Effects of grape pomace supplementation on the diet of lactating ewes as compared to vitamin E on the meat shelf life of suckling lambs

C. Vieira[a,*] vieallce@itacyl.es, C. Guerra-Rivas[b], B. Martínez[a], B. Rubio[a], T. Manso[b]

[a]Estación Tecnológica de la Carne, Instituto Tecnológico Agrario de Castilla y León (ITACyL), Guijuelo, Salamanca, Spain

[b]Escuela Universitaria Superior de Ingeniería Agraria, Universidad de Valladolid, Spain

*Corresponding author.

Abstract

Forty-eight Churra ewes and their suckling lambs were assigned to four dietary treatments: control (CTRL), VIT-E (500 mg kg-1 TMR vitamin E), GP-5 (5% grape pomace) and GP-10 (10% grape pomace). After slaughter (11.5 kg live weight), longissimus muscle of lambs was sliced, packaged under modified atmosphere (80,20%/O 2–:CO 2–) and stored in retail conditions. At each sampling point (0, 3, 7, 10, 14 days), microbiological, physicochemical and sensory characteristics were analysed. Vitamin E and GP-5 were found to be effective (–$p < 0.05$) at preventing enterobacteria growth as of day 10. After day 10, vitamin E and grape pomace in the ewe-'s supplementation reduced metmyoglobin ($p < 0.05$) lipid oxidation ($p < 0.05$) and sensory spoilage throughout the storage period. An effect of the grape pomace dosage was observed, with the supplementation at 5% being more effective. Therefore, we can conclude that grape pomace was just as effective as vitamin E in preventing spoilage during retail storage.

Keywords:

Grape pomace, Antioxidant, Meat shelf life, Modified atmosphere, Microbial quality

~~1.~~1 Introduction

Changes in shopping and consumer habits have made shelf life a central issue in the sale of meat. Shelf life is influenced by oxidative processes, which are affected by temperature, oxygen exposure, light and microbial growth. Muscle pigment, protein and lipid oxidation affect essential sensory qualities, producing undesirable flavours and colours. For the meat industry, retailers, and consumers, extending fresh meat shelf life is essential for increasing sales (Luciano et al., 2009; Possamai et al., 2018). Retailers attempt to extend the shelf life of meat through storage practices such as modified atmosphere packaging (MAP). It has been reported that optimum colour stability in red meat may be obtained through the use of gas mixtures with high oxygen concentrations together with low carbon dioxide levels, which exhibit antimicrobial activity and thereby restrict the growth of aerobic spoilage bacteria (Jeremiah, 2001; Karabagias, Badeka, & Kontominas, 2011; Vieira, Rubio, Martínez, Mantecón, & Manso, 2019).

Changes in meat during storage are also influenced by chemical composition, with higher intramuscular fat concentration having a detrimental impact on lipid oxidation and colour stability of lamb meat (Calnan, Jacob, Pethick, & Gardner, 2019). In addition to a long expiration date, consumers consider nutritional characteristics of meat to be important. Therefore, an improved nutritional value of lamb represents a market opportunity for the lamb industry. Over recent decades, the scientific community has focused on obtaining increased levels of polyunsaturated fatty acids (PUFA) and other functional fatty acids, such as conjugated linoleic acid (CLA) or n-3 PUFA, in ruminant products. However, the increase in unsaturated fatty acids (UFA) in meat and milk makes them more susceptible to lipid oxidation and haeminic pigment oxidation, since both reactions are closely related. Therefore, to extend the shelf life of suckling lamb meat, it should be enriched with components having antioxidant properties, to counteract the effect of oxidation and microbial spoilage (Calnan et al., 2019; Chikwanha et al., 2019).

One of the most common ways for reducing oxidation in animal products is to feed synthetic antioxidants. The positive effect of vitamin E on shelf life has been widely reported. It is the most frequently used antioxidant. Recently, Calnan et al. (2019) reported that dietary vitamin E supplementation improved the display colour of highly marbled lamb meat. However, the effect of synthetic compounds on consumer health remains controversial. The use of natural substances to improve the oxidative stability of animal products is of growing interest, given consumer demand for natural products and their willingness to pay for them. The re-use of agro-industrial by-products is of growing importance as it mitigates the impact of their waste on the environment. Furthermore, the use of these by-products in ruminant feeding has been shown to be effective in reducing feeding costs (Cunha et al., 2018; Guerra-Rivas et al., 2016; Natalello et al., 2020). Numerous plant secondary compounds, such as essential oils and other substances rich in phenolic compounds, have antioxidant and antimicrobial properties. Therefore, their use in animal feeding has been encouraged (Embuscado, 2015 ; Guerrini et al., 2020; Muela, Alonso, Campo, Sañudo, & Beltrán, 2014; Ranucci et al., 2019). Polyphenols have demonstrated ability to prevent meat lipid oxidation when added to the diets of fattening lambs (Chikwanha et al., 2019 ; Flores et al., 2021; Karami, Alimon, & Goh, 2011; Serrano, Jordán, & Bañón, 2014) or lactating ewes (Argov-Argaman et al., 2020; Nieto, Díaz, Bañón, & Garrido, 2010a; Santos et al., 2014) or when used in milk replacers for suckling lambs (Morán et al., 2014). Grape pomace is the main residue generated by the wine industry, consisting of grape seeds, skin and pulp. In areas of wine production great quantities of residues are generated, causing problems from an economic and environmental point of view. Due to incomplete extraction during the winemaking process, seeds and skins of crushed grapes are very rich in phenolic compounds (Chikwanha et al., 2019; Yi et al., 2009) and may play a significant role as antioxidants. Some studies have examined the effects of including wine extracts in growing lamb diets (Guerra-Rivas et al., 2016; Jerónimo et al., 2010; Mu et al., 2020; Muíño et al., 2014). A transfer of phenolic compounds from diets to milk has been reported in goats (Jordán, Moñino, Martínez, Lafuente, & Sotomayor, 2010). Therefore, the phenolic compounds and antioxidant activity of milk could be transferred to suckling lamb muscle, potentially exerting beneficial effects on their meat quality. Few studies, however, are available on the effects of the inclusion of whole grape pomace in lactating ewe diets on suckling lamb meat quality and meat shelf life stored under retail conditions.

The aim of this study was to examine the effects of adding grape pomace from red wine to the diet of ewes on the shelf life of suckling lamb meat during storage in retail sale conditions. In order to do so, lamb was packaged in a modified atmosphere gas mixture of 80:20% / O_2:CO_2, and grape pomace was compared to a control group and a vitamin E group (positive control).

~~2.~~2 Material and methods

~~2.1.~~2.1 Experimental design, animal management and sample collection

The experiment was conducted on a farm in Palencia, located in north-central Spain. The study was carried out according to commercial farm conditions, with no additional handling. Notwithstanding, all the conditions and handling practices followed the recommendations of the Directive 2010/63/EU of the European Parliament. The experiment was also subjected to evaluation and was approved by the ITACYL Animal Ethics Committee (Protocol 77 number 2021/01/CEEA). The procedures were also approved by the Institutional Animal Care and Use Committee (AEC) of the University of Valladolid (Spain).

Forty-eight pregnant Churra ewes (mean live body weight, LBW, 59.2 ± 4.91 kg) were selected before lambing and were fed the same diet. The ewes were aged between 3 and 5 years, and parity ranged from 4 to 6, with all of them

giving birth 3–4 days before the experiment began. After lambing, each ewe was randomly 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, 6 males and 6 females), in accordance with the Protected Geographical Indication "Lechazo de Castilla y León", were housed with their respective mothers all day long and were nourished exclusively by suckling for the entire experimental period (from birth until reaching approximately 11.5 kg LBW).

Experimental diets consisted of a total mixed ration (TMR) containing 2.7% (on a DM basis) of linseed oil, forage and concentrate, at a 40:60 ratio. The four dietary treatments were: CTRL (without grape pomace), VIT-E (500 mg of vitamin E per kg TMR, DM basis), GP-5 (5% grape pomace from red wine production, DM basis), and GP-10 (10% grape pomace from red wine production, DM basis). The vitamin E and polyphenols from grape pomace levels which had been shown to exert positive antioxidant effects on meat from different species without affecting the animal performance were selected (Guerra-Rivas et al., 2016; Karami et al., 2011; Lauzurica et al., 2005). In this experiment, isoenergetic and isoproteic diets were formulated to meet the energy and protein requirements of the dairy ewes. Fresh grape pomace was supplied and mixed daily with the TMR. The ingredients and chemical composition of the experimental diets are shown in Table 1. The chemical composition of the TMR was determined using the procedures described by the AOAC (2012). The sequential analysis of NDF and ADF was performed as described by Van Soest, Robertson, and Lewis (1991) using an ANKOM 220 Fibre Analyzer unit (Ankom Technology Corp., Fairport, New York, United States of America). Two weeks before lambing ewes were fed the same control diet they would receive during the experimental period without oil added. During the first week after lambing, ewes were adapted to the dietary treatments mixing the control diet and the corresponding dietary treatment. TMR was supplied twice a day and fresh water was always available. The ewes were individually fed during the entire experimental period and each intake was recorded. The amounts of diet offered (and refused) were weighed daily for each ewe, and samples were collected for subsequent analyses (data shown in a previously published work (Gómez-Cortés et al., 2018).

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~~Table 1.~~Table 1

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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

Table Footnotes

[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 identified fatty acids): 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.

[3] Grape pomace composition (g kg^{-1} DM): DM, 955 g kg^{-1}; OM (organic matter), 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 identified fatty acids): C16:0, 11.1; C18:0, 4.41; C18:1, 16.0; C18:2, 61.3; C18:3, 3.69.

At the end of the trial, when suckling lambs reached approximately 11.5 kg LBW, they were transported (2 km) to a commercial EU-licensed abattoir and were slaughtered at 27.1 ± 4.8 days of age. The lambs were stunned and then severing the jugular. After slaughter, skin and all internal organs were removed and carcasses were immediately weighed (6.29 kg on average) and transferred to a cooler and stored at 4 °C for 24 h. Carcass performance, proximate meat composition and fatty acid profile of *longissimus thoracis et lumborum* (LTL) muscle have been thoroughly detailed in Gómez-Cortés et al. (2018). The authors reported that diet did not have a major effect on chemical composition or fatty acid profile.

~~2.2.~~2.2 Meat shelf-life analysis

~~2.2.1.~~2.2.1 *Sample preparation*

After cooling the carcasses, the *longissimus thoracis et lumborum* (LTL) muscles from each carcass were removed and sliced (about 3 cm thick). Slices were placed in trays and randomly assigned to different storage periods (0, 3, 7, 10 and 14 days). Since microbial analysis is destructive, the trays used for shelf life study were prepared in duplicate, one set for microbial analyses and the other one for sensory and physicochemical analysis. The trays were then flushed with the selected gas mixture (80,20% / O_2:CO_2), closed by heat-sealing with a packer (TECNOTRIP mod. TSB-100) with high barrier film (55 µm thick, O_2 permeability 5 cc/m^2/24h/bar at 23 °C/50% RH and steam permeability 19 g/m^2/24 h at 23 °C/90%RH). Finally, the trays were randomly placed in a refrigerated open front display cabinet illuminated with white fluorescent light (620~~-~~ luxlx) at 4 ± 1 °C. The cabinet was set to a 12-h light-dark cycle to simulate retail display conditions. Trays were rotated daily to minimise light intensity differences. On each sampling day, the corresponding trays were removed for subsequent analysis.

~~2.2.2.~~2.2.2 *Microbiological analysis*

On each sampling day, microbiological testing was carried out. After pack opening, 10 g of meat were aseptically Q5 removed and homogenised with 90 ml of peptone water for 2 min in a sterile plastic bag in a PK 400 Masticator (IUL, S.A., Barcelona, Spain). Serial decimal dilutions were made in sterile peptone water and, in duplicate, 1 ml or 0.1 ml samples of appropriate dilutions were poured or spread onto total count and selective agar plates. The microbiological analyses performed on the samples were: total viable counts (TVC) determined on 3 M Petrifilm Aerobic Count Plate (Bioser, Barcelona, Spain) incubated at 30 °C for 72 h; enterobacteria on 3 M Petrifilm *Enterobacteriaceae* Count Plate (Bioser, Barcelona, Spain) incubated at 42 °C for 24 h; lactic acid bacteria (LAB) on MRS Agar (Scharlau, Spain) incubated at 30 °C for 72 h; *Pseudomonas* spp. on Pseudomonads Agar (Oxoid, Spain) supplemented with Cetrimide Fucidine and Cephaloridine (CFC, Oxoid, Spain) incubated at 30 °C for 48 h; and *Brochothrix thermosphacta* on STAA Agar (Oxoid, Spain) supplemented with STAA selective supplement (Oxoid, Spain) incubated at 25 °C for 48 h. Presumptive colonies were differentiated from pseudomonads by performing an oxidase test using Oxidase Test

Sterile Swabs (Scharlau, Spain). When counts were above 7 log cfu g^{-1} the product was considered unfit for consumption (ICMSF, 1986).

~~2.2.3.~~2.2.3 *Instrumental colour measurement*

When the modified atmosphere packages were opened, a reflectance spectrophotometer (Konica Minolta CM-2600d; Osaka, Japan) was used to measure the surface colour of LTL muscle slices after retail display, after 30 ~~minutes~~min of blooming at room temperature (20 °C ± 2 °C). Measures were taken in the CIE 1976 L*a*b* space under D65 illumination with a 10-degree observer visual angle, 11 mm aperture for illumination and 8 mm for measurement, and SCI mode conditions, previously calibrated against a white plate supplied by the manufacturer. For colour determination, the spectrophotometer directly touched the muscle cross-section surfaces. Three different areas were measured on each tray and the results were expressed as an average of the three measurements. Colour results were expressed as L^* (lightness), a^* (redness), b^* (yellowness), h^* (hue angle) and C^* (chroma) values.

~~2.2.4.~~2.2.4 *Myoglobin and lipid oxidation*

To determine myoglobin oxidation, wavelength range of 400–760 nm data provided by Minolta CM-2600d (Minolta Co., Osaka, Japan) were used. Metmyoglobin percentage was estimated spectrophotometrically by measuring the reflectance (A) at 525, 572 and 730 nm, in accordance with Krzywicki (1979). The metmyoglobin relative percentage was calculated as follows: % MMb = [1.395 – (A 572 – A 730) / (A 525 – A 730) x 100].

Each sample was minced and the extent of lipid oxidation in LTL muscle during storage was evaluated by thiobarbituric acid reactive substance (TBARS) assay using a UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). This method is based on the reaction between malondialdehyde and thiobarbituric acid and the production of a coloured pigment, as determined by Maraschiello, Sarraga, and García-Regueiro (1999), TBARS values were expressed as micrograms of malondialdehyde per gram of meat.

~~2.2.5.~~2.2.5 *Shelf-life analysis*

Shelf-life studies were carried out on raw meat. On each sampling day, samples were assessed, based on appearance, display of muscle oxidation, presence of off-odours and overall sample rating by a panel of six individuals with long experience in shelf-life studies of meat and meat products. Panellist who were selected and trained in accordance with the International Standard method for selection, training and monitoring of assessors (UNE-EN ISO 8586:2014, 2014). The panel performed the trial under controlled conditions, in booths at 22 °C. Measurements to evaluate the shelf life were performed in one session in each corresponding sampling day (0, 3, 7, 10 and 14 days). The reliability of the scores was assessed with coefficient of variation (CV%) considering acceptable those below 20% of CV. Also, the scores given by panellists were validated according to deviation index within session. In all cases, scores and panellist were valid. The appearance of the meat was assessed in unopened trays, using a structured scale with numerical scores ranging from 1 (excellent, fresh meat) to 5 (extremely undesirable). Likewise, in intact trays, display of muscle oxidation, measured as the percentage of discoloured or brownish meat was scored using a 5-point scale (1, none; 2, 1–10%; 3, 11–20%; 4, 21–60% and 5, 61–100%). Once the film was removed, the panellists were asked to score odour, using a 5-point scale (1, no off-odours; 2, slight off-odours; 3, minor off-odours but not spoiled; 4, clearly recognizable off-odours and 5, extremely strong off-odours). Overall rating was also evaluated using a 5-point scale (1, excellent; 2, good; 3, acceptable; 4, fair; 5, unacceptable). A score of 3, were used as threshold to reject a sample for each parameter, since this point is situated in the middle of the structured 5-point scale.

~~2.3.~~2.3 Statistical analysis

Data were analysed as repeated measures by the MIXED procedure of the SAS 9.2. package, according to the following model: $Y_{ijk} = \mu + T_i + D_k + T_i D_k + B_j + \varepsilon_{ijk}$; where Y_{ijk} is the response variable, μ the overall mean, T_i the dietary treatment (T) effect (CTRL, VIT-E, GP-5 and GP-10), D_k the sampling day (D), $T_i D_k$ the interaction (T × D), B_j the block effect and ε_{ijk} the residual error. When the interaction between diet and storage period was significant, planned comparisons between means were performed using Duncan's method (comparing diets for each storage period and storage period for each diet, $P < 0.05$). For shelf-life studies data, the model included treatment (T) and sampling day (D) as fixed effects and the interaction (T × D). Tasting order, panellist number and replication were

considered as random effects. Data were presented as least square means and Duncan's method were used to identify significant differences between means ($P < 0.05$).

3.3 Results

3.1.3.1 Microbial counts

Table 2 summarizes the results of the microbial analysis of LTL muscle slices packaged under a gas mixture of 80:20% / O_2:CO_2 during refrigerated storage, from suckling lambs assigned to different experimental ewe dietary treatments. Initially, no differences were observed between treatments. In general, the microbial populations increased with refrigerated storage time. Total viable counts (TVC), increase significantly ($P < 0.05$) at 14 days of storage, except in VIT-E, which remained stable during storage. Total viable counts were not different ($P > 0.05$) between experimental treatments for any time.

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Table 2. Table 2

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Effect of experimental ewe diets and storage time on microbial counts (log cfu g^{-1}) on m. *longissimus thoracis et lumborum* from suckling lambs during refrigerated storage at 2 °C.

Microorganisms	Days	Treatments[1]				SED[2]	*P. value*[3]		
		CTRL	VIT-E	GP-5	GP-10		T	D	T × D
Total viable counts	0	$^{AB}3.17$	2.60	$^{A}3.10$	$^{A}2.70$	0.457	0.799	<0.001	0.986
	3	$^{A}2.69$	2.85	$^{A}2.74$	$^{A}3.18$				
	7	$^{AB}3.14$	3.05	$^{AB}3.76$	$^{A}3.23$				
	10	$^{AB}3.87$	3.92	$^{AB}4.08$	$^{A}3.68$				
	14	$^{B}4.92$	4.67	$^{B}5.21$	$^{B}6.28$				
Enterobacteria	0	2.39^{a}	1.65^{ab}	1.00^{b}	1.60^{ab}	0.267	0.039	0.230	0.199
	3	1.78	1.60	1.96	1.60				
	7	2.00^{ab}	1.60^{a}	2.57^{b}	1.60^{a}				
	10	3.01^{a}	1.69^{b}	1.60^{b}	1.92^{ab}				
	14	2.97^{a}	1.97^{b}	1.30^{b}	3.02^{a}				
Lactic acid bacteria	0	$^{A}2.74$	$^{A}2.04$	$^{A}1.96$	$^{A}1.98$	0.424	0.496	<0.001	0.837
	3	$^{A}2.43$	$^{A}2.30$	$^{A}2.12$	$^{AB}2.62$				
	7	$^{A}2.93$	$^{A}2.57$	$^{AB}3.40$	$^{AB}2.79$				
	10	$^{A}2.77$	$^{AB}3.08$	$^{AB}3.86$	$^{B}4.16$				
	14	$^{B}4.18$	$^{B}4.56$	$^{B}4.80$	$^{C}6.07$				
Pseudomonads	0	2.00	2.00	2.00	2.00	0.245	0.435	0.641	0.463
	3	2.30	2.00	2.00	2.00				
	7	2.00	2.30	2.30	2.30				
	10	2.00	2.00	2.00	2.00				
	14	2.00	2.62	2.00	1.8				
Brochothrix thermosphacta	0	2.52	2.00	2.00	2.00	0.247	0.180	0.418	0.882

	3	2.30	2.00	2.00	2.00			
	7	2.66	2.30	3.24	1.80			
	10	2.30	2.00	2.00	2.00			
	14	2.48	2.30	2.78	2.00			

[a, b] Means with different letter in the same row are significantly different ($P < 0.05$).
[A, B, C] Means for each parameter with different letter in the same column are significantly different ($P < 0.05$).

Table Footnotes

[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] SED: standard error of the difference.

[3] Probability of significant effects due to the dietary treatment (T), sampling day (D) and their interaction (T × D).

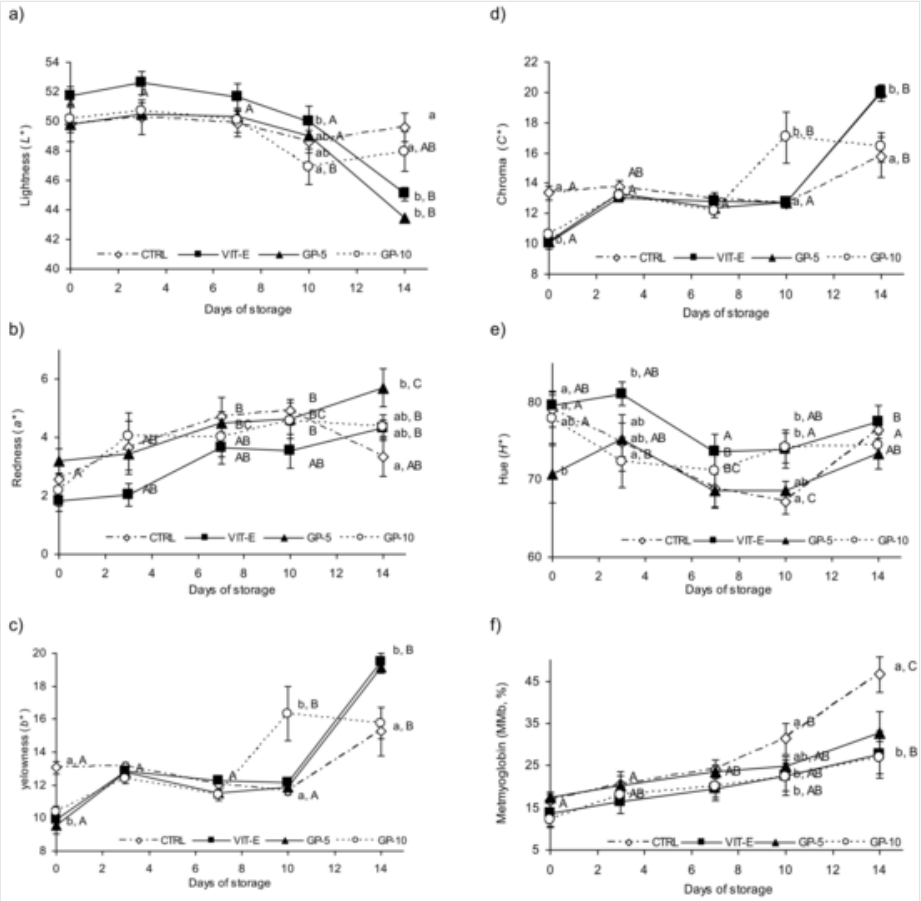
Enterobacteria counts, considered a hygiene indicator, revealed lower values in GP-5 than CTRL and lower than VE and GP-10 at 7 days of storage ($P < 0.05$), but at the last two sampling points (days 10 and 14) meat from GP-5 and from VIT-E revealed significantly lower values than meat from CTRL and GP-10 ($P < 0.05$). As for lactic acid bacteria, which behave as facultative anaerobes and can grow under relatively high concentrations of CO_2, they began to increase significantly ($P < 0.05$) as of 10 days of storage in GP-10 and as of 14 days of storage in the other experimental treatments. With respect to the effect of the ewe's diet on lactic acid bacteria counts, no statistical differences were found at any sampling time. *Pseudomonas* spp. counts remained stable during storage ($P > 0.05$) in all groups, with counts ranging from 2.00 to 2.62 log cfu g^{-1}. *Brochothrix thermosphacta* remained nearly stable during storage in all treatments, with counts ranging from 1.80 to 3.24 log cfu g^{-1}. In short, microbial counts showed lower values of Enterobacteria of VE and G-P at 10 and 14 days. However, grape pomace was not found to be effective in preventing any microbial development at the 10% incorporation level. And considering that shelf life is defined as the time (in days) needed to reach mean values at the limit of 7 log cfu g^{-1} of TVC (ICMSF, 1986), the microbiological shelf life of the meat in all of the experimental treatments did not reach that level. Therefore, the shelf life of lamb meat under high O_2:CO_2 modified atmosphere packaging was not limited by microbial spoilage.

~~3.2.~~3.2 Colour coordinates, myoglobin and lipid oxidation

Results of colour measurements and metmyoglobin percentage are presented in Fig. 1. Lightness (L*) remained stable during the first 10 days of storage for all treatments. However, VIT-E and GP-5 began to decrease from of day 10 until the end of the trial, presenting lower L* values at 14 days as compared to the CTRL and GP-10 groups ($P < 0.05$). However, grape pomace inclusion at the 10% level did not affect the L* values ($P > 0.05$), as compared to the CTRL group. Redness (a*) is one of the most important colour parameters for evaluating meat oxidation, and in our study, it began to increase as of 10 days, except in VIT-E, which showed a significant increase at 14 days of storage ($P < 0.05$). At this sampling point, GP-5 showed higher values than CTRL, with the VIT-E and GP-10 values being intermediate. Yellowness (b*) remained stable in all treatments except GP-10 until day 10 while in GP-10, b* values increased from day 7. In addition, higher values were observed in the VIT-E and GP-5 treatments, as compared to the CTRL and GP-10 groups, at the end of storage period. The saturation of red colour, chroma, follows the same behaviour as yellowness and significant differences were found between treatments at the end of the trial, with VIT-E and GP-5 treatments having higher values, indicating a very vivid colour, but with no differences found between GP-10 and CTRL at this time ($P < 0.05$). With regard to hue angle, which has been related to the visual appraisal of meat discolouration, it exhibited a slight decrease during storage; however, no differences were observed between treatments at the end of the storage ($P > 0.05$). Despite the lack of a clear evolution of colorimetric coordinates, the metmyoglobin (MMb) percentage was clearly influenced by the presence of vitamin E and grape pomace (Fig. 1). The accumulation of MMb during storage is responsible for meat browning, and the inclusion of vitamin E and grape pomace in the ewes' diets inhibited MMb formation as of day 10. It is interesting to note that the effect of grape pomace supplementation (GP-5 and GP-10) was similar to that of vitamin E.

alt-text: Fig. 1

~~Figure 1.~~Fig. 1

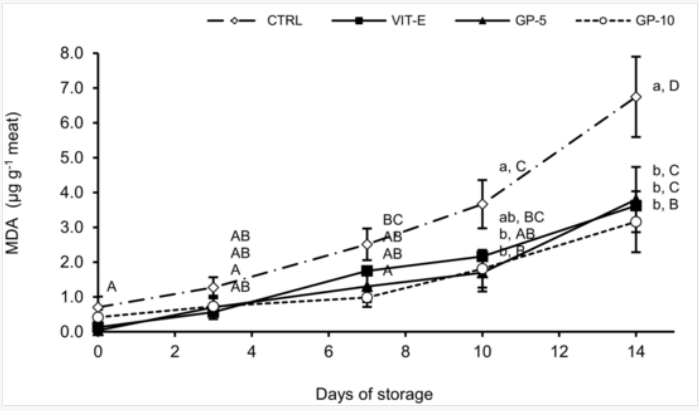


Effect of experimental ewe diets (CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of TMR, DM basis; GP-5, 5% of grape pomace from red wine, DM basis; GP-10, 10% of grape pomace from red wine, DM basis) and storage time on evolution of colour parameters: lightness (a), redness (b), yellowness (c), chroma (d), hue (e) and metmyoglobin haem pigment (f) in m. *longissimus thoracis et lumborum* from suckling lambs stored at 4 °C. Different small letters mean significant differences ($P < 0.05$) between treatments within time and capital letters mean significant differences ($P < 0.05$) between times within treatment. The error bars represent standard error. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

As expected, meat lipid oxidation increased considerably with storage time (Fig. 2). Initially, TBARS concentrations were not significantly different in the four experimental treatments, ranging from 0.40 to 0.70 μg MDA g^{-1} muscle. However, throughout the storage period, the muscle oxidative processes, reflected in TBARS values, were significantly affected by dietary treatment ($P < 0.05$). In this study, the inclusion of both grape pomace (GP-5 and GP-10) and vitamin E supplementation were effective in preventing muscle MDA formation during storage under modified atmosphere packaging, since significantly lower ($P < 0.05$) TBARS values were found as of day 10 with respect to CTRL treatment. According to our results, inclusion of vitamin E or grape pomace have similar effects on delaying meat lipid oxidation, apparently confirming the antioxidant effects of wine derivative compounds.

alt-text: Fig. 2

~~Figure 2.~~ Fig. 2



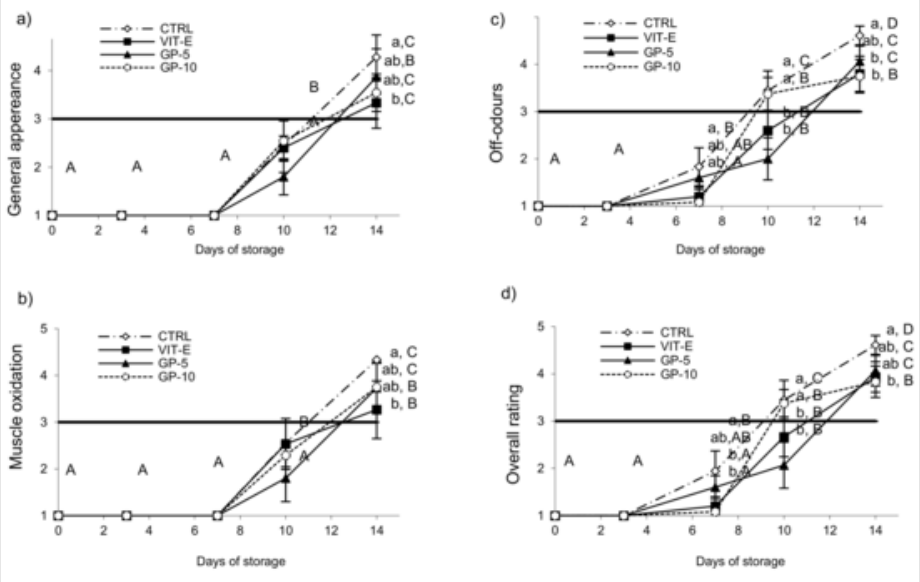
Effect of experimental ewe diets (CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of TMR, DM basis; GP-5, 5% of grape pomace from red wine, DM basis; GP-10, 10% of grape pomace from red wine, DM basis) and storage time on evolution of TBARS (μg-/g- + meat) in m. *longissimus thoracis et lumborum* from suckling lambs stored at 4 °C. Different small letters mean significant differences ($P < 0.05$) between treatments within time and capital letters mean significant differences ($P < 0.05$) between times within treatment. The error bars represent standard error. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

~~3.3.~~3.3 Shelf-life analysis

Fig. 3 shows results of shelf-life studies of LTL slices packaged under a gas mixture of 80:20% / O_2:CO_2. As expected, no differences were found between treatments ($P > 0.05$) in the recently cut meat (day 0), with all samples having a score of 1. In general shelf-life stability decreased gradually throughout the storage period, presenting the highest scores for appearance, off-odours, muscle oxidation, and overall rating on the last day of display. Although the perception of lamb meat discolouration, reflected in meat appearance and display of muscle oxidation parameters, increased gradually during storage in all groups, the behaviour differed between treatments. At the 14-day sampling point, VIT-E had lower scores in these sensory parameters ($P < 0.05$) than CTRL, although all treatments reached scores above 3 at this sampling point. Similarly, the presence of off-odour increased gradually and significantly during storage in all groups. Nevertheless, as of 10 days of display, VIT-E and GP-5 presented significantly lower values ($P < 0.05$), with scores of less than 3, the established threshold for rejection. The overall rating of the samples reflects the changes in the other sensory attributes studied. Thus, according to the panellists, CTRL and GP-10 samples received values above 3 after 10 days of storage, while VIT-E and GP-5 treatments reached that threshold at day 14.

alt-text: Fig. 3

~~Figure 3.~~Fig. 3



Effect of experimental ewe diets (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) and storage time on general appearance (a), display of muscle oxidation (b), off-odours (c) and overall rating (d) evaluated by a panel of trained members in m. *longissimus thoracis et lumborum* from suckling lambs stored at 2 °C. Different small letters mean significant differences ($P < 0.05$) between treatments within time and capital letters mean significant differences ($P < 0.05$) between times within treatment. The error bars represent standard error. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

~~4.~~4 Discussion

Firstly, authors acknowledge that as pointed out previously by Gómez-Cortés et al. (2018), experimental diets did not affect dry matter intake, milk yield and composition and sucking lamb growth. Differences in the results obtained in this experiment can only be attributed to the effect of the treatments used.

Regarding shelf life of meat, microbial growth is one of the main causes of fresh meat spoilage. Therefore, the shelf life of meat is highly influenced by the initial bacterial load. Thus, high numbers of microorganisms in meat prior to storage

shorten its shelf life since the microorganism limit will be reached more rapidly (Blixt & Borch, 2002, Karabagias et al., 2011; Vieira et al., 2019). As expected, no differences between treatments were initially observed (values ranged 2.00–3.17 log cfu/g) as found in other studies on lamb meat prior to storage (Lauzurica et al., 2005; Nieto, Bañón, & Garrido, 2011).

The bacteria that are usually responsible for meat spoilage are *Brochotrix thermospacta*, *Enterobacteriaceae*, *Lactobaciluus* spp., *Leuconostoc* spp., *Pseudomonas* spp. and *Shewanella putrefaciens*. In the case of modified atmosphere packaging, a high concentration of oxygen permits the growth of aerobic microorganisms, although CO_2 controls the growth of bacteria associated with meat spoilage (Buys, Krüger, & Nortjé, 1994; Fernandes et al., 2014). Levels of 20–60% CO_2 are required for effectiveness against aerobic spoilage organisms by penetrating membranes and lowering intracellular pH (Smith, Ramaswamy, & Simpson, 1990). The experimental diets did not affect TVC counts for a considerable amount of time, beginning to increase as of 10 days of storage. However, the inclusion of both vitamin E and grape pomace at 5% was found to decrease enterobacteria counts at the last two sampling periods (days 10 and 14). Our results appear to indicate that vitamin E was effective in preventing enterobacteria development as of day 10. This data was unexpected and the authors offer no explanation for the same, since all studies evaluating the effect of α-tocopherol on microbial growth have concluded that vitamin E lacks antimicrobial activity (Bellés, del Mar Campo, Roncalés, & Beltrán, 2019; Lauzurica et al., 2005). The inclusion of grape pomace at the 5% level was effective in preventing enterobacteria growth as of day 10 of storage. It has been demonstrated that dietary polyphenols do not undergo substantial metabolic modifications, thus enriching the meat with bioactive molecules originated from the diet (Branciari et al., 2017; Gladine, Rock, Morand, Bauchart, & Durand, 2007). Several studies have indicated a transfer of dietary phenolic compounds to the ewes' milk and subsequently a transfer to their suckling lamb muscles (Chiofalo, Liotta, Fiumanò, Benedetta, & Chiofalo, 2012; Gómez-Cortés et al., 2018). In this regard, some studies have shown that supplementation of ewe diets with rosemary (Nieto et al., 2010a; Serrano et al., 2014) and thyme (Karabagias et al., 2011; Nieto, Díaz, Bañón, & Garrido, 2010b), rich in phenolic compounds, reduced microbial populations responsible for lamb meat spoilage during storage due to the accumulation of these compounds in the muscles during the animal's life. Likewise, other authors (Reddy et al., 2013) have reported that the addition of grape seed extract to lamb diets significantly reduced total psychrophilic and coliform counts in meat during refrigerated storage. Therefore, grape by-products could exert a protective effect against microbiological spoilage. The antimicrobial effect of polyphenols could be attributable to be highly potent DNA gyrase enzyme inhibitors, essential in the transcription, replication of DNA and chromosome segregation processes of bacteria (Khan et al., 2018). Papuc, Goran, Predescu, Nicorescu, and Stefan (2017) also indicated that the effect of polyphenols on microbial counts is related to the inhibition of biofilms, chelation of metal ions required for energy production, or even to the alteration of the phospholipid layer, which increases the loss of components necessary for the bacteria's survival. Despite the antibacterial effect observed in grape pomace included at 5%, in our study, grape pomace was not effective in preventing microbial development at the 10% level of incorporation. Most studies assessing dose effects, particularly concerning the degree of degradation of active polyphenol compounds in the feed given to lambs, are not consistent in their results. Polyphenols can have either beneficial or detrimental effects on feed intake, nutrient digestibility and growth performance. However, the importance of rearing conditions on the dose-response effect has been highlighted (Chikwanha et al., 2019; Gladine et al., 2007).

Given that shelf life is defined as the time (in days) needed to reach mean values at the limit of 7 log cfu g^{-1} of TVC (ICMSF, 1986), the shelf life of the lamb meat studied was not limited by microbial spoilage as none of the experimental treatments reached this level. This is consistent with Serrano et al. (2014), who concluded that the shelf life of lamb fillets under high O_2:CO_2 MAP was not limited by microbial spoilage, although extracts rich in phenolic compounds contributed to controlling the meat's microbial load.

Meat colour has been reported to be the most important factor when consumers assess meat quality, since they relate colour to freshness and it deteriorates rapidly under retail display conditions. Colour coordinates and metmyoglobin values reflected the dietary effect on colour stability during storage. The lowest L^* *values* founded in VIT-E and GP-5 at the end of storage period are in line with the results of Nieto et al. (2010b) who observed lower L^* lamb meat values when phenols (from thyme) were included in the dams' diets. However, grape pomace inclusion at the 10% level did not affect the L^* index, as compared to the CTRL group. Consumers (Corlett, Pethick, Kelman, Jacob, & Gardner, 2021) prefer fresh and red-coloured meat, associated with higher a^* values, with the myoglobin oxidation process being responsible for meat browning over storage time, which is generally associated with a decrease in a^* values (

Bodas et al., 2012). However, in this trial, the redness values (a^*) increased during storage, especially in the GP-5 group which revealed the highest values. This is consistent with the results of Nieto et al. (2010a) and more recently, with Flores et al. (2021), who found a positive effect on redness in lamb meat from dams fed with a phenol-rich source. In our study, the behaviour of yellowness values (b^*), like redness values (a^*), remained stable during the 10 days of storage, except in CTRL which increased at this time. At the end of the storage, VIT-E and GP-5 treatments presented higher values than CTRL and GP-10. Our results are in line with those of Karami et al. (2011), who reported higher b^* values in vitamin E-supplemented growing lambs. However, Gallardo, Manca, Mantecón, Nudda, and Manso (2015a, 2015b) reported a lower b* value in suckling lamb meat from dietary vitamin E-supplemented ewes. As for the effect of polyphenols, lower b^* values were reported by Nieto et al. (2010a) in lamb meat from ewes fed different polyphenol sources. In our study, the different behaviour of GP-5 and GP-10 may be due to the effect of different doses of polyphenol sources. Likewise, the inclusion of vitamin E, and grape pomace at the 5% level resulted in higher chroma values ($p < 0.05$) at day 14. This could be related to the lower meat decolouration induced by polyphenols. In our study, however, no differences were found between GP-10 and CTRL at the end of storage period. Regarding hue angle which has been related to the visual appraisal of meat discolouration (Luciano et al., 2011), although slight differences were observed between groups, our data suggested that h^* remained virtually stable throughout the trial for all treatments.

High-oxygen modified atmosphere packaging improves meat colour due to oxymyoglobin formation, which maintains the desirable bright red colour of meat. However, oxygen promotes numerous deteriorative reactions during meat storage, such as the conversion of myoglobin to its oxidised form (Natalello et al., 2020). The accumulation of MMb during storage is responsible for meat browning. According to our results, the inclusion of both vitamin E and grape pomace in the ewes' diets inhibited MMb formation as of day 10. This is because their antioxidant properties avoid reaching an MMb level of 40%, which has been reported to be a level that causes meat rejection (Greene & Cumuze, 1971). According to Descalzo et al. (2007) and Bellés et al. (2019), vitamin E can improve the overall muscle antioxidant status, by two mechanisms, neutralising oxidative free radicals that trigger myoglobin oxidation and by lowering the formation of some oxidation markers (Jose, Jacob, Pethick, & Gardner, 2016; Lauzurica et al., 2005). The direct antioxidant action of α-tocopherol on protecting membrane lipids would reduce the formation of primary and secondary compounds of lipid oxidation, which indirectly could delay myoglobin oxidation. On the other hand, the conclusions obtained in the study of Ponnampalam, Butler, McDonagh, Jacobs, and Hopkins (2012) highlighted the key role of heme iron and tocopherol in extending the desirable meat colour. Vitamin E has been demonstrated to Q6 reduce the conversion of myoglobin to metmyoglobin and therefore, to prevent meat from colour fading (Bellés et al., 2018; Jose et al., 2016; Kerry, O'Sullivan, Buckley, Lynch, & Morrissey, 2000; Lauzurica et al., 2005; Leal et al., 2018 ; Ripoll, Joy, & Muñoz, 2011). The effect of vitamin E has also been observed on suckling lamb meat colour stabilization throughout ewes' dietary vitamin E supplementation (Gallardo et al., 2015a, 2015b). Vitamin E is stable in the ruminal environment and before absorption, needs to be hydrolysed, thus little if any absorption in the rumen would be expected (Hymøller & Jensen, 2010). According to Belles et al. (2019), tocopherol esters are largely hydrolysed in the intestinal lumen by pancreatic esterase and is absorbed in combination with lipid micelles. Vitamin E, protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction, acting throughout a chain-breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the systems. Thus, converting it to a hydroperoxide, which prevents propagation of peroxidation (Bellés et al., 2019). It is interesting to note that the antioxidant effect of grape pomace (GP-5 and GP-10) was similar to that of vitamin E, probably due to the transfer of dietary phenolic compounds from milk to suckling lamb muscle, which may have contributed to the protective effect of these substances against meat discolouration, preventing the formation of MMb. Phenolic acids are small molecules with known antioxidant activity, acting as free radical acceptors and chain breakers. In addition to being phenolic compounds, flavonoids have also been reported to have antioxidant activity, free radical scavenging capacity, metal chelation activity and human health-promotion effects. Similar results in meat discolouration were obtained by Nieto et al. (2010a) with different plants rich in phenolic compounds. The effect of these phenolic-rich extracts on haeminic pigment oxidation was previously explained by Samman et al. (2001), who associated this effect with the fact that they are iron chelating agents, promoting the inhibition of iron absorption and therefore, resulting in lower red blood cell counts or haemoglobin levels (Bodas et al., 2012). Because grape pomace was just as effective as vitamin E in delaying meat colour deterioration, this may be attributed to the antioxidant activity of this by-product. Of the agro-industrial by-products used in animal feeding due to their antioxidant activity, grape pomace has the highest polyphenol content, and is a good natural preservative when included in ruminant finishing diet (Tayengwa et al., 2020). According to Gladine et al. (2007), contrary to monogastrics, ruminants can make polymeric

proanthocyanidins bioavailable and benefit from the strong antioxidant capacity of grape derivate compounds, due to the hydrolysis of proanthocyanidin polymers into bioavailable and bioactive monomers (catechin or epicatechin) by rumen micro-organisms.

In meat packaging under aerobic conditions, discolouration and microbial spoilage occur before lipid oxidation occurs. In contrast, in packaging under high-oxygen atmospheres and moderate carbon dioxide levels, that maintain the desired bright colour and inhibits the growth of aerobic meat spoiling-bacteria respectively, shelf life is commonly limited by lipid oxidation (Renerre & Labadie, 1993). Lipid oxidation results from the production of free radicals, which may cause the oxidation of meat pigments and the generation of rancid odours and flavours (Faustman & Cassens, 1990). As expected, meat lipid oxidation increased considerably with storage time, reflecting the decreased ability of the meat to resist lipid oxidation during storage under retail display conditions. Thus, the high MDA values observed are likely to be the result of the oxidizing conditions taking place during storage (high O_2:CO_2 atmosphere and intense lighting), since lipid oxidation in meat packed in high-oxygen modified atmospheres can be enhanced by the high O_2 level. Values around 2.0 mg MDA/kg have been reported in several works in beef for detecting off-odours. However, studies such as the work carried out by Soldatou, Nerantzaki, Kontominas, and Savvaidis (2009) in lamb meat mark a value of 4.4 mg MDA/ kg to stablish the initiation of lipid oxidation/rancidity. As expected, initially, the TBARS concentrations in meat were not significantly different in the four experimental treatments. However, the muscle oxidative processes throughout the storage, as reflected in the TBARS values, were significantly reduced when vitamin E or grape pomace were added to the diet ($p < 0.05$).

Although the meat's resistance to oxidation is affected by the complex balance and interaction between antioxidant and pro-oxidant factors in muscles, in general, dietary strategies promoting the deposition of vitamin E in muscles improve meat oxidative stability (Faustman, Chan, Schaefer, & Havens, 1998; Luciano et al., 2019). Vitamin E is the primary lipid-soluble antioxidant in biological systems, and it breaks the lipid oxidation chain in cell membranes (Buckley, Morrissey, & Gray, 1995). In this study, vitamin E supplementation on the diet was effective in preventing muscle MDA formation during storage under modified atmosphere packaging, since significantly lower ($p < 0.05$) TBARS values were found as of day 10, as compared to the CTRL treatment. These findings are consistent with those of other authors on meat from Churra breed suckling lambs that were also fed only on maternal milk, employing α-tocopherol acetate in ewe diets (Gallardo et al., 2015a, 2015b), and in lambs fed with milk replacers (Morán et al., 2014). Gallardo et al. (2015a, 2015b) showed concentrations of vitamin E in muscle of 1.3 μg/g when the ewes were supplemented with 400 mg·kg^{-1} of synthetic vitamin E. These authors reported that dietary vitamin E powerfully inhibited MDA formation in lamb meat, especially during longer retail display periods. As for the inclusion of dietary phenolic compounds, although several studies (Luciano et al., 2011; Moniño, Martínez, Sotomayor, Lafuente, & Jordan, 2008) have suggested that these compounds favour the antioxidant stability of meat, their mechanisms of action have not been fully established. However, it has been reported that many plant secondary compounds, such as phenolic compounds, can attenuate oxidative damage of tissue, since they can interfere with the propagation reaction, in addition to inhibiting the enzymatic systems involved in initiation reactions (You, Jong, & Kim, 1999). Phenolic compounds contain conjugated ring structures and hydroxyl groups that stabilize free radicals, and carboxylic acid groups that inhibit lipid oxidation by metal chelation (Decker, 1995). The direct antioxidant activity of dietary polyphenols would imply their absorption through the gastrointestinal tract and their transfer to tissues (Luciano et al., 2009). It has been also suggested that the polyphenols present in wine by-products are absorbed, distributed, and remain active modulating antioxidant activity in muscle tissue (Nardoia et al., 2018). In the case of polymeric and high molecular weight substances, such as condensed tannins, their absorption could be limited and it is unlikely that oligomers larger than trimers could be absorbed in the small intestine in their native form (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). Recently, Jerónimo et al. (2020) observed that the addition of *Cistus ladanifer*, rich in condensed tannins, had a protective effect against lipid oxidation by increasing α-tocopherol concentration in the muscle. Hydrolysis of polymers into compounds with a low degree of polymerization, or monomers would permit their absorption. Nevertheless, the effect of dietary polyphenols on meat oxidative stability may be indirect, through the interaction between phenols and other antioxidant compounds or pro-oxidant compounds found in meat.

The antioxidant effect of phenolic compounds has been confirmed by our results, since lower TBARS values were observed as of day 10 in grape pomace treatments (GP-5 and GP-10) (Fig. 2), suggesting that grape pomace inclusion at the two levels employed was effective in preventing lipid peroxidation as compared to the CTRL. Although the accumulation of grape pomace polyphenols in the meat was not determined in the present study, the subsequent transfer

of milk phenolic compounds to the muscles could be the cause of this reduction in MDA formation when grape pomace was added to the ewes' diets. Previous studies on suckling lambs have demonstrated improved lipid oxidation of meat during storage when different polyphenol sources were included in the lactating ewes' diet (Nieto et al., 2010a, 2010b; Serrano et al., 2014), or in milk replacers for suckling lambs (Morán et al., 2014). As with vitamin E, the improvement in lamb meat TBARS from grape pomace treatments could relate to the lower numerical value of ewe plasma TBARS reported in animals fed with grape pomace. Our results suggest that the inclusion of both vitamin E and grape pomace has similar effects on delaying meat lipid oxidation, apparently confirming the antioxidant effects of wine derivative additives. In fact, while the meat from the antioxidant treatments (VIT-E, GP-5 and GP-10) did not reach the established rancidity threshold (4.4 μg MDA g^{-1} meat), this level was exceeded by the CTRL treatment at the end of storage period.

As expected, according to our trained shelf-life panel, the shelf-life stability of lamb meat decreased gradually during the entire storage period, presenting the highest scores at the end of the experimental period. Meat spoilage is associated with oxidizing phenomena, such as lean browning, exudation, loss of metallic blood odour and increasing rancid odour and flavour (Calnan et al., 2019). Perception of discolouration is consistent with the relative MMb percentage observed, but statistical differences between treatments were only detected at the end of storage period, when they all presented scores above 3, the established threshold for meat rejection (Guerra-Rivas et al., 2016; Vieira et al., 2019). Only after 14 days, the appearance was rejected in all treatments, but after only 10 days, the CTRL and GP-10 treatments had off-odour scores exceeding the threshold for rejection. This discrepancy between the muscular appearance and off-odour was also observed by Mancini and Hunt (2005) who reported that, although high-oxygen atmospheres prolong the time before metmyoglobin is visible on the muscular surface, rancidity often develops while colour is still desirable. However, after 10 days of display, VIT-E and GP-5 showed significantly lower values, with only minor off-odours but not spoiled.

The effect of antioxidant supplementation was also reflected at the end of trial since CTRL off-odour score was slightly higher than the other treatments, though all treatments had values over 3. The shelf-life panel corroborated the TBARS values since lipid oxidation is linked to rancid odours. Thus, CTRL presented off-odour to a greater extent, as compared to the treatments that included antioxidant supplementation. However, it should be noted that panellists described off-odour as “rancid”, but “acid”, or “putrid” off-odours were barely detected, reflecting the low microbial counts observed. The overall rating of the samples reveals the changes in the other shelf-life attributes studied. Thus, according to the panellists, the CTRL and GP-10 samples had values above 3 at 10 days, while the VIT-E and GP-5 treatments attained that score on day 14. According to Ripoll et al. (2011), the use of dietary vitamin E in lamb diets extended their shelf life an additional 4 days. This is also in line with the findings of Muíño et al. (2014), who observed that a trained panel judged the colour, flavour and overall appearance of a dietary vitamin *E*-supplemented fattening lamb meat more favourably than non-supplemented meat. In our study, the presence of polyphenols at 5% grape pomace in ewes' diet resulted equally effective as vitamin E at the end of storage period, with lower scores on off-odours and a lower overall rating. These results are supported by studies that have suggested improved sensory attributes with regard to meat colour and odour under display conditions when including polyphenols in lamb diets (Nieto et al., 2010a, 2010b). As in our study, Serrano et al. (2014) reported that ewes' diet supplementation with rosemary, rich in polyphenols, led to an improved shelf life for the meat of their suckling lambs when packed under high O_2 MAP and kept under retail conditions.

~~5.~~5 Conclusions

Results suggest feeding ewes fresh grape pomace, a by-product of red wine production, is just as effective as vitamin E in preventing suckling lamb meat spoilage, when packaged under high oxygen atmospheres and exposed to retail storage conditions. In fact, lambs from ewes that were fed grape pomace and vitamin E showed lower metmyoglobin percentage, lower lipid oxidation and improved scores on sensory perception, as compared to ewes that were not supplemented. However, it should be noted that grape pomace supplementation at 5% was more effective than supplementation at 10%. Given the positive results observed on sensory parameters, it is recommended that additional research be conducted on the use of these wine by-products in sheep feeding, particularly to test different doses and means of supplementation of winery by-products in lamb diets.

Author ~~C~~contributions

For research articles with several authors, a short paragraph specifying their individual contributions is provided. The following statements should be used.

Vieira, C:

- Affiliation: Estación Tecnológica de la Carne. Instituto Tecnológico Agrario de Castilla y León (ITACyL). Guijuelo (Salamanca-Spain)
- Contribution to article: this author is the researcher in charge of the project in ITACYL. She has investigated about the subjects involved in the article, has participated in the conceptualization, execution of the project, formal analysis in farm and in sensory analysis, data collection and resources at the laboratory. She also has been the writing—review and editing of the article.

Guerra-Rivas:

- Affiliation: Escuela Universitaria Superior de Ingeniería Agraria. Universidad de Valladolid (Spain)
- Contribution to article: this author has contributed to methodology and has been performed the formal analysis.

Martínez, B:

- Affiliation: Estación Tecnológica de la Carne. Instituto Tecnológico Agrario de Castilla y León (ITACyL)..Guijuelo (Salamanca-Spain)
- Contribution to article: this author has been the researcher responsible of the shelf life experiment. Also, she has performed the methodology, data collection and formal resources at the laboratory of sensory analysis.

Rubio, B.:

- Affiliation: Estación Tecnológica de la Carne. Instituto Tecnológico Agrario de Castilla y León (ITACyL). Guijuelo (Salamanca-Spain)
- Contribution to article: this author has implemented the methodology and has been performed the formal analysis, data collection and validation of microbial analysis. She has controlled the and resources at the microbial laboratory.

Manso, T.

- Affiliation: Escuela Universitaria Superior de Ingeniería Agraria. Universidad de Valladolid (Spain)
- Contribution to article: this author is the researcher in charge of the project in Universidad de Valladolid. She has investigated about the subjects involved in the article, has participated in the conceptualization, execution of the project, formal analysis in farm and in sensory analysis, data collection and resources at the laboratory. She also has been the writing—review and editing of the article.

CRediT authorship contribution statement

C. Vieira: Conceptualization, Investigation, Methodology, Resources, Supervision, Writing – review & editing. **C. Guerra-Rivas:** Investigation, Methodology, Formal analysis. **B. Martínez:** Investigation, Formal analysis, Funding acquisition, Resources, Writing – original draft. **B. Rubio:** Formal analysis, Methodology, Resources. **T. Manso:** Conceptualization, Investigation, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

None.

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The corrections made in this section will be reviewed and approved by a journal production editor. The newly added/removed references and its citations will be reordered and rearranged by the production team.

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Q4 HIGHLIGHTSighlights

- Addition of VIT-E and grape pomace to ewe diet affects shelf life of suckling lamb meat packaged under modified atmospheres and stored in retail conditions.
- Ewes fed VIT-E and grape pomace delayed myoglobin oxidation, lipid oxidation and sensory spoilage of suckling lamb meat during storage.
- Grape pomace was as effective as vitamin E in extending the shelf life of lamb meat.
- Supplementation of 5% grape pomace in ewe diets was more effective at extending shelf life of suckling lamb meat than 10%”

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Answer: Addition VIT-E and grape pomace to ewe diet affects shelf life of suckling lamb meat packaged and stored in retail conditions. 155Ewes fed VIT-E and grape pomace delayed myoglobin, lipid oxidation and sensory spoilage of suckling lamb meat during storage. 127Grape pomace was as effective as vitamin E in extending the shelf life of lamb meat.Supplementation of 5% grape pomace in ewe diets was more effective at extending shelf life of suckling lamb meat than 10% 122

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