trans*-7,*cis*-9 + *trans*-8,*cis*-10 CLA isomers with prevailing (about 90%) content of *cis*-9,*trans*-11 CLA (Blasko et al. 2009).

The data set of selected 25 FA milk fat contents was subjected to analysis of variance (ANOVA) for a randomized block design with three repeated measures using a Linear Mixed Models procedure (IBM SPSS Advanced Statistics 21, 2012; Statistical Analysis System, Version 16.0, 2006) The model included three types of diet (D) and twelve sampling days (T) as fixed effects and measures from bulk milk samples from each group as a random effect, and an interaction between the type of diet and the sampling days (D × T). All variables were considered significant at $P < 0.05$. Pair-wise means comparisons in selected data sets were evaluated *post-hoc* by Scheffé's method.

RESULTS AND DISCUSSION

Temporal changes in FA composition in ewes' milk during winter basal diet supplementation with sunflower or linseed oils

The time-dependent changes in milk FA content during the supplementation period followed moderately different temporal patterns between both oil treatments. The responses of individual FA to sunflower and linseed oil supplementation and to a lesser degree to WBR (except for 18:0 and oleic acid (OA)) varied according to the time on a diet. Figures 1 and 2 show the time-dependent changes in SFA, MUFA, and PUFA contents in milk fat of ewes fed uWBR, and WBR supplemented with sunflower or linseed oils during the 12-day experiment. Final results are summarized in Table 3.

Figures 1 and 2 show that temporal milk responses in ewes fed WBR alternately increased and decreased for the first 8 days of experiment. Approximately from day 9, the milk FA showed more systematic trends, i.e. an increased content of 6:0 to 16:0, *cis*-9 14:1 and 16:1, and LA, and a decreased content of 18:0 and OA, and they remained relatively stable for CLA, VA, ALA, *trans*-10 18:1; the differences of means of these FA for the last four days were statistically insignificant. As the FA responses to uWBR also varied according to time on a diet, the temporal changes in sunflower and linseed supplementation were related to the same day of the experiment.

The changes in FA content in milk were apparent immediately the next morning following the addition of sunflower or linseed oils to WBR. When compared with uWBR, both supplementations increased the contents of CLA, VA, LA, *trans*-10 18:1, and decreased 6:0, 8:0, 10:0, LA, MA, PA, and *cis*-9 16:1 milk contents (Table 3). The data set of 25 FA including potentially health beneficial FA (CLA, VA, ALA) and maleficent FA (LA, MA, PA acids and *trans*-10 18:1) were subjected to ANOVA. The results are summarized in Table 3. It is obvious that dietary treatments (uWBR, sunflower oil

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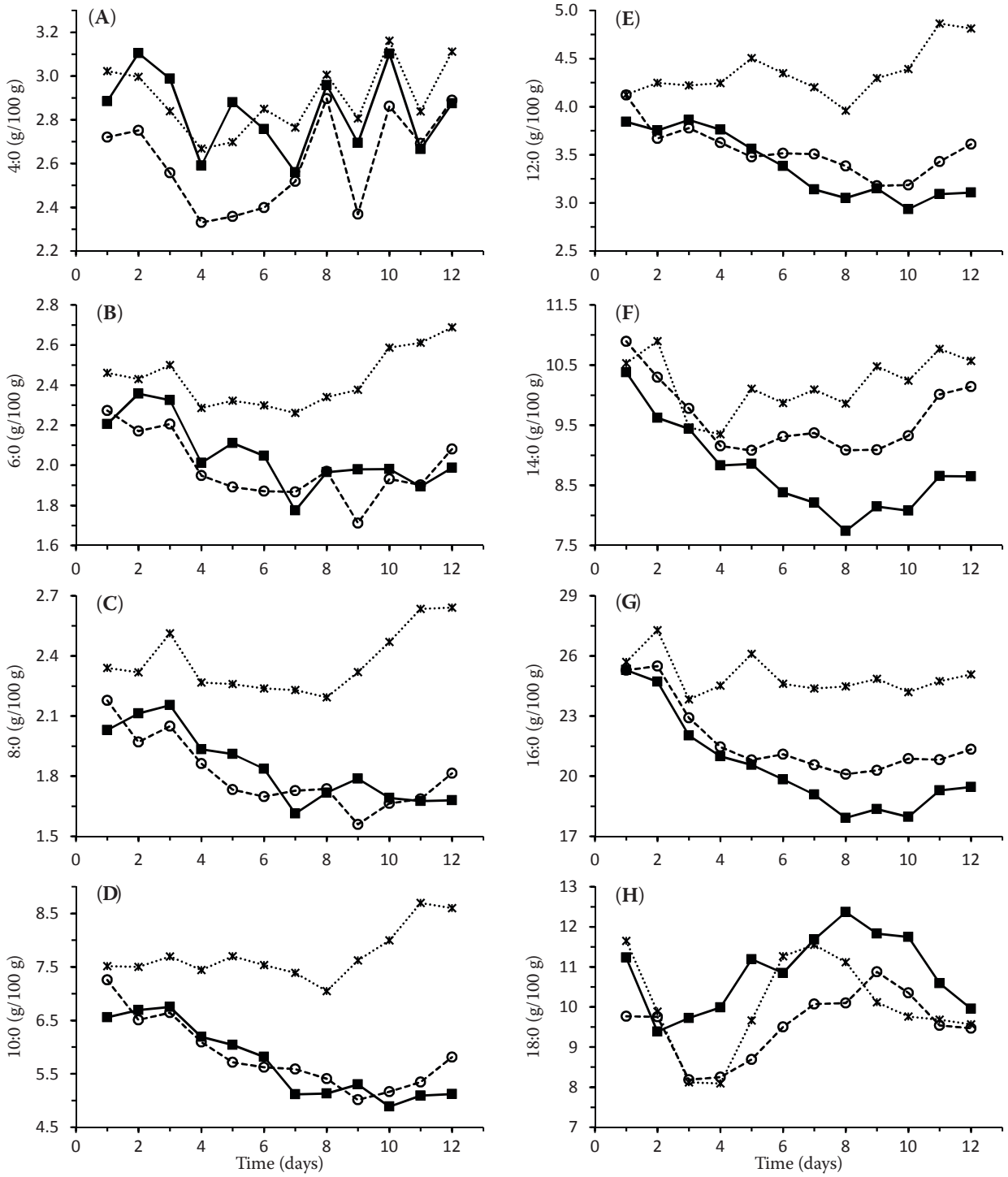

Figure 1. Temporal changes in 4:0 to 18:0 saturated fatty acids content in milk from ewes fed a winter ration (×), winter ration supplemented with linseed (■) and sunflower (○) oils. Values represent the means from six ewes

(SO) or linseed oil (LO) supplemented WBR) on sampling days (days 1–12) and their interactions were significant at $P < 0.05$ for almost all FA, except for D × T for 4:0 and 17:0 anteiso, and T for 17:0 anteiso. The variation > 80% in the evaluated FA can be ascribed to the statistical model used (D, T, D × T). Within the model the major effect was attributed to the diet, except for 4:0, 17:0 iso, 17:0 anteiso, 18:0, and OA and the effect of diet was significant for all the 25 FA. Therefore the pair effect diet was further evaluated and significant pairs are given in Table 3. The pair effect diets

doi: 10.17221/8241-CJAS

Table 3. Effect of diet composition and days of plant oils supplementation on selected milk fatty acids content of ewes fed unsupplemented winter basal ration (uWBR) at the start (1) and end (2) of the experiment, and winter basal ration (WBR) supplemented with sunflower oil (SO) and linseed oil (LO) (g/100 g fatty acid methyl esters (FAME)) at the end of the experiment

Fatty acid	uWBR 1	uWBR 2	SO	LO	SE	Significance			
						D	D × D	T	D × T
4:0	3.02[a]	2.97[a]	2.79[a]	2.77[a]	0.159	*	uWBR-SO LO-SO	*	ns
6:0	2.46[a]	2.65[a]	1.99[b]	1.94[b]	0.118	*	uWBR-SO uWBR-LO SO-LO	*	*
8:0	2.34	2.64	1.75	1.68	0.089	*	uWBR-SO uWBR-LO	*	*
10:0	7.52	8.65	5.58	5.11	0.298	*	uWBR-SO uWBR-LO	*	*
12:0 (LAA)	4.13	4.84	3.52	3.10	0.170	*	uWBR-SO uWBR-LO	*	*
14:0 (MA)	10.53[a]	10.67[a]	10.08	8.65	0.205	*	uWBR-SO uWBR-LO SO-LO	*	*
cis-9 14:1	0.12[a]	0.15[ab]	0.17[b]	0.13[a]	0.006	*	uWBR-SO uWBR-LO SO-LO	*	*
15:0 iso	0.30[a]	0.32[a]	0.24[b]	0.20[c]	0.015	*	uWBR-SO uWBR-LO SO-LO	*	*
15:0 anteiso	0.43[a]	0.48[a]	0.35[b]	0.35[b]	0.008	*	uWBR-SO uWBR-LO SO-LO	*	*
15:0	0.68[a]	0.82	0.71[a]	0.72[a]	0.016	*	uWBR-SO uWBR-LO SO-LO	*	*
16:0 iso	0.34[a]	0.35[a]	0.24[b]	0.39[a]	0.010	*	uWBR-SO uWBR-LO SO-LO	*	*
16:0 (PA)	25.71	24.91	21.08	19.37	0.126	*	uWBR-SO uWBR-LO SO-LO	*	*
cis-9 16:1	0.73[a]	0.83	0.74[a]	0.64	0.032	*	uWBR-SO uWBR-LO SO-LO	*	*
17:0 iso	0.41[a]	0.46[a]	0.39[a]	0.40[a]	0.033	*	uWBR-SO uWBR-LO	*	*
17:0 anteiso	0.45[a]	0.48[a]	0.34[b]	0.41[ab]	0.056	*	uWBR-SO uWBR-LO	ns	ns
17:0	0.59[a]	0.55[a]	0.42[b]	0.44[b]	0.005	*	uWBR-SO uWBR-LO SO-LO	*	*
18:0	11.65	9.63[a]	9.50[a]	10.27	0.224	*	uWBR-SO uWBR-LO SO-LO	*	*

doi: 10.17221/8241-CJAS

Table 3 to be continued

Fatty acid	uWBR 1	uWBR 2	SO	LO	SE	Significance			
						D	D × D	T	D × T
trans-10 18:1	0.46[a]	0.64[a]	4.31	3.39	0.114	*	uWBR-SO uWBR-LO SO-LO	*	*
trans-11 18:1 (VA)	0.98[a]	1.23[a]	3.83	4.08	0.145	*	uWBR-SO uWBR-LO SO-LO	*	*
cis-9 18:1 (OA)	17.79[a]	16.64[b]	16.62[b]	17.99[a]	0.221	*	uWBR-SO uWBR-LO SO-LO	*	*
cis-9,*cis*-12 18:2 (LA)	2.24	2.65	4.48	4.14	0.141	*	uWBR-SO uWBR-LO SO-LO	*	*
cis-9,*cis*-12,*cis*-15 18:3 (ALA)	0.37[a]	0.48	0.40[a]	1.42	0.045	*	uWBR-SO uWBR-LO SO-LO	*	*
cis-9,*trans*-11 18:2 (CLA)	0.53	0.67	1.99	1.77	0.089	*	uWBR-SO uWBR-LO SO-LO	*	*
20:5 n-3 (EPA)	0.04[a]	0.03[ab]	0.02[b]	0.06	0.005	*	uWBR-SO uWBR-LO SO-LO	*	*
22:6 n-3 (DHA)	0.05[a]	0.05[ab]	0.03[b]	0.04[ab]	0.006	*	uWBR-SO uWBR-LO SO-LO	*	*
ΣLA + MA + PA	40.36[a]	40.42[a]	34.68	31.12	1.762	*	uWBR-SO uWBR-LO SO-LO	*	*
ΣVA + ALA + CLA	1.88[a]	2.38[a]	6.22[b]	7.27[b]	0.307	*	uWBR-SO uWBR-LO	*	*

SE = standard error, D = type of diet, T = sampling day, D × D = significant diet pair, D × T = interaction between type of diet and sampling days, LAA = lauric acid, MA = myristic acid, PA = palmitic acid, VA = vaccenic acid, OA = oleic acid, ALA = α-linolenic acid, CLA = conjugated linoleic acid, LA = linoleic acid

[a–c]means not connected by the same letter are considered different at $P < 0.05$, SE and significance effect were assessed for each day of the 12-day experiment, *$P < 0.001$, ns = not significant

WBR-SO and WBR-LO were significant for all FA (except WBR-LO for 4:0). The data for 4:0 could have been affected by its relatively high volatility and higher variance of measurements. The pair effect diet LO-SO was found not to be significant for SFA (8:0–12:0 and isomers 17:0).

Milk saturated fatty acids. In contrast to uWBR, the milk fat contents of 6:0 to 12:0 upon oil supplementations declined steadily up to day 7 to relatively sustainable values. In the case of PA this pattern was observed for all dietary treatments. Greater reductions in SFA content were observed for 6:0 to 16:0 after supplementation with linseed oil, compared to sunflower oil. SFA with an even number of carbon atoms in a molecule 6:0 to 14:0 showed gradual changes in their content for sunflower oil compared with linseed oil supplementation, and the differences in their contents increased with the increasing number of carbon atoms in a molecule (Figure 1). However, the difference in PA content in milk fat between the linseed and sunflower oil supplementation decreased ($P < 0.01$). The total amount of three unfavourable SFA (LAA, MA, and PA) represented 40.4 g/100 g FAME of milk fat content of ewes fed uWBR. This content dropped to 31.1 and 34.7 g/100 g FAME

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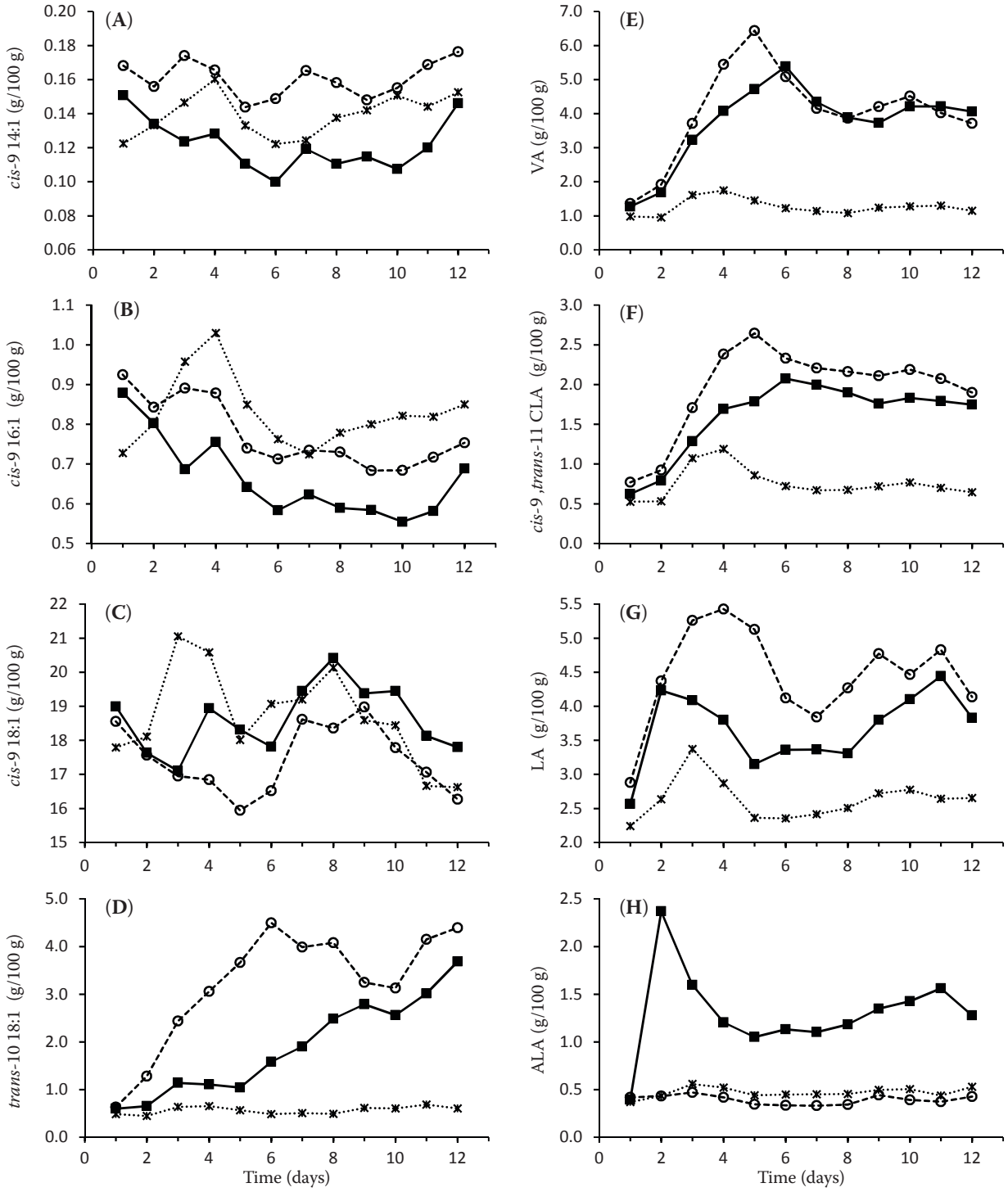

Figure 2. Temporal changes in C14 to C18 mono- and poly-unsaturated fatty acids content in milk from ewes fed a winter ration (×), winter ration supplemented with linseed (■) and sunflower (○) oils. Values represent the means from six ewes

VA = vaccenic acid, CLA = conjugated linoleic acid, LA = linoleic acid, ALA = α-linolenic acid

after the linseed oil and sunflower supplementation ($P < 0.001$), respectively (Table 3). In contrast to 6:0–16:0, the content of 18:0 increased from day 3 to days 7–9 and then it decreased for all the three diets. The 18:0 content in milk fat was higher after linseed oil addition ($P < 0.001$), and it was not

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statistically significantly different after uWBR and sunflower oil addition. Zhang et al. (2006) found the lowest 18:0 content in ewe's milk after the control diet probably due to a different basal diet composition.

The lower content of short- and medium-chain SFA in milk from the uWBR supplemented ewes compared to the content in milk from WBR supplemented ewes can be associated with the inhibitory effect of the increased availability of long-chain FA to the mammary gland on *de novo* synthesis of FA (Grummer 1991, Shingfield et al. 2006). The reduced 6:0 to 16:0 SFA content after lipid oil supplementation is consistent with the previously published data for dairy cows, goats, and ewes (Sanz Sampelayo et al. 2007; Shingfield et al. 2008). The temporal changes of 18:0 content in milk are different from those of other SFA. The 18:0 milk fat content rapidly dropped down reaching the nadir on day 3, then it gradually rose on days 8–10, and then decreased again until the end of experiment. The lower 18:0 milk fat content from sunflower oil compared with linseed oil supplementation (9.5 vs 10.3 g/100 g FAME, $P<0.001$) can be associated with the inhibitory effect of LA on the biohydrogenation of C18 UFA to 18:0 in the rumen (Shingfield et al. 2008).

Similarly to SFA with an even number of carbon atoms in a molecule, WBR supplementation with linseed and sunflower oil resulted in lower contents of all odd- and branch-chain 15:0 to 17:0 having positive health effects. The mean decrease in the total content of these FA was 21%, i.e. similar as in SFA with the even number of carbon atoms. Nevertheless, the content of these FA in milk fat is substantially lower (up to 0.86%) than that of SFA with the even number of carbon atoms. In addition, Vlaeminck et al. (2006) reported lower milk fat contents of odd- and branch-chain SFA after supplementation of cows' diet with oils rich in LA or ALA.

Milk unsaturated fatty acids. The effect of dietary treatment on the content of *cis*-9 14:1 to 18:1 in ewes' milk fat is presented in Figures 2A–C. When comparing linseed and sunflower oil supplementations, the variance in MUFA content gradually decreased with the increasing number of FA carbon atoms, from the higher variation for *cis*-9 14:1 and 16:1 to the lower variation and a reverse content ratio for *cis*-9 18:1. In contrast to *cis*-9 14:1 and *cis*-9 16:1, but similarly to 18:0, the content of *cis*-9 18:1 increased from day 5 to day 8 of experiment, and then it decreased for all the three diets. At the end of the experiment, the milk OA content increased by 1.4 g/100 g FAME for linseed oil ($P < 0.001$), and it remained unchanged for sunflower oil if compared with uWBR. The higher content of *cis*-9 18:1 after linseed oil and the lower content after sunflower oil supplementation compared with uWBR can stem from the combination of ruminal biohydrogenation and 18:0 desaturation in the mammary gland by the action of Δ9-desaturase enzyme (Dhiman et al. 2000).

The time-dependent changes of OA/18:0 content ratio are presented in Figure 3A. The 18:0 desaturation ratio was markedly higher for WBR in days 3–4 ($P < 0.001$) and reached the nadir on day 8 of experiment. The OA/18:0 ratio after sunflower oil was higher than that after linseed oil supplementation, however, it was statistically identical with all the three diets at the end of experiment. Thus, unlike in cows (Chilliard and Ferlay 2004), it was only slightly affected by lipid supplementation, although dietary lipids are putative inhibitors of Δ9-desaturase.

WBR supplementation with sunflower or linseed oils significantly increased the CLA content in ewes' milk compared with uWBR ($P < 0.001$). The CLA content increased as early as after the first day of supplementation if compared with uWBR ($P < 0.05$). Addition of sunflower oil to WBR led to the highest CLA content (2.64 g/100 g FAME) on day 5 of supplementation corresponding to a 4-fold increase compared with uWBR (0.67 g/100 g FAME) ($P < 0.001$). Nevertheless, such an increase was only transient, followed by a gradual decline to almost sustainable average CLA content of 2.0 g/100 g FAME despite a 4-fold increase in *trans*-10 18:1 content for the rest of experiment. Similarly, Chilliard et al. (2007) found a relatively stable CLA content in goats' milk despite increasing the *trans*-10 18:1 content 5–8 times above its content in a control diet. The time-dependent changes of CLA and VA content during supplementation with peak values on days 4–5 and nearly sustainability from day 8 are in agreement with the data for cows fed a ration with a higher content of grass hay and supplemented with linseed oil (Roy et al. 2006). The variability in CLA content during supplementation is associated with its synthesis in the mammary gland by the action of Δ9-desaturase. The CLA milk fat content after sunflower oil supplementation found in our study was slightly lower than that presented by Zhang et al. (2006) (2.0 vs 2.5 g/100 g FAME) probably

doi: 10.17221/8241-CJAS

due to different WBR composition as both studies found a similar increase of CLA content in milk fat after sunflower supplementation (by 1.4 g/100 g FAME) compared with uWBR.

Figure 2F shows that the CLA content in milk fat of the ewes from the group still fed uWBR differed on the first day (uWBR group 0.53, LO group 0.62, and SO group 0.77 g/100 g FAME) suggesting the effect of individual ewes on the CLA production in milk (Sojak et al. 2013). Unexpectedly, the CLA content in milk fat for uWBR increased from 0.5 to 1.2 g/100 g and correspondingly for VA from 0.8 to 1.7 g/100 g FAME from day 1 to 4, and then it steeply decreased toward the initial values on day 6 (Figure 2F). The temporal relation of CLA/VA milk fat content ratio after uWBR between days 1 and 4 showed a steep increase from 0.54 to 0.68 on day 4 ($P < 0.05$) and then a steep decrease toward initial values. Such a fluctuating Δ9-desaturase activity could be due to the lack of homogeneity in the ALA content of meadow hay in the course of the study period. Previous analyses showed a broad range of the ALA content (15–54 g/kg DM) in meadow hay samples from Slovakia (unpublished data).

The temporal changes in CLA content in milk fat were associated with the changes in the content of other FA. Most significantly, the time dependence of CLA milk fat content correlated with VA milk fat content changes during the supplementation period ($R^2 = 0.97$, $P < 0.001$) (Figure 2E, F). The VA formation in the rumen reflected an overall increase in *trans* 18:1 accumulation in the rumen. The VA milk fat content increased from 1.7 to 6.4 g/100 g FAME during the first 5 days of sunflower oil supplementation ($P < 0.001$), and then it gradually decreased to the content of 3.9 g/100 g FAME ($P < 0.001$). Thus, a higher CLA milk fat content after sunflower oil supplementation was in agreement with ruminal biohydrogenation that was shifted toward the formation of VA as a major intermediate because the milk fat content of *trans*-10 18:1 was lower during the entire supplementation period except for the last day of experiment.

The changes in the content of CLA, VA, and *trans*-10 18:1 in milk fat during the supplementation period (Figure 2D–F) followed moderately different temporal patterns between both oil treatments. The CLA content after linseed oil addition was lower compared with sunflower oil addition with the peak of 2.1 g/100 g FAME on day 6 of supplementation ($P < 0.05$), and then it decreased to nearly sustainable value of 1.8 g/100 g FAME ($P < 0.01$). When compared with sunflower oil supplementation, markedly different temporal changes were observed after linseed addition for *trans*-10 18:1 milk fat content, which gradually increased only after the 5th day of supplementation from 1.0 to 3.4 g/100 g FAME at the end of experiment ($P < 0.001$). In contrast to sunflower oil supplementation, the CLA content in milk fat after linseed oil supplementation was slightly higher than that reported by Zhang et al. (2006) (1.8 vs 1.5 g/100 g FAME), although its relative change compared to WBR was remarkable (1.2 vs 0.4 g/100 g FAME in the mentioned study). These differences can be attributed to the variations in the basal diet composition.

The lower milk fat contents of VA and CLA were observed after linseed oil supplementation up to day 5 ($P < 0.001$). In goats fed a basal diet with a higher content of concentrate (67%), Martinez Marin et al. (2013) found weaker responses of VA and CLA to diets with linseed oil as compared to sunflower oil supplemented diets (50 vs 10%). With linseed oil the biohydrogenation of dietetic PUFA passes slowly and more completely thus the formation of saturated FA is more enhanced than that of CLA and VA (Sanz Sampelayo et al. 2007). A different temporal alteration of lipid metabolism in the rumen through plant oil effect on the VA/*trans*-10 18:1 content ratio was most notable for linseed oil with a maximum on day 5 of supplementation ($P < 0.001$, Figures 2D, E and 3B). The VA/*trans*-10 18:1 ratio was higher for linseed oil compared with sunflower oil on day 2 ($P < 0.001$), however, it was similar and relatively stable for both plant oils after 8 days, and lower compared with WBR ($P < 0.001$) (Figure 3B). There was no marked *trans*-11 to *trans*-10 18:1 formation shift at the beginning of linseed oil supplementation because ruminal microflora needed 5 days for adaptation of biohydrogenation to linseed oil. In contrast, sunflower oil addition caused an apparent increase in *trans*-10 18:1 content after the first day of supplementation ($P < 0.001$).

The highest VA content of 6.4 g/100 g FAME after sunflower oil supplementation observed on day 5 of supplementation corresponded to the peak CLA content of 2.6 g/100 g FAME with the VA/CLA content ratio of 2.43. The highest content ratio of 2.54 was found on day 2 of supplementation for VA content (1.7 g/100 g FAME) and CLA

doi: 10.17221/8241-CJAS

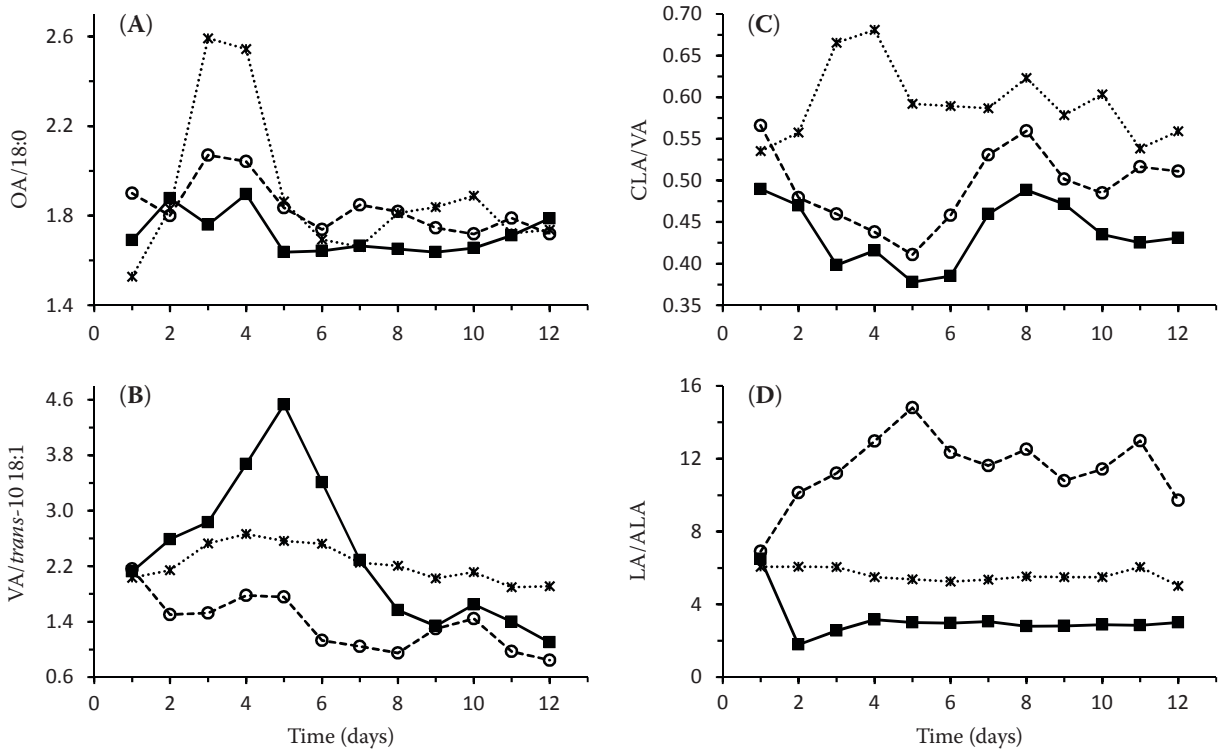

Figure 3. Temporal changes in *cis*-9 18:1 (oleic acid, OA)/18:0 (**A**), *trans*-11 18:1 (vaccenic acid, VA)/*trans*-10 18:1 (**B**), *cis*-9,*trans*-11 18:2 (conjugated linoleic acid, CLA)/*trans*-11 18:1 (VA) (**C**), and *cis*-9,*cis*-12 18:2 (linoleic acid, LA)/*cis*-9,*cis*-12,*cis*-15 18:3 (α-linolenic acid, ALA) (**D**) content ratio in milk from ewes fed a winter ration (×), winter ration supplemented with linseed (■) and sunflower (○) oils

content (0.68 g/100 g FAME). Thus, the linearity of CLA and VA relationship was found across the full range of VA contents suggesting that the Δ9-desaturation capacity of the mammary gland was not exceeded during the supplementation period. Because of the low content of concentrate in supplemented WBR (19.2 g/100 g DM), the oil supplementation favoured the rumen accumulation of VA with an increasing secretion of VA and CLA in milk. A decreased ewe's sensitivity to the shift of biohydrogenation to the *trans*-10 18:1 can also contribute to high and sustainable CLA content in milk fat (Sanz Sampelayo et al. 2007).

The temporal changes in CLA/VA milk content ratio presented in Figure 3C show peak values in days 3–4 for uWBR. Unlike uWBR, the CLA/VA milk content ratio after plant oil addition was decreased till day 5, then it increased till day 8, and thereafter it declined to relatively stable values at the end of experiment. The CLA/VA ratio was the highest for uWBR, medium for sunflower oil, and the lowest for linseed oil supplementation, and it was relatively stable during the last days of experiment. These data reflect the changing activity of the mammary gland Δ9-desaturase for conversion of VA to CLA, which decreased in the following order: uWBR < sunflower oil < linseed oil addition. The trends of temporal changes in CLA/VA and OA/18:0 ratios were similar (Figure 3A, C) ($R^2 = 0.911$, $P < 0.001$).

The inclusion of plant oils also altered the profiles of other 18:2 and 18:3 FA in milk. The CLA content in milk fat can be increased using the rations containing LA or ALA, which serve as precursors for VA formation. These PUFA are not synthetized by ruminant tissue, and the content of LA and ALA in milk is dependent on the amount of these PUFA absorbed and partitioned toward the mammary gland (Shingfield et al. 2008). The content of LA and ALA in milk also depends on the extent of their metabolism in rumen. The temporal variation of LA and ALA content in milk fat after sunflower and linseed oil supplementation and uWBR are presented in Figure 2G, H. If compared with uWBR, supplementation with sunflower and linseed oils increased the LA content in milk fat by 1.8 and 1.5 g/100 g FAME ($P < 0.001$), respectively. The LA content in milk was significantly ($P < 0.001$) higher after sunflower supplementation partly

doi: 10.17221/8241-CJAS

because of the higher LA content in sunflower oil compared with linseed oil. The lowest LA content was found in the milk of ewes fed uWBR ($P < 0.001$). With linseed oil supplementation, the ALA content in milk fat sharply rose on day 2, decreased in days 2–5, and then it moderately increased toward the end of experiment ($P < 0.001$), whereas for sunflower oil it changed only slightly, statistically insignificantly throughout the experiment. The linseed oil supplementation led to 0.9 g/100 g FAME increase in the ALA content in milk fat compared with uWBR ($P < 0.001$). In contrast, sunflower oil supplementation resulted in negligibly lower, statistically insignificant, but steady ALA content of –0.1 g/100 g FAME, compared with uWBR. These differences in the milk ALA content are also associated with the higher ALA content in linseed oil.

The temporal changes in LA/ALA milk content ratio was presented in Figure 3D. The LA/ALA ratio was sharply increased up to day 5 ($P < 0.001$) of supplementation with sunflower oil, whereas supplementation with linseed oil led to a steep decrease on day 2 ($P < 0.001$) and then a very slight increase up to day 4 ($P < 0.05$). Furthermore, this parameter decreased moderately for sunflower oil and insignificantly for linseed oil to relatively stable values. The LA/ALA ratio for uWBR is higher ($P < 0.001$) than that for linseed oil and lower ($P < 0.001$) than that for sunflower oil addition. At the end of experiment, the LA/ALA ratio was ideal (equal to 3) for linseed oil, but unfavourable with regard to human health for sunflower oil (equal to 11).

The effect of WBR supplementation on FA composition must have also been affected by randomization of the ewe groups studied. Three experimental groups of ewes were randomly chosen from the herd of 328 ewes fed a winter diet. Later in the summer season the CLA content in milk fat of individual grazing ewes of this herd varied in a wide range (0.5–2.6 g/100 g FAME) for milk sampled on the same day (Sojak et al. 2013). The selection of ewes into more homogeneous experimental groups based on a similar CLA content in milk fat should lead to more representative results of the effect of oil supplementation on milk FA composition.

CONCLUSION

The study data suggest that addition of 3% linseed oil or sunflower oil to a standard winter diet with a relatively low concentrate content is a perspective nutritional intervention to achieve ewes' winter milk with a higher content of health-promoting fatty acids *cis*-9,*trans*-11 18:2, and *trans*-11 18:1, and a lower content of lauric, myristic, and palmitic acid showing health adverse effects. The fatty acid composition in milk fat of the plant oil supplemented ewes was similar to that of the ewes grazed on a lowland pasture in the middle of a pasture season (Meluchova et al. 2008), however, the content of unhealthy *trans*-10 18:1 was significantly higher. Optimization of the amount and fatty acid profile of the supplemented plant oils as well as the composition of winter basal ration can further improve milk fatty acid composition of ewes fed a winter diet.

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Received: 2014–03–31
Accepted after corrections: 2014–10–30

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