J. Dairy Sci. TBC:1–10
http://dx.doi.org/10.3168/jds.2015-9981

JDS9981

Influence of dietary grape pomace combined with linseed oil on fatty acid profile and milk composition

T. Manso,* B. Gallardo,* A. Salvá,† C. Guerra-Rivas,* A. R. Mantecón,‡ P. Lavín,‡ and M. A. de la Fuente[1]†
*ETS Ingenierías Agrarias, Universidad de Valladolid, 34004 Palencia, Spain
†Instituto de Investigación en Ciencias de la Alimentación (CSIC-UAM), Nicolás Cabrera, 9. Universidad Autónoma de Madrid, 28049 Madrid, Spain
‡Instituto de Ganadería de Montaña (CSIC-ULE), 24346 Grulleros, León, Spain

ABSTRACT

Grape pomace is a by-product resulting from the winery industry that is rich in phenolic compounds. It could play a role as an antioxidant and, owing to its high fiber concentration, it would be an alternative ingredient to partially replace forage in the diet of small ruminants. The objective of this study was to evaluate the effect of dietary supplementation of vitamin E or different doses of grape pomace associated with linseed oil on milk fatty acid **[AU1: Note: To be consistent with IUPAC nomenclature, the terms and abbreviations free fatty acids (FFA) and nonesterified fatty acids (NEFA) are redundant and no longer in use in JDS; the term "fatty acid(s)" should be used instead and not abbreviated.]** profile, composition, and yield. Forty-eight Churra ewes were fed with experimental diets consisting of a total mixed ration (TMR) containing 2.7% [on a dry matter (DM) basis] of linseed oil, forage, and concentrate at a 40:60 ratio. Ewes were assigned to 1 of 4 treatments: control (without grape pomace), vitamin E (with 500 mg/kg of TMR of vitamin E), grape pomace-5 (5 g/100 g of TMR of DM of grape pomace), and grape pomace-10 (10 g/100 g of TMR of DM of grape pomace). Experimental diets did not affect DM intake and milk yield and composition. The vitamin E supplementation had only a moderate effect on milk concentration of fatty acids (increase in α-linolenic acid and 16:0 and decrease in *cis*-9 18:1). Grape pomace supplementation did not affect the percentages of total saturated, monounsaturated, and polyunsaturated fatty acids. Levels of α-linolenic acid reached about 1% of total fatty acids as a consequence of the presence of linseed oil in the diets, were not modified with vitamin E, and remained unaltered in grape pomace-5 and -10 treatments. Linoleic acid was increased by the highest dose of grape pomace, but this ingredient did not modify the *cis*-9,*trans*-11 18:2 milk fat content. The concentration of total odd- and branched-chain fatty acids did not diminish in grape pomace-5 and pomace-10 treatments. The presence of grape residue did not modified the *trans*-11 18:1 and *trans*-10 18:1 contents, which might indicate that, under the conditions assayed, this winery by-product would not alter the pathways of rumen conversion of dietary unsaturated fatty acids.
Key words: grape pomace, fatty acid, milk, linseed oil, ewe

INTRODUCTION

It is well established that dietary ingredients have a noticeable ability to modify the fatty acid profile of ruminant milk fat, especially supplementation with a lipid source (Shingfield et al., 2013; Nudda et al., 2014). Compared with results from a control diet, the inclusion of 3 different unsaturated plant oils (linseed, sunflower, and olive) results in the highest contents of MUFA, CLA, and PUFA, and the lowest proportion of SFA (Bodas et al., 2010). The best option of these 3 oils for improving milk fat fatty acid composition from a nutritional point of view was linseed oil, because it allows for the highest n-3 fatty acid content as well as the lowest atherogenicity index and n-6-to-n-3 PUFA ratio (Bodas et al., 2010; Martínez Marín et al., 2011).

Increasing the content of unsaturated fatty acids in milk could also increase susceptibility to oxidation. Therefore the addition of antioxidants to PUFA-supplemented diets to improve milk quality seems to be an advisable practice. Vitamin E is the major antioxidant used to supplement ruminant diets (Atwal et al., 1990; Zened et al., 2012; Casamassima et al., 2014). It does not modify milk yield, milk fat, or protein percentage, causes only moderate effects on milk fatty acid concentration (Kay et al., 2005; Ferlay et al., 2010), and can prevent milk fat depression in ruminants fed with high doses of PUFA-supplemented diets (Pottier et al.,

Received June 18, 2015.
Accepted October 20, 2015.
[1]Corresponding author: mafl@if.csic.es

2006). The antioxidant effect of synthetic vitamin E could be replaced by natural antioxidants, such as plant polyphenols from fruits, which might induce an effect on PUFA rumen biohydrogenation (**BH**) and, consequently, on the milk fatty acid profile.

Grape pomace is a by-product resulting from the winery industry that is costly to dispose of. Seeds and skins of crushed grapes are very rich in phenolic compounds (Yi et al., 2009) and therefore could play an important role as antioxidants. Furthermore, because of its low cost and high fiber concentration, grape residue could be an alternative feed ingredient to partially replace the forage portion in the diet of ruminants. Grape pomace has traditionally been used in the Mediterranean area as an alternative feed for sheep during periods of scarce feed supplies. Furthermore, Santos et al. (2014) reported that grape residue silage improved antioxidant activity in milk, Moate et al. (2014) found that ensiled grape marc reduced methane emissions by approximately 20%, and Nudda et al. (2015) suggested that grape seed might have an immunomodulatory effect on dairy ewes.

The ruminal bacteria of dairy cows were altered by dietary supplementation with grape marc (Moate et al., 2014), and the rumen metabolism of lactating dairy ewes was influenced by dietary supplementation with grape seed, alone or mixed with linseed (Correddu et al., 2015). However, evaluation of the effect of grape residue as a feed ingredient for lactating ruminants on the detailed milk fatty acid profile, including fatty acids derived from the ruminal BH process, has received less attention (Ferlay et al., 2010). The aim of our study was to determine the effect of dietary inclusion of vitamin E or different doses of grape pomace mixed with linseed oil on dairy performance and milk fatty acid profile during the first month of lactation.

MATERIALS AND METHODS

Animals and Dietary Treatments

Forty-eight pregnant Churra ewes (mean BW = 64.3 ± 0.92**[AU2: Plus-minus SD, here and throughout?]** kg) were selected 2 wk before lambing and were fed the same control diet they would receive during the experimental period without oil added (Table 1). The ewes, aged 3 to 5 yr (4.1 ± 0.61), whose parity ranged from 4 to 6 (4.9 ± 0.91), all gave birth 3 to 4 d before starting the experiment. According to a continuous experimental design, after lambing, each ewe was housed in an individual tiestall with its respective newborn lamb and randomly assigned to 1 of 4 treatments (12 ewes per treatment) based on their milk production, age, initial BW, and parity. The newborn lambs (12 per treatment) were housed with their respective mothers and were fed exclusively by suckling throughout the whole experimental period. The trial lasted for 4 wk and all animal handling practices followed the recommendations of the European Council Directive 2010/63/EU for the protection of animals used for experimental and other scientific purposes. The experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Valladolid.

The experimental diets consisted of a TMR containing 2.7% (on a DM basis) of linseed oil and forage and concentrate at a 40:60 ratio. The 4 dietary treatments were control (**CTRL**; without grape pomace), vitamin E (**VIT-E**; with 500 mg/kg of TMR of vitamin E), 5 g of grape pomace (**GP-5**; 5 g/100 g of TMR of DM of grape pomace), and 10 g of grape pomace (**GP-10**; 10 g/100 g of TMR of DM of grape pomace). The grape pomace (*Vitis vinifera* sp.) was collected from 3 wineries of red wine belonging to the Ribera de Duero designation of origin (Valladolid, Spain) and vitamin E (α-tocopherol acetate) was obtained from Inatega S.L. (León, Spain). Diets were formulated to meet the energy and protein requirements of the dairy ewes using INRA (2007) and FEDNA (2010). The composition of the experimental diets and the ingredients are given in Tables 1 and 2, respectively. The chemical composition was determined by using the procedures described by the AOAC International (2003). Dried feed samples were analyzed for NDF, ADF, and ADL using filter bag equipment (Ankom Technology Corp., Fairport, NY). Total mixed ration was supplied twice a day and fresh water was always available. The ewes were fed individually during the whole experimental period and each intake was recorded. The amounts of diet offered and refusals were weighed daily for each ewe, and samples were collected for subsequent analyses. Milk yield and composition were recorded weekly during the first month of lactation.

Milk Sampling and Composition

During the suckling period, as is common for Churra sheep, ewes were machine-milked once a day at 0900 h in a 2 × 24 low-line Casse**[AU3: Add manufacturer name and location for Casse.]** system milking parlor with 12 milking units and 2 milkers during the entire experimental period. The milking machine (Alfa-Laval Iberia, S.A., Madrid, Spain) was set to provide 180 pulsations per minute in a 50:50 ratio with a vacuum level of 36 kPa.

Once a week, individual ewe milk production was recorded and samples were taken in milk collection

Table 1. Ingredients and chemical composition of the experimental diets

Item	Diet[1]			
	CTRL	VIT-E	GP-5	GP-10
Ingredients (g/100 g of DM)				
Dehydrated alfalfa hay	35.2	35.2	33.2	31.2
Barley straw	8.45	8.45	8.02	7.54
Whole corn grain	10.1	10.1	9.52	8.96
Oat grain	9.25	9.25	8.72	8.20
Whole barley grain	6.95	6.95	6.55	6.16
Soybean meal	15.7	15.7	15.1	14.4
Beet pulp	6.99	6.99	6.59	6.20
Molasses	3.67	3.67	3.47	3.27
Grape pomace[2]			5.17	10.32
Linseed oil[3]	2.67	2.67	2.72	2.73
Vitamin-mineral premix	1.00	1.00	1.00	1.00
Chemical composition, (g/100 g of DM)				
OM	92.1	92.4	92.3	92.4
CP	18.9	18.7	18.6	18.3
NDF	34.8	34.7	34.8	34.9
ADF	22.7	22.6	23.1	23.5
ADL	4.40	4.40	5.20	5.99
Ether extract	5.13	5.13	5.42	5.68
Fatty acid profile (g/100 g of total fatty acids)				
C14:0	0.00	0.00	0.00	0.00
C16:0	0.17	0.17	0.21	0.25
C16:1	0.00	0.00	0.00	0.01
C18:0	0.13	0.13	0.15	0.16
C18:1	0.60	0.60	0.66	0.71
C18:2	0.41	0.41	0.62	0.83
C18:3	1.37	1.37	1.41	1.43
C >20	0.01	0.01	0.01	0.02

[1]Diets: CTRL = without grape pomace (GP); VIT E = with 500 mg/kg of TMR of vitamin E; GP-5 = 5 g of GP/100 g of TMR (% DM); and GP-10 = 10 g of GP/100 g of TMR (% DM).

[2]Fatty acid composition (% of total FAME): 14:0 (0.3); C16:0 (11.1), C16:1 (0.6), C17:0 (0.1), C18:0 (4.4), C18:1 (16.0), C18:2 (61.3), C18:3 (3.7), C20:0 (0.5), C20:1 (0.4), C24:0 (0.2). Phenolic compounds (g/kg of DM): extractable polyphenols (42.8), condensed tannins (54.6), anthocyanins (4.1).

[3]Fatty acid composition (% of total FAME): C12:0 (<0.01), C14:0 (0.10), C15:0 (<0.01), C16:0 (6.20), C16:1 (0.10), C18:0 (4.90), C18:1 (21.90), C18:2 (14.80), C18:3 (51.30), C20:0 (0.20); C22:0 (0.10).

jars. For this purpose, milk production was recorded by the oxytocin technique: in the morning, before milking, each ewe was injected with 0.35 mL of oxytocin (Oxiton, Laboratorios Ovejeros, S.A., Spain) and then immediately milked. Ewes were returned to their paddock for 6 h while the lambs were confined and were then milked again for milk sampling. One subsample of milk was kept at 4°C until analyzed for fat and protein, in accordance with International Dairy Federation recommendations (IDF, 2000), using a MilkoScan-400

Table 2. Chemical composition of main dietary ingredients[1]

Ingredient	Chemical composition (g/100 g of DM)					
	OM	CP	NDF	ADF	ADL	Ether extract
Dehydrated alfalfa hay	88.2	19.3	42.2	31.7	8.4	3.0
Barley straw	92.1	4.0	78.5	50.6	9.2	1.7
Whole corn grain	98.6	8.7	9.2	3.5	1.0	4.2
Oat grain	96.8	9.7	34.9	19.3	2.9	5.4
Whole barley grain	97.6	12.5	18.8	7.0	1.2	2.2
Soybean meal	93.0	50.0	14.5	8.2	0.5	2.2
Beet pulp	92.7	10.2	47.6	25.5	1.9	0.9
Molasses	86.3	5.8	0.0	0.0	0.0	0.1
Grape pomace	86.6	12.2	37.6	31.7	20.7	6.39
Linseed oil	100.0	0.0	0.0	0.0	0.0	99.9

analyzer (Foss Electric, Hillerød, Denmark). Another subsample of each ewe belonging to the second and fourth week of milk sampling was stored at –80°C for subsequent fatty acid analysis.

Fatty Acid Analysis

Milk fat separation was carried out using the method proposed by Luna et al. (2005a). Separated lipids were stored in amber vials, blanketed with a stream of N_2, and stored at –20°C until analysis. Fatty acid methyl esters were prepared by base-catalyzed methanolysis of glycerides with KOH in methanol (ISO-IDF, 2002).

Gas chromatography with 2 different columns was used to determine the FAME profile in accordance with the method proposed by De la Fuente et al. (2015). An Agilent model 6890 N Network Gas Chromatograph (Agilent, Palo Alto, CA) equipped with autoinjector, fitted with a flame-ionization detector on a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm i.d., Varian, Middelburg, the Netherlands), was used. Injector and detector temperature was 250°C. Helium was the carrier gas at an inlet pressure of 193.9 kPa and a split ratio of 1:100. Initial oven temperature was 45°C. After 4 min, oven temperature was raised at 13°C/min to 165°C and held for 35 min, then increased to 215°C at 4°C/min and maintained for 30 min. Another Agilent gas chromatograph, model 7820A GC System equipped with auto-injector and flame-ionization detector, was fitted with an SLB-IL111 capillary column (100 m × 0.25 mm i.d., Supelco, Bellefonte, PA). Injector and detector temperature was 250°C. The column inlet pressure was set at 241 kPa, resulting in He gas flow rates of 0.8 mL/min. Sample was injected at a split ratio of 1:100. Initial oven temperature was isothermal (168°C), and after 45 min it was raised at 5°C/min to 210°C and held for 36.6 min. Identification of unknown fatty acids, as well as C18:1 and C18:2 isomers, was based on a previous study undertaken in identical chromatographic conditions (Luna et al., 2005b; De la Fuente et al., 2015).

Statistical Analysis

Data regarding individual DMI, milk production, and chemical and fatty acid composition were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The model took into account the fixed effects of dietary treatment, time on diet, and their interaction. Time on diet was considered a repeated factor, and animal nested within treatment was subjected to a compound symmetry-covariance structure. As the interaction was not significant in most cases, only means for the principal effects are presented in the tables.

RESULTS AND DISCUSSION

Experimental diets did not affect DMI. These results agree with previous studies on dairy cows (Belibasakis et al., 1996; Moate et al., 2014; Santos et al., 2014) and ewes (Correddu et al., 2015), supporting the idea that the inclusion of grape residue, with or without linseed oil, in the ruminant diet does not depress food intake. Milk yield was not modified by the various treatments, and the period of sampling did not have a significant influence on animal performance. Although the presence of grape pomace in the diet decreased ($P < 0.01$) lactose concentration (from 5.24 in control to 5.02 and 5.09**[AU4: Percent?]** in the GP-5 and GP-10 diets, respectively), fat and protein in milk were not affected. These results confirm previous research on this issue. For instance, in dairy cows the replacement of maize silage with ensiled grape marc in the diet had no effect on milk production and milk fat and protein concentration (Belibasakis et al., 1996), and no detrimental effects of the dietary inclusion of grape residue silage on animal performance were observed (Santos et al., 2014). In dairy ewes it was reported that grape seed in the diet did not reduce milk yield or yields of milk constituents when compared with a control diet (Nudda et al., 2015). Sheep BW and body condition were not affected ($P > 0.05$) by the conditions of the experiment (data not shown), so changes in milk composition and fatty acid profile would only be due to the effect of the diets.

The composition of milk fatty acids with the different treatments is shown in Table 3. The inclusion of vitamin E did not modify total PUFA content, but it produced an increase in the total content of SFA ($P < 0.05$) at the expense of MUFA, particularly *cis* isomers ($P < 0.10$). In contrast, grape pomace supplementation did not affect the percentages of total SFA, MUFA, and PUFA (Table 3). This behavior was similar to that observed by Santos et al. (2014) when a similar proportion of grape residue silage was included in dairy cow diets.

The effect of GP-5 and GP-10 diets on the individual SFA contents was variable. The supplementation with grape pomace did not modify the percentages of 4:0, 6:0, 14:0, and 18:0 in milk fat, a tendency to increase ($P < 0.10$) was observed for 16:0, whereas a significant decrease ($P < 0.05$) of 8:0, 10:0, and 12:0 was found (Table 3). This decrease is probably grape pomace dose-dependent, because only these fatty acid percentages were significantly different with the GP-10

treatment. Feeding high proportions of grape marc also lowered 8:0, 10:0, and 12:0 in dairy cows (Moate et al., 2014). Those authors attributed this result principally to the presence in the grape residue of a great amount of lignin, which is not fermented in the rumen. Fatty acids 8:0, 10:0, and 12:0 are almost exclusively produced de novo in the mammary gland, and their synthesized amount has been shown to be related to the dietary intake of fermentable carbohydrate (Moate et al., 2008). The lack of changes in the levels of 8:0, 10:0, and 12:0 with the VIT-E diet (Table 3) would support this argument.

Odd- and branched-chain fatty acids (**OBCFA**), as a whole, were not modified as a consequence of the inclusion of vitamin E or grape pomace in the control diet (Table 3). The contents of most of the individual OBCFA in GP-5 and GP-10 milk fat were not different ($P > 0.05$) among treatments except for 17:0 *iso* and 17:0, which diminished ($P < 0.05$) or showed a tendency to decrease ($P < 0.10$), respectively. Although OBCFA were found in amounts lower than 2% of total fatty acids, they influenced milk fat melting point and have shown anticarcinogenic effects (Vlaeminck et al., 2006). Odd- and branched-chain fatty acids are largely derived from bacteria leaving the rumen and serve as biomarkers of rumen function and also as a diagnostic tool to predict shifts in microorganism populations associated with diet changes. The lack of variation in total OBCFA content among treatments (Table 3) would suggest no shifts in the rumen microbial ecosystem, indicating that, under the conditions assayed, the diets did not affect the activity and growth of ruminal microorganisms. A similar behavior was reported by Correddu et al. (2015) in rumen liquor of ewes fed grape seed, alone or associated with linseed-supplemented diets. Those authors observed that inclusion of grape seed in a diet enriched with linseed oil hardly modified the total OBCFA content in the rumen fluid. In contrast, significant decreases of some individual OBCFA as a consequence of lipid supplementation in comparison with unsupplemented diets were found (Correddu et al., 2015). This reduction in milk OBCFA due to linseed oil supplementation is well documented in lactating ewes (Luna et al., 2008; Gallardo et al., 2015) and is based on the role exerted by the oil supplements used as sources of PUFA, which have detrimental effects on ruminal microflora (Maia et al., 2007).

Oleic acid (*cis*-9 18:1) was the most abundant MUFA (about 15% of total FAME) in all treatments, and the other mono *cis* isomers were found in amounts lower than 0.5% (Table 4). Although a tendency to decrease ($P < 0.10$) with the incorporation of vitamin E was observed, statistical difference does not necessarily mean biological difference. The lack of significant differences found between control and grape pomace diets for *cis*-9 18:1 can be justified by the origin of this fatty acid in milk. Part of milk fat oleic acid comes from the diet, but endogenous synthesis in the mammary gland via Δ^9-desaturase using 18:0 as substrate is the major source of *cis*-9 18:1, accounting for more than 60% of its content in ewe milk fat (Bichi et al., 2012). As can be seen in Table 3, Δ^9-desaturase activities were not affected by the treatments, and increases of oleic acid by this route should not be expected. The diet also had no effect on the milk oleic acid content because the presence of this MUFA in grape pomace is not very high (Table 1).

Trans MUFA represents about 8% of the total fatty acids, and *trans*-11 18:1 [vaccenic acid (**VA**)] was quantitatively the most important isomer (Table 4). The remarkable levels of VA in the milk fats analyzed (more than 3% of total FAME) are attributable to the presence of linseed oil in all the diets, because VA is the main BH intermediate of unsaturated fatty acid substrates, such as α-linolenic acid (Loor et al., 2004, 2005). The high content of linoleic acid (61.3%) in grape pomace (Table 1) could also potentially enhance the VA content in milk, as has previously been demonstrated in dairy ewes and cows (Tsiplakou and Zervas, 2008; Moate et al., 2014). However, this VA level enhancement did not occur in the present study (Table 4) as well as in ruminal fluid from sheep fed linseed oil when grape seed was incorporated in the diet (Correddu et al., 2015). The explanation for this probably lies in the dose of grape residues. The proportion of grape marc in the studies of Tsiplakou and Zervas (2008) and Moate et al. (2014) was higher than 20% on a DM basis, whereas in the present research (Table 1), as well as in the study with grape seed by Correddu et al. (2015), it did not exceed 11%.

After VA, *trans*-15 and *trans*-13 + *trans*-14 were the most abundant *trans*-MUFA isomers in all the treatments (Table 4). Various authors have pointed out that these fatty acids with a double bond to carbon between positions 13 and 15 can be considered specific to α-linolenic acid metabolism (Loor et al., 2004; 2005). In contrast, *trans*-10, an isomer characteristic of rumen enzymatic conversion of oleic and linoleic acids, was detected at percentages not exceeding 0.5% and did not significantly vary with diet and time. The presence of high amounts of *trans*-10 18:1 fatty acid in milk fat is usually a reliable indicator of changes in the ruminal ecosystem and modifications in the routes of conversion of dietary PUFA. Sharp increases of *trans*-10 18:1 in milk fat at the expense of VA indicate a shift in the BH pathways that, under certain conditions, might lead to the development of milk fat depression. The absence of changes in percentages of *trans*-10 and *trans*-11 18:1

Table 3. Effect of the supplementation of dairy ewe diet with vitamin E and different doses of grape pomace and the duration of supplementation on milk fatty acids (g/100 g of total FAME) profile

	Diet[1]				Time			*P*-value[3]	
Fatty acid	CTRL	VIT-E	GP-5	GP-10	Week 2	Week 4	SED[2]	D	T
SFA									
4:0	4.46[a]	4.73[b]	4.46[a]	4.64[ab]	4.62	4.52	0.078	*	NS
5:0	0.03	0.04	0.03	0.04	0.03	0.04	0.002	NS	†
6:0	3.58[a]	3.83[b]	3.48[ac]	3.41[ac]	3.54	3.61	0.080	**	NS
7:0	0.05	0.05	0.04	0.04	0.04	0.05	0.003	NS	†
8:0	3.20[ac]	3.41[c]	3.05[ab]	2.83[b]	3.06	3.19	0.104	**	NS
9:0	0.07	0.07	0.06	0.06	0.06	0.07	0.004	NS	*
10:0	8.25[a]	8.73[a]	8.10[a]	7.17[b]	7.76	8.37	0.325	**	†
11:0	0.08	0.08	0.07	0.07	0.07	0.08	0.005	NS	*
12:0	4.17[a]	4.30[a]	4.05[a]	3.63[b]	3.87	4.22	0.174	*	*
13:0	0.09	0.09	0.09	0.09	0.08	0.10	0.005	NS	**
14:0	8.45	8.80	8.87	8.39	8.33	8.92	0.217	NS	†
15:0	0.79	0.82	0.78	0.79	0.77	0.82	0.026	NS	†
16:0	19.35[b]	20.41[a]	20.47[a]	20.52[a]	19.82	20.55	0.383	†	†
17:0	0.59[a]	0.51[b]	0.54[ab]	0.52[b]	0.59	0.49	0.023	†	***
18:0	10.78	10.26	10.44	10.70	11.26	9.83	0.414	NS	***
19:0	0.06	0.07	0.06	0.06	0.07	0.06	0.002	NS	†
20:0	0.18	0.18	0.18	0.19	0.18	0.18	0.004	NS	NS
22:0	0.08	0.09	0.08	0.08	0.08	0.09	0.004	NS	NS
23:0	0.04[a]	0.04[a]	0.03[b]	0.03[b]	0.04	0.04	0.002	†	NS
24:0	0.04[a]	0.03[a]	0.03[b]	0.03[ab]	0.03	0.03	0.002	†	NS
Keto-10 18:0	0.03	0.03	0.03	0.03	0.03	0.03	0.002	NS	NS
13:0 *iso*	0.01	0.01	0.01	0.01	0.01	0.01	0.001	NS	NS
14:0 *iso*	0.07	0.07	0.06	0.07	0.07	0.07	0.003	NS	NS
15:0 *iso*	0.16	0.17	0.17	0.16	0.17	0.16	0.006	NS	NS
15:0 *anteiso*	0.32	0.31	0.28	0.30	0.30	0.31	0.012	NS	NS
16:0 *iso*	0.20	0.21	0.18	0.19	0.21	0.19	0.009	NS	*
17:0 *iso*	0.25[a]	0.24[a]	0.24[a]	0.22[b]	0.25	0.22	0.007	*	***
17:0 *anteiso*	0.30	0.29	0.28	0.26	0.30	0.27	0.012	†	**
18:0 *iso*	0.06	0.04	0.05	0.05	0.06	0.04	0.004	NS	***
MUFA									
10:1	0.20	0.21	0.18	0.17	0.17	0.22	0.013	NS	***
cis-9 12:1	0.03	0.03	0.03	0.02	0.02	0.03	0.002	NS	**
cis-11 12:1	0.02[a]	0.03[a]	0.03[a]	0.01[b]	0.02	0.03	0.003	*	*
cis-9 14:1	0.09	0.09	0.09	0.09	0.08	0.11	0.007	NS	***
cis-7 16:1	0.17[a]	0.14[b]	0.16[c]	0.17[ac]	0.16	0.16	0.005	***	NS
cis-9 16:1	0.50	0.45	0.49	0.49	0.47	0.49	0.022	NS	NS
cis-12 16:1	0.03[a]	0.02[a]	0.02[b]	0.02[b]	0.02	0.02	0.001	*	NS
cis-13 16:1	0.04	0.04	0.04	0.03	0.03	0.04	0.003	NS	**
cis-9 17:1	0.18[a]	0.13[b]	0.16[a]	0.16[a]	0.18	0.14	0.012	*	**
cis-11 20:1	0.04	0.04	0.04	0.04	0.04	0.04	0.002	NS	NS
trans 15:1	0.09[ac]	0.09[a]	0.08[bc]	0.08[b]	0.08	0.09	0.004	*	NS
trans-5 16:1	0.01	0.01	0.01	0.01	0.01	0.01	0.001	NS	*
trans-6 + *trans*-7 16:1	0.02	0.01	0.02	0.02	0.02	0.01	0.001	NS	**
trans-8 16:1	0.04	0.04	0.04	0.04	0.04	0.04	0.001	NS	**
trans-9 16:1	0.18	0.20	0.23	0.24	0.21	0.22	0.019	NS	NS
trans-10 16:1	0.04	0.03	0.03	0.04	0.03	0.04	0.002	NS	NS
trans-13 16:1	0.03[a]	0.03[a]	0.03[a]	0.04[b]	0.03	0.03	0.001	***	*
trans-14 16:1	0.02	0.02	0.02	0.02	0.02	0.02	0.001	NS	NS
trans-10 17:1	0.03	0.04	0.04	0.04	0.03	0.04	0.004	NS	NS
UFA									
16:2	0.01	0.02	0.02	0.02	0.02	0.02	0.002	NS	NS
18:3n-6	0.02	0.02	0.02	0.02	0.02	0.02	0.002	NS	NS
18:3 (*trans, trans, cis* + *cis, cis*)	0.01[a]	0.01[a]	0.01[ab]	0.01[b]	0.01	0.01	0.001	†	NS
trans-9,*cis*-12,*cis*-15 18:3	0.01[a]	0.02[ab]	0.01[a]	0.02[b]	0.01	0.02	0.001	†	†
18:3*n*-3	1.09[ab]	1.18[b]	1.01[a]	0.96[a]	1.03	1.10	0.056	*	NS
cis-9,*trans*-11,*trans*-15 18:3	0.06	0.07	0.06	0.07	0.06	0.07	0.006	NS	†
cis-9,*trans*-11,*cis*-15 18:3	0.16	0.20	0.20	0.18	0.17	0.20	0.017	NS	NS
20:2n-6	0.01	0.01	0.01	0.01	0.01	0.01	0.001	NS	NS
20:3n-6	0.02[a]	0.02[a]	0.02[a]	0.02[b]	0.02	0.02	0.001	**	Ns
20:3n-3	0.03	0.03	0.03	0.02	0.03	0.03	0.003	NS	NS
20:4n-6	0.13[a]	0.13[a]	0.14[ab]	0.15[b]	0.15	0.13	0.006	†	***
22:2n-6	0.01[a]	0.01[ab]	0.01[b]	0.01[b]	0.01	0.01	0.000	†	NS

Continued

Table 3 (Continued). Effect of the supplementation of dairy ewe diet with vitamin E and different doses of grape pomace and the duration of supplementation on milk fatty acids (g/100 g of total FAME) profile

	Diet[1]				Time			*P*-value[3]	
Fatty acid	CTRL	VIT-E	GP-5	GP-10	Week 2	Week 4	SED[2]	D	T
20:5n-3 (eicosapentaenoic acid)	0.06	0.06	0.06	0.06	0.06	0.06	0.002	NS	NS
22:4n-6	0.01	0.01	0.01	0.01	0.01	0.01	0.001	NS	*
22:5n-3 (docosapentaenoic acid)	0.12	0.12	0.12	0.11	0.12	0.11	0.005	NS	†
22:6n-3 docosahexaenoic acid)	0.04	0.04	0.04	0.04	0.04	0.03	0.002	NS	***
Σ SFA	65.75^{a}	67.91^{b}	66.23^{a}	64.59^{a}	65.69	66.55	0.692	*	NS
Σ OBCFA[4]	1.37	1.35	1.28	1.27	1.37	1.27	0.035	NS	**
Σ MUFA	27.04^{a}	24.44^{b}	26.18^{a}	27.34^{a}	26.95	25.55	0.656	*	*
Σ MUFA *cis*	19.33^{a}	16.56^{b}	18.24^{ab}	18.61^{a}	19.05	17.32	0.736	†	*
Σ MUFA *trans*	7.71	7.89	7.94	8.73	7.90	8.23	0.354	NS	NS
Σ PUFA	6.97	7.41	7.34	7.84	7.13	7.66	0.274	NS	†
cis-9 14:1/(14:0 + *cis*-9 14:1)	0.01	0.01	0.01	0.01	0.01	0.01	0.001	NS	***
cis-9 16:1/(16:0 + *cis*-9 16:1)	0.03^{a}	0.02^{b}	0.02^{ab}	0.02^{ab}	0.02	0.02	0.001	*	NS
cis-9 18:1/(18:0 + *cis*-9 18:1)	0.60	0.58	0.60	0.60	0.59	0.60	0.008	NS	NS
RA/(VA + RA)[4]	0.26	0.26	0.26	0.27	0.25	0.28	0.008	NS	***
n-6/n-3	1.55^{ac}	1.43^{a}	1.67^{c}	1.91^{b}	1.68	1.60	0.067	***	NS
De novo synthesis fatty acids[5]	43.19^{a}	45.38^{b}	43.59^{ab}	41.67^{a}	42.38	44.54	0.881	*	*

$^{a–c}$Means within a row with different superscripts differ significantly.

[1]Diets: CTRL = without grape pomace (GP); VIT-E = with 500 mg/kg of TMR of vitamin E; GP-5 = 5 g GP/100 g of TMR (% DM); and GP-10 = 10 g GP/100 g of TMR (% DM).

[2]SED = standard error of the difference;

[3]Probability of significant effects due to experimental diet (D) and time on diet (T). There were no statistical differences ($P > 0.1$) due to interaction effects (D × T). NS indicates $P > 0.1$.

[4]OBCFA = odd- and branched-chain fatty acids; VA = *trans*-11 18:1; RA = *cis*-9 *trans*-11 18:2.

[5]De novo synthesized fatty acids = sum of 4:0–14:0 and half of 16:0.

†$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

with grape pomace supplementation (Table 4) is in agreement with an identical finding by Correddu et al. (2015) in ruminal liquid, and is due to the fact that the conditions assayed would not induce drastic changes in rumen microbiota. A shift in BH pathways from VA to *trans*-10 18:1 occurs with diets containing high amounts of fat, which was not the case in the present research. It is likely that only a higher proportion of grape residues in the diets would generate more substantial changes in the *trans* fatty acid profile (Moate et al., 2014). This statement would also be valid and applicable to the VIT-E treatment, which also did not affect the percentages of *trans*-10 and *trans*-11 18:1 in the milk.

Table 4 shows the contents of 18:2 isomers in milk fat with the various treatments assayed. As can be seen, linoleic acid (*cis*-9,*cis*-12) is quantitatively the most abundant 18:2 in all the diets. The other nonconjugated 18:2 isomers were found in lower proportions and corresponded to intermediates of ruminal BH of dietary PUFA, mainly α-linolenic acid (Loor et al., 2004; Bodas et al., 2010). The presence of vitamin E did not significantly modify the linoleic acid content. In contrast, the incorporation of grape pomace in the diet could increase the percentage of this fatty acid in milk fat. This increase is dose dependent because significant modifications were only found with 10 g of this supplement in the diet (Table 4). Because its origin is exclusively exogenous, this increase is attributable to the presence of linoleic acid in the grape pomace (Table 1). In this regard, Moate et al. (2014) succeeded in tripling the linoleic acid content in milk when more than 25% (on a DM basis) of the diet was based on ensiled grape marc.

The content of *cis*-9,*trans*-11 18:2 [rumenic acid (**RA**)], the most important CLA isomer, showed a tendency to increase with time ($P < 0.10$), but no statistically significant effect due to the experimental diets was observed (Table 4). Rumenic acid in ewe milk fat is mostly produced in the mammary gland by Δ9-desaturase from VA formed in the rumen (Bichi et al., 2012). It is also a direct intermediate in rumen BH of linoleic acid, and a portion of it is absorbed to provide the remainder of the RA in milk fat. Thus, the GP-5 and GP-10 diets, which contain a high proportion of linoleic acid (Table 1), should lead to an increase in the levels of RA in the rumen and, consequently, in the milk. In fact, in previous research (Tsiplakou and Zervas, 2008; Moate et al., 2014) it was reported that the inclusion of a variety of winery industry residues in ruminant feeding could be a good way of multiplying the levels of RA in milk fat. The higher presence of grape marc in those studies (Tsiplakou and Zervas, 2008; Moate et

Table 4. Effect of the supplementation of dairy ewe diet with vitamin E and different doses of grape pomace and duration of supplementation on the *cis* and *trans* 18 MUFA and the conjugated and nonconjugated 18:2 isomers (g/100 g of total FAME) profile

	Diet[1]				Time			*P*-value[3]	
Fatty acid	CTRL	VIT-E	GP-5	GP-10	Week 2	Week 4	SED[2]	D	T
Cis and *trans* 18:1									
cis-9 18:1	16.61^{a}	14.05^{b}	15.61^{ab}	15.75^{a}	16.45	14.57	0.718	†	*
cis-11 18:1	0.54	0.52	0.56	0.55	0.56	0.52	0.020	NS	*
cis-12 18:1	0.49^{a}	0.45^{a}	0.48^{a}	0.67^{b}	0.48	0.57	0.030	***	**
cis-13 18:1	0.04^{a}	0.04^{b}	0.04^{b}	0.04^{b}	0.04	0.04	0.001	**	*
cis-14 18:1	0.05^{a}	0.04^{a}	0.04^{a}	0.05^{b}	0.04	0.05	0.002	**	**
cis-15 18:1	0.13^{a}	0.13^{a}	0.12^{a}	0.16^{b}	0.13	0.14	0.012	†	NS
cis-16 18:1	0.04^{a}	0.04^{a}	0.04^{a}	0.04^{b}	0.04	0.04	0.002	*	NS
trans-4 18:1	0.02	0.02	0.02	0.02	0.02	0.02	0.001	NS	NS
trans-5 18:1	0.02	0.02	0.02	0.02	0.02	0.02	0.001	NS	NS
trans-6 + *trans*-7 18:1	0.13	0.13	0.13	0.13	0.13	0.13	0.005	NS	NS
trans-8 18:1	0.18^{a}	0.18^{a}	0.17^{a}	0.22^{b}	0.18	0.20	0.008	***	†
trans-9 18:1	0.32^{a}	0.32^{a}	0.31^{a}	0.36^{b}	0.30	0.34	0.012	*	**
trans-10 18:1	0.44	0.46	0.42	0.50	0.43	0.47	0.026	NS	NS
trans-11 18:1	3.17	3.49	3.58	3.93	3.52	3.56	0.273	NS	NS
trans-12 18:1	0.62^{a}	0.63^{a}	0.61^{a}	0.71^{b}	0.59	0.69	0.031	†	**
trans-13 + *trans*-14 18:1	0.77^{a}	0.73^{ab}	0.64^{b}	0.77^{a}	0.67	0.78	0.042	†	*
trans-15 18:1	1.23	1.10	1.25	1.20	1.23	1.16	0.055	NS	NS
trans-16 18:1	0.37^{a}	0.33^{b}	0.30^{b}	0.36^{a}	0.32	0.36	0.011	***	**
Nonconjugated 18:2									
trans-11,*trans*-15	0.13	0.16	0.16	0.17	0.15	0.16	0.014	NS	NS
trans-9,*trans*-12	0.01	0.01	0.00	0.01	0.00	0.01	0.000	NS	*
cis-9,*trans*-13 + *trans*-8,*cis*-12	0.46^{ac}	0.42^{cb}	0.40^{b}	0.49^{a}	0.40	0.49	0.020	**	***
trans-8,*cis*-13	0.10^{a}	0.08^{b}	0.08^{b}	0.10^{a}	0.08	0.10	0.004	***	***
cis-9,*trans*-12	0.03	0.03	0.03	0.04	0.03	0.04	0.002	NS	***
trans-9,*cis*-12	0.03^{a}	0.03^{a}	0.03^{a}	0.04^{b}	0.03	0.03	0.002	*	*
trans-10,*cis*-15	0.06	0.06	0.06	0.07	0.05	0.07	0.006	NS	*
trans-11,*cis*-15	0.80^{a}	1.06^{b}	1.07^{b}	1.09^{b}	0.97	1.04	0.085	†	NS
cis-9,*cis*-12	1.85^{a}	1.83^{a}	1.89^{a}	2.04^{b}	1.92	1.89	0.041	**	NS
cis-9,*cis*-15	0.06^{a}	0.06^{a}	0.06^{a}	0.08^{b}	0.06	0.07	0.007	†	NS
cis-12,*cis*-15	0.15	0.16	0.15	0.22	0.15	0.20	0.025	NS	†
Conjugated 18:2									
cis-9,*trans*-11 (RA)	1.11	1.21	1.26	1.41	1.15	1.35	0.099	NS	†
trans-7,*cis*-9	0.07	0.06	0.07	0.07	0.06	0.07	0.003	NS	*
trans-9,*cis*-11	0.01^{a}	0.01^{ab}	0.01^{b}	0.01^{b}	0.01	0.01	0.001	†	NS
trans-8,*trans*-10 + *trans*-9,*trans*-11 + *trans*-10,*cis*-12	0.04	0.04	0.04	0.04	0.03	0.04	0.002	NS	*
trans-11,*cis*-13	0.11	0.11	0.12	0.11	0.11	0.12	0.011	NS	NS
trans-13,*trans*-15 + *trans*-12,*trans*-14	0.05^{a}	0.04^{a}	0.05^{a}	0.06^{b}	0.05	0.05	0.005	†	†
trans-11,*trans*-13	0.04	0.04	0.04	0.04	0.04	0.04	0.003	NS	NS
Other *trans*-*trans*	0.04^{a}	0.04^{b}	0.03^{b}	0.04^{a}	0.03	0.04	0.002	*	**

$^{a-c}$Means within a row with different superscripts differ significantly.

[1]Diets: CTRL = without grape pomace (GP); VIT-E = with 500 mg/kg of TMR of vitamin E; GP-5 = 5 g GP/100 g of TMR (% DM); and GP-10 = 10 g GP/100 g of TMR (% DM).

[2]SED = standard error of the difference.

[3]Probability of significant effects due to experimental diet (D) and time on diet (T). There were no statistical differences ($P > 0.1$) due to interaction effects (D × T). NS indicates $P > 0.1$.

†$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

al., 2014) in comparison with the diets assayed in the present research (Table 1) could justify this discrepancy. Moreover the addition of grape seed to linseed-supplemented diets at similar levels to those reported in the present research hardly modified the content of RA in the ruminal fluid of dairy ewes (Correddu et al., 2015), as occurred with VA. Therefore, to produce drastic changes in the metabolism of dietary fatty acids in the rumen of dairy ewes an intake of higher proportions of grape pomace would be required. Apart from RA, other minor CLA isomers were detected in the milk fat, but in very low or negligible amounts. Most of them were not modified by the treatments (Table 4). *Trans*-11,*cis*-13 was quantitatively the second most abundant CLA, an isomer characteristic of milk of ewes fed with linseed-oil enriched diets (Luna et al., 2008; Bodas et al., 2010).

The α-linolenic acid contents in all the samples analyzed can be regarded as high (around 1%) (Table 3) in comparison with the usual values of ruminant milks

(Shingfield et al., 2013; Nudda et al., 2014). This is a consequence of the supplementation of all the diets with linseed oil, a substrate very rich in this fatty acid (Table 1), and due to the fact that part of 18:3 n-3 can escape BH and be subsequently transferred to milk. Time did not modify ($P > 0.1$) the α-linolenic acid in milk. The α-linolenic acid content increased in the VIT-E treatment ($P < 0.05$), supporting the view that vitamin E can play a part as an antioxidant in ewe diets. In contrast, the presence of grape pomace in the diets did not modify the percentage of this n-3 fatty acid in milk fat. The contents of other minor 18:3 isomers in milk fat were also not modified by the various treatments (Table 3). In this regard, it is noteworthy that the percentages of *cis*-9,*trans*-11,*cis*-15 18:3 and *cis*-9,*trans*-11,*trans*-15 18:3, both intermediates of α-linolenic acid in the rumen, remained unmodified with all the diets (Table 3), again supporting the idea that the treatments assayed did not affect lipid ruminal metabolism. Other intermediates of the dietary α-linolenic conversion pathways, such as *trans*-11,*cis*-15 18:2, VA, or *trans*-15 18:1, were also not affected (Table 4). Overall, these data provide additional evidence supporting the view that, under the conditions assayed (Table 1), dietary PUFA digestive conversion pathways and rumen microflora were not altered.

Other PUFA with more than 18 carbon atoms were detected in milk fat but in very low amounts (Table 3). The contents of the remaining n-3 PUFA (20:5, 22:5, and 22:6) reported in our study did not vary with the presence of vitamin E or the inclusion of different levels of grape pomace in the diets. The n-6-to-n-3 ratio did not vary with time ($P > 0.1$), but was significantly affected by feeding ($P < 0.001$). However, the n-6-to-n-3 values in all the treatments may be considered very favorable from the human health point of view because they are clearly below 4, which is the recommended maximum value in dietary fat (Simopoulos, 2008).

CONCLUSIONS

The results of this work clearly demonstrate that supplementation with grape pomace of diets enriched with linseed oil under the conditions assayed is not detrimental for animal performance and milk yield. This study confirms that the addition of vitamin E to the diet had only a moderate effect on milk fatty acid content. The presence in the diet of grape pomace at the doses assayed did not substantially modify the milk fatty acid profile. Consequently, the use of grape pomace as feed in dairy sheep area could be adopted as a strategy to reduce feeding cost and also to cope with the need to recycle waste material, which is costly to dispose of without adverse effects on animal performance and milk yield and composition.

ACKNOWLEDGMENTS

This work was subsidized by INIA**[AU5: Spell out INIA.]** (RTA2010-0068-C02) and the Consejería de Educación de la Junta de Castilla y León (Spain; VA196A11-2). Cristina Guerra-Rivas was supported by a FPU grant (FPU AP-2010-0571) under the Spanish Ministry of Education**[AU6: Spell out FPU and add a location for this ministry.]**.

REFERENCES

AOAC International. 2003. Official Methods of Analysis. 17th ed. AOAC Int., Gaithersburg, MD.

Atwal, A. S., M. Hidiroglou, J. K. G. Kramer, and M. R. Binns. 1990. Effects of feeding α-tocopherol and calcium salts of fatty-acids on vitamin-E and fatty-acid composition of cow's milk. J. Dairy Sci. 73:2832–2841.

Belibasakis, N. G., E. Progia, A. Papaioannou, and G. Skulidis. 1996. Effects of ensiled wet grape marc on milk production, milk composition and blood components of dairy cows. Czech J. Anim. Sci. 41:307–310.

Bichi, E., P. G. Toral, G. Hervás, P. Frutos, P. Gómez-Cortés, M. Juárez, and M. A. de la Fuente. 2012. Inhibition of Δ9-desaturase activity with sterculic acid: effect on the endogenous synthesis of *cis*-9 18:1 and *cis*-9,*trans*-11 18:2 in dairy sheep. J. Dairy Sci. 95:5242–5252.

Bodas, R., T. Manso, A. R. Mantecón, M. Juárez, M. A. de la Fuente, and P. Gómez-Cortés. 2010. Comparison of the fatty acid profile in cheeses from ewes fed diets supplemented with different plant oils. J. Agric. Food Chem. 58:10493–10502.

Casamassima, D., M. Nardoia, M. Palazzo, F. Vizzarri, A. G. D'Alessandro, and C. Corino. 2014. Effect of dietary extruded linseed, verbascoside and vitamin E supplements on yield and quality of milk in Lacaune ewes. J. Dairy Res. 81:485–493.

Correddu, F., A. Nudda, G. Battacone, R. Boe, A. H. D. Francesconi, and G. Pulina. 2015. Effects of grape seed supplementation, alone or associated with linseed, on ruminal metabolism in Sarda dairy sheep. Anim. Feed Sci. Technol. 199:61–72.

De la Fuente, M. A., V. Rodríguez-Pino, and M. Juárez. 2015. Use of extremely polar 100-m column in combination with cyanoalkyl polysiloxane column to complement the study of milk fats with different fatty acid profiles. Int. Dairy J. 47:52–63.

FEDNA. 2010. Tablas de composición y valor nutritivo de los alimentos para la fabricación de piensos compuestos. C. de Blas, G. G. Mateos and P. García-Rebollar, ed. FEDNA, Madrid, Spain.**[AU7: Spell out FEDNA.]**

Ferlay, A., B. Martin, S. Lerch, M. Gobert, P. Pradel, and Y. Chilliard. 2010. Effects of supplementation of maize silage diets with extruded linseed, vitamin E and plant extracts rich in polyphenols, and morning v. evening milking on milk fatty acid profiles in Holstein and Montbéliarde cows. Animal 4:627–640.

Gallardo, B., M. G. Manca, A. R. Mantecón, A. Nudda, and T. Manso. 2015. Effects of linseed oil and natural or synthetic vitamin E supplementation in lactating ewes′ diets on meat fatty acid profile and lipid oxidation from their milk fed lambs. Meat Sci. 102:79–89.

IDF. 2000. Determination of milk fat, protein and lactose content. Guidance on the operation of mid-infrared instruments. International IDF standard 141C:2000. International Dairy Federation, Brussels, Belgium.

INRA. 2007. Alimentation des bovins, ovins et caprins. Besoins des animaux-valeurs des aliments. Éditions Quae, Versailles Cedex, France.

ISO-IDF. 2002. Milk Fat—Preparation of Fatty Acid Methyl Esters. International Standard ISO 15884-IDF 182:2002. International Organization for Standardization, Geneva, Switzerland.

Kay, J. K., J. R. Roche, E. S. Kolver, N. A. Thomson, and L. H. Baumgard. 2005. A comparison between feeding systems (pasture and TMR) and the effect of vitamin E supplementation on plasma and milk fatty acid profiles in dairy cows. J. Dairy Res. 72:322–332.

Loor, J. J., A. Ferlay, A. Ollier, K. Ueda, M. Doreau, and Y. Chilliard. 2005. High-concentrate diets and polyunsaturated oils alter *trans* and conjugated isomers in bovine rumen, blood, and milk. J. Dairy Sci. 88:3986–3999.

Loor, J. J., K. Ueda, A. Ferlay, Y. Chilliard, and M. Doreau. 2004. Biohydrogenation, duodenal flow, and intestinal digestibility of *trans* fatty acids and conjugated linoleic acids in response to dietary forage:concentrate ratio and linseed oil in dairy cows. J. Dairy Sci. 87:2472–2485.

Luna, P., A. Bach, M. Juárez, and M. A de la Fuente.. 2008. Influence of diets rich in flax seed and sunflower oil on the fatty acid composition of ewes' milk fat especially on the content of conjugated linoleic acid, n-3 and n-6 fatty acids. Int. Dairy J. 18:99–107.

Luna, P., M. A. de la Fuente, and M. Juárez. 2005b. Conjugated linoleic acid in processed cheeses during the manufacturing stages. J. Agric. Food Chem. 53:2690–2695.

Luna, P., M. Juárez, and M. A. de la Fuente. 2005a. Validation of a rapid milk fat separation method to determine the fatty acid profile by gas chromatography. J. Dairy Sci. 88:3377–3381.

Maia, M. R., L. C. Chaudhary, L. Figueres, and R. J. Wallace. 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Antonie van Leeuwenhoek 91:303–314.

Martínez Marín, A. L., P. Gómez-Cortés, G. Gómez Castro, M. Juárez, L. Pérez Alba, M. Pérez Hernández, and M. A. de la Fuente. 2011. Animal performance and milk fatty acid profile of dairy goats fed diets added differently unsaturated plant oils on fatty acid profile of goat milk. J. Dairy Sci. 94:5359–5368.

Moate, P. J., W. Chalupa, R. C. Boston, and I. A. Lean. 2008. Milk fatty acids II: Prediction of the production of individual fatty acids in bovine milk. J. Dairy Sci. 91:1175–1188.

Moate, P. J., S. R. O. Williams, V. A. Torok, M. C. Hannah, B. E. Ribaus, M. H. Tavendale, R. J. Eckard, J. L. Jacobs, M. J. Auldist, and W. J. Wales. 2014. Grape marc reduces methane emissions when fed to dairy cows. J. Dairy Sci. 97:5073–5087.

Nudda, A., G. Battacone, O. Boaventura Neto, A. Cannas, A. H. D. Francesconi, A. S. Atzori, and G. Pulina. 2014. Feeding strategies to design the fatty acid profile of sheep milk and cheese. Rev. Bras. Zootec. 43:445–456.

Nudda, A., F. Correddu, A. Marzano, G. Battacone, P. Nicolussi, P. Bonelli, and G. Pulina. 2015. Effects of diets containing grape seed, linseed, or both on milk production traits, liver and kidney activities, and immunity of lactating dairy ewes. J. Dairy Sci. 98:1157–1166.

Pottier, J., M. Focant, C. Debier, G. de Buysser, C. Goffe, E. Mignolet, E. Froidmont, and Y. Larondelle. 2006. Effect of dietary vitamin E on rumen biohydrogenation pathways and milk fat depression in dairy cows fed high-fat diets. J. Dairy Sci. 89:685–692.

Santos, N. W., G. T. D. Santos, D. C. Silva-Katama, P. A. Grande, P. M. Pinto, F. E. de Marchi, C. C. Jobin, and H. V. Petit. 2014. Production, composition and antioxidants in milk of dairy cows fed diets containing soybean oil and grape residue silage. Livest. Sci. 159:37–45.

Shingfield, K. J., M. Bonnet, and N. D. Scollan. 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal 7:132–162.

Simopoulos, A. P. 2008. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp. Biol. Med. (Maywood) 233:674–688.

Tsiplakou, E., and G. Zervas. 2008. The effect of dietary inclusion of olive trees leaves and grape marc on the content of conjugated linoleic acid and vaccenic acid in the milk of dairy sheep and goats. J. Dairy Res. 75:270–278.

Vlaeminck, B., V. Fievez, A. R. J. Cabrita, A. J. M. Fonseca, and R. J. Dewhurst. 2006. Factors affecting odd- and branched-chain fatty acids in milk: A review. Anim. Feed Sci. Technol. 131:389–417.

Yi, C., J. Shi, J. Kramer, S. Xue, Y. M. Jiang, M. W. Zhang, Y. Ma, and J. Pohorly. 2009. Fatty acid composition and phenolic antioxidants of winemaking pomace powder. Food Chem. 114:570–576.

Zened, A., A. Troegeler-Meynadier, T. Najar, and F. Enjalbert. 2012. Effects of oil and natural or synthetic vitamin E on ruminal and milk fatty acid profiles in cows receiving a high-starch diet. J. Dairy Sci. 95:5916–5926.