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Changes in milk and plasma fatty acid profile in response to fish and soybean oil supplementation in dairy sheep

Eleni Tsiplakou* and George Zervas

Department of Nutritional Physiology and Feeding, Agricultural University of Athens, Iera Odos 75, GR-11855 Athens, Greece

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An effective strategy for enhancing the bioactive fatty acids (FA) in sheep milk could be dietary supplementation with a moderate level of a combination of soybean oil with fish oil (SFO) without negative effects on milk yield and its chemical composition. Thus, the objective of this study was to determine the effects of a moderate forage diet supplementation with SFO on milk chemical composition and FA profile, as well as on plasma FA. Twelve dairy sheep were assigned to two homogenous sub-groups. Treatments involved a control diet without added oil, and a diet supplemented with 23·6 g soybean oil and 4·7 g fish oil per kg dry matter (DM) of the total ration. The results showed that SFO diet had no effect on milk yield and chemical composition. In blood plasma the concentrations of *trans-11* $C_{18:2}$ (VA), $C_{18:2n\text{-}6}$, $C_{20:5n\text{-}3}$ (EPA) and $C_{22:6n\text{-}3}$ (DHA) were significantly higher while those of $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ were lower in sheep fed with SFO diet compared with control. The SFO supplementation of sheep diet increased the concentrations of VA, *cis-9, trans-11* $C_{18:2}$ CLA, *trans-10, cis-12*, $C_{18:2}$ CLA, EPA, DHA, monounsaturated FA (MUFA), polyusaturated fatty acids (PUFA) and *n*-3 FA and decreased those of short chain FA (SCFA), medium chain FA (MCFA), the saturated/unsaturated ratio and the atherogenicity index value in milk compared with the control. In conclussion, the SFO supplementation at the above levels in a sheep diet, with moderate forage to concentrate ratio, improved the milk FA profile from human health standpoint without negative effects on its chemical composition.

Keywords: Fish oil, soybean oil, milk, fatty acids, sheep.

Introduction

Scientific progress in understanding the relationship between nutrition and health has an increasingly profound impact on consumers approach to nutrition which has resulted in the development of the concept of functional foods (Bhat & Bhat, 2011). Milk and dairy products can be used as functional foods due to their bioactive lipids such as: butyrate, branched-chain fatty acids, conjugated linoleic acid (CLA), vaccenic acid (VA = *trans-11* $C_{18:2}$) and *n*-3 polyunsaturated fatty acids (PUFA) (Shingfield et al. 2008).

Taking into account that diet strongly influences the overall milk fat content and chemical composition, many studies have been performed in sheep to ascertain the effects of diet on milk fatty acid (FA) profile (Tsiplakou et al. 2006; Tsiplakou & Zervas, 2008). Supplementation of sheep diets with vegetable oils, rich in linoleic acid, induce an increase in milk CLA content (Hérvas et al. 2008; Castro et al. 2009; Gómez–Cortés et al. 2011a, b) while, the inclusion of marine lipids, such as fish oil (Capper et al. 2007), and marine algae (Reynolds et al. 2006; Toral et al. 2010b) enhance the *n*-3 PUFA content in milk fat. Despite the promising results obtained from the separate inclusion of vegetable and fish oil in sheep diets, to date there is scarce information concerning the dietary supplementation with a combination of soybean oil and fish oil. On the other hand, in dairy cows a lot of research has been conducted to investigate the combination of fish oil with sunflower oil in bovine milk FA profile giving results with a considerable variation in this response, based on a number of factors including the type of forage (AbuGhazaleh et al. 2007), the level of oil added (Cruz-Hernandez et al. 2007) and the forage/concentrate ratio (F/C) (AbuGhazaleh et al. 2003; Shingfield et al. 2006). To our knowledge, in sheep, only Toral et al. (2010a) used fish oil in combination with sunflower oil in a low forage diet (F/C = 20/80) and induced milk fat depression which affects cheese yield and quality. Gómez–Cortés et al. (2011b) using a moderate level of plant oil only, in low – to moderate – concentrate diets, confirmed better milk FA profile with no reduction in milk fat content. Due to those different responses of sheep to dietary oil supplementation, it was

*For correspondence; e-mail: eltsiplakou@aua.gr

Table 1. Ingredients, chemical composition and mean daily fatty acids intake (g) of the diets used throughout the experimental period

	Alfalfa hay	Concentrates	
		Control	Treated
Ingredients (g/kg)			
Corn grain		670	300
Soybean meal		200	—
Sunflower meal		—	280
Sugar beet pulp		—	70
Wheat middlings		100	260
Minerals and vitamins premix		30	30
Soybean oil		—	50
Fish oil		—	10
Chemical composition (g/kg)			
Dry matter	860	887	892
Organic matter	775	836	824
Crude protein	130	158	157
Ether extracts	14	32	83
NDF	446	129	264
ADF	340	53	120
Energy content†, MJ NEL/kg	3·70	7·41	7·45

Fatty acids intake (Alfalfa hay and concentrates)	Control	Treated
$C_{14:0}$	0·87	1·10
$C_{16:0}$	12·06	18·47
$C_{18:0}$	2·23	4·13
cis-9 $C_{18:1}$	8·56	16·70
cis-9 cis-12 $C_{18:2}$	23·92	45·42
*cis-9 cis-12 cis-15*$C_{18:3}$	4·96	7·18
$C_{20:5n\text{-}3}$	–	1·98
$C_{22:6n\text{-}3}$	–	0·49
Starch/NDF	0·643	0·294

†Calculated

hypothesized that the inclusion of soybean oil in combination with fish oil in dairy sheep diet, with a moderate F/C ratio will potentially increase the CLA and *n*-3 PUFA content which results in a milk FA profile more beneficial to human health, with no reduction in either milk fat content or milk yield. Thus, this work was conducted with the aim to study the effect of diet supplementation with soybean oil in combination with fish oil (SFO) on milk chemical composition and on milk and plasma FA profile in dairy sheep.

Materials and Methods

Twelve 3-years-old Friesian crossbred dairy sheep at 90–98 d in milk were maintained at the Agricultural University of Athens. The mean initial body weight (BW) of the animals was 63 ± 2·1 kg. Housing and care of animals conformed to Ethical Committee guidelines of the Faculty of Animal Science. The sheep were assigned to 2 homogeneous sub-groups (n = 6) that were balanced by their BW and milk yield. Throughout the experimental period each sheep of each group was fed individually according to its requirements (Zervas, 2007). The animals of both groups were fed after two weeks adaptation period with a ration consisting of alfalfa hay and concentrates with an F/C = 53/47 which was offered to the animals twice a day (two equal parts at 0800 and 1600 h). The concentrate of the control group had no added oil, while that of the treated group was supplemented with 23·6 g soybean oil and 4·7 g fish oil per kg dry matter (DM) of the total ration. The choice for the levels of oils used in this study were based on the results of Gómez–Cortés et al. (2011a) who found that a supplementation level in sheep diets between 17 and 34 g sunflower oil/kg DM gave better milk FA profile from the consumers point of view in comparison with a supplementation with 51 g sunflower oil/kg DM of a TMR ration.

The concentrate diets were prepared every week for both groups and were formulated to be isoenergetic and isoproteic. In order to have isoenergetic and isoproteic concentrate diets, due to oil inclusion in the concentrate diet of treated group, two different raw ingredients (sugar beet pulp and sunflower meal) were used which resulted in concentrate chemical composition differences between control and treated groups (Table 1). That was unavoidable with the feedstuffs available for the experiment.

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The quantities of food offered to the animals were adjusted at the 0, 7, 14 and 28 experimental day according to their individual requirements based on their BW and milk fat corrected yield. Ingredients and chemical composition of alfalfa hay and concentrates and the FA intake of the whole ration are presented in Table 1. The average daily DM intake throughout the experimental period for the control and the treated groups was 1·81 and 1·85 kg respectively. The whole experimental period lasted 42 d. Animals had free access to fresh water.

Samples collection

All animals were milked twice a day at 8 am and 6 pm by a milking machine. Individual milk samples were collected from sheep at day 0, 7, 14, 28 and 42 for chemical analyses, and at day 28 and 42 for FA determination after mixing the yield from the evening and the morning milking on a percent volume (5%). Blood samples were collected at day 28 and 42 for FA determination from the jugular vein into EDTA-containing tubes and subsequently centrifuged at 2700 ***g*** for 15 min to separate plasma from the cells. Both milk and blood samples used for FA analyses were stored at − 80 °C, prior to analysis.

Milk, plasma and feeds analyses

The milk samples were analysed for FA according to Nourooz-Zadeh & Appelqvist (1998) method as described by Tsiplakou et al. (2012). A 17 ml milk sample was transferred into a separating funnel, where 30 ml isopropanol were added. After vigorous shaking, 22·5 ml hexane were added, and the mixture was shaken for another 3 min. The mixture was then centrifuged at 2520 ***g*** for 5 min at 5 °C, and the upper layer was transferred to a second separating funnel. The lower layer was extracted twice with 22·5 ml hexane, and the supernatants were pooled with the previous hexane layer. After addition of 15 ml 0·47 M aqueous Na_2SO_4 the hexane layer was collected into a flask and evaporated with a rotary evaporator at 30 °C. In 40 mg lipid, 2 ml hexane was added followed by 40 µl methylacetate. After vortexing, 40 µl methylation reagent (1·75 ml methanol/0·4 ml 5·4 mol/l sodium methylate) was added. The mixture was vortexed and allowed to react for 10 min, then 60 µl termination reagent (1 g oxalic acid/30 ml diethylether) was added. The sample was then centrifuged for 5 min at 2400 ***g*** at 5 °C and the liquid layer was removed and used directly for chromatographic determination.

The plasma FA analysis was carried out by the method of Bondia-Pons et al. (2004). One hundred microlitres plasma samples were saponified in PTFE screw-capped Pyrex tubes containing 20 µg tridecanoic acid, by adding 1 ml sodium methylate (0·5% w/v) and heating to 100 °C for 15 min. After cooling to 25 °C, samples were esterified with 1 ml boron trifluoride-methanol reagent (also at 100 °C) for 15 min. Once the tubes were cooled, FAME were isolated by adding 500 µl *n*-hexane. After shaking for 1 min, 1 ml of a saturated sodium chloride solution was added. Finally, the tubes were centrifuged for 8 min at 2200 ***g***. After drying with anhydrous sodium sulphate, the clear *n*-hexane top layer was transferred into a vial and used directly for chromatographic determination.

Alfalfa hay and concentrate samples were analysed for FA according to Sánchez-Machado et al. (2002) method. For the determination of FA concentration an Agilent 6890 N gas chromatograph equipped (Agilent Technologies 2850, Centerville Road, Wilmington, USA) with an HP-88 capillary column (60 m × 0·25 mm i.d. with 0·20 µm film thickness, Agilent) and a flame ionization detector, was used.

The different groups of FA and the atherogenicity index (AI) were defined according to Stockdale et al. (2003) and by Ulbricht & Southgate (1991) respectively, while the Δ^{-9} desaturase activity index was calculated according to the following four ratios: $C_{14:1}/C_{14:0}$, $C_{16:1}/C_{16:0}$, $C_{18:1}/C_{18:0}$ and *cis-9, trans-11* $C_{18:2}$/*trans-11* $C_{18:1}$. The milk samples were also analysed for fat, protein, lactose, total solids (TS), and solids-not-fat (SNF), with IR spectrometry (Milkoscan 133/; Foss Electric, Hllerod, Demark), after appropriate calibration of the instrument according to Gerber (BSI, 1955) and Kjeldahl (IDF, 1993). Alfalfa hay and concentrate samples were also analysed for organic matter (OM; 7·009), dry matter (DM; 7·007), N (7·016) and ether extract (7·060) according to AOAC (1984) and for neutral detergent fibre (NDF) and acid detergent fibre (ADF) according to Van Soest et al. (1991).

Statistical analysis

Data are presented as least squares (LSs) means. Experimental data were analysed using the SPSS statistical package (version 16.0). The BW, the milk chemical composition and the FA profile of milk and blood plasma were analysed using a general linear model (GLM) for repeated measures analysis of variance (ANOVA) with dietary treatments (Control, Treatment) and sampling time (T) as fixed effects and their interactions ($D \times T$) according to the model:

$$Y_{ijk} = \mu + D_i + T_j + (D \times T)_{ij} + e_{ijk}$$

where Y_{ijk} is the dependent variable, µ the overall mean, D_i the effect of dietary treatment, T_j the effect of sampling time ($j = 5$ for milk chemical composition, 2 for milk and plasma FA profile) $(D \times T)_{ij}$ the interaction between dietary treatments and sampling time and e_{ijk} the residual error.

Post hoc analyses were performed when appropriate using Duncan's multiple range test and significance was set at 0·05.

Results and Discussion

The inclusion of soybean and fish oil (SFO) in sheep diet induces important changes in milk FA profile in the absence of detrimental effects on milk fat content and on milk yield. In this respect, it should be highlighted that the SFO

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Table 2. Body weight (Kg), milk yield (g/d/animal) and milk chemical composition (%) of the milk of sheep fed the two diets throughout the experimental period

	Diets (D)			Sampling time (*T*) at day						Effects		
	Control (n = 6)	Treated (n = 6)	SEM	0 (n = 12)	7 (n = 12)	14 (n = 12)	28 (n = 12)	42 (n = 12)	SEM	*D*	*T*	*D* × *T*
Body weight	63·81	63·01	5·738	63·04	63·25	63·59	64·86	64·25	0·364	NS	***	*
Milk yield	986	1·137	128	1·089	1·111	1·122	1·055	932	46	NS	**	NS
MFC†	1109	1236	116	1·114	1·316	1·243	1·175	1·028	43	NS	**	NS
Fat	7·04	6·73	0·573	6·19	7·54	6·90	6·95	6·86	0·204	NS	***	*
Protein	5·67	5·60	0·250	5·93	5·83	5·59	5·37	5·46	0·091	NS	***	*
Lactose	5·21	5·24	0·084	5·42	5·30	5·19	5·20	5·03	0·051	NS	***	*
Total solids	18·72	18·14	0·825	18·32	19·47	18·24	18·14	17·97	0·270	NS	***	**
Solids non fat	11·68	11·65	0·301	12·15	11·93	11·58	11·36	11·30	0·087	NS	***	NS

† MFC = Milk fat corrected yield in 6% with the equation $Y_{6\%} = (0{\cdot}28 + 0{\cdot}12F)M$ where F = fat% and M = milk yield in g

supplementation did not negatively affect the milk fat content (Table 2) which is an important parameter in cheese yield for the dairy sheep breeders, because ovine milk is mostly transformed into cheese. It has been shown that moderate forage diets, as in this study, when supplemented with plant oils had no effect on milk fat content (Gómez–Cortés et al. 2011b) in sheep, while the opposite happened in dairy cows (Cruz-Hernández et al. 2007). Although the reasons are still uncertain, some authors have reported that lactating sheep might be less sensitive than cows to some milk fat depression inducing factors, probably because of their ability to maintain rumen function (Pulina et al. 2006). Further to that, in relation to milk fat content there is scarce information in sheep, compared with dairy cows, concerning the inclusion of plant oils in combination with fish oil in their diets. In fact, recently, Toral et al. (2010a) has found a decrease in milk fat content of sheep fed a diet supplemented with sunflower and fish oil. The contradictory results of Toral et al. (2010a) and our study, concerning the milk fat content, could be mostly accounted for by differences in the F/C ratio, the basal diet composition and to the lipid dosage, concerning mainly the FO supplementation.

From a nutritional point of view the significant decrease in the concentrations of $C_{6:0}$, $C_{8:0}$, $C_{11:0}$, $C_{14:0}$, $C_{15:0}$, $C_{15:1}$, $C_{16:0}$, $C_{16:1}$, $C_{17:1}$ FA, short chain FA (SCFA) and medium chain FA (MCFA) in sheep milk of the treated group compared with controls (Table 3) can be seen as a positive effect, because accumulated evidence shows a strong link between the intake of some saturated FA (SFA) ($C_{12:0}$, $C_{14:0}$ and $C_{16:0}$) and the incidence of cardiovascular diseases (Hu et al. 2001; Mensink et al. 2003). SCFA and MCFA are synthesized *de novo* from acetate and β-hydroxybutyrate in the mammary gland (Chilliard et al. 2000). Dietary fat modifies ruminal fermentation and decreases the availability of acetate and β-hydroxybutyrate, both of which are precursors of mammary lipogenesis (Schmidely & Sauvant, 2001). Further to that the decrease in the percentage of these FA in milk could also be due to the fact that the long chain FA (18 or more carbon atoms) alter the lipogenic gene networks in mammary epithelial cells. In fact, dietary polyunsaturated FA (PUFA) are biohydrogenated in the rumen into *trans*-FA, some of which, such as *trans-10* $C_{18:1,}$ are recognized as potent inhibitors of lipogenesis in the udder (Bauman et al. 2008; Kadegowda et al. 2009).

The SFO treatment resulted in a significant increase of milk *trans-11* $C_{18:1}$ (VA) content (Table 3) which was probably caused by the higher daily intake of linoleic acid from the sheep fed with the SFO diet (Table 1). This was also evident from the significantly higher concentration of this FA which was observed in their plasma (Table 4), since changes in plasma FA profile largely reflect the FA composition of the dietary treatments. Linoleic acid is the predominant FA in soybean oil and the main source of VA in the rumen. Indeed, measurements of FA flow in the omasum in response to incremental amounts of sunflower oil in the diet (Shingfield et al. 2008) demonstrated that the major pathway of ruminal linoleic acid biohydrogenation involves the formation of VA

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Table 3. Fatty acids profile (% of total FA), atherogenicity and Δ^{-9} desaturase indexes of the milk from sheep fed the two diets at the two sampling times

Fatty acids	Diets (*D*)			Sampling time (*T*)			Effects		
	Control ($n = 6$)	Treated ($n = 6$)	SEM	28 d ($n = 12$)	42 d ($n = 12$)	SEM	*D*	*T*	*D* × *T*
$C_{4:0}$	3·82	3·86	0·183	3·97[a]	3·71[b]	0·111	NS	*	NS
$C_{6:0}$	2·81[a]	2·16[b]	0·205	2·55[a]	2·43[b]	0·051	*	*	NS
$C_{8:0}$	2·52[a]	1·78[b]	0·306	2·19	2·12	0·046	*	NS	NS
$C_{10:0}$	7·73	5·26	1·151	6·60	6·39	0·157	NS	NS	NS
$C_{11:0}$	0·34[a]	0·19[b]	0·042	0·26	0·27	0·013	**	NS	NS
$C_{12:0}$	4·22	3·18	0·623	3·72	3·68	0·073	NS	NS	NS
$C_{13:0}$	0·10	0·10	0·014	0·09	0·10	0·006	NS	NS	NS
$C_{14:0}$	11·88[a]	10·06[b]	0·646	11·09	10·84	0·165	*	NS	NS
$C_{14:1}$	0·49	0·35	0·084	0·48[a]	0·37[b]	0·030	NS	**	**
$C_{15:0}$	1·06[a]	0·85[b]	0·051	0·93	0·97	0·022	**	NS	NS
$C_{15:1}$	0·26[a]	0·18[b]	0·020	0·22	0·22	0·013	**	NS	NS
$C_{16:0}$	27·78[a]	25·15[b]	1·154	26·95[a]	25·98[b]	0·245	*	**	NS
$C_{16:1}$	1·74[a]	1·41[b]	0·097	1·54	1·61	0·036	**	NS	NS
$C_{17:1}$	0·22[a]	0·13[b]	0·016	0·18	0·17	0·016	***	NS	NS
$C_{18:0}$	8·34	7·01	0·931	7·49	7·86	0·191	NS	NS	NS
trans-$C_{18:1}$	0·50[a]	1·55[b]	0·114	0·89[a]	1·15[b]	0·109	***	*	*
trans-11 $C_{18:1}$	1·58[a]	9·81[b]	0·751	5·54	5·85	0·539	***	NS	NS
Cis-9 $C_{18:1}$	18·34	15·59	1·560	16·63	17·30	0·322	NS	NS	*
trans-10 $C_{18:1}$	0·51[a]	0·85[b]	0·049	0·68	0·68	0·019	***	NS	NS
$C_{18:2n6t}$	0·24	0·35	0·057	0·29	0·31	0·010	NS	NS	NS
$C_{18:2n6c}$	3·10	3·73	0·351	3·47	3·35	0·110	NS	NS	*
$C_{18:3n6}$	0·07	0·07	0·003	0·07	0·07	0·002	NS	NS	NS
$C_{20:0}$	0·26	0·35	0·046	0·30	0·31	0·011	NS	NS	NS
$C_{18:3n3}$	0·42	0·38	0·049	0·39[a]	0·42[b]	0·013	NS	*	NS
Cis-9, trans-11 $C_{18:2}$	0·99[a]	4·72[b]	0·571	2·69	3·02	0·153	***	NS	NS
trans-10 , cis-12 $C_{18:2}$	0·11[a]	0·20[b]	0·018	0·13[a]	0·17[b]	0·018	***	*	**
$C_{20:3n3}$	0·10[a]	0·08[b]	0·009	0·09	0·09	0·005	*	NS	*
$C_{20:5n3}$	0·00[a]	0·11[b]	0·003	0·05	0·05	0·002	**	NS	NS
$C_{21:0}$	0·10	0·08	0·011	0·09	0·09	0·006	NS	NS	NS
$C_{22:0}$	0·16	0·15	0·021	0·15	0·15	0·010	NS	NS	NS
$C_{22:2n6}$	0·02[a]	0·09[b]	0·018	0·05	0·05	0·019	*	NS	NS
$C_{22:6n3}$	0·00[a]	0·10[b]	0·002	0·04	0·05	0·001	**	NS	NS
$C_{23:0}$	0·12	0·09	0·015	0·10[a]	0·10[b]	0·006	NS	*	NS
$C_{24:0}$	0·06	0·06	0·005	0·06	0·06	0·003	NS	NS	NS
SCFA†	17·23[a]	13·25[b]	1·579	15·56	14·92	0·296	*	NS	NS
MCFA‡	45·03[a]	39·33[b]	1·297	42·79[a]	41·57[b]	0·429	***	*	NS
LCFA§	9·05	7·73	0·980	8·20	8·58	0·211	NS	NS	NS
PUFA¶	5·04[a]	9·82[b]	0·781	7·28	7·59	0·225	***	NS	NS
MUFA‖	23·65[a]	29·87[b]	1·473	26·18[a]	27·34[b]	0·414	**	*	NS
S/U††	2·49[a]	1·52[b]	0·169	2·00[a]	1·86[b]	0·057	***	*	NS
AI‡‡	2·77[a]	1·73[b]	0·218	2·24[a]	2·09[b]	0·071	***	*	NS
n-3	0·52[a]	0·66[b]	0·056	0·57	0·61	0·045	**	NS	NS
n-6	3·40[a]	4·15[b]	0·256	3·83	3·73	0·301	**	NS	NS
$C_{14:1}/C_{14:0}$	0·04	0·04	0·010	0·05[a]	0·04[b]	0·003	NS	*	**
$C_{16:1}/C_{16:0}$	0·07	0·06	0·005	0·06[a]	0·06[b]	0·002	NS	**	NS
$C_{18:1}/C_{18:0}$	2·34	2·69	0·300	2·51	2·52	0·038	NS	NS	NS
cis-9, trans-11 $C_{18:2}$/*trans-11* $C_{18:1}$	0·64[a]	0·48[b]	0·059	0·56	0·57	0·017	*	NS	NS

† **SCFA** = $C_{6:0} + C_{8:0} + C_{10:0} + C_{11:0}$

‡ **MCFA** = $C_{12:0} + C_{13:0} + C_{14:0} + C_{15:0} + C_{16:0}$

§ **LCFA** = $C_{18:0} + C_{21:0} + C_{20:0} + C_{22:0} + C_{23:0} + C_{24:0}$

¶ **PUFA** = cis-9, *trans* − 11 $C_{18:2}$CLA + *trans* − 10, cis-12, $C_{18:2}$CLA + $C_{18:2n-6} + C_{18:3n-3} + C_{18:3n-6} + C_{20:2} + C_{20:3n-3} + C_{20:3n-6} + C_{20:4n-6} + C_{20:5n-3} + C_{22:2n-6} + C_{22:6n-3}$

‖ **MUFA** = $C_{14:1} + C_{15:1} + C_{16:1} + C_{17:1}$ + cis-9 $C_{18:1}$ + *trans* $C_{18:1}$ + *trans* − 11 $C_{18:1}$(VA) + *trans* − 10$C_{18:1} + C_{20:1}$

†† **S/U**: (SCFA + MCFA + LCFA)/(PUFA + MUFA) and *trans* − 11 $C_{18:1}$ This value is not included in the $C_{18:1}$content

***n*-3** = $C_{18:3n-3} + C_{20:3n-3} + C_{20:5n-3} + C_{22:6n-3}$

***n*-6** = $C_{18:2n-6} + C_{18:3n-6} + + C_{20:3n-6} + C_{20:4n-6} + C_{22:2n-6}$

‡‡ **AI** = $(C_{12:0} + 4 \times C_{14:0} + C_{16:0})$/(PUFA + MUFA)

Means with different superscript (a,b) in each row (between the two diets, and between the two sampling time) for each fatty acid differ significantly ($P \leqslant 0{\cdot}05$)

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Table 4. The mean fatty acids concentrations (% of total FA) of total lipids of sheep plasma fed the two diets at the two sampling times

Fatty acids	Diets (*D*)			Sampling time (T)			Effects		
	Control (n = 6)	Treated (n = 6)	SEM	28 d (n = 12)	42 d (n = 12)	SEM	*D*	*T*	*D* × *T*
$C_{14:0}$	0·49[a]	0·32[b]	0·059	0·39	0·42	0·052	*	NS	NS
$C_{14:1}$	0·16	0·13	0·028	0·12	0·17	0·031	NS	NS	*
$C_{15:0}$	0·68[a]	0·51[b]	0·063	0·54	0·64	0·065	*	NS	NS
$C_{15:1}$	0·20	0·19	0·038	0·15	0·24	0·044	NS	NS	NS
$C_{16:0}$	17·34[a]	14·20[b]	0·455	15·78	15·76	0·302	***	NS	**
$C_{16:1}$	0·79	0·66	0·065	0·84[a]	0·62[b]	0·100	NS	*	NS
$C_{17:0}$	1·02	0·87	0·072	0·91	0·97	0·113	NS	NS	NS
$C_{17:1}$	0·23	0·24	0·055	0·23	0·25	0·068	NS	NS	NS
$C_{18:0}$	24·68[a]	20·71[b]	1·554	22·35	22·04	0·720	*	NS	*
trans-$C_{18:1}$	0·50[a]	0·76[b]	0·113	0·69	0·56	0·076	*	NS	NS
trans-11 $C_{18:1}$	1·26[a]	5·75[b]	0·994	3·82	3·19	0·848	***	NS	NS
cis-9 $C_{18:1}$	12·89	10·51	1·139	11·18	12·22	0·791	NS	NS	NS
trans-10 $C_{18:1}$	1·04	0·93	0·078	1·01	0·96	0·060	NS	NS	NS
$C_{18:2n\text{-}6t}$	0·43	0·33	0·050	0·41	0·35	0·053	NS	NS	NS
$C_{18:2n\text{-}6c}$	29·88[a]	35·03[b]	1·221	32·94	31·98	0·721	**	NS	NS
$C_{18:3n\text{-}6}$	0·39	0·30	0·066	0·33	0·35	0·071	NS	NS	NS
$C_{18:3n\text{-}3}$	1·62	1·62	0·112	1·60	1·63	0·088	NS	NS	NS
cis-9. trans-11 $C_{18:2}$	0·17	0·13	0·114	0·26	0·04	0·105	NS	NS	NS
trans-10. cis-12 $C_{18:2}$	0·18	0·16	0·142	0·24	0·10	0·120	NS	NS	NS
$C_{20:3n\text{-}6}$	0·38[a]	0·22[b]	0·059	0·34	0·26	0·086	*	NS	NS
$C_{20:5n\text{-}3}$	0·03[a]	0·07[b]	0·030	0·00[a]	0·10[b]	0·021	**	**	*
$C_{20:3n\text{-}3}$	2·47[a]	1·63[b]	0·153	2·15[a]	1·94[b]	0·081	***	*	NS
$C_{20:4n\text{-}6}$	0·21	0·17	0·086	0·24	0·13	0·082	NS	NS	NS
$C_{21:0}$	0·03[a]	0·37[b]	0·063	0·16	0·23	0·049	***	NS	NS
$C_{22:0}$	0·56	0·47	0·096	0·57	0·46	0·089	NS	NS	NS
$C_{22:2n\text{-}6}$	0·01	0·02	0·002	0·02	0·01	0·001	NS	NS	NS
$C_{22:6n\text{-}3}$	0·82[a]	1·28[b]	0·302	0·99	1·11	0·224	**	NS	NS
$C_{23:0}$	0·21[a]	0·17[b]	0·146	1·07	1·19	0·059	***	NS	**
$C_{24:0}$	0·62	0·39	0·142	0·40	0·62	0·148	NS	NS	NS
$C_{24:1}$	0·41	0·21	0·105	0·23	0·38	0·120	NS	NS	NS

Means with different superscript (a,b) in each row (between the two diets, and between the two sampling time) for each fatty acid differ significantly ($P \leqslant 0·05$)

as an intermediate metabolite (Griinari et al. 1998). On the other hand, the increase in VA content in sheep milk fed with the SFO diet may be also due to the action of long-chain *n*-3 PUFA present in FO which are potent inhibitors of *trans*-$C_{18:1}$ ruminal reduction (Loor et al. 2005). Indeed, supplementation of sheep diets with fish oil has been shown to increase the plasma *trans*-$C_{18:1}$ concentrations by incomplete ruminal biohydrogenation of PUFA (Capper et al. 2007), results that concur with the increase of *trans*-$C_{18:1}$ and VA FA in sheep blood plasma conferred by SFO supplementation within the current study (Table 4). VA is the major *trans* $C_{18:1}$ in dairy products and recently its protective role in cardiovascular disease has been proved (Tyburczy et al. 2009).

The inhibitory effect of FO long-chain *n*-3 PUFA on *trans*-$C_{18:1}$ ruminal reduction had as a consequence not only to increase their concentration in milk fat, as described previously, but also to decrease the $C_{18:0}$ and *cis-9* $C_{18:0}$ concentrations in milk and blood plasma of the treated sheep compared with controls with the results being significant only for the $C_{18:0}$ content in plasma (Table 4). The inhibitory action of FO on $C_{18:0}$ FA, which is produced from the biohydrogenation of dietary lipids in the rumen and is the main source on milk $C_{18:0}$ content, has already been proved in cows (Lee et al. 2008; Or-Rashid et al. 2008). Accordingly, with our results, Toral et al. (2010a) observed also a significant decrease in $C_{18:0}$ milk fat content of sheep fed with FO alone or in combination with sunflower oil while Capper et al. (2007) found a significant reduction on plasma $C_{18:0}$ content in sheep plasma fed with fish oil.

The concentration of *trans-10* $C_{18:1}$ significantly increased in milk of sheep fed with SFO diet (Table 3) which is in agreement with the results of Gulda et al. (2012) who observed, in continuous culture fermenters, that fish oil (1% DM) with soybean oil (2% DM) when added in a high forage diet (F/C = 70/30) caused an increase in both VA and *trans-10* $C_{18:1}$ concentrations. It is remarkable that high levels of *trans-10* $C_{18:1}$ in sheep milk appear to be accompanied by similar or even higher contents of VA (Reynolds et al. 2006), whereas in dairy cows there is a pronounced inverse

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relationship between these two FA. So the *trans-10* $C_{18:1}$ content may amply exceed that of VA (Boeckaert et al. 2008). In addition, in contrast to cows, an increase in *trans-10* $C_{18:1}$ levels as a consequence of SFO supplementation was not followed by a concomitant decrease in sheep milk fat as mentioned earlier. The reasons for this discrepancy between ovine and bovine responses to oils supplementation in milk fat remain uncertain.

The principal dietary factors that affect the production of *trans-10* $C_{18:1}$ content in milk have been classified in two categories: 1. diets that alter rumen microbial activity associated with PUFA hydrogenation and 2. the presence of a source of linoleic acid in the rations (Chilliard & Ferlay 2004; Palmquist et al. 2005). Further to these it has been observed that an increase in the dietary starch:NDF ratio caused a relative decrease of cellulotic bacteria abundance and also shifts the rumen biohydrogenation of PUFA toward the production of *trans-10* $C_{18:1,}$ but this is related to the proportions of the readily degradable carbohydrates (Griinari et al. 1998). In the present study the starch: NDF ratio was higher in the control diet (0·643 *vs.* 0·294), compared with SFO, but the opposite was observed for the linoleic acid intake (Table 1). This indicates that in this study the alterations in milk FA profile, as far as the *trans-10* $C_{18:1}$ was concerned, were due to the oil inclusion in the SFO sheep diet.

The significance of the increasing VA concentration also comes from its role as substrate for *cis-9, trans-11* $C_{18:2}$ CLA endogenous synthesis not only in the ruminant mammary gland but also in human tissues (Palmquist et al. 2005). Thus, in our study the SFO inclusion in a moderate forage diet (F/C = 53/47) caused a significant increase on *cis-9, trans-11* $C_{18:2}$ CLA (Table 3) in sheep milk to a higher level compared with that observed by Toral et al. (2010a) with a high concentrate diet (F/C = 20/80). In agreement with our results, supplementation of high forage diets with 10 g FO in combination with 20 g sunflower oil per kg DM has been shown to result in higher levels of *cis-9, trans-11* $C_{18:2}$ CLA in cow milk (6·1 g/100 g FA) in relation to that found when the same amount of these oils was offered in a high concentrate diet (1·7 g/100 g FA). In addition, comparisons between sheep and cows fed with moderate forage diets supplemented with a combination of sunflower oil with fish oil reveal two fold higher *cis-9, trans-11* $C_{18:2}$ CLA and VA levels in sheep milk fat compared with cows which underlines the animal species differences (AbuGhazaleh & Holmes, 2007; AbuGhazaleh et al. 2007). Further to *cis-9, trans-11* $C_{18:2}$ CLA concentration, significant increase was also observed in the *trans-10, cis-12* $C_{18:2}$ CLA (Table 3) in the milk fat of sheep fed with SFO diet compared with controls, in contrast to the observations of Toral et al. (2010a). From the above it becomes clear that even though the combination of high linoleic vegetable oil with marine lipids is considered to be a good nutritional strategy for enhancing CLA content in milk fat, there is a considerable variation in this response. It has been reported that the composition of the basal diet and the F/C ratio in dairy cows are also important factors which determine the extent to which changes in ruminal biohydrogenation occur when combination of FO with plant oil (rich in $C_{18:2n6c}$) are given (Shingfield et al. 2006).

The diet supplementation with SFO caused also some additional changes in milk, pointed toward an enhancement in PUFA concentration and a decrease in S/U, AI and *cis-9, trans-11* $C_{18:2}$ CLA/*trans-11* $C_{18:1}$ Δ^{-9} desaturase activity index values (Table 3). In the recent years, PUFA have received much attention from the nutritionists, consumers and researches because of their beneficial function on human health including antiatherogenic, immune system stimulation, etc (Ruxton et al. 2005). The reduction in *cis-9, trans-11* $C_{18:2}$ CLA/*trans-11* $C_{18:1}$ Δ^{-9} desaturase activity index in sheep milk by the SFO diet may suggest enzyme saturation due to the substrate (*trans-11* $C_{18:1}$) increases and/or that other FA which are produced from the rumen biohydrogenation inhibit its activity. On the other hand these results may also suggests that Δ^{-9} desaturase activity was inhibited by the high levels of linoleic acid contained in the SFO diet (Table 1) as suggested by Sessler & Ntambi (1998). A decrease in *cis-9, trans-11* $C_{18:2}$ CLA/ *trans-11* $C_{18:1}$ Δ^{-9} desaturase activity index in milk was also found by Angulo et al. (2012) in cows fed a diet containing 2·5% sunflower in combination with 0·4% DHA from algae.

Conclusions

The results of this work have shown that the supplementation of a moderate F/C sheep diet with soybean oil in combination with fish oil (SFO) could be an effective nutritional strategy which enhances milk FAs which may be beneficial to human health without any effect on milk yield and fat content. They also show that sheep respond in a different way to SFO inclusion in their diets compared with cows concerning milk chemical composition which underlines the differences between ruminant species.

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