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Grape pomace in ewes diet: Effects on meat quality and the fatty acid profile of their suckling lambs

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ABSTRACT

Grape pomace (GP) is an inexpensive natural antioxidant promising as animal feed supplement due to its high content of phenolic compounds. In order to evaluate its effect in lactating ewe rations on meat quality and fat composition of their suckling lambs, 48 Churra ewes were divided into 4 treatments. All animals were fed a ration containing linseed oil (Control) supplemented with Vitamin E or two levels of GP. Lambs were nourished exclusively by suckling until they were slaughtered. Dietary GP did not generate adverse effects on carcasses or lambs meat quality when compared with Control or Vit-E diets. GP improved the water holding capacity of the meat. In addition, lambs meat FA profile was not nutritionally affected with the diets assayed. Hence, the use of GP as a dietary supplement in ewe rations would not have negative effects on meat from suckling lambs.

1. Introduction

In the last few decades, the scientific community has focused on increasing bioactive lipids contents, such as conjugated linoleic acid (CLA) or omega-3 polyunsaturated fatty acids (PUFA n-3) in ruminant products (Shingfield, Bonnet, & Scollan, 2013). However, higher levels of unsaturated fatty acids in meat and milk from ruminants makes them more susceptible to oxidation. One of the most common methods for reducing lipid oxidation in animal products is the use of dietary synthetic antioxidants, but the effect of synthetic compounds on consumer health is still controversial. The use of natural ingredients to improve the oxidative stability of animal products is of increasing interest owing to consumer demand for healthier products and their willingness to pay premium prices for organic foods (Brewer, 2011).

A number of plant secondary compounds possess antioxidant and antimicrobial properties, and therefore their use in animal feeding could be promoted (Brenes, Viveros, Chamorro, & Arija, 2016; Vasta & Luciano, 2011). Indeed, polyphenols have demonstrated their ability to prevent meat lipid oxidation when they are incorporated in diets of fattening lambs (Andrés et al., 2014; Karami, Alimon, & Goh, 2011) or lactating ewes (Nieto, Díaz, Bañón, & Garrido, 2010; Santos et al., 2014) or incorporated in milk replacers for suckling lambs (Morán et al., 2014). On the other hand, it has also been documented that polyphenols reduce ruminal biohydrogenation (BH) and consequently they may increase contents of lipid BH intermediates on milk and meat from ruminants (Vasta, Mele, Makkar, & Priolo, 2009). Grape pomace (GP) is a rich source of polyphenols which results from the wine and juice industry and that is costly to dispose of ((Crupi, Dipalmo, Clodoveo, Toci, & Coletta, 2018; Yi et al., 2009). Besides, due to high fibre concentration, grape residue could be an alternative feed ingredient to partially replace the forage portion in the diet of ruminants.

Suckling lamb meat is highly representative of Mediterranean countries, being a traditionally consumed food in certain regions. The high quality of this meat is related to its tenderness, juiciness and palatability (Gorraiz, Beriain, Chasco & Iraizoz, 2000). Suckling lambs covered by a Protected Geographical Indication, are reared with their dams, fed exclusively on maternal milk and slaughtered after a suckling period of 30–35 days. Polyphenols of GP are transferred from diet to milk of cows (O'Connell & Fox, 2001), ewes (Chiofalo, Liotta, Fiumanò, Benedetta, & Chiofalo, 2012) and goats (Jordán, Moñino, Martínez, Lafuente, & Sotomayor, 2010), improving the antioxidant activity of

Abbreviations: ADF, acid detergent fibre; BH, biohydrogenation; CCW, cold carcass weight; CLA, conjugated linoleic acid; FA, fatty acid; FAME, fatty acid methyl ester; HCW, hot carcass weight; GP, grape pomace; LTL, *longissimus thoracis et lumborun*; LBW, live body weight; MUFA, monounsaturated fatty acids; NDF, neutral detergent fibre; OBCFA, odd and branched chain fatty acids; PUFA, polyunsaturated fatty acids; RA, rumenic acid; SFA, saturated fatty acids; TMR, total mixed ration; VA, vaccenic acid; WHC, water holding capacity.

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their milk (Santos et al., 2014). In addition, the phenolic compounds and antioxidant activity of milk could be transferred to suckling lamb muscle and exert beneficial effects on their meat quality. Some research have been carried out to study the effects of including wine or grape seeds extracts in growing lamb diets (Jerónimo et al., 2010; Muíño et al., 2014). However, to our knowledge, no studies have investigated the effects of the inclusion of whole GP in lactating ewe diets on milk composition and suckling lamb performance, meat quality and meat fatty acid profile. The aim of this study was to determine the effect of dietary inclusion of different doses of GP mixed with linseed oil in lactating ewe diets on animal performance, meat and fatty acid composition of suckling lambs.

2. Material and methods

2.1. Animals and experimental design

Forty-eight pregnant Churra ewes (mean live body weight, LBW, 59.2 ± 4.91 kg) were selected before lambing and fed on the same diet. The ewes, aged 3–5 years, whose parity ranged from 4 to 6, gave birth 3–4 days before starting the experiment. After lambing, each ewe was assigned to one of four treatments (12 ewes per treatment) based on their milk production, age, initial LBW and parity. The newborn lambs (12 per treatment, six males and six females), covered by the Protected Geographical Indication 'Lechazo de Castilla y León', were housed with their respective mothers all day long and nourished exclusively by suckling during the whole experimental period (from birth until they reached 11.5 kg LBW, approximately). 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 (Spain).

The experimental diets consisted of a total mixed ration (TMR) containing 2.7% (on a DM basis) of linseed oil, and 40:60 forage:concentrate ratio. The four dietary treatments were: CTRL (without GP, negative control), VIT-E (500 mg of vitamin E per kg TMR, DM basis, positive control), GP-5 (5% of GP from red wine production, DM basis), and GP-10 (10% of GP from red wine production, DM basis). The grape pomace (*Vitis vinifera* sp.) was collected from three wineries of red wine belonging to the Ribera de Duero designation of origin (Valladolid, Spain). In this experiment, vitamin E was used as positive control because it is the antioxidant most frequently employed in animal nutrition while GP was supplied in fresh form. TMR was offered twice a day and fresh water was always available. 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.

The ingredients and chemical composition of the experimental diets are given in Table 1. The chemical composition of the TMR was determined using the procedures described by the AOAC (2012). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the sequential procedure using an ANKOM^{200} fibre analyser (Ankom Technology Corporation, Macedon, NY, USA) and the method of Van Soest, Robertson, and Lewis (1991). The quantification of phenolic compounds, tannins and anthocyanins were carried out according to Guerra-Rivas, Gallardo, Mantecón, Del Álamo-Sánza, and Manso (2017).

The lambs were weighed twice a week until they reached the intended LBW (approximately 11.5 kg). At the end of the trial, suckling lambs were transported (2 km) to an EU-licensed abattoir and slaughtered. Lambs were stunned and slaughtered by section of the jugular vein in the neck. After slaughter, the carcasses were immediately weighed (hot carcass weight, HCW) and transferred to a cooler at 4 °C. After 24 h, the carcasses were weighed again (cold carcass weight, CCW). Kidney knob fat was removed from the carcass and weighed as fatness measurement.

Table 1
Ingredients and chemical composition of the experimental ewe diets.

	Treatments[1]			
	CTRL	VIT-E	GP-5	GP-10
Ingredients (g kg^{-1} DM)				
Dehydrated alfalfa hay	352	352	332	312
Barley straw	84.5	84.5	80.2	75.4
Whole corn grain	101	101	95.2	89.6
Oat grain	92.5	92.5	87.2	82.0
Whole barley grain	69.5	69.5	65.5	61.6
Soybean meal	157	157	151	144
Beet pulp	69.9	69.9	65.9	62.0
Molasses	36.7	36.7	34.7	32.7
Vitamin-mineral premix	10.0	10.0	10.0	10.0
Linseed oil [2]	26.7	26.7	27.2	27.3
Grape pomace [3]	-	-	51.7	103
Vitamin E (mg kg^{-1} DM)	50.0	500	50.0	50.0
Chemical composition (g kg^{-1} DM)				
Dry matter (DM)	889	888	828	775
Organic matter	921	924	923	924
Neutral detergent fibre	348	347	348	349
Acid detergent fibre	227	226	231	235
Crude protein	189	187	186	183
Ether extract	51.3	51.3	54.2	56.8

[1] Treatments: CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of TMR, DM basis; GP-5, 5% of grape pomace from red wine, DM basis; GP-10, 10% of grape pomace from red wine, DM basis.

[2] Linseed oil fatty acid composition (% of total fatty acids): C14:0, 0.1; C16:0, 6.2; C16:1, 0.1; C18:0, 4.9; C18:1, 21.9; C18:2, 14.8; C18:3, 51.3; C20:0, 0.2; C22:0, 0.1.

[3] Grape pomace composition (g kg^{-1} DM): DM, 955 g kg^{-1}; MO, 866; NDF, 376, ADF, 317; CP, 122; EE, 63.9; extractable polyphenols, 42.8; condensed tannins, 54.6; anthocyanins, 4.10. Fatty acid composition (% of total fatty acids): C16:0, 11.1; C18:0, 4.41; C18:1, 16.0; C18:2, 61.3; C18:3, 3.7.

2.2. Meat quality and chemical composition measurements

At 24 h *post mortem*, the pH value of *longissimus thoracis* muscle was measured at the 6th rib site with a pHmeter, equipped with penetrating electrode and temperature probe. At the same time, *rectus abdominis* muscle and subcutaneous dorsal fat colorimetric parameters were measured directly on the carcass surface at three different locations, using a reflectance spectrophotometer (Konica Minolta CM-2600d; Osaka, Japan). The illuminant used was D65 (colour temperature of 6504 K) and the standard observer position was 10°. Colour results were expressed as CIE $L^*a^*b^*$ values (CIE, 1986): L^* (lightness), a^* (redness) and b^* (yellowness). The hue angle (H^*), which defines colour was calculated as arctangent (b^*/a^*), and the chroma (C^*) was computed as $(a^{*2} + b^{*2})^{1/2}$.

The *longissimus thoracis et lumborun* (LTL) muscle was excised from both sides of the carcasses, stored at −80 °C and used to carry out meat analysis. Meat water holding capacity (WHC) was determined as cooking losses using the method described by Honikel (1998). Samples of LTL, weighing 150 g, were thawed overnight at cooler temperature (4 °C). Cooking losses were determined after cooking the samples in open polyethylene bags in a water bath (Precisterm, JP Selecta, Spain) at 75 °C until they reached an internal temperature of 70 °C, measured with a digital thermometer with a temperature probe (Hanna Instruments, Woonsocket, RI, US) in the centre of the sample. Cooked samples were allowed to cool under running water for 30 min and blotted dry until they reached 20–25 °C. The cooking loss values were calculated on the basis of the difference in weight before and after cooking.

After cooking losses measurement, the same samples were used for the determination of shear force. Ten parallelepipeds measuring approximately 1 × 1 × 2 cm (height × width × length) from each sample were cut parallel to the long axis of the muscle fibres. They were sheared perpendicular to the fibre, with a Warner-Bratzler shear blade attached to a TA-XTplus texture analyser (Stable Micro Systems, Godalming, Surrey, UK). The crosshead speed was 5 mm s^{-1}. For each sample the maximum shear force was recorded, and the value reported for each steak was the mean of all the evaluated strips.

Dry matter, ash, crude protein and fat were determined as AOAC (2012) described. Meat fat extraction and fatty acid methyl esters (FAME) were prepared according to Gallardo et al. (2014). FAME analyses in hexane were carried out by gas chromatography with 2 different columns in accordance to the methodology described by De la Fuente, Rodríguez-Pino, and Juárez (2015). All peaks were identified by comparison of their retention times with analogous fat samples and standard mixtures purchased from Nu-Chek Prep. Inc. (Elysian, MN, USA).

2.3. Statistical analysis

Average lamb daily weight gain (ADG) was estimated as the regression coefficient (slope) of LBW against time using the following simple linear regression (REG) model: $y = \beta_0 + \beta_1 x + \varepsilon$, where y is the final body weight; β_0 the ADG; β_1 the initial body weight; x the time and ε the error.

Lamb carcass and meat data were subjected to analysis of variance using the general linear model (GLM) procedure of the SAS 9.2. package (SAS Inst. Inc. Cary, NC, USA), according to the model $Y_i = \mu + T_i + \varepsilon_i$; where Y is the dependent variable; μ the overall mean; T_i the fixed effect of dietary treatment (four levels, CTRL, VIT-E, GP-5 and GP-10) and ε_i the error. The LSD test was used to assess the significance between treatment means where the effect was significant. The CORR procedure of SAS was used to calculate the correlation coefficients of selected FA between milk and suckling lamb meat. Additionally, a binomial distribution was used for the results of the sensory analysis (triangle test).

For all statistical procedures the statistical significance of differences was defined as $P < 0.05$.

3. Results and discussion

As it has been detailed in a previous work (Guerra-Rivas et al., 2017), grape pomace is of a lignocellulosic nature, with high cell wall, ADF and lignin contents, which reveals its low energy content and low digestibility. The presence of extractable polyphenols, CT and anthocyanins in the grape pomace used in this study showed in Table 1, was comparable to reported values for various wine by-products (Molina-Alcaide et al., 2008; Spanghero et al., 2009).

Milk production and composition, suckling lamb performance and carcass traits are shown in Table 2. No differences attributable to any experimental treatment were observed for suckling lamb performance ($P > 0.05$). As there were no differences in ewe intake and milk production (Manso et al., 2016), it was expected that ADG, live weights of sucking lambs and carcass performance, carcass weights and fatness would not be affected. The lack of differences in lamb performance and carcass traits could be due to the fact that the suckling lambs were fed exclusively on maternal milk, and the milk yield was not limiting to lamb growth. Thus, the absence of changes in milk yield and composition would explain a similar behaviour on lamb performance. Similar results to those in this study were reported in suckling lambs from ewes fed diets supplemented with different types of fats (Gallardo et al., 2014; Manso, Bodas, Vieira, Mantecón, & Castro, 2011), with linseed oil plus vitamin E (Gallardo, Manca, Mantecón, Nudda, & Manso, 2015), and with thyme (Nieto, Bañón, & Garrido, 2011).

3.1. Meat quality and chemical composition

Meat pH, colour, meat shear force, cooking losses (WHC) and chemical composition parameters are shown in Table 3. Muscular pH did not present significant differences ($P > 0.05$) between experimental treatments (Table 3). These results are in agreement with findings by other authors employing vitamin E or phenolic compounds in milk replacers for suckling lambs (Morán et al., 2014), and in ewe diets (Nieto, 2013; Nieto, Bañón, & Garrido, 2012). Our results reflected a regular trend of the *post mortem* glycolysis in muscle, and, according to Inserra et al. (2014), they were supported by the fact that the dietary treatments did not affect the growth rate (ADG), which may have an impact on the pool of muscle glycogen or the colour of muscle between treatments.

Table 2
Effect of experimental ewe diets on milk yield and composition, growth performance and carcass traits of sucking lambs.

	Treatments[1]				RSD[2]	P. value
	CTRL	VIT-E	GP-5	GP-10		
Milk yield (g)	2557	2449	2291	2397	164.1	0.427
Milk composition (%)						
Fat	5.92	6.13	6.29	6.42	0.371	0.540
Protein	4.33	4.44	4.40	4.39	0.078	0.512
Total solids	16.4	16.6	16.6	16.8	0.34	0.650
Animal performance						
Birth body weight (kg)	4.19	4.45	4.53	4.37	0.699	0.674
Slaughter weight (kg)	11.8	11.5	11.6	11.3	0.69	0.291
Average daily gain (g $animal^{-1}$ day^{-1})	295	256	283	258	45.8	0.116
Slaughter age (days)	27.8	27.6	24.6	28.6	4.66	0.096
Carcass traits						
Cold carcass weight (kg)	6.23	6.14	6.09	5.91	0.418	0.160
Carcass yield (%)	53.1	53.4	52.7	52.3	1.99	0.426

[a,b,c]Means with different letter in the same row are significantly different ($P < 0.05$).

[1] Treatments: CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of TMR, DM basis; GP-5, 5% of grape pomace from red wine, DM basis; GP-10, 10% of grape pomace from red wine, DM basis.

[2] RSD: residual standard deviation.

Table 3
Effect of experimental ewe diets on pH, colour, cooking losses, texture and chemical composition of suckling lamb meat.

	Treatments[1]				RSD[2]	P. value
	CTRL	VIT-E	GP-5	GP-10		
Meat pH 24 h post slaughter *L. thoracis*	5.74	5.65	5.68	5.77	0.019	0.100
R. abdominis colour						
*L**	47.8	48.6	46.7	47.0	2.45	0.136
*a**	4.66^{a}	4.08^{a}	6.18^{b}	5.06^{a}	1.800	0.013
*b**	5.16	5.14	5.07	4.74	0.846	0.446
*H**	49.4	52.0	40.1	45.2	13.48	0.073
*C**	7.18^{a}	6.75^{a}	8.10^{b}	7.15^{a}	1.072	0.006
L. thoracis et lumborum colour						
*L**	49.3	51.7	49.8	50.6	2.35	0.410
*a**	2.82	1.85	3.22	2.47	1.333	0.445
*b**	13.4^{a}	9.91^{b}	9.54^{b}	10.2^{b}	1.08	<0.001
*H**	13.8^{a}	10.1^{b}	10.1^{b}	10.3^{b}	0.939	<0.001
*C**	78.2	79.6	70.7	76.2	7.46	0.291
Subcutaneous fat colour						
*L**	74.4^{a}	74.7^{a}	73.4^{ab}	72.7^{b}	2.10	0.032
*a**	1.01	1.28	1.41	1.52	0.928	0.432
*b**	7.63	8.85	8.90	8.15	1.917	0.178
*H**	72.8	59.8	70.3	68.7	45.32	0.858
*C**	7.74	8.98	9.04	8.32	1.972	0.189
Warner-Bratzler shear force (kgF cm^{-2})	6.85	5.71	5.83	5.38	1.474	0.150
Cooking losses (%) (WHC) [3]	24.1^{a}	18.9^{c}	21.4^{b}	19.1^{bc}	2.76	<0.001
Chemical composition						
Moisture (%)	74.8	74.7	74.5	75.1	0.69	0.114
Fat (% DM)	12.3	11.5	11.7	11.0	3.38	0.731
Protein (% DM)	84.8	84.8	83.2	85.3	3.35	0.326

$^{a, b, c}$Means with different letter in the same row are significantly different ($P < 0.05$).

[1] Treatments: CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of TMR, DM basis; GP-5, 5% of grape pomace from red wine, DM basis; GP-10, 10% of grape pomace from red wine, DM basis.

[2] RSD: residual standard deviation.

[3] WHC: water holding capacity determined as cooking losses (Honikel, 1998).

Colour is a highly variable parameter which affects consumer decisions concerning the purchase of red meat (Morrissey, Buckley, Sheehy, & Monahan, 1994). The redness descriptor (a^*) and chroma (C^*) values were higher ($P < 0.05$) in *R. abdominis* muscle from GP-5 lambs compared with the other treatments. In LTL muscle, the yellowness (b^*) and hue angle (H^*) parameters were lower in the supplemented groups ($P < 0.05$) than in CTRL animals. With regard to subcutaneous fat colour, the L^* parameter was lower in GP-10 animals than in the other treatments (Table 3). Several authors have suggested that vitamin E supplementation as well as tannins or other polyphenols can reduce meat colour degradation during storage over time owing to their ability to prevent oxidation of myoglobin (Gallardo et al., 2015; Luciano et al., 2009; Priolo, Lanza, Biondi, Pappalardo, & Young, 1998). However, these changes in meat and fat colour associated with oxidation processes are generally not detected at 24 h after slaughter. They could be attributed to deposition of colourful compounds such as tannins, anthocyanins and even carotenoids in each tissue.

Some authors (Morán et al., 2012) have associated reductions in meat shear force with the protection exerted by vitamin E or polyphenols against the oxidation of endogenous proteases during the ageing process. However, in this study meat shear force measurements in suckling lambs from ewes fed VIT-E or GP were according to the values obtained in suckling-lamb meat covered by the Protected Geographical Indication (Miguélez et al., 2008) and no statistical differences were found between treatments ($P > 0.05$) (Table 3). A significant reduction in shear force has been reported in muscles with high ultimate pH (Devine, Graafhuis, Muir, & Chrystall, 1993) and with high WHC (Morán et al., 2012). In contrast, our results showed a meat pH between 5.6 and 5.8, and no differences between experimental treatments, which may be related to the lack of differences in shear force for muscle. At the same time, these results do not disagree with the differences in meat WHC (Table 3). It has been stated that toughening of meat would be related to the oxidation of myofibrillar proteins (Estévez, 2011).

Meat oxidation reduces WHC between muscle myofibrils, which increases juice loss from the meat and, as a result, meat lightness (Huff-Lonergan & Lonergan, 2005). It has been reported that the use of antioxidants might improve WHC, avoiding the loss of membrane integrity and protein cross-link (Estévez, 2011). In accordance with that statement, WHC measured by cooking losses (Table 3) was higher in lambs fed with VIT-E and GP compared with the Control group ($P < 0.001$). This result is in agreement with Morán et al. (2012), who found higher meat WHC when antioxidants (vitamin E or natural polyphenols) were included in fattening lamb diets.

Chemical composition of the suckling lamb LTL samples and was within the range of reported values (Manso et al., 2011) and, as expected, it was not affected ($P > 0.05$) by ewe dietary treatments (Table 3). These results would be related to the lack of differences in milk yield and composition (Table 2), since lambs were fed exclusively with maternal milk. Morán et al. (2014) also did not report statistical differences ($P > 0.05$) in meat protein, fat and moisture values from suckling lambs fed with milk replacer enriched with vitamin E or carnosic acid. Neither Nieto (2013) found differences in muscle lamb chemical composition when thyme and rosemary were included in ewe diets.

3.2. Fatty acid composition

The influence of different dietary ingredients rich in polyphenols on fattening lambs meat FA profile has been recently evaluated to some extent (Morán et al., 2013; Muíño et al., 2014). However, studies of the effects of antioxidants in ewe rations on the intramuscular FA profile of suckling lambs are very scarce. In principle, the FA composition of intramuscular fat of suckling lambs would be strongly conditioned to the

fat profile of dams milk (Gallardo et al., 2014). Thus, differences observed among treatments in sucking lambs meat tend to reflect those present in their mothers' milk. Table 4 shows the FA composition of LTL muscle for the 4 experimental treatments assayed. As expected, saturated fatty acids (SFA) and *cis* monounsaturated fatty acids (MUFA) constituted the majority of the FA quantified, oleic acid (*cis-9* C18:1) being the most abundant, followed by palmitic acid (C16:0) and stearic acid (C18:0). Values of most FA determined in this trial (Table 4) were within the reference ranges obtained in similar breeding and production systems (Manso et al., 2011).

In general terms, incorporation of vitamin E or GP into the ewes' diet did not cause great changes on intramuscular FA contents. The most remarkable effect between diets was the reduction of oleic acid levels by 10% in rations supplemented with antioxidants and, consequently, total MUFA content (Table 4). This decrease was statistically identical ($P < 0.05$) between VIT-E, GP-5 and GP-10 diets meaning that they exert a similar effect on oleic acid reduction in lamb meat. The origin of oleic acid resides in both, diet and endogenous synthesis from C18:0 via Δ-9 desaturase enzyme. As desaturase indexes were not affected by experimental treatments (Table 4), these changes are related to dams milk composition (Manso et al., 2016). Ewes milk presented lower levels of oleic acid when diet was supplemented with vitamin E, but alterations with dietary GP were less clear (Manso et al., 2016). Oleic acid could be diminished due to an alteration of ruminal BH processes by dietary polyphenol. In fact, a previous study (Correddu et al., 2015) reported significant decreases of oleic acid in ewes rumen liquor when feeding a diet supplemented with linseed oil plus grape seed.

With the exception of *iso* C14:0, values of odd and branched chain fatty acids (OBCFA) were not affected in any treatment. Correddu et al. (2015) and Manso et al. (2016) in ruminal liquid and milk, respectively, from ewes fed diets supplemented with different winery by-products, did not find differences in OBCFA contents that are mostly formed from rumen microorganisms. Neither Gallardo et al. (2015) detected significant variations of most of the individual OBCFA in suckling lamb intramuscular fat when natural or synthetic vitamin E was used to supplement lactating ewe diet.

Regarding *trans* FA, no significant differences were observed between treatments for either total *trans*-18:1 content or any of the individual isomers ($P > 0.05$, Table 4). Vaccenic acid (*trans*-11 C18:1, VA) was the most abundant *trans* FA in all treatments rising the highest value in GP-10 (2.21% of total FA). This abundance should be attributed to the presence of a source of α-linolenic acid (linseed oil) in the dam diet. Supplementation of small ruminants rations with linseed oil multiplies the levels of VA in milk as a consequence of BH processes (Bodas et al., 2010; Gallardo et al., 2015; Martínez Marín et al., 2012). On the other hand, the low levels of *trans*-10 C18:1 found in all experimental treatments diets would suggest the absence of a shift on ruminal BH pathways, promoting the accumulation of VA and RA in the ewes milk and consequently in lambs meat.

Among conjugated linoleic acid isomers, *trans*-7 *cis*-9 C18:2 and mainly *cis*-9 *trans*-11 C18:2 (rumenic acid, RA) were quantitatively the most important. The major source of both FA is the endogenous synthesis from *trans*-7 C18:1 and VA respectively, through Δ-9 desaturation (Bichi et al., 2012). In the current research, a positive correlation between VA and RA was observed in lamb intramuscular fat ($r = 0,99$, $P < 0.001$). The values of RA in treatments GP-5 (1% of total FA) and GP-10 (1.2% of total FA) were the highest detected but they did not significantly differ from CTRL ($P > 0.05$). This pattern was also reported by Gallardo et al. (2015) in intramuscular fat of suckling lamb after natural and synthetic vitamin E incorporation in lactating ewes diets. These results could be explained taking into account the fact that dam milk is the only source of nutrients of suckling lambs and VA and RA in mother milks did not differ among diets (Manso et al., 2016).

Omega-3 comprised about 5% of total FA in LTL muscle. Levels of α-linolenic acid reached more than 1.6% as a consequence of the presence of linseed oil in maternal diet. α-linolenic acid content was not significantly modified with the addition of vitamin E, and remained unaltered in GP treatments (Table 4). As stated above, suckling lamb rumen would not be functional and PUFA BH would not occur. Accordingly, the α-linolenic acid measured in lambs meat would correspond to the fact that part of α-linolenic acid of the ewes diets are transferred to milk. Antioxidants did not affect the contents of α-linolenic acid metabolites (Table 4). Neither *cis*-9 *trans*-11 *trans*-15 18:3, *trans*-11 *cis*-15 C18:2, *trans*-10 *cis*-15 C18:2, *trans*-11 *cis*-13 C18:2, *trans*-11 C18:1 nor *cis*-15 C18:1 were altered by dietary antioxidants, whereas *cis*-9 *trans*-11 *cis*-15 C18:3 showed changes but without a clear trend. Concerning long chain n-3 PUFA, C22:5 was the most abundant followed by C20:5 and C22:6 in all experimental diets. No statistical differences ($P > 0.05$) were found in GP treatments when compared with CTRL and VIT-E, following the same pattern as previous researchs (Gallardo et al., 2015; Manso et al., 2016).

4. Conclusions

In summary whole dried GP at inclusion levels of 5% and 10% of TMR can be included in lactating ewe diets without adverse effects on animal performance, carcass characteristics and meat quality of their suckling lambs when compared with control diet or vitamin E supplementation. GP and VIT-E improved the WHC of the meat. In addition, the presence of GP in the diet at the doses assayed did not negatively affect the nutritional quality of lambs meat fat profile. Therefore, GP constitutes an inexpensive source of polyphenols that could be used in early lactating ewe diets for reducing the environmental impact and waste disposal of the wine industry.

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Table 4
Effect of experimental ewe diets on intramuscular fatty acid composition of suckling lamb (% of identified fatty acids).

	Treatments[1]				RSD[2]	P. value
	CTRL	VIT-E	GP-5	GP-10		
Saturated (SFA)						
C10:0	0.24	0.30	0.29	0.29	0.081	0.284
C11:0	0.02	0.02	0.03	0.02	0.009	0.087
C12:0	0.38	0.43	0.41	0.42	0.120	0.850
C13:0	0.03	0.03	0.03	0.02	0.008	0.954
C14:0 *iso*	0.19^{a}	0.16^{ab}	0.14^{b}	0.08^{c}	0.052	<0.001
C14:0	4.03	4.36	4.08	4.36	0.807	0.709
C15:0 *iso*	0.08	0.08	0.08	0.07	0.014	0.453
C15:0 *anteiso*	0.12	0.13	0.12	0.11	0.017	0.330
C15:0	0.25	0.29	0.29	0.29	0.045	0.241
C16:0	17.3	17.9	17.7	18.1	1.40	0.632
C16:0 *iso*	0.11	0.12	0.13	0.11	0.024	0.188
C17:0 *iso*	0.24	0.23	0.23	0.221	0.031	0.413
C17:0 *anteiso*	0.30	0.27	0.27	0.30	0.066	0.603
C17:0	0.61	0.59	0.61	0.58	0.050	0.664
C18:0 *iso*	0.09	0.09	0.09	0.08	0.028	0.803
C18:0	11.5	11.4	11.5	11.0	0.686	0.248
C19:0	0.03	0.04	0.04	0.03	0.014	0.499
C20:0	0.13	0.13	0.13	0.13	0.015	0.938
C21:0	0.07	0.08	0.09	0.08	0.020	0.149
C22:0	0.10	0.11	0.11	0.10	0.026	0.461
C23:0	0.29	0.29	0.31	0.25	0.067	0.328
Monounsaturated (MUFA)						
C10:1	0.02	0.02	0.02	0.02	0.010	0.528
cis-11 C12:1	0.02	0.02	0.02	0.01	0.011	0.250
cis-9 C14:1	0.15	0.16	0.16	0.18	0.036	0.488
C15:1	0.09	0.11	0.12	0.10	0.035	0.328
cis-7 C16:1	0.18	0.17	0.19	0.20	0.023	0.066
trans-3 C16:1	0.04^{b}	0.03^{b}	0.06^{a}	0.04^{b}	0.015	0.011
trans-4 C16:1	0.02	0.03	0.04	0.03	0.014	0.113
trans-5 C16:1	0.04	0.04	0.05	0.03	0.012	0.189
trans (6 + 7) C16:1	0.04	0.05	0.05	0.04	0.011	0.371
trans-8 C16:1	0.04	0.04	0.05	0.04	0.011	0.253
trans-9 C16:1	0.13	0.16	0.19	0.18	0.057	0.077
trans-13 C16:1	0.12	0.14	0.11	0.12	0.028	0.133
trans-14 C16:1	0.04	0.03	0.04	0.04	0.016	0.392
cis-8 C16:1	0.02	0.02	0.02	0.02	0.007	0.069
cis-9 C16:1	1.21	1.18	1.21	1.26	0.187	0.802
cis-12 C16:1	0.03	0.03	0.04	0.04	0.011	0.366
cis-9 C17:1	0.31^{a}	0.27^{b}	0.31^{a}	0.29^{ab}	0.032	0.008
trans (6 + 7) C18:1	0.09	0.10	0.11	0.10	0.032	0.462
trans-8 C18:1	0.06	0.07	0.07	0.08	0.032	0.750
trans-9 C18:1	0.21	0.23	0.22	0.24	0.037	0.471
trans-10 C18:1	0.23	0.24	0.25	0.25	0.058	0.740
trans-11 C18:1	1.75	2.11	2.10	2.21	0.611	0.366
trans-12 C18:1	0.45	0.51	0.50	0.51	0.081	0.351
trans (13 + 14) C18:1	0.32	0.36	0.33	0.34	0.065	0.605
trans-16 C18:1	0.16	0.16	0.15	0.17	0.020	0.189
cis (9 + 10) + *trans*-15 C18:1	27.7^{a}	24.9^{b}	24.7^{b}	25.0^{b}	2.575	0.040
cis-11 C18:1	2.05^{a}	1.94^{ab}	1.82^{b}	1.76^{b}	0.219	0.031
cis-12 C18:1	1.11	1.23	1.23	1.34	0.233	0.194
cis-13 C18:1	0.02	0.02	0.02	0.02	0.006	0.598
cis-14 C18:1	0.03^{b}	0.04^{ab}	0.05^{a}	0.04^{ab}	0.013	0.026
cis-15 C18:1	0.08	0.09	0.09	0.10	0.025	0.216
cis-16 C18:1	0.04	0.05	0.05	0.05	0.011	0.211
C19:1	0.01	0.01	0.01	0.01	0.005	0.708
cis-11 C20:1	0.17	0.21	0.19	0.19	0.074	0.629
C22:1	0.03	0.04	0.04	0.03	0.011	0.104
cis-15 C24:1	0.49^{a}	0.39^{ab}	0.29^{bc}	0.23^{c}	0.144	0.001

	Treatments[1]				RSD[2]	P. value
	CTRL	VIT-E	GP-5	GP-10		
Non-conjugated C18:2						
trans-11 *trans*-15 C18:2	0.04	0.05	0.05	0.05	0.016	0.887
Other *trans trans* C18:2	0.10	0.11	0.11	0.10	0.021	0.686
cis-9 *trans*-13 + *trans*-8 *cis*-12 C18:2	0.49	0.49	0.47	0.52	0.078	0.544
trans-8 *cis*-13 C18:2	0.14	0.14	0.14	0.16	0.024	0.304
cis-9 *trans*-12 C18:2	0.06	0.06	0.06	0.06	0.009	0.871

Table 4 (*Continued*)

	Treatments[1]				RSD[2]	P. value
	CTRL	VIT-E	GP-5	GP-10		
trans-9 *cis*-12 C18:2	0.04	0.05	0.05	0.05	0.008	0.131
trans-10 *cis*-15 C18:2	0.05	0.07	0.07	0.05	0.031	0.152
trans-11 *cis*-15 C18:2	0.49	0.66	0.59	0.61	0.195	0.281
cis-9 *cis*-12 C18:2	9.25	9.56	9.85	9.71	1.459	0.820
cis-9 *cis*-15 C18:2	0.05	0.05	0.06	0.06	0.014	0.465
cis-12 *cis*-15 C18:2	0.17	0.20	0.17	0.21	0.071	0.504
Conjugated C18:2						
cis-9 *trans*-11 C18:2	0.87	1.00	1.00	1.20	0.332	0.183
trans-7 *cis*-9 C18:2	0.07	0.09	0.09	0.05	0.053	0.304
trans-9 *cis*-11 C18:2	0.01[b]	0.01[b]	0.02[a]	0.01[b]	0.008	0.015
trans-10 *cis*-12 C18:2	0.01	0.01	0.01	0.01	0.006	0.241
trans-11 *cis*-13 C18:2	0.01	0.01	0.01	0.01	0.004	0.368
trans-13 *trans*-15 + *trans*-12 *trans*-14 C18:2	0.04	0.04	0.04	0.04	0.011	0.550
trans-11 *trans*-13 C18:2	0.03	0.03	0.03	0.03	0.014	0.497
Other polyunsaturated (PUFA)						
C18:3 n-6 (γ-linolenic acid)	0.09	0.10	0.11	0.09	0.026	0.396
C18:3 n-3 (α-linolenic acid)	1.62	2.11	1.88	1.66	0.436	0.059
cis-9 *trans*-11 *trans*-15 C18:3	0.07	0.09	0.08	0.09	0.025	0.451
cis-9 *trans*-11 *cis*-15 C18:3	0.97[b]	1.05[ab]	1.17[a]	0.96[b]	0.168	0.032
Other C18:3	0.07	0.08	0.08	0.06	0.022	0.064
cis-9 *trans*-12 *cis*-15 C18:3 n-3 + *cis*-8 C20:1	0.07	0.08	0.08	0.07	0.015	0.104
C20:2 n-6	0.07[b]	0.08[ab]	0.09[a]	0.07[b]	0.017	0.029
C20:3 n-6	0.37	0.35	0.39	0.37	0.077	0.816
C20:3 n-3	0.05	0.06	0.07	0.06	0.015	0.184
C20:4 n-6 AA[3]	5.59	5.27	5.79	5.11	0.922	0.368
C20:5 n-3 EPA[4]	1.51	1.66	1.65	1.40	0.327	0.266
C22:2 n-6	0.06	0.07	0.06	0.04	0.026	0.130
C22:4 n-6	0.40	0.36	0.41	0.38	0.075	0.492
C22:5 n-6	0.15	0.16	0.18	0.15	0.044	0.281
C22:5 n-3 DPA[5]	1.96	2.02	2.07	1.87	0.336	0.599
C22:6 n-3 DHA[6]	1.35	1.45	1.51	1.31	0.345	0.568
Total SFA	36.1	37.1	36.6	38.0	2.78	0.496
Total MUFA	37.6[a]	35.3[b]	34.9[b]	35.4[b]	2.14	0.038
Total PUFA	26.3	27.6	28.4	26.6	3.41	0.491
Trans C18:1	0.23	0.27	0.30	0.19	0.793	0.336
Total CLA	1.03	1.19	1.21	1.34	0.332	0.235
C14:1 desaturase index[7]	0.03	0.03	0.04	0.04	0.006	0.177
C16:1 desaturase index[8]	0.07	0.06	0.06	0.07	0.007	0.687
C18:1 desaturase index[9]	0.90	0.88	0.89	0.88	0.021	0.390
CLA desaturase index[10]	0.78	0.80	0.79	0.82	0.037	0.167
Total n-3	4.92	5.22	5.34	4.68	0.919	0.377
Total n-6	6.33	6.05	6.61	5.83	1.03	0.374
n-6/n-3	1.29	1.18	1.25	1.26	0.168	0.499

[a,b,c]Means with different letter in the same row are significantly different ($P < 0.05$).

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

[2] RSD: residual standard deviation;

[3] AA: arachidonic acid;

[4] EPA: eicosapentaenoic acid;

[5] DPA: docosapentaenoic acid;

[6] DHA: docosahexaenoic acid.

[7] C14:1 desaturase index = *cis*-9 C14:1/(C14:0 + *cis*-9 C14:1);

[8] C16:1 desaturase index = C16:1/(C16:0 + C16:1);

[9] C18:2 desaturase index = *cis*-9 C18:1/(C18:0 + *cis*-9 C18:1);

[10] CLA desaturase index = *cis*-9 *trans*-11 C18:2/(*trans*-11 C18:1 + *cis*-9 *trans*-11 C18:2).

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