Universidad de Valladolid

Escuela Técnica Superior de Ingenierías Agrarias

Programa de Doctorado en Investigación en Ingeniería
para el Desarrollo Agroforestal

TESIS DOCTORAL

Grape pomace in the feeding of sheep

Empleo de orujo de uva en la alimentación del ganado ovino

Presentada por Cristina Mª Guerra Rivas para optar
al grado de Doctor con mención “Doctor Internacional”
por la Universidad de Valladolid

Dirigida por:
Dra. Teresa Manso Alonso
Dra. Ceferina Vieira Aller

Grape pomace in the feeding of sheep

Empleo de orujo de uva en la alimentación del ganado ovino

Memoria presentada para optar al grado de
Doctor con mención “Doctor Internacional”
por la Universidad de Valladolid

Fdo. Cristina Mª Guerra Rivas
29 de Julio de 2015

Universidad de Valladolid

Departamento de
Ciencias Agroforestales

AUTORIZACIÓN DE LOS DIRECTORES DE LA TESIS PARA SU PRESENTACIÓN

Las Dras. Dña. Teresa Manso Alonso y Dña. Ceferina Vieira Aller como directoras de la Tesis Doctoral titulada “Grape pomace in the feeding of sheep (Empleo de orujo de uva en la alimentación del ganado ovino)” realizada por Dña. Cristina Mª Guerra Rivas en la Escuela Técnica Superior de Ingenierías Agrarias de la Universidad de Valladolid dentro del Programa de Doctorado en Investigación en Ingeniería para el Desarrollo Agroforestal, autorizan su presentación dado que reúne las condiciones necesarias para su defensa.

Y para que conste a los efectos oportunos, lo firman en Palencia a 29 de Julio de 2015.

Fdo. Dra. Dña. Teresa Manso Alonso
Profesora Titular de Universidad
Universidad de Valladolid

Fdo. Dra. Dña. Ceferina Vieira Aller
Investigadora Estación Tecnológica de la Carne
Instituto Tecnológico Agrario de Castilla y León

La autora de esta Tesis Doctoral ha sido beneficiaria de una beca y contrato de Formación de Profesorado Universitario (FPU) del Programa Nacional de Formación de Recursos Humanos de Investigación (Orden EDU/61/2011) en el marco del Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica 2008–2011 (Referencia: AP–2010–0571), así como de una ayuda para la Formación de Profesorado Universitario para la realización de una Estancia Breve en la Éscola Agrária de Bragança (Instituto Politécnico de Bragança) (Portugal) del Programa Estatal de Promoción del Talento y su Empleabilidad en el marco del Plan Estatal de Investigación Científica y Técnica y de Innovación 2013–2016 en I+D+i (BOE 279/2013).

Este trabajo se ha realizado dentro de un Convenio de Colaboración entre la Universidad de Valladolid y la Diputación de Palencia, y ha sido financiado por el proyecto RTA2010–0068–C02–01/02 del Ministerio de Educación y Ciencia (I+D), subprograma de proyectos de investigación fundamental orientada a los recursos y tecnologías agrarias en coordinación con las CCAA (2010–2013), y por el proyecto VA196A11–2 de la Consejería de Educación de la Junta de Castilla y León (2011–2013).

A mi padre

Agradecimientos

Quiero agradecer a todas aquellas personas e instituciones que han puesto a mi disposición sus conocimientos y los medios necesarios para la realización de esta Tesis Doctoral.

Deseo mostrar mi gratitud a las directoras de esta Tesis Doctoral, Teresa Manso Alonso y Ceferina Vieira Aller. Especialmente, quiero expresar mi agradecimiento a Teresa, por creer en mí, por estar ahí día a día, por sus consejos e indicaciones, por su generosidad, su infinita paciencia en mis momentos de crisis y por su apoyo incondicional.

En segundo lugar, a todos aquellos organismos sin cuya financiación, colaboración o infraestructuras no hubiera sido posible. Entre ellos quiero destacar a la Universidad de Valladolid, al Instituto de Ganadería de Montaña del CSIC (Grulleros, León) y a la Estación Tecnológica de la Carne del ITACyL (Guijuelo, Salamanca), así como a la Diputación Provincial de Palencia y a la Escuela de Capacitación Agraria de Viñalta (Palencia), porque gracias a las personas que allí trabajan se ha podido llevar a cabo la parte experimental de este trabajo.

A los compañeros, familia y amigos que durante estos años han sufrido los efectos de escribir una tesis, por saber escuchar y por ofrecerme su apoyo y ayuda en todo momento, en especial a Beatriz Gallardo por estar siempre disponible.

También a todo el equipo del laboratorio de Qualidade da Carcaça e da Carne, de la Éscola Agrária de Bragança (Portugal) por su buena acogida durante mi estancia, y en especial al profesor Alfredo Teixeira, por su amabilidad y atención.

Y a todas aquellas personas, que de algún modo u otro me animaron a emprender este camino y me ayudaron durante el viaje.

Gracias.

Índices

Índice general

Índice de tablas

Índice de figuras

Abreviaturas

Abreviaturas

AGV	ácidos grasos volátiles
Azúc.	azúcares
BF	músculo *bicesps femoris*
BH	biohidrogenación
BHA	butilhidroxianisol
BHT	butilhidroxitolueno
CLA	ácido linoleico conjugado
CRA	capacidad de retención de agua
D	músculo *deltoideus*
EDTA	ácido etiléndiaminotetraacético
EE	extracto etéreo
EM	energía metabolizable
FAD	fibra ácido detergente
FB	fibra bruta
FND	fibra neutro detergente
GM	músculo *gluteus medius*
LAD	lignina ácido detergente
LD	músculo *longissimus dorsi*
LL	músculo *longissimus lumborum*
LT	músculo *longissimus thoracis*
LTL	músculo *longissimus thoracis et lumborum*
MMb	metamioglobina
MDA	malonaldehído
MO	materia orgánica
MS	materia seca
MUFA	ácidos grasos monoinsaturados
OEA	músculo *obliquus externus abdominis*
PB	proteína bruta
PB-FAD	proteína bruta ligada a la fibra
PG	propilgalato
PUFA	ácidos grasos poliinsaturados
RA	ácido ruménico
ROS	especies reactivas del oxígeno
TMR	ración total mezclada
SFA	ácidos grasos saturados
TBARS	sustancias reactivas al ácido tiobarbitúrico
TBHQ	terbutilhidroquinona
TMR	ración total mezclada
UNSFA	ácidos grasos insaturados
VA	ácido vacénico

Abreviations

ADF	acid detergent fibre
ADG	average daily weight gain
ADICP	acid detergent insoluble crude protein
ADL	acid detergent lignin
BH	biohydrogenation
CCW	cold carcass weight
CLA	conjugated linoleic acid
CP	crude protein
CT	condensed tannins
DHA	docosahexaenoic acid
DM	dry matter
DMI	dry matter intake
DPA	docosapentaenoic acid
ED	effective degradability
EE	ether extract
EP	extractable polyphenols
EPA	eicosapentaenoic acid
FA	fatty acid
FID	flame ionization detector
GC	gas chromatograph
GLM	general linear model
HCW	hot carcass weight
HP	hydrolysable polyphenols
LAB	lactic acid bacteria
LBW	live body weight
LSD	least significant difference
LTL	*longissimus thoracis et lumborum* muscle
MDA	malonaldehyde
MMb	metmyoglobin
MUFA	monounsaturated fatty acids
NDF	neutral detergent fibre
NDSC	neutral detergent-soluble carbohydrates
NIRS	near-infrared spectroscopy
OM	organic matter
PD	potential degradability
PDI	protein truly digestible in the small intestine
PUFA	polyunsaturated fatty acids
RA	rumenic acid
ROS	reactive oxygen species
RSD	residual standard deviation
SED	standard error of the difference

SFA	saturated fatty acid
TA	total anthocyanins
TBARS	thiobarbituric acid-reactive substances
TMR	total mixed ration
TVC	total viable counts
UFV	feed unit for maintenance and meat production
VFA	volatile fatty acids
UNSFA	unsaturated fatty acid
VA	vaccenic acid
WHC	water-holding capacity

Resúmenes

Resumen

El orujo de uva, que está formado por semillas y hollejos (piel y pulpa), es el principal residuo de la industria del vino y representa aproximadamente el 20% del volumen total de las uvas procesadas para la producción de vino. Este subproducto no está suficientemente aprovechado, y su empleo como alimento en dietas de rumiantes, podría reducir los costes de alimentación y reciclar un residuo que es costoso de eliminar.

Durante el proceso de elaboración del vino, parte de los compuestos fenólicos de las uvas son transferidos al vino, pero una alta proporción permanecen en el orujo. Varios estudios han demostrado que los compuestos fenólicos poseen muchas propiedades, entre las que destaca su capacidad antioxidante, que podría tener efectos beneficiosos sobre la calidad de los productos, minimizando la oxidación y aumentando la vida útil de la carne. Por otra parte, el orujo de uva cuenta con la presencia de altas cantidades de ácido linoleico y ácido oleico, que pueden ejercer efectos beneficiosos sobre el perfil de ácidos grasos de la carne y de la leche, con la hipótesis de obtener productos con grasa más insaturada. En consecuencia, el orujo de uva constituye una fuente barata de polifenoles y ácidos grasos insaturados para alimentación de rumiantes, cuyo empleo podría reducir el impacto ambiental que genera la acumulación residuos de la industria del vino.

El empleo de orujo como alimento es poco habitual debido a su bajo valor nutritivo para rumiantes, su alto contenido en fibra y lignina, especialmente en las semillas, su baja digestibilidad y por la presencia de compuestos secundarios, como los taninos condensados, que pueden presentar efectos nutricionales adversos o beneficiosos en los rumiantes, dependiendo de su estructura química y de su concentración en la dieta.

Teniendo en cuenta las consideraciones anteriores, los objetivos de este trabajo fueron evaluar el valor nutritivo del orujo de uva procedente de la elaboración de vino tinto producido en Castilla y León (España) como alimento para ovejas, conocer los efectos de la inclusión de orujo de uva en la dieta de ovejas en inicio de lactación sobre los rendimientos de los animales, la composición de la leche y la calidad de la carne de los corderos lechales, y estudiar los efectos de la inclusión de orujo de uva en la dieta de corderos en crecimiento-cebo sobre los rendimientos de los animales y la calidad de la carne.

La parte experimental de la presente memoria de tesis doctoral se estructura en tres pruebas experimentales diferentes diseñadas de acuerdo con los objetivos experimentales planteados.

La composición química y las propiedades nutricionales de los subproductos de la uva varían con el método de producción de vino, la variedad de uva y con las proporciones relativas de semillas y hollejos, lo que hace que a veces sea difícil extrapolar los resultados obtenidos por otros autores. En la Prueba experimental I, se determinó el valor nutritivo de las fracciones (semillas y hollejos) del orujo de uva procedente de diferentes bodegas de vino tinto representativas de Castilla y León (España), a través de su composición química, digestibilidad *in vitro* y cinética de degradación ruminal. Para la determinación de los parámetros de fermentación ruminal (pH; amoníaco-N; ácidos grasos volátiles, AGV) y degradabilidad *in sacco* de las semillas y los hollejos, se emplearon ocho ovejas provistas de una cánula ruminal, que se asignaron a dos tratamientos experimentales consistentes en una ración total mezclada (TMR), compuesta por heno de alfalfa y concentrado en proporción 50:50, del siguiente modo: CTRL (control, sin orujo de uva) y GP-7,5 (7,5% de orujo de uva de vino tinto, %MS). También se midió la oxidación lipídica del plasma de las ovejas a través de la cuantificación del nivel de malonaldehído (MDA). La composición química del orujo de uva fue variable dependiendo de su proporción de semillas y de hollejos. La fracción correspondiente a las semillas presentó mayor contenido en compuestos fibrosos y en extracto etéreo (EE). Sin embargo, los contenidos en proteína bruta (PB) y en proteína ligada a la fibra fueron mayores en los hollejos. El perfil lipídico de las semillas fue menos saturado (12,6 *vs.* 31,4%), y más poliinsaturado que el de los hollejos (69,6 *vs.* 53,3%). Los ácidos grasos mayoritarios fueron C18:2, C18:1 y C16:0. Las semillas presentaron mayor contenido en compuestos fenólicos que los hollejos, siendo los flavonoles como la quercetina y los flavanoles como la catequina los más abundantes. Los hollejos mostraron un importante contenido en antocianinas. El estilbeno *trans* resveratrol sólo fue detectado en los hollejos (1,08 mg kg^{-1} MS). La digestibilidad *in vitro* verdadera, y la degradabilidad efectiva *in sacco* de la materia seca y de la materia orgánica fueron mayores en los hollejos. Sin embargo, la degradabilidad efectiva *in sacco* de la PB fue mayor en las semillas. La concentración de amoníaco-N y de AGV totales en el líquido ruminal fue significativamente menor en las ovejas que consumieron orujo de uva. A pesar su bajo valor nutritivo, el interés del orujo de uva en alimentación ovina podría estar relacionado con su poder antioxidante, ya que se obtuvieron valores numéricos inferiores en el nivel de MDA del plasma (22%, 25% y 25% a las 3, 6 y 9 h después de la alimentación respectivamente) en comparación con el tratamiento CTRL, y con su contenido en ácidos grasos poliinsaturados, ya que proporciona el sustrato para la producción de ácidos grasos bioactivos en el rumen.

En la segunda prueba experimental, se formuló una dieta para ovejas en inicio de lactación suplementada con 2,7% (en MS) de aceite de linaza, forraje y concentrado en una

proporción 40:60, para estudiar la inclusión de distintos niveles de orujo de uva procedente de la elaboración de vino tinto, sobre los rendimientos de los animales, la composición de la leche y la calidad y la vida útil de la carne de los corderos lechales. Cuarenta y ocho ovejas de raza Churra con sus respectivos corderos lactantes fueron asignadas a cuatro tratamientos experimentales en función de la TMR que consumieron: CTRL (control, sin orujo de uva), VIT-E (500 mg de vitamina E por kg de MS), GP-5 (5% de orujo de uva de vino tinto, %MS) y GP-10 (10% de orujo de uva de vino tinto, %MS). La oxidación lipídica del plasma de las ovejas se midió a través de la cuantificación del nivel de MDA. Los corderos fueron sacrificados cuando alcanzaron 11,5 kg de peso vivo y se tomaron muestras del músculo *longissimus thoracis et lumborum* (LTL). Para el análisis de la vida útil de la carne, el músculo LTL se cortó, se envasó en atmósfera modificada (80:20% / O_2:CO_2) y se almacenó en condiciones de venta durante 14 días. En cada día (0, 3, 7, 10, 14) de muestreo se analizaron las características microbiológicas, físico-químicas y sensoriales de la carne. En comparación con el control, los tratamientos VIT-E, GP-5 y GP-10 no afectaron negativamente al consumo de materia seca de las ovejas, y la presencia de orujo de uva en las dietas no fue perjudicial para la producción de leche o su contenido en grasa y en proteína. La suplementación con vitamina E sólo ejerció un efecto moderado sobre la concentración de ácidos grasos de la leche (aumentó en el ácido α-linolénico y el C16:0, y disminuyó el C18:1). La suplementación con orujo de uva no afectó a los porcentajes de ácidos grasos saturados totales (SFA), monoinsaturados (MUFA) y poliinsaturados (PUFA) en la leche. El nivel de ácido α-linolénico alcanzó aproximadamente el 1% del total de ácidos grasos de la leche como consecuencia de la presencia de aceite de linaza en las dietas, incrementándose con la vitamina E, y permaneció inalterado para los tratamientos GP-5 y GP-10. El ácido linoleico se incrementó al incluir orujo de uva al 10%, pero a este nivel no se modificó el *cis-9 trans-11* C18:2 (RA, ácido ruménico) de la grasa de la leche. Tampoco la inclusión de orujo de uva modificó el contenido en *trans-11* C18:1 (VA, ácido vacénico) en la leche, lo que podría indicar que, en las condiciones ensayadas, este subproducto de bodega no alteró las rutas ruminales de los ácidos grasos insaturados. El orujo de uva integral no mostró efectos adversos sobre los rendimientos de los corderos lechales, las características de la canal y la calidad de la carne (pH, color, textura y composición química), en comparación con la vitamina E. El orujo de uva y la vitamina E incrementaron la capacidad de retención de agua (CRA) de la carne. La inclusión de orujo de uva no afectó al contenido intramuscular de PUFA, sin embargo aumentó las concentraciones de RA y VA en la grasa del músculo. Con respecto a la evaluación sensorial, los resultados de la prueba triangular indican que ninguno de los tratamientos fue identificado como diferente. Los tratamientos VIT-E y GP-5 fueron eficaces previniendo el

crecimiento de enterobacterias a partir del día 10 de almacenamiento. A partir del día 10, la suplementación de la dieta con vitamina E y orujo de uva redujo la formación de metamioglobina y previno la formación de MDA en el músculo en comparación con el tratamiento CTRL, lo cual podría estar relacionado con la disminución del valor numérico de TBARS en el plasma de las ovejas suplementadas con vitamina E y orujo de uva en comparación con el tratamiento control. Por último, el tratamiento VIT-E y los tratamientos con orujo de uva fueron eficaces reduciendo el deterioro sensorial en los últimos días de almacenamiento. Por lo tanto, podemos concluir que el orujo de uva resultó tan eficaz como la vitamina E en prevenir el deterioro de la vida útil de la carne.

En el tercer experimento (Prueba experimental III) se evaluó el efecto de la inclusión de orujo de uva en la dieta de corderos en crecimiento-cebo sobre los rendimientos y la calidad y la vida útil de la carne. Cuarenta y ocho corderos machos de raza Merina (14,3 ± 2,05 kg peso inicial), alojados de forma individual y alimentados *ad libitum* con concentrado y paja de cebada, fueron asignados a cuatro tratamientos experimentales: CTRL (control, sin orujo de uva), VIT-E (500 mg de vitamina E por kg de concentrado), GSE (50 mg de extracto de semilla de uva por kg de concentrado) y GP-5 (5% de orujo de uva de vino tinto desecado). Los corderos fueron sacrificados cuando alcanzaron 27 kg de peso vivo y se tomaron muestras de músculo LTL. Para el análisis de la vida útil de la carne, el músculo LTL fue cortado, envasado en atmósfera modificada (80:20% / O_2:CO_2) y se almacenó en condiciones de venta durante 14 días. En cada día (0, 4, 7, 11, 14) de muestreo, se analizaron las características microbiológicas, físico-químicas y sensoriales de la carne. No hubo diferencias significativas en los rendimientos de los corderos y las características de la canal entre los tratamientos experimentales. Sin embargo, el valor de ingestión de materia seca mostró una diferencia numérica a favor del tratamiento GP-5, observándose un aumento del 4% en el consumo de concentrado para alcanzar el mismo nivel de consumo de energía. Aparentemente, no se observaron problemas de palatabilidad respecto al orujo de uva. Las dietas experimentales no afectaron el pH, el color, la textura y la composición química de la carne. La CRA de la carne fue mayor en corderos alimentados con vitamina E que los otros tratamientos, y GSE y GP-5 no afectaron a este parámetro. El contenido de grasa de la carne presentó un valor numéricamente inferior en los animales del grupo GP-5. La carne de los corderos del tratamiento GP-5 tendió a incrementar el contenido en PUFA en comparación con CTRL, lo que podría ser debido a la alta concentración de *cis-9 cis-12* C18:2 del orujo de uva. Además, el nivel de RA mostró un valor numéricamente más alto en grasa del músculo LTL en los corderos pertenecientes al tratamiento GP-5. Los contenidos de la carne en SFA y MUFA no se vieron afectados por los tratamientos experimentales. Con respecto a la evaluación sensorial, los resultados de la prueba triangular muestran que,

en comparación con el grupo control, el único tratamiento identificado como diferente fue el GSE. En relación con los resultados de vida útil, la carne de los animales del grupo VIT-E presentó recuentos microbianos más bajos que los grupos CTRL, GSE y GP-5, sin que se observasen diferencias entre los tratamientos con polifenoles (GSE y GP-5) y CTRL. La vitamina E redujo la decoloración de la carne y la oxidación lipídica (TBARS) desde el día 7 en comparación con los otros tratamientos. Aunque no fue significativo, se observó una mejora en los valores de TBARS de alrededor del 20% con los tratamientos GSE y GP-5 en comparación con CTRL a partir de 7 días de almacenamiento. El tratamiento VIT-E fue más eficaz en la prevención de deterioro sensorial que los otros tratamientos.

Por último, esta memoria incluye una discusión general que integra todas las pruebas experimentales realizadas y se presentan las conclusiones obtenidas.

Abstract

Grape pomace, consisting of seeds, skin and pulp, is the main residue left in the wine industry and accounts for approximately 20% of the total volume of grapes used for wine production. This material is under-exploited, and the possibility of using this winery by-product as feed in ruminant diets could be adopted as a strategy to reduce feeding costs and also to cope with the need to recycle waste material whose disposal is costly.

During the winemaking process, part of the phenolic compounds in grapes is transferred to the wine, but a large proportion still remains in the wine's by-products. Several studies have shown that these phenolic compounds possess many properties, such as an antioxidant capacity, which could have beneficial effects on product quality, minimising oxidative rancidity and increasing meat shelf life. In another direction, the fatty acids (FA) of grape pomace have been examined and the presence of large amounts of linoleic and oleic acids reported in winery wastes may also have beneficial effects on the meat and milk FA profile, with the hypothesis of obtaining a more unsaturated ruminant product. Consequently, grape pomace is an inexpensive source of polyphenols and unsaturated FA that can be used in ruminant nutrition while reducing the environmental impact of waste disposal in the wine industry.

Grape pomace is seldom used as a feedstuff owing to its low nutritive value for ruminants, its high fibre and lignin content, especially in seeds, its low digestibility and secondary compounds, such as phenolics like tannins that may have adverse or beneficial nutritional effects on ruminants, depending on their chemical structure and dietary concentration.

The objectives of this work were to evaluate the nutritive value of grape pomace from red wine produced in Castilla y León (Spain) as feed for sheep, to find out the effects of dietary grape pomace in lactating ewes on animal performance, milk composition and meat quality of suckling lambs, and to study the effects of the inclusion of grape pomace in growing lamb diets on animal performance and meat quality. The experimental part of the present thesis consists of three experiments, in order to respond to the experimental objectives.

The chemical composition and nutritional properties of grape by-products vary according to the method of wine production, type of grape and the relative ratios of seeds and pulp plus skin in the pomace, which sometimes makes it difficult to extrapolate the results obtained by other authors. In the first experiment, the nutritive value of grape pomace fractions (seeds

and pulp plus skin) from different representative red wine winerys of Castilla y León (Spain) was determined on the basis of their chemical composition, *in vitro* digestibility and rumen fermentation kinetics. In order to determine ruminal parameters (pH; ammonia-N; volatile fatty acids, VFA) and *in sacco* degradability of seeds and pulp plus (hereafter referred to as 'pulp'), eight ruminally cannulated ewes were assigned to two dietary treatment consisting of a TMR of lucerne hay and concentrate with a ratio of 50:50 as follows: CTRL (control, without grape pomace) and GP-7.5 (7.5% of grape pomace from red wine, DM basis). Plasma lipid oxidation of ewes was measured as MDA quantification. Grape pomace chemical composition was variable depending on its seeds and pulp proportion. Seed fraction presented higher contents of fibre compounds and ether extract (EE). However, crude protein (CP) and acid detergent in soluble crude protein (ADICP) were higher in pulp. Seed lipid profile was less saturated (12.6 *vs.* 31.4%), and more polyunsaturated than pulp (69.6 *vs.* 53.3%). The major FAs were C18:2, C18:1 and C16:0. Seed fraction presented higher content of phenolic compounds than pulp, flavonols as quercetin and flavanols as catechin being the most abundant ones. Pulp fraction also showed an important content in anthocyanins. *Trans* resveratrol stilbene was only detected in pulp (1.08 mg kg^{-1} MS). *In vitro* true digestibility, dry matter and organic matter *in sacco* effective degradability was higher in pulp. However, *in sacco* CP effective degradability was higher in seeds. Ammonia-N concentration and total VFA in ruminal liquid was significantly lower in sheep which? consumed grape pomace. In spite of their low nutritive value, the interest of that by-product in sheep feeding could be related to its antioxidant power evidenced as lower numerical sheep plasma MDA values (accounting for 22%, 25% and 25% for 3, 6 and 9 h after feeding, respectively) compared to CTRL, and its polyunsaturared FA content, which provide the substrate for the production of bioactive FA in the rumen.

In the second experiment, a diet formulated for early lactating ewes supplemented with 2.7% (on a DM basis) of linseed oil, forage, and concentrate at a 40:60 ratio, was used to study the inclusion of different levels of red grape pomace on animal performance, milk composition and meat quality and shelf life of suckling lambs. Forty-eight Churra ewes with their suckling lambs were assigned to four dietary treatments: CTRL (control, 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) and GP-10 (10% of grape pomace from red wine, DM basis). Plasma lipid oxidation of ewes was measured as MDA quantification. Suckling lambs were slaughtered when they reached 11.5 kg live weight and samples were taken from *longissimus thoracis et lumborum* (LTL) muscle. For meat shelf life analysis, LTL was sliced, packaged under modified atmosphere (80:20% / O_2:CO_2) and stored in retail conditions for 14 days. On each sampling day (0, 3, 7, 10, 14), microbiological, physico-chemical and

sensory characteristics were analyzed. Compared to the control, the VIT-E, GP-5, and GP-10 treatments did not negatively affect dry matter intake (DMI), and the presence of grape pomace in the diets was not detrimental for milk yield or fat and protein concentration. The vitamin E supplementation had only a moderate effect on milk concentration of FA (increase in α-linolenic acid and C16:0 and decrease in C18:1). Grape pomace supplementation did not affect the percentages of total saturated (SFA), monounsaturated (MUFA), or polyunsaturated (PUFA) milk FA. Levels of α-linolenic acid reached about 1% of total milk FA as a consequence of the presence of linseed oil in the diets, increased with vitamin E, and remained unaltered in GP-5 and GP-10 treatments. Linoleic acid was increased by the highest dose of grape pomace, but this ingredient did not modify the *cis-9 trans-11* C18:2 (RA, rumenic acid) milk fat content. Nor did the presence of grape residue modify the *trans-11* C18:1 (VA, vaccenic acid) content, which might indicate that, under the conditions assayed, this winery by-product would not alter the pathways of rumen conversion of dietary unsaturated FA. Whole grape pomace did not show adverse effects on suckling lambs performance, carcass characteristics and meat quality (pH, colour, texture and chemical composition) compared to VIT-E. Grape pomace and vitamin E seems to improve meat water-holding capacity (WHC) of meat. Grape pomace inclusion did not affect intramuscular PUFA content, although it increased RA and VA intramuscular concentrations. With regard to sensory assessment, results of the triangle test suggest that any treatment identified was different. Vitamin E and GP-5 were effective in preventing enterobacteria growth from day 10. From that day, vitamin E and grape pomace addition to the ewes' diet reduced metmyoglobin formation and prevented MDA lamb muscle formation compared to CTRL, which could be connected to lower numerical values in ewe plasma TBARS reported in supplemented vitamin E and grape pomace treated animals. Finally, VIT-E and grape pomace treatments were effective in preventing sensory spoilage in the last days of storage. Therefore, we can conclude that grape pomace resulted in being as effective as vitamin E in preventing shelf life spoilage.

In the third experiment, the effect of dietary grape pomace inclusion on lamb performance and meat quality and shelf life was evaluated in fattening lambs. For that purpose, forty-eight male Merino lambs (14.3 ± 2.05 kg initial body weight) housed in individual pens and fed *ad libitum* with concentrate and barley straw were assigned according to the four dietary treatments: CTRL (control, without grape pomace), VIT-E (500 mg of vitamin E per kg of concentrate), GSE (50 mg of grape seed extract per kg of concentrate) and GP-5 (5% of dry grape pomace from red wine, as fed). Lambs were slaughtered when they reached 27 kg live weight and samples were taken from LTL muscle. For meat shelf life analysis, LTL was sliced, packaged under modified atmosphere (80:20% /

O_2:CO_2) and stored under retail conditions for 14 days. On each sampling day (0, 4, 7, 11, 14), microbiological, physico-chemical and sensory characteristics were analyzed. There were no significant differences in lamb performance and carcass traits between experimental treatments. However, the DMI value showed a numerical difference in favour of GP-5 treatment, resulting in a 4% increase in DMI concentrate to reach the same energy intake level. No apparent palatability problems with grape pomace were observed. The experimental lamb diets did not affect pH, colour, texture or chemical composition of meat. WHC was higher in lambs fed vitamin E than the other dietary treatments, and GSE and GP-5 did not have any significant effect on WHC. Meat fat content presented a numerically lower value in GP-5 animals. Meat from GP-5 lambs tended to increase the PUFA content in comparison with CTRL, which could be due to the high concentration of *cis-9 cis-12* C18:2 in grape pomace. In addition, RA showed a numerically higher value in LTL fat from GP-5 lambs. The SFA and MUFA contents were not affected by the treatments. With regard to sensory assessment, results of the triangle test show that, in comparison with the control group, the only treatment identified as being different was GSE. As regards meat shelf life results, meat from VIT-E presented lower microbial counts than CTRL, GSE and GP-5, without differences between polyphenol treatments (GSE and GP-5) and CTRL. Vitamin E reduced meat discolouration and lipid oxidation (TBARS values) from day 7 with respect to the other treatments. Although not significant, an improvement in TBARS values of about 20% was observed for GSE and GP-5, compared to CTRL from day 7 of storage. VIT-E dietary treatment was more effective in preventing sensory spoilage than the other treatments.

Finally, the dissertation includes a general discussion integrating all the experiments and the conclusions of this study.

1. Introducción general

La producción ovina constituye una actividad económica de gran importancia en Castilla y León. De hecho, Castilla y León es una de las comunidades con mayor censo de ganado ovino de España, ocupando además, el primer puesto a nivel nacional en cuanto a número de ovejas de ordeño (más de un 50% del censo nacional) y producción de leche de oveja, así como en carne de cordero, especialmente de lechazo.

En la alimentación de los rumiantes en general, y del ganado ovino en particular, es muy habitual utilizar subproductos de la industria agroalimentaria, ya que esto permite, no sólo aprovechar productos que de otra manera serían únicamente residuos, con la importancia que este tema presenta desde un punto de vista medioambiental, sino también reducir el coste de la ración y/o proporcionar sustancias bioactivas con efectos beneficiosos sobre la calidad de los productos y la salud de los consumidores.

Dentro del sector agroalimentario en Castilla y León, el vitivinícola se ha convertido en determinante para el desarrollo territorial y económico de esta región. En el año 2013, en Castilla y León se produjeron más de dos millones de hectolitros de vino, consolidándose como una de las Comunidades Autónomas más importantes en cuota de mercado nacional de vino.

Durante el proceso de elaboración del vino se generan distintos subproductos, muchos de ellos con un interesante contenido en compuestos fenólicos. Así, en la vinificación de tintos, tras la fermentación alcohólica, se generan entre 10 y 20 kg de orujo formado por hollejos, semillas y lías por cada 100 kg de uva procesada. La vinificación en tinto se realiza en contacto con las semillas y piel de la uva, que es donde se localiza el mayor contenido en compuestos fenólicos, y solamente un 35% de compuestos fenólicos de la uva son transferidos al vino, quedando presentes en el orujo una amplia gama de estos compuestos con propiedades importantes entre las que destaca su poder antioxidante. Por todo ello, el orujo puede ser considerado como un subproducto de alto valor añadido.

La composición del orujo varía en función de la variedad de uva y proceso de vinificación, pero globalmente se pueden encontrar además de compuestos mayoritarios como los antocianos (responsables del color de los vinos) y taninos (responsables de la astringencia de los vinos), otros compuestos como flavanoles (catequina y epicatequina), flavonoles como quercetina, mirecitina y entre los estilbenos, el resveratrol como compuesto más importante.

El perfil de compuestos fenólicos de los subproductos generados durante la elaboración del vino, ha sido objeto de numerosos trabajos publicados en medios especializados que constatan las propiedades antioxidantes y antimicrobianas de los residuos, además de otras propiedades beneficiosas para la salud humana relacionadas con su actividad protectora cardiovascular, vaso relajante y antitumoral. Por esta razón, el empleo de subproductos del vino presenta interés desde el punto de vista biológico, farmacológico y nutricional.

En los últimos años se han realizado numerosos trabajos dirigidos a mejorar el perfil de ácidos grasos de los productos del ganado ovino. Estos trabajos han permitido el desarrollo de estrategias de alimentación dirigidas a la obtención de productos con un perfil de ácidos grasos más saludable desde el punto de vista de la alimentación humana (mayor contenido en ácidos grasos poliinsaturados, ácido linoleico conjugado y mejor relación n3:n6). Sin embargo, el aumento en el grado de insaturación de la grasa de los corderos, la hace también más susceptible a la oxidación y, por tanto, con vida útil mas corta. Este último aspecto, entra en conflicto con los nuevos deseos de los consumidores ya que, además de buscar productos beneficiosos para su salud, han cambiado sus hábitos de compra decantándose por la adquisición de carne con menor frecuencia y por tanto con vida útil más larga.

Una de las estrategias mas comúnmente utilizadas para prevenir la oxidación lipídica y aumentar la vida útil de la carne es la utilización de antioxidantes en las raciones animales. En muchas ocasiones se utilizan antioxidantes de síntesis, cuyo uso esta bastante restringido en algunos países debido a sus efectos tóxicos y carcinogénicos. El antioxidante más utilizado es el α-tocoferol acetato de síntesis, con una eficacia bastante limitada en algunas ocasiones, por lo que existe un gran interés por desarrollar antioxidantes de origen natural con posible utilización en alimentación animal.

En los últimos años, el efecto de la incorporación de compuestos fenólicos como antioxidantes naturales en las dietas de rumiantes ha generado un gran interés en la comunidad científica. Así, existen evidencias de efectos beneficiosos de la incorporación en dietas de rumiantes de compuestos fenólicos puros (ácido carnósico, naringina, quercetina etc..), de extractos purificados (extracto de romero, extracto de semilla de uva, etc.), de plantas enteras (hojas de plantas aromáticas, arbustos como *Cistus ladanifer*…) y subproductos agroindustriales (residuos de plantas aromáticas, residuos de granada, pulpa de cítricos…) sobre la producción y calidad de los productos obtenidos (incremento en el nivel de ácidos grasos poliinsaturados y de algunos ácidos grasos funcionales como el ácido linoleico conjugado en la carne, mejoras en la estabilidad oxidativa y el color de la carne,

retraso del desarrollo microbiano de la carne, etc.). Sin embargo, y a pesar del potencial que presentan los subproductos de la elaboración del vino en cuanto a su riqueza en compuestos fenólicos, los trabajos realizados sobre su inclusión en dietas de ganado ovino en condiciones prácticas de alimentación son muy escasos. Aunque es indudable que la utilización de estos subproductos de vinificación presentan un enorme interés en rumiantes, su utilización debe realizarse con precaución debido a su bajo valor nutritivo, a la presencia de fibra muy lignificada y al contenido en compuestos secundarios como los taninos condensados con posibles efectos negativos en la fermentación ruminal.

El empleo de orujo de uva en alimentación del ganado ovino presenta gran interés desde el punto de vista económico para una región como Castilla y León, donde tanto la producción de vino como de ganado ovino, supone un peso importante dentro de la riqueza de la misma. Los resultados que se deriven del trabajo propuesto resultarán de gran utilidad para el sector agroalimentario en general y para los productores de ganado ovino de Castilla y León, para las bodegas, empresas de alimentación animal, industrias transformadoras y para los consumidores. En definitiva, se trata de ofrecer alternativas a los alimentos habitualmente utilizados en ganado ovino que redunden en mejoras de los sistemas de alimentación del ganado. El aprovechamiento del potencial que presentan residuos poco empleados en alimentación ovina y cuya eliminación es costosa, podría permitir la obtención de productos (carne y leche) de mayor calidad nutricional y aumentar su consumo.

La hipótesis de partida de la tesis doctoral es que la utilización de orujo de uva en las raciones del ganado ovino podría mejorar las características funcionales y tecnológicas de la carne de cordero y de lechazo y, así, dar salida a unos residuos ampliamente producidos en las bodegas de Castilla y León y que suelen plantear problemas a los productores de vino. Actualmente, no se dispone de suficiente información para realizar recomendaciones concretas en este sentido, ya que los trabajos sobre los efectos de la utilización de residuos de vinificación sobre la calidad de la leche y de la carne de cordero son muy escasos. Además, existen pocos trabajos en los que se hayan realizado pruebas prácticas de alimentación con ganado ovino en los que se estudie, de forma específica, el efecto de la incorporación de residuos de la elaboración del vino sobre los rendimientos productivos y características tecnológicas y funcionales de la carne de cordero.

La memoria que se presenta, después de la revisión bibliográfica y de la redacción de los objetivos y el planteamiento experimental, se estructura en tres apartados de acuerdo con las tres pruebas experimentales realizadas. Cada una de las pruebas experimentales planteadas (valoración nutritiva del orujo de uva, prueba con ovejas en inicio de lactación y

prueba de cebo de corderos) se presenta en inglés e incluye introducción, material y métodos, resultados y discusión y conclusiones. Es preciso señalar que la metodología se presenta en cada prueba experimental con el objetivo de facilitar la lectura, conscientes de que en algunas ocasiones pueda resultar coincidente.

2. Revisión bibliográfica

2.1. Los antioxidantes

2.1.1. Definición y mecanismo de acción

Las especies reactivas del oxígeno (ROS) son un conjunto de sustancias producidas en algunos procesos metabólicos, que centran su actividad en un átomo de oxígeno. Dentro de ellas se incluyen los iones de oxígeno, los peróxidos y los radicales libres, que son cualquier especie química (molécula, átomo o ión) que, pudiendo existir de manera independiente, tienen un electrón desapareado en su orbital más externo, dotándoles de gran reactividad que les hace reaccionar con otras moléculas orgánicas en procesos de oxido-reducción.

Cuando la producción de ROS no excede a los mecanismos de defensa antioxidante del organismo, éstas ejercen una actividad beneficiosa, ya que participan en diversos procesos biológicos esenciales, como el control de la expresión genética, el control de la señalización y regulación intracelular, los procesos inmunitarios, etc. (Falowo *et al.,* 2014). Los animales domésticos, en especial los explotados en sistemas de producción intensiva, frecuentemente se encuentran expuestos al estrés oxidativo (Aurosseau, 2002), que se produce cuando la formación de ROS supera la capacidad de defensa antioxidante de las células para eliminar especies tóxicas, generándose un desequilibrio que hace que la presencia de ROS se vuelva perjudicial debido a su capacidad para provocar daños en las moléculas (daño oxidativo) y procesos patológicos (Alfadda y Sallam, 2012).

Los lípidos, las proteínas y los ácidos nucleicos son las principales moléculas afectadas por el estrés oxidativo a causa de las ROS, por ello el daño oxidativo está implicado en procesos como mutagénesis, carcinogénesis, daños en la membrana por peroxidación lipídica y oxidación proteica, etc.

Los lípidos son las moléculas más susceptibles de ser oxidadas por las ROS, especialmente si en su estructura existe la presencia de dobles enlaces. La oxidación lipídica consiste en una reacción en cadena que consta de tres fases: iniciación, propagación y terminación. La reacción de iniciación comienza con la participación de una especie reactiva capaz de sustraer un átomo de hidrógeno de un grupo metilo de la cadena del ácido graso insaturado, lo que convierte a éste en un radical alquilo (R•), el cual reacciona con oxígeno para formar radicales peroxilo (ROO•) en la fase de propagación. Los radicales peroxilo a su vez, reaccionan con el siguiente ácido graso formando hidroperóxidos (ROOH), generando una reacción en cadena a través de la matriz lipídica.

La lipoperoxidación altera de forma severa la estructura y función celular de los mamíferos y produce metabolitos tóxicos (Esterbauer, 1993). Los animales domésticos son propensos a la oxidación lipídica debido, además de su exposición al estrés oxidativo, a su alimentación. La suplementación con dietas ricas en ácidos grasos poliinsaturados (PUFA) se ha convertido en una práctica frecuente para mejorar la calidad nutricional de la grasa en los productos animales. Esta estrategia nutricional incrementa la proporción de PUFA en los tejidos y la susceptibilidad a la lipoperoxidación (Gladine *et al.,* 2007a).

Las proteínas también son moléculas susceptibles de ser oxidadas por las ROS. El proceso de oxidación proteica, que afecta a la estructura de las proteínas, comienza con la sustracción de un átomo de hidrógeno (PH) por las ROS, dando lugar a la generación de un radical proteico (P•). Dicho radical es convertido a su vez en presencia de oxígeno en radical peroxilo (POO•) y de ahí se genera un alquilperóxido (POOH) que extrae el hidrógeno de otra molécula proteica. A su vez los alquilperóxidos pueden reaccionar con agua para generar radicales alcoxi (PO•) o su derivado hidroxilo (POH).

Los antioxidantes son sustancias que, estando en bajas concentraciones con respecto a las biomoléculas que protegen, previenen o reducen el daño que sufren las mismas debido a la oxidación (Halliwell, 1990). Mediante la captación de especies tóxicas (generalmente radicales libres), generan un radical más estable y menos dañino (RH) después de reaccionar con la especie radical (R•) (Cos *et al.,* 2003). Esta reacción se basa en una transición redox en la que está implicada la donación de un electrón (o átomo de hidrógeno) a la especie radicalaria (Figura 2.1.). Como resultado de esta transferencia, se formará un radical derivado del antioxidante (A•) que puede tener carácter inerte, estable o presentar cierta reactividad (Cadenas, 1997).

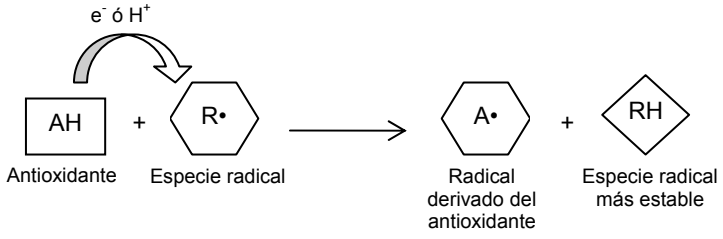


Figura 2.1. Mecanismo antiradicalario de las moléculas antioxidantes.

El empleo de compuestos antioxidantes en alimentación animal presenta un doble interés. Por una parte, evitar problemas derivados del estrés oxidativo que pueden originar

diversas patologías, provocando descensos en los rendimientos productivos. En segundo término, los antioxidantes se utilizan para mejorar la estabilidad oxidativa de los productos obtenidos, como son la carne y la leche. Los antioxidantes pueden ser modificados por el tracto digestivo, ser o no absorbidos y acumularse en unos tejidos y no en otros, por lo que un compuesto que presenta propiedades antioxidantes *in vitro* puede mostrar otros efectos cuando se administra directamente en la dieta de los animales. Además, si las dosis *in vivo* no son adecuadas puede producirse el efecto contrario incrementando el daño oxidativo (Rietjens *et al.,* 2002).

2.1.2. Clasificación

Los antioxidantes se clasifican en función de su naturaleza (enzimáticos o no enzimáticos), sus propiedades físico-químicas (hidrosolubles o liposolubles), su estructura, su mecanismo de acción (primarios o secundarios) o su origen (sintético o natural) (Vertuani *et al.,* 2004), siendo esta última, la clasificación más empleada en alimentación animal.

Los antioxidantes sintéticos, mayoritariamente derivados de estructuras fenólicas con diferentes grados de sustitución alquílica, se incluyen de forma habitual en los piensos compuestos. Sin embargo, debido a sus posibles efectos perjudiciales sobre la salud animal y humana (Botterweck *et al.,* 2000), su uso se está cuestionando desde el punto de vista de su seguridad. Los antioxidantes sintéticos más empleados son: el butilhidroxianisol (BHA), el butilhidroxitolueno (BHT), el propilgalato (PG), el palmitato de ascorbilo, la terbutilhidroquinona (TBHQ), el ácido etiléndiaminotetraacético (EDTA), los ésteres del ácido gálico y la vitamina E de síntesis.

Actualmente la industria alimentaria y los consumidores demandan el empleo de ingredientes o aditivos naturales en sustitución de los denominados sintéticos. Por ello existe un claro interés de búsqueda, reconocimiento y aplicación de antioxidantes extraídos de fuentes naturales en forma de compuestos puros, extractos y/o aceites esenciales. El reino vegetal ofrece una gran variedad de compuestos con propiedades antioxidantes, son las llamadas sustancias fitoquímicas, que incluyen grupos de compuestos de naturaleza química diversa y propiedades muy variadas, y juegan un papel importante en el mantenimiento del equilibrio redox y en la disminución de la incidencia del daño producido por los radicales libres. Los principales antioxidantes de origen natural son las vitaminas, los carotenoides y los compuestos fenólicos. La Tabla 2.1. recoge los antioxidantes de origen natural más importantes, así como su procedencia, características y mecanismo de acción.

Tabla 2.1. Procedencia, características y mecanismo de acción de los principales antioxidantes de origen natural.

Compuesto	Procedencia	Características y mecanismo de acción	Referencia
Vitamina C o ácido ascórbico	Frutas y vegetales frescos	Actúa en medio acuoso. Reacciona de forma directa con radicales superóxido, hidroxilo e hidroperóxidos lipídicos y neutraliza los radicales peroxilo. Actúa sinérgicamente con el α-tocoferol (Vitamina E).	Chihuailaf *et al.,* 2002
Vitamina E o α-tocoferol	Aceites vegetales, frutos secos, verduras, forrajes y pastos frescos	El término vitamina E incluye ocho moléculas de origen vegetal, siendo el α-tocoferol el que presenta mayor actividad biológica. Se trata de una sustancia de naturaleza lipofílica con alta capacidad antioxidante debido a su efecto inhibitorio sobre la propagación en cadena de radicales libres, mediada por su propia conversión en productos oxidados. Actúa de forma sinérgica con otras vitaminas (A, C, β-carotenos), enzimas y otros antioxidantes. El α-tocoferol también está disponible comercialmente para alimentación animal en forma sintética.	Bramley *et al.,* 2000; Tucker y Townsend, 2005
Carotenoides	Frutas, verduras, hortalizas, forrajes y pastos frescos, algas y algunas clases de hongos y bacterias	Son pigmentos orgánicos de carácter lipídico. Los más conocidos son el β-caroteno, que es precursor de la vitamina A, y el licopeno. Pueden captar oxígeno singlete y radicales peroxilo, mediante transferencia de electrones o sustracción de átomos de hidrógeno. También pueden presentar efectos sinérgicos con otros antioxidantes, como la vitamina E o la vitamina C.	El-Agamey *et al.,* 2004
Aceites esenciales	Plantas aromáticas y medicinales, frutas, semillas	Son insolubles en agua y están constituidos mayoritariamente por terpenos mezclados con otros compuestos como fenoles, alcaloides y/o aminoácidos. Su efecto antioxidante se debe principalmente a sus componentes fenólicos.	Acamovic y Brooker, 2005; Leopoldini *et al.,* 2011
Ácidos orgánicos	Frutas y vegetales	Son moléculas de bajo peso molecular que contienen uno o más grupos carboxilo, que les confiere actividad antioxidante mediante el secuestro de radicales libres. Algunas de ellas son el ácido cítrico, ácido málico, ácido tartárico, ácido láctico, ácido sórbico, etc.	López-Bucio *et al.,* 2000
Minerales antioxidantes	Cereales, ajo, champiñones, semillas de girasol, frutas, fuentes minerales	Algunos minerales contribuyen a la protección de las células frente al daño oxidativo contra los radicales libres. Entre ellos se encuentran el selenio, que aumenta la actividad de algunas enzimas antioxidantes, presentando además, un mecanismo de acción estrechamente relacionado con el de la vitamina E. Otros minerales con efecto antioxidante son el manganeso, el cobre y el zinc.	Ripoll *et al.,* 2011
Compuestos fenólicos	Raíces, tallos, troncos, hojas, frutas y arbustos	Su actividad antioxidante se basa en su capacidad secuestradora de radicales libres y quelación de metales. Se clasifican habitualmente en no flavonoides y flavonoides.	Urquiaga y Leighton, 2000

2.2. Los compuestos fenólicos

Los compuestos fenólicos o polifenoles son sustancias procedentes del metabolismo secundario de las plantas, que constituyen uno de los grupos de antioxidantes naturales más abundantes. Se han identificado más de 8.000 compuestos que se encuentran ampliamente distribuidos en el reino vegetal (Dreosti, 2000).

Estos compuestos agrupan un amplio conjunto de moléculas caracterizadas por poseer un anillo aromático (benceno) con al menos un grupo hidroxilo (grupo fenol) (Figura 2.2.).

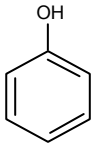
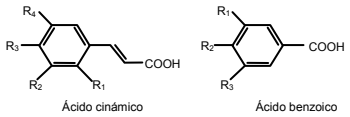
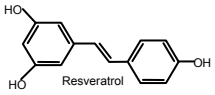
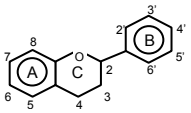
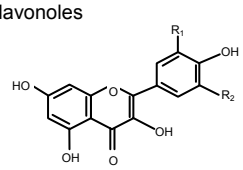
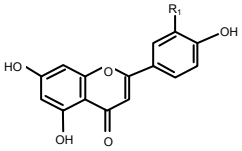
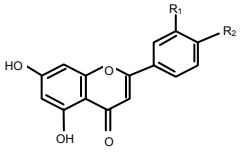
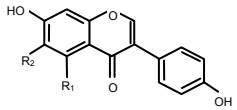
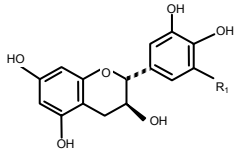
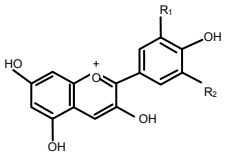


Figura 2.2. Grupo fenol de los polifenoles.

La estructura de los polifenoles varía de moléculas simples como los ácidos fenólicos, a estructuras complejas, como los taninos condensados. Se clasifican en función del número de anillos fenólicos y de los elementos estructurales unidos a esos anillos (Carratù y Sanzini, 2005) en no flavonoides y flavonoides. Los compuestos no flavonoides incluyen los ácidos fenólicos, estilbenos y lignanos. Los compuestos flavonoides se pueden clasificar en seis subclases: flavonoles, flavonas, flavanonas, isoflavonas, flavanoles, que a su vez se subdividen en catequinas y en taninos catequíticos (procianidinas y prodelfinidinas), y antocianidinas (Heim *et al.,* 2002) (Tabla 2.2).

La actividad antioxidante de los compuestos polifenólicos se basa en su capacidad secuestradora de radicales libres y quelación de metales (Urquiaga y Leighton, 2000; Heim *et al.,* 2002), inhibiendo la peroxidación de lípidos. Su estructura química es ideal para reaccionar con los radicales libres y formar un radical intermedio más estable y menos reactivo, ya que la presencia de anillos aromáticos y grupos hidroxilo permiten que se deslocalicen los electrones (Sang *et al.,* 2002). Algunos polifenoles, además de su propia acción antioxidante, pueden potenciar la actividad de enzimas antioxidantes (Metin-Donma y Donma, 2005), así como inhibir las enzimas responsables de la producción de superóxidos (Ursini *et al.,* 1994), e interaccionar con otras enzimas relacionadas con la oxidación (Kandaswami y Middleton, 1994).

Tabla 2.2. Clasificación, origen y características de los compuestos fenólicos.

Compuesto fenólico	Origen	Características
Compuestos no flavonoides		
- Ácidos fenólicos 	Uvas, vino, té, hierba, hojas de árboles, arbustos y leguminosas herbáceas	Presentan un solo anillo bencénico. Se dividen en ácidos cinámicos y ácidos benzoicos. Los ácidos hidroxibenzoicos son componentes de estructuras complejas como los taninos hidrolizables.
- Estilbenos 	Uvas y vino	Poseen dos ciclos bencénicos unidos por una cadena de etano o de etileno. Entre ellos destaca el resveratrol y el piceido.
- Lignanos	Semillas y bayas	Se consideran fitoestrógenos. Algunos de ellos son la enterolactona y el enterodiol.
Compuestos flavonoides 		Los flavonoides son los compuestos polifenólicos más comunes. Tienen bajo peso molecular y están formados por dos ciclos bencénicos (A y B) unidos por un anillo heterocíclico oxigenado (C).
- Flavonoles 	Vino tinto, té, verduras, cebolla, escarola y brócoli	Son los flavonoides más abundantes en los alimentos. Se presentan en forma glicosilada. Los principales son el kaempferol, el quercetol, el miricetol, y el isoramnetol.
- Flavonas 	Perejil y apio	No son tan abundantes como los flavonoles. Las más representativas son la luteolina y la apigenina.
- Flavanonas 	Cítricos, tomate y plantas aromáticas	Se presentan glicosiladas y las más importantes son la naringenina en el pomelo, la hesperedina en las naranjas y el eriodictiol en los limones.
- Isoflavonas 	Leguminosas como la soja, la alfalfa o el trébol	Se las clasifica como fitoestrógenos. Las más importantes son la genisteína y la daidzeína.
- Flavanoles 	Té, vino tinto, chocolate, frutas, hojas de árboles, arbustos y leguminosas herbáceas	Se presentan en forma monomérica (catequinas) y en forma polimerizada constituyendo los taninos catequíticos o taninos condensados, que se subdividen en proantocianidinas y prodelfinidinas.
- Antocianidinas o antocianos 	Frutas, vino tinto, cereales, vegetales	Son los pigmentos responsables de la coloración de las flores y frutos. Químicamente se caracterizan por dos anillos bencénicos unidos por un anillo heterocíclico, del tipo pirano o pirilio. Hay seis comunes: malvidina, delfinidina, peonidina, cianidina, petunidina y pelargonidina.

Además de sus propiedades antioxidantes, los polifenoles poseen otras actividades biológicas específicas derivadas o no de su acción antioxidante. Se les atribuye propiedades antimicrobianas y antimutagénicas, inhiben *in vitro* la oxidación de las lipoproteínas de baja densidad relacionadas con enfermedades coronarias y protegen el ADN del daño oxidativo relacionado con algunos cánceres (Yang *et al.,* 2001; Hou *et al.,* 2004). También inhiben la agregación plaquetaria y presentan efectos antiinflamatorios (Middleton *et al.,* 2000), antiapoptóticos, antialérgicos (Piñeiro *et al.,* 2006), acciones vasodilatadoras y vasoprotectoras, acciones antitrombóticas, antilipémicas, antiateroscleróticas (Quiñones *et al.,* 2013), propiedades beneficiosas para la diabetes (De Pascual-Teresa y Sánchez-Ballesta, 2008) y enfermedades oftalmológicas (Lee *et al.,* 2005). Además, ha sido descrito que poseen actividad antiVIH, que actúan como protectores frente a la peroxidación lipídica en los glóbulos rojos (Urquiaga y Leighton, 2000), y que presentan efectos positivos en relación con enfermedades neurodegenerativas como el alzheimer, etc.

2.3. Compuestos fenólicos y nutrición de rumiantes

La literatura disponible refleja que un amplio número de compuestos fenólicos, entre los que destacan los flavonoides, especialmente los taninos condensados, y los aceites esenciales, presentes en numerosas plantas y frutas, y por tanto también en diversos residuos agroindustriales, están relacionados con variaciones en los procesos fermentativos y en la utilización digestiva de los alimentos, afectando a la salud y a los rendimientos productivos de los animales que los consumen (Hervás *et al.,* 2003; McAllister *et al.,* 2005). De este modo, se han señalado mejoras en el crecimiento, en el peso y rendimiento a la canal y en la producción de leche, de proteína y de lactosa. También se han encontrado mejoras en la prolificidad de algunos rebaños. Además, el efecto antimicrobiano de los compuestos fenólicos puede evitar las reducciones de los rendimientos productivos de los animales que se producen cuando se presentan distintas enfermedades infecciosas y parasitarias (Min *et al.,* 2003).

Los compuestos fenólicos más estudiados son los taninos. En la naturaleza existen dos tipos distintos de taninos: los taninos condensados y los taninos hidrolizables, con estructuras químicas (Tabla 2.2), y efectos distintos sobre los animales que los consumen. Así, el efecto de los taninos en la nutrición de los rumiantes puede ser beneficioso o perjudicial dependiendo del tipo de tanino, de su estructura química y de su peso molecular, así como de la dosis ingerida y de la fisiología de la especie que los consuma (Frutos *et al.,* 2004).

Los taninos se han asociado a cambios en la ingestión voluntaria, en la fermentación ruminal, en la digestibilidad y, como consecuencia, en los rendimientos productivos de los animales.

En general, la ingestión voluntaria se reduce, o incluso puede detenerse con consumos de taninos condensados superiores a 50 g por kg de materia seca (MS) y no parece verse afectada con ingestiones medias o bajas (inferior a 50 g por kg de MS). Los mecanismos sugeridos para explicar el efecto de los taninos sobre el consumo voluntario son la reducción de la palatabilidad, la ralentización de la digestión y el desarrollo de aversiones condicionadas (Frutos *et al.,* 2004).

La característica principal de los taninos es su capacidad para formar complejos reversibles con las proteínas. Estos complejos, estables e indegradables a pH ruminal, se disocian por efecto del pH ácido del abomaso (3,5) o pH básico del duodeno (8) permitiendo mayor disponibilidad de aminoácidos (muchos de ellos esenciales) en el intestino delgado. De hecho, el empleo de taninos se ha propuesto como método de protección de la proteína de la dieta frente a la degradación ruminal (Frutos *et al.,* 2000). La reducción de la degradación proteica a nivel ruminal depende de la dosis de taninos empleada y también ha sido relacionada con las propiedades antimicrobianas de los taninos (Davidson y Naidu, 2000).

Aunque la acción de los taninos en el rumen es ejercida principalmente sobre las proteínas, también se han observado reducciones en la degradación de los hidratos de carbono particularmente sobre la celulosa, la hemicelulosa, el almidón y las pectinas (Makkar *et al.,* 1995; Bento *et al.,* 2005). La formación de macromoléculas glucídicas, la inhibición de enzimas microbianas y la acción directa sobre los microorganismos del rumen han sido señalados como posibles mecanismos por los que los taninos pueden reducir la degradación ruminal de la fibra (Björck y Nyman, 1987; McSweeney *et al.,* 2001).

El efecto de los taninos sobre degradación de la fibra y la proteína puede verse reflejado en la producción de ácidos grasos volátiles (AGV) y en sus proporciones molares. En este sentido, en algunos casos ha sido evidenciada una menor producción de AGV al incluir taninos en la dieta (Frutos *et al.,* 2004; Besharati y Taghizadeh 2009).

Algunos autores también han señalado menor digestibilidad al incorporar taninos en las raciones. Sin embargo, la menor digestibilidad no siempre impide la retención de nitrógeno en los animales, ya que es posible que el mayor flujo de proteína del rumen que se produce

al incorporar taninos condensados compense la reducción en la capacidad de absorción de aminoácidos a nivel del intestino delgado.

Además de los taninos, otros tipos de compuestos fenólicos pueden ejercer efectos variables sobre los rendimientos productivos de los animales. En general, al igual que ocurre con los taninos, el efecto de otros compuestos fenólicos como los aceites esenciales varía dependiendo del tipo, de la dosis empleada, de la composición de la dieta y de las condiciones experimentales en las que se suministren a los animales (Castrillejos *et al.,* 2007; Carro *et al.,* 2014). No obstante, existe poca información acerca de su efecto sobre la ingestión y los rendimientos productivos en rumiantes, mostrando en ocasiones, resultados muy dispares.

En este sentido, Chaves *et al.* (2008) observaron mayores rendimientos productivos en corderos en crecimiento al incluir diferentes aceites esenciales en sus dietas sin que la ingestión se viese afectada. En otras ocasiones, no ha sido señalado ningún efecto sobre la producción al incluir en dietas de rumiantes aceites esenciales (Simitzis *et al.,* 2008), subproductos ricos en flavonoides como cítricos (Inserra *et al.,* 2014), flavanonas purificadas como la naringina (Bodas *et al.,* 2012), ó la hesperidina (Simitzis *et al.,* 2013), flavonoles como la quercetina (Andrés *et al.,* 2013), u otros compuestos fenólicos procedentes de plantas aromáticas como romero, tomillo u orégano (Moñino *et al.,* 2008; Nieto *et al.,* 2011; Bampidis *et al.,* 2005).

También han sido encontradas evidencias de efectos beneficiosos o no perjudiciales al incorporar este tipo de compuestos fenólicos sobre los rendimientos lecheros de vacas (Ferlay *et al.,* 2010; Hristov *et al.,* 2013), ovejas (Chiofalo *et al,* 2012; Toral *et al.,* 2013) y cabras (Jordán *et al.,* 2010).

2.4. Calidad de carne

2.4.1. Concepto de calidad de carne y parámetros que definen la calidad de la carne

El concepto de “calidad” es un término relativo, comparable y subjetivo, por lo que resulta difícil conmensurar una definición. Este término, aplicado a la producción cárnica, depende del punto de vista desde donde se enfoque, ya que cada uno de los eslabones de la cadena alimentaria lo considera diferente. El concepto de “calidad” ha ido evolucionando dentro de la industria productora de alimentos de origen animal, siguiendo el desarrollo del

conocimiento científico, los avances en los métodos analíticos y las experiencias de los consumidores. De este modo, “calidad” podría definirse como “el conjunto de características cuya importancia relativa le confiere al producto un mayor grado de aceptación y un mayor precio frente a los consumidores o frente a la demanda del mercado” (Colomer-Rocher, 1988). Asimismo, Hammond (1955) definió calidad como “aquello que gusta al consumidor y por lo que está dispuesto a pagar más que el precio medio”.

Debido al aumento de la utilización de aditivos alimentarios, fármacos y nuevos ingredientes, a esta definición se le han ido incorporando cada vez más conceptos relacionados con la salud del consumidor, como la seguridad del alimento, el bajo contenido energético o la presencia de vitaminas y minerales. Como consecuencia, durante la década de los 90 el concepto de calidad en los productos alimentarios se definió como “calidad total” (Nardone y Valfrè, 1999), lo cual implica el análisis de las características nutricionales, tecnológicas, organolépticas e higiosanitarias. La composición química y el perfil de ácidos grasos determinan la calidad nutricional; el pH y la capacidad de retención de agua (CRA) definen la calidad tecnológica; el color, la textura y el flavor describen la calidad sensorial y organoléptica, y la microbiología determina la calidad higiosanitaria.

Los parámetros que determinan la calidad de la carne dependen de factores inherentes al animal (genética, raza, sexo, edad y peso de sacrifico, tipo de músculo, etc.), así como de factores extrínsecos, relativos al manejo, especialmente a la alimentación que reciben y a factores *post mortem*.

Los dos principales factores que limitan la calidad de la carne y los productos cárnicos, son el deterioro microbiano y la oxidación. Estos procesos están interconectados entre sí y relacionados con la degradación del color y con la aparición de olores y sabores desagradables, que hacen al producto indeseable para el consumo humano (Ercolini *et al*., 2006). La vida útil de la carne se puede prolongar, entre otras estrategias, mediante la adición de antioxidantes y/o antimicrobianos a la carne (Ahn *et al.,* 2002). La única forma aceptada actualmente para incorporar antioxidantes a la carne pasa necesariamente por su inclusión en la dieta de los animales. La inclusión de antioxidantes en las raciones, especialmente de vitamina E, presenta gran influencia sobre los parámetros que definen la calidad de la carne. De hecho, ésta ha sido la estrategia habitualmente empleada para mejorar la estabilidad oxidativa y evitar el deterioro posterior de la carne. La vitamina E juega un papel importante en la prevención de los efectos negativos que los radicales libres causan en los tejidos, particularmente en la oxidación de los lípidos de las membranas de las células, en la reducción de las pérdidas por goteo y en la estabilización del color de la

carne (López-Bote *et al.,* 2001). La tendencia actual se dirige hacia la búsqueda de compuestos naturales de origen vegetal, con propiedades antioxidantes, susceptibles de ser incluidos en raciones animales.

2.4.2. Efecto de los compuestos fenólicos sobre la calidad de la carne

Composición química

La carne es un alimento con alto valor nutritivo, ya que proporciona gran cantidad de energía y nutrientes esenciales. La composición química de la carne permite establecer su calidad nutritiva y está íntimamente relacionada con sus características tecnológicas, higiénicas y organolépticas. De acuerdo con la Tabla 2.3, el componente mayoritario de la carne es el agua que oscila entre el 70 y el 80%. El contenido en proteína presenta un valor medio del 20% y es de gran importancia desde el punto de vista nutricional debido a su contenido en aminoácidos esenciales y a su alto valor biológico. La grasa es el componente más variable y se encuentra en el tejido adiposo y en el tejido muscular. La proporción de grasa del músculo aumenta a medida que lo hace el peso de sacrificio y la edad del animal, presentando valores medios del 2,5% en el músculo de corderos lechales y del 8% en ovino mayor. Esta tendencia es más notoria en el caso del depósito de grasa subcutánea que en el de la grasa intramuscular. Los triglicéridos, los fosfolípidos y el colesterol son los componentes lipídicos de mayor interés y con mayor importancia desde el punto de vista cuantitativo. Además, la carne contiene un gran número de elementos en menor proporción pero no menos importantes, como son las vitaminas, los minerales y los ácidos grasos esenciales (Díaz *et al.,* 2005).

Tabla 2.3. Composición química del músculo *longissimus* de las distintas categorías comerciales de ganado ovino.

%	Lechal [1]	Cordero ligero [2]	Ovino mayor [3]
Agua	76,3	73,3	70,6
Proteína	22,8	20,6	20,6
Grasa	2,47	3,87	8,68
Cenizas	1,10	1,71	0,11

[1] D'Alessandro *et al*. (2012); [2] Rodríguez (2005); [3] Murphy *et al*. (1994)

Los procesos oxidativos que tienen lugar durante el almacenamiento de la carne pueden modificar su composición química. El contenido en agua de la carne y la retención de ésta durante su almacenamiento o tras el cocinado, tiene gran influencia sobre la textura y la jugosidad. En este sentido, la oxidación proteica provoca pérdida de parte del agua y genera una percepción de "sequedad" en el consumidor (Lund *et al.,* 2011). Asimismo, la oxidación proteica también puede perjudicar la biodisponibilidad de ciertos aminoácidos (Davies, 1987).

En general la carne es una buena fuente de minerales, a excepción del calcio, siendo muchos de ellos cofactores de enzimas antioxidantes, que también pueden actuar como catalizadores de la oxidación y, por tanto, como pro-oxidantes. Además, la carne es una excelente fuente de vitaminas hidrosolubles del grupo B y vitaminas liposolubles A, D, E y K que se encuentran principalmente en la grasa. En relación con la oxidación, muchas vitaminas juegan un papel importante como antioxidantes no enzimáticos.

Para evitar los efectos negativos de la oxidación sobre la composición de la carne, se emplean antioxidantes en las raciones que pueden ser de origen sintético (cómo la vitamina E) o natural. En este sentido, el efecto de la inclusión de compuestos fenólicos en dietas de rumiantes ha sido poco estudiado, siendo los resultados obtenidos en muchos casos contradictorios. Así, Morán *et al*. (2012a) al incluir ácido carnósico procedente del romero en dietas de corderos observó mayores niveles de proteína bruta en el músculo. Sin embargo, Nieto *et al*. (2012) al incluir hojas de tomillo en ovejas, observo menor contenido en proteína y mayor de grasa en la carne de cordero y Vasta *et al.* (2007) encontró mayor contenido en proteína, pero menor de grasa al añadir taninos en dietas de corderos en comparación con la carne de corderos cuyas mismas dietas fueron suplementadas con polietilenglicol (compuesto que presenta gran afinidad con los taninos y es capaz de prevenir la formación de complejos tanino-proteína).

En general, las variaciones en la composición química de la carne al incluir en dietas de ganado ovino diferentes fuentes de compuestos fenólicos como los taninos (Priolo *et al.,* 2000; 2005), el flavonol quercetina (Andrés *et al.,* 2013) o extractos de romero y tomillo (Nieto, 2013) han sido mínimas, y tampoco se han observado variaciones en el contenido mineral de la carne (Ca, Zn, Fe y Cu) (Norouzian y Ghiasi, 2012) a pesar del efecto quelante de los polifenoles sobre algunos metales (Zhou *et al.,* 2006).

Perfil de ácidos grasos

El perfil lipídico de la carne es un factor que presenta gran importancia debido a su relación con la salud humana. La carne de rumiantes en general, y la del ganado ovino en particular, se caracteriza por presentar un perfil de ácidos grasos altamente saturado, siendo el ácido oleico (C18:1), el ácido palmítico (C16:0) y el ácido esteárico (C18:0) los más abundantes (Bas y Morand-Fehr, 2000; Castro *et al.,* 2005) (Tabla 2.4).

Tabla 2.4. Perfil de ácidos grasos mayoritarios (% de ácidos grasos totales) de la carne de las distintas categorías comerciales de ganado ovino.

	Lechal [1]	Ternasco [2]	Ovino mayor [3]
C14:0	4,11	2,64	-
C16:0	20,5	25,2	24,0
C18:0	12,0	16,4	11,5
trans-11 C18:1	1,48	-	0,54
cis-9 C18:1	29,3	37,0	35,1
C18:2 n6	11,0	5,99	9,61
cis-9 trans -11 C18:2	1,13	0,35	0,05
C18:3 n3	1,95	0,44	0,56
C20:5	1,65	0,51	0,25
C22:5	2,34	0,80	0,63
C22:6	1,25	0,42	0,14
SFA[4]	37,7	43,0	35,8
MUFA[5]	34,9	43,7	42,9
PUFA[6]	27,4	13,3	16,7
n6/n3	2,61	14,9	9,86

[1] Músculo *longissimus dorsi* (Lanza *et al.,* 2006). [2] Músculo *longissimus lumborum* (Manso *et al.,* 2009). [3] Músculo *extensor carpi radialis* (Berthelot *et al.,* 2010).

[4] SFA, ácidos grasos saturados; [5] MUFA: ácidos grasos monoinsaturados, [6] PUFA, ácidos grasos poliinsaturados.

La grasa de los rumiantes difiere notablemente de la grasa que consumen, ya que los ácidos grasos que abandonan el rumen son distintos de los presentes en la dieta. Este hecho se produce como consecuencia del metabolismo de los lípidos en el rumen. La digestión ruminal de la grasa incluye una fase de hidrólisis o lipolisis y otra de hidrogenación.

La hidrólisis de los lípidos de la dieta tiene lugar en el rumen por la acción de lipasas, galactosidasas y fosfolipasas de origen microbiano y, posteriormente, los ácidos grasos insaturados que se liberan en el rumen son biohidrogenados. La biohidrogenación (BH) se

produce en dos etapas por la acción de enzimas generadas por bacterias celulolíticas, tales como *Butyrivibrio fibrisolvens* (Kepler y Tove, 1967). En primer lugar, las enzimas isomerasas transforman los dobles enlaces con configuración *cis*, mayoritarios en los ácidos grasos de los alimentos de origen vegetal, en enlaces *trans*, más estables a nivel ruminal, y por último, las reductasas se encargan de hidrogenar progresivamente las insaturaciones.

Los ácidos grasos poliinsaturados (PUFA) mayoritarios en los alimentos que consumen los rumiantes (forrajes, cereales, semillas de oleaginosas y subproductos) son el ácido linoleico (*cis-9 cis-12* C18:2) y el α-linolénico (*cis-9 cis-12 cis-15* C18:3 n3). Por ello, las rutas de BH de éstos ácidos grasos han sido las más estudiadas y los intermediarios producidos mejor identificados. Así, tal y como muestra la Figura 2.3, la BH del ácido linoleico tiene lugar en 3 pasos: uno inicial de isomerización mediante el cual el doble enlace *cis* en posición 12 del ácido linoleico pasa a configuración *trans-11* y da lugar al ácido ruménico (*cis-9 trans-11* C18:2, RA). Posteriormente, tiene lugar una primera y rápida hidrogenación del enlace *cis-9* para dar lugar a ácido vacénico (*trans-11* C18:1, VA) que, después de una segunda hidrogenación, origina ácido esteárico (C18:0) (Shingfield *et al.,* 2010). La gran estabilidad que posee la configuración del enlace *trans-11* a nivel ruminal hace que la conversión de VA a ácido esteárico sea la etapa más limitante de todo el proceso, lo cual provoca, bajo determinadas condiciones, una acumulación de dicho ácido graso en el fluido ruminal antes de su paso al intestino delgado para ser absorbido. La BH del ácido α-linolénico también pasa por la formación de VA, sin embargo, no genera RA, sino intermediarios distintos (*cis*-9 *trans-11 cis-15* C18:3 y *trans-11 cis-15* C18:2). La isomerización del enlace *cis-12* y la conversión de VA procedente del ácido α-linolénico a esteárico es idéntica a la descrita para el ácido linoleico.

Existe un gran interés por modificar el perfil lipídico de la carne y adaptarlo a las recomendaciones para la salud humana. Por ello, en los últimos años, numerosos esfuerzos de investigación se han dirigido a incrementar el contenido en la carne de ácidos grasos insaturados asociados a efectos saludables (PUFA n3, RA y VA) (Simopoulos, 1999).

En numerosos estudios en ganado ovino se ha comprobado que, cuando se incorporan materias primas ricas en ácido linoleico y α-linolénico se favorece la formación de ácidos grasos funcionales en el rumen (RA y VA) y su presencia en la leche y en la carne. La suplementación de rumiantes con dietas ricas en PUFA n3 también se ha convertido en una práctica frecuente para mejorar la calidad nutricional de la grasa de los productos animales, incrementando de este modo, la proporción de PUFA n3 en los tejidos.

Uno de los problemas asociados a la mayor insaturación de la grasa, es su mayor susceptibilidad a la lipoperoxidación (Gladine *et al.,* 2007a), que provoca problemas durante el almacenamiento, procesado y cocinado de la carne, contribuyendo a disminuir la aceptación del producto por parte del consumidor.

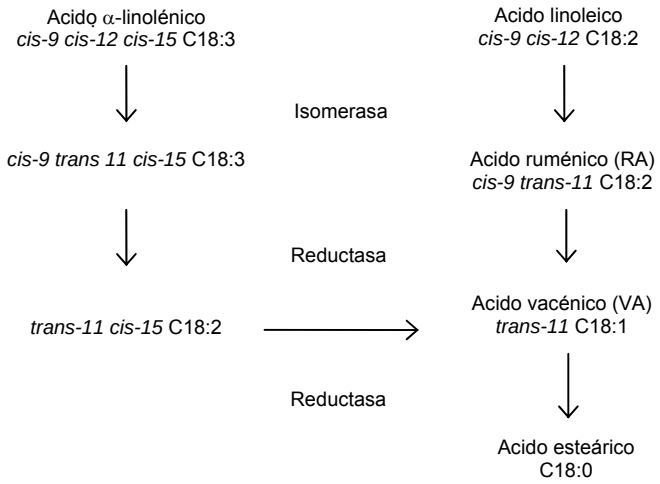


Figura 2.3. Biohidrogenación del ácido linoleico y α-linolénico a nivel ruminal. Adaptada de Harfoot y Hazlewood (1997) y Griinari y Bauman (1999).

La incorporación en dietas de rumiantes de antioxidantes en general, puede interferir sobre el perfil de ácidos grasos de la carne. En este sentido algunos autores han indicado cambios en los procesos de BH a nivel ruminal al incluir vitamina E en la dieta por su efecto inhibitorio sobre *Butyrivibrio fibrisolvens*, provocando un descenso en el nivel de algunos ácidos grasos *trans* perjudiciales para la salud humana como es el caso del *trans-10* C18:1 (Pottier *et al.,* 2006) generados en rutas alternativas del proceso de BH.

Los compuestos fenólicos son un interesante ejemplo de metabolitos secundarios procedentes de plantas con prometedoras posibilidades de utilización en alimentación animal en relación con su efecto sobre el perfil lipídico de los productos generados (Vasta y Luciano, 2011). Dentro de la familia de los compuestos fenólicos, los taninos condensados han sido ampliamente estudiados.

La inclusión de diversas fuentes de taninos condensados en la dieta de rumiantes ha sido planteada como una estrategia efectiva para la modulación de la BH de los PUFA a

nivel ruminal y para mejorar el valor nutricional de la grasa de los rumiantes. En este sentido, ha sido indicado que estos polifenoles son capaces de obstaculizar el último paso de la BH (conversión de *trans-11* C18:1 a C18:0), favoreciendo la acumulación de intermediarios como el VA y el RA y del contenido de ácidos grasos poliinsaturados totales, en detrimento de los ácidos grasos saturados (SFA). Estos efectos han sido corroborados al incluir diversas fuentes de taninos en dietas de pequeños rumiantes, en el líquido ruminal en estudios *in vitro* (Vasta *et al.,* 2009a), así como en el músculo de cordero en trabajos *in vivo* (Priolo *et al.,* 2005; Vasta *et al.,* 2009b).

El efecto de los taninos condensados sobre el perfil lipídico de la carne depende de la dosis empleada y del tipo de tanino. En la actualidad no es posible predecir con exactitud el efecto dosis-respuesta de los taninos ya que su mecanismo de acción no ha sido completamente aclarado (Vasta y Luciano, 2011). Así, algunos trabajos han indicado que los taninos además de interferir sobre la población microbiana del rumen responsable de la BH, también pueden incrementar la actividad de la enzima Δ^9-desaturasa y favorecer la síntesis endógena de RA en los tejidos animales por desaturación del VA (Vasta *et al.,* 2009b).

Además de los taninos, otros tipos de compuestos fenólicos, que habitualmente están presentes en subproductos de la industria alimentaria y en derivados de plantas aromáticas han sido estudiados. En este sentido, al incluir subproductos de romero y tomillo (Nieto*,* 2013) en raciones de ovejas en lactación también se ha observado un descenso de SFA y un aumento de ácidos grasos insaturados (UNSFA) y PUFA en la carne de los corderos, similar a lo que ocurre con los taninos. Asimismo, Vasta *et al.* (2013) observaron que el aceite esencial de artemisia en dietas de corderos de cebo induce la acumulación de VA, RA, y C18:3 con interés desde el punto de vista de la salud humana. Sin embargo, otros estudios (Chaves *et al.,* 2008; Vasta *et al.,* 2013) al incluir otros aceites esenciales en dietas de cordero apenas han observado efecto sobre el perfil lipídico de la carne. La actividad antimicrobiana de los distintos compuestos fenólicos y el hecho de que los compuestos fenólicos puedan ser degradados por los microorganismos podrían explicar la disparidad de resultados encontrados (Broudiscou *et al.,* 2007).

De forma general, el efecto de los polifenoles de la dieta sobre el perfil lipídico de la carne de cordero varía dependiendo del tipo de compuesto fenólico, de la dosis de inclusión y de la composición de las raciones. En este sentido, ha sido señalado que el efecto de los polifenoles sobre el perfil lipídico de la carne es más evidente cuando se emplean grasas muy insaturadas en las raciones. Así, Andrés *et al.* (2014a) al incluir el flavonol quercetina

observó un incremento en el nivel de RA de la carne de cordero únicamente cuando la dieta fue suplementada con aceite de linaza.

pH

El pH de la carne está relacionado con los procesos bioquímicos de transformación del músculo en carne. Por ello, su evolución *post mortem* y su valor final influye sobre las características organolépticas de la carne. Después de la muerte del animal cesa el aporte de oxígeno y las reservas de energía son utilizadas para la síntesis ATP y para mantener la estructura del músculo. A medida que se agota el ATP, aparece fosfato inorgánico que estimula la glucólisis anaerobia. Como resultado, se forma ácido láctico y otros ácidos inorgánicos que provocan el descenso del pH muscular hasta que se agotan las reservas de glucógeno o hasta la inactivación de las enzimas celulares (Lawrie, 1983). En ovino, el pH del músculo está entre 7,08 y 7,30, mientras que el pH a las 24h *post mortem* en el músculo de referencia (*longissimus dorsi*) oscila entre 5,4 y 5,8 (Sen *et al.,* 2004; Vieira *et al.,* 2012).

De forma general, el pH no varía con los niveles de oxidación ni viceversa. Sin embargo, la inclusión de subproductos o extractos vegetales ricos en polifenoles en dietas de rumiantes puede afectar al pH final de la carne, ya que estos compuestos pueden interferir en el estatus nutritivo de los animales y en las reservas de glucógeno del músculo antes del sacrificio. Así, Priolo *et al*. (2000) y Simitzis *et al.* (2008) encontraron valores más altos en el pH de la carne de cordero asociados con déficits nutritivos y situaciones de estrés al incluir taninos condensados y aceites esenciales en la dieta. En otros trabajos, al incluir taninos condensados (Vasta *et al.,* 2007), flavanonas como la hesperidina (Simitzis *et al.,* 2013) o derivados de romero y/o tomillo en dietas de corderos (Bañón *et al.,* 2012; Morán *et al.,* 2012a; Nieto, 2013) no se observaron variaciones en el crecimiento de los animales y el pH tampoco se vio afectado.

Color

El color es una de las principales características de la carne fresca mediante la cual los consumidores juzgan su frescor y calidad. Esta coloración viene determinada por la cantidad de pigmentos que contenga el músculo y por el estado químico en que éstos se encuentren. La mioglobina es el pigmento hemínico mayoritario y el que le confiere un color rojo. Se

caracteriza por la existencia de un grupo hemo con un átomo central de hierro, que es susceptible de unirse con un número limitado de compuestos, e interviene directamente en el proceso de oxidación. La forma reducida de la mioglobina es de color púrpura, pero durante la exposición al aire, se combina con el oxígeno y se forma la oximioglobina de color rojo brillante, tonalidad que es característica de carne fresca y considerada atractiva por los consumidores. Sin embargo, la exposición prolongada de la mioglobina al medio, da lugar a la transformación del hierro desde su forma reducida (Fe^{2+}) a su forma oxidada (Fe^{3+}), siendo el compuesto resultante de dicha oxidación, la metamioglobina, que no puede unirse con el oxígeno y presenta un desagradable color marrón.

Por tanto, el color de la carne fresca está definido por la cantidad relativa de tres derivados de la mioglobina: la mioglobina reducida, la oximioglobina y la metamioglobina (Mancini y Hunt, 2005). Para su valoración en la carne se utilizan diversos métodos, tanto objetivos como subjetivos. Entre los métodos objetivos, se encuentran las técnicas que utilizan colorímetros triestímulo o espectrofotómetros de reflectancia, ya que las medidas obtenidas presentan buena correlación con los métodos de evaluación sensorial del color. Estos equipos realizan las medidas en diferentes espacios de color, siendo el espacio CIE*L*a*b* el más frecuentemente utilizado. La metodología CIE*L*a*b** mide el color de tal manera que, el espacio del espectro visible se divide en los ejes cartesianos x, y, z, los cuales se corresponden con las coordenadas tricromáticas: luminosidad (*L**) (blanco-negro), índice de rojo (*a**) (rojo-verde) e índice de amarillo (*b**) (amarillo-azul). Estos valores, a su vez, sirven para calcular los parámetros de tono (Hue; $H^* = \text{arctg } b^*/a^*$) y de saturación o intensidad de color (Chroma; $C^* = (a^{*2}+b^{*2})^{1/2}$). Por otro lado, a partir de los valores de reflexión obtenidos por espectrofotometría, se han propuesto fórmulas útiles para el cálculo de las proporciones de los distintos pigmentos (Krzywicki, 1979).

El color de la carne se debe principalmente al estado oxidativo de la mioglobina. Por ello, una de las estrategias empleadas para el mantenimiento de la estabilidad del color ha sido el empleo de antioxidantes (Robbins *et al.,* 2003).

Aunque no siempre ocurre de este modo, y en ocasiones pueden encontrarse resultados contradictorios, de forma general a medida que avanzan los días de almacenamiento de la carne, los valores de *L** y *b** aumentan, mientras que los de *a** y *C** disminuyen, provocando su decoloración (McKenna *et al.,* 2005) y una apariencia desagradable para el consumidor. La coordenada *H** aumenta a lo largo del almacenamiento y resulta un buen indicador del pardeamiento y, por tanto, de la proporción de metamioglobina (MMb) en la carne (Liu *et al.,* 1996). Por ello, es especialmente

importante retrasar la formación de MMb en la carne y alargar el periodo en que la superficie del músculo no muestra evidencias de decoloración (Arnold *et al.,* 1993).

Una de las estrategias empleadas para el mantenimiento de la estabilidad del color de la carne ha sido el empleo de antioxidantes (Robbins *et al.,* 2003). Como se ha indicado, en carne fresca, la forma de incorporar antioxidantes es su uso en alimentación animal. La vitamina E es el antioxidante más ampliamente utilizado. Sin embargo, el origen sintético de la buena parte de la vitamina E empleada en nutrición animal, ha despertado el interés por estudiar el efecto antioxidante de otros compuestos de origen natural.

Los polifenoles, presentes en plantas y en algunos residuos agroindustriales, han sido asociados con efectos positivos sobre la estabilidad del color de la carne. Este efecto varía con factores extrínsecos (tipo de compuesto fenólico, la dosis de inclusión, la alimentación del ganado) y con factores intrínsecos al animal como el tipo de músculo de que se trate. Así, los músculos con mayor cantidad de fibras rojas en su composición presentan mayor contenido en mioglobina, mayor actividad oxidativa y mayor inestabilidad en el color (O'Keefe y Hood, 1982).

Entre los compuestos fenólicos que han demostrado ser efectivos en la prevención del deterioro del color de la carne y en su estabilización a lo largo del almacenamiento destacan los taninos condensados (Priolo *et al*., 2000; 2005), otros flavonoides como la quercetina (Andrés *et al.,* 2013) y derivados de romero o tomillo (Moñino *et al.,* 2008; Nieto *et al.,* 2010a; Morán *et al.,* 2012a).

Al incluir taninos condensados de distintas procedencias en dietas de corderos, se han observado mayores valores de luminosidad (L^*) (Priolo *et al.,* 2000; 2005) y una correlación negativa con el hierro del músculo (Zembayashy *et al.,* 1999). Estos resultados han sido atribuidos a la acción quelante de los taninos y a la reducción que se produce en el contenido en hierro del músculo. Aunque los mecanismos de acción no están muy claros, ha sido señalado que los taninos no afectan a la absorción de hierro en los rumiantes, pero sí pueden obstaculizar su utilización para la síntesis de mioglobina. Por otra parte, existen evidencias *in vitro* que señalan a los taninos como responsables de reducciones de la biosíntesis microbiana de vitamina B_{12}, precursora de la síntesis de mioglobina y del color más luminoso en la carne (Priolo y Vasta, 2007).

También ha sido demostrado que los taninos condensados mejoran la estabilidad oxidativa y previenen la decoloración de la carne, extendiendo su vida útil (Vasta y Luciano, 2011). En este sentido, Luciano *et al.* (2009; 2011) observaron que la adición de taninos en

la dieta de corderos incrementó el valor de *a** y redujo el de *b**, provocando un menor incremento del descriptor del pardeamiento *H**, así como menor formación de MMb durante el almacenamiento, prolongando la estabilidad de color.

El mecanismo de acción por el que los taninos previenen la decoloración de la carne durante su almacenamiento no está del todo claro, ya que se han encontrado resultados contradictorios. De forma general, está ampliamente aceptado que la oxidación de los lípidos y la oxidación de la mioglobina de la carne están asociados entre sí. En general, ambos procesos aumentan simultáneamente durante el almacenamiento (Luciano *et al.,* 2009) y son contrarrestados por el efecto protector de moléculas antioxidantes, como la vitamina E (Faustman *et al.,* 2010), o los taninos en este caso. Sin embargo, recientemente, otros estudios han indicado una falta de relación entre la oxidación lipídica y la estabilidad del color (Luciano *et al.*, 2011; Monoley *et al.,* 2012), de manera que algunos autores han observado efecto de protección de los polifenoles sobre la decoloración de la carne, pero no sobre la oxidación lipídica (Luciano *et al.,* 2009). Luciano *et al.* (2011) sugieren que el efecto protector de los polifenoles de la dieta contra la oxidación de la mioglobina, podría ser resultado de su capacidad de mejora del estatus antioxidante del músculo sin que la oxidación lipídica se vea afectada.

A diferencia de lo señalado anteriormente, también se han obtenido resultados negativos por efecto de los taninos y de otros compuestos fenólicos. En este sentido, Larraín *et al.* (2008) encontró que la inclusión de taninos condensados en dietas de terneros perjudicó la estabilidad del color de la carne como consecuencia de una menor deposición de vitamina E en el músculo. Del mismo modo, al emplear flavanonas como la naringina (Bodas *et al.,* 2012) o la hesperidina (Simitzis *et al.,* 2013) y extractos de romero (Bañón *et al.*, 2012), tampoco se encontraron efectos positivos sobre los parámetros de color. De hecho, en algunos trabajos con corderos (Inserra *et al.,* 2014) tampoco han sido hallados resultados positivos sobre el color de la carne al incluir en la dieta pulpa de cítricos (rica en flavonoides).

La variabilidad en los resultados obtenidos al incluir distintos compuestos fenólicos en raciones de corderos hace difícil poder predecir con exactitud su efecto sobre el color de la carne. De manera que es preciso realizar estudios concretos que nos permitan conocer el efecto que plantas y subproductos ricos en compuestos fenólicos y con interés en alimentación animal tienen sobre la calidad de los productos obtenidos.

Capacidad de retención de agua (CRA)

La capacidad de retención de agua (CRA) puede definirse como la aptitud de la carne para retener el agua de constitución (ligada o libre) o, eventualmente añadida, cuando se aplica sobre ella fuerzas externas, o a lo largo de un determinado proceso industrial y de ella depende, en buena medida, la jugosidad (Honikel, 1998). El agua libre comprende el agua que se encuentra unida a las cargas de la superficie de las proteínas y el agua extracelular, que se libera fácilmente al aplicar una fuerza externa, y es la que origina el llamado "drip loss" o "pérdidas por goteo". Según diferentes estudios, la evolución de la CRA de la carne durante su almacenamiento es variable. Algunos autores han observado un incremento de la capacidad de retención de agua (Lesiow y Ockerman, 1998), fenómeno que está asociado a la liberación de iones de sodio, que contribuyen a una mayor retención del agua libre. Sin embargo, otros autores indican que la oxidación lipídica que tiene lugar durante el almacenamiento de la carne, afecta claramente a la CRA, ya que la oxidación de los fosfolípidos y del colesterol así como de las proteínas que componen la membrana celular, genera cambios en su fluidez, permeabilidad y estabilidad causando la reducción de la CRA de la carne (Guardiola *et al.,* 1996; Lund *et al.,* 2011).

El empleo de vitamina E (Castellini *et al.,* 1998) o de sustancias naturales con efecto antioxidante en alimentación de rumiantes mejora la CRA de la carne. Albertí *et al.* (2005) empleando flavonoides en dietas de terneros, observaron menores pérdidas de líquido de la carne, consiguiendo una carne más jugosa para el consumidor. Sin embargo, otros autores no han encontrado diferencias sobre la CRA al incluir en dietas de corderos taninos condensados (Priolo *et al.,* 2000), flavonoles como la quercetina (Andrés *et al.,* 2014b), flavanonas como la hesperidina (Simitzis *et al.,* 2013) y polifenoles de romero (Morán *et al.,* 2012a). En cualquier caso es un parámetro cuya variabilidad se ha atribuido en muchos casos a la diversidad de métodos empleados para su determinación.

Textura

La textura se define como “la manifestación sensorial de la estructura del alimento frente a la aplicación de fuerzas” (Szczesniak, 1963), englobando al atributo de terneza o su inverso, la dureza. Los cambios provocados por la oxidación sobre la CRA afectan a la textura de la carne y, como consecuencia a la jugosidad y la terneza. Si bien durante el almacenamiento de la carne tiene lugar el proceso de maduración, que se asocia con un incremento de la terneza, la oxidación proteica también puede afectar negativamente a la

integridad y textura de la carne ya que la reacción entre lípidos oxidados y proteínas, puede generar entrecruzamientos entre estas moléculas y causar una disminución de la solubilidad de las proteínas, e incluso su desnaturalización (Kanner, 1994). Las reducciones de la proteolisis por inactivación de la enzima μ-calpaína (Rowe *et al.,* 2004) o la formación de uniones proteicas, con fortalecimiento de la estructura de las miofibrillas (Lund *et al.,* 2007) también son mecanismos que pueden explicar los efectos indeseables de la oxidación sobre la textura de la carne, así como sobre la pérdida de jugosidad o disminución de la terneza.

Algunos trabajos han estudiado el efecto de la inclusión en dietas animales de diferentes antioxidantes como vitamina E, sustancias naturales o diferentes metabolitos secundarios de las plantas, sobre la textura de la carne. En este sentido, ha sido observado una carne mas tierna al incluir taninos condensados (Priolo *et al.,* 2000), flavonoides (Albertí *et al.,* 2005), extractos de romero (Morán *et al.,* 2012b) y pulpa de cítricos (Scerra *et al.,* 2001) en alimentación de rumiantes. Este efecto ha sido asociado con la protección ejercida por los compuestos fenólicos frente a la oxidación de proteasas endógenas durante el proceso de maduración de la carne.

Flavor

El flavor o sensación olfato-gustativa es otra característica sensorial de la carne que puede estar influenciada por la dieta de los animales (Prescott *et al.,* 2001) y que resulta importante en relación con su aceptación por los consumidores. En la expresión del flavor de una pieza de carne contribuyen tanto el músculo como la grasa. El aroma del músculo es relativamente constante, salvo por la presencia de aminoácidos, bases nitrogenadas y compuestos azufrados que pueden conferir características peculiares. La grasa, por su contenido en compuestos volátiles liposolubles, liberados por efecto del calor, es la que proporciona el aroma característico de la carne. Por lo tanto, las diferencias en la cantidad y composición de la grasa intramuscular explican el efecto que, factores como la raza, el sexo o los cambios durante el almacenamiento *post mortem*, causan en el flavor (Reineccius, 1994).

La oxidación lipídica y el deterioro microbiano perjudican la calidad de la carne ya que afectan negativamente, entre otros aspectos, a su aroma y sabor. De hecho, estos procesos son responsables de la aparición de off-flavors indeseables (sensaciones olfato-gustativas no características, generalmente asociadas con el deterioro o transformación de la muestra), que provocan el rechazo por parte del consumidor.

A pesar del interés que en los últimos años ha despertado el efecto de sustancias antioxidantes y/o antimicrobianas en alimentación animal sobre la calidad de la carne, han sido pocos los trabajos que han estudiado la influencia de estas sustancias en general, y de los compuestos fenólicos en particular, en la dieta de los animales sobre las propiedades olfato-gustativas de la carne.

Algunos estudios en rumiantes han sugerido que una dieta suplementada con taninos condensados, afecta a la biosíntesis de compuestos volátiles responsables del aroma de la carne, como el escatol y el indol, sintetizados en el rumen por los microorganismos. Estos compuestos confieren connotaciones de flavor desagradables a la carne de cordero, relacionadas con olor fecal. Varios autores han indicado que la inclusión de taninos en alimentación de rumiantes mejora el flavor de los productos, reduciendo la biosíntesis de escatol y su acumulación en la carne (Schreurs *et al.,* 2007; Priolo *et al.,* 2009), obteniendo una valoración sensorial más positiva en cuanto a este tipo de flavores desagradables.

Otro tipo de flavores no deseados son los relacionados con la rancidez. Diversos estudios al emplear en dietas de cordero plantas aromáticas o extractos derivados de éstas, ricos en flavonoides y aceites esenciales (Chaves *et al.,* 2008; Nieto *et al.,* 2010b; Serrano *et al.,* 2014), observaron reducción de algunos compuestos volátiles de la carne, disminuyendo la percepción de off-flavors como el flavor a rancio, demostrando ser efectivas frente a la oxidación y proporcionando una carne más aceptable para los consumidores.

Aunque en general, los compuestos fenólicos presentan efectos positivos sobre las características olfato-gustativas de la carne, la efectividad de estos compuestos no es del todo clara y requiere mayor investigación ya que se han observado algunos efectos negativos. Así, Priolo *et al.* (2005) observaron efectos desfavorables de los taninos sobre la reducción del escatol en corderos. Staerfl *et al.* (2011) en terneros alimentados con aceite esencial observaron intensos off-flavors.

Oxidación lipídica

Los lípidos son las moléculas sobre las cuales recae el mayor daño derivado del estrés oxidativo durante el proceso de peroxidación lipídica, alterando la adhesión, la fluidez, la permeabilidad y la función metabólica de las membranas celulares. Los cambios asociados a la oxidación lipídica constituyen la principal causa de deterioro en la carne, ya que el aumento de la rancidez desencadena la aparición de olores y flavores extraños, disminución

de la CRA, alteración del color y, en general, una reducción de la calidad organoléptica de la carne (Morrissey *et al.,* 1998). Todo ello está unido a la generación de compuestos potencialmente nocivos y relacionados con el riesgo de padecer diversas patologías, como enfermedades cardiovasculares, cáncer, envejecimiento, etc. (Bou *et al.,* 2009).

La oxidación de la carne depende de factores intrínsecos al animal, como son la genética, el sexo, la edad y el peso al sacrificio y el tipo de músculo así como de factores extrínsecos relacionados con el manejo y la alimentación. La oxidación lipídica de la carne puede controlarse, o al menos minimizarse, mediante la adición de antioxidantes de forma exógena (Zhang *et al.,* 2010), o bien adoptando sistemas de alimentación del ganado capaces de mejorar el estatus antioxidante del músculo (Descalzo y Sancho, 2008) y prevenir el ataque de los PUFA por las ROS a lo largo de la vida del animal. La suplementación de la dieta permite introducir uniformemente antioxidantes en las membranas de los fosfolípidos con el objetivo de inhibir de forma eficaz las reacciones de oxidación *in situ* (Lauridsen *et al.,* 1997).

El α-tocoferol o vitamina E es el antioxidante que se utiliza habitualmente y también ha sido el más estudiado. Sin embargo, su coste, su origen sintético, su limitada bioeficiencia cuando la ingestión de PUFA es muy elevada (Allard *et al.,* 1997), su potencial acción pro-oxidante (Mukai *et al.,* 1993) y su distribución poco homogénea en los tejidos (Bjorneboe *et al.,* 1986) ha generado un creciente interés por la búsqueda de suplementos naturales a precios competitivos. En este sentido, los polifenoles presentes en algunas plantas, en sus extractos o en algunos residuos agroindustriales han suscitado un gran interés por sus propiedades antioxidantes y antimicrobianas, y por ser una alternativa a otros aditivos sintéticos con vistas a la mejora de la calidad y vida útil de la carne (Gladine *et al*., 2007a).

Los polifenoles presentan gran variedad de estructuras químicas y en algunos casos muestran baja biodisponibilidad. Por este motivo, en la bibliografía existe disparidad de resultados, pero en general los compuestos bioactivos de carácter fenólico han mostrado ser útiles para prevenir la lipoperoxidación y extender la vida útil de los productos cárnicos. La administración en la dieta de polifenoles ha demostrado ser capaz de mejorar la estabilidad oxidativa de diferentes tejidos en varias especies animales, habiéndose observado este efecto en plasma e hígado de ratas (Gladine *et al.,* 2007a; 2007b), en músculo de aves (Giannenas *et al.,* 2005) y conejo (Gai *et al.,* 2009), etc. Asimismo, tal y como recoge la Tabla 2.5, el efecto protector de los polifenoles de distintas procedencias, frente a la lipoperoxidación del músculo también ha sido observado en pequeños rumiantes, al ser incorporados en las dietas.

Tabla 2.5. Relación de algunos trabajos sobre la inclusión de compuestos fenólicos procedentes de distintas fuentes vegetales en la dieta de pequeños rumiantes, donde se han observado mejoras en la estabilidad oxidativa y/o en el estatus antioxidante de la carne.

Fuente natural	Dosis [1]	Compuestos fenólicos presentes	Especie	Periodo experimental (días)	Músculo[2]	Referencia
Compuestos puros						
Ácido carnósico (extraído de *Rosmarinus officinalis*)	600 y 1200 ppm	Ácido carnósico (diterpeno fenólico)	Corderos	49	LL, GM	Morán *et al.,* 2012b
Hesperidina	1500 y 3000 ppm	Hesperidina (flavanona)	Corderos	35	LT	Simitzis *et al.,* 2013
Quercetina (extraída de *Sophora japonica*)	2000 ppm	Quercetina (flavonol)	Corderos	35	BF	Andrés *et al.,* 2014b
Extractos purificados						
Aceite esencial de orégano (*Origanum vulgare*)	1 ppm	Ácidos fenólicos (carvacrol, thymol)	Corderos	60	LD	Simitzis *et al.,* 2008
Extracto de quebracho (*Schinopsis lorentzii*)	8,96%	Taninos condensados	Corderos	60	LD	Luciano *et al.,* 2011
Extracto de semilla de uva (*Vitis vinifera*)	2,50%	Taninos condensados	Corderos	42	LD	Jerónimo *et al.,* 2012
Extracto de romero (*Rosmarinus officinalis*)	600 ppm	Ácido carcósico y carnosol	Corderos	60 ± 5	LD	Bañón *et al.,* 2012
Extracto de romero (*Rosmarinus officinalis*)	600 ppm	Ácido carcósico y carnosol	Ovejas y corderos	240	LD	Serrano *et al.,* 2014
Extracto de romero (*Rosmarinus officinalis*)	640 y 685 ppm	Ácido carcósico y carnosol	Corderos	56 ± 11	D, OEA	Jordán *et al.,* 2014
Plantas enteras						
Hojas y tallos desecados de jara (*Cistus ladanifer*)	25%	Taninos condensados, ácidos fenólicos	Corderos	42	LD	Jerónimo *et al.,* 2012
Hojas desecadas de *Andrographis paniculata*	0,50 %	Flavonoides	Cabras	100	LD	Karami *et al.,* 2011
Hojas de tomillo (*Thymus zygis*) en forma de pellets	3,75 y 7,5%	Ácidos fenólicos (thymol, carvacrol, eugenol)	Ovejas en lactación	240	LD	Nieto *et al.,* 2010a
Subproductos agroindustriales						
Hojas del destilado del romero (*Rosmarinus officinalis*)	10 y 20%	Diterpenos fenólicos (ácido carnósico, carnosol, etc.) y ácidos fenólicos (ácido rosmarínico, etc.)	Ovejas en lactación	240	LD, D, OEA	Moñino *et al.,* 2008; Nieto *et al.,* 2010b
Pulpa de cítricos desecada	24 y 35%	Flavonoides (hesperidina, narirutina, naringina y eriocitrina)	Corderos	68	LTL	Inserra *et al.,* 2014
Granada (*Punica granatum*) desecada	5, 10 y 15%	Antocianinas, ácido elágico, punicalín, punicalagín, pedunculagín y diferentes flavonoles	Cabras	84	LL	Emami *et al.,* 2015

[1] ppp, mg por kg de concentrado; %, porcentaje de la MS de la dieta.

[2] BF, *biceps femoris;* GM, *gluteus medius;* LD, *longissimus dorsi;* LL, *longissimus lumborum;* LT, *longissimus thoracis;* LTL, *longissimus thoracis et lumborum;* D, *deltoideus; OEA, obliquus externus abdominis*

Cuando los polifenoles son incluidos en la dieta de rumiantes, su efecto sobre la estabilidad oxidativa de la carne resulta difícil de predecir (Jordán *et al.,* 2014). Algunos autores han señalado una correlación positiva entre la concentración de polifenoles y la estabilidad oxidativa del músculo y han relacionado su absorción a lo largo del tracto gastrointestinal con su transferencia a los tejidos. De hecho, existen trabajos en pequeños rumiantes que han demostrado la transmisión de compuestos fenólicos de la dieta al músculo y su efecto protector frente a de la oxidación (Gladine *et al.,* 2007c; Moñino *et al.,* 2008; Luciano *et al.,* 2011).

Los compuestos fenólicos monoméricos son absorbidos fácilmente a través del intestino, mientras que en el caso de polifenoles complejos (como los taninos condensados), su naturaleza polimérica y su alto peso molecular, limitan su absorción y, por tanto, su biodisponibilidad, perdiendo parte de su capacidad antioxidante *in vivo*. De hecho, es poco probable que oligómeros mayores que trímeros sean absorbidos en el intestino delgado en su forma original (Manach *et al.,* 2004). Algunos autores (Déprez *et al.,* 2000) han señalado una despolimerización intestinal de este tipo de compuestos complejos, en partes más pequeñas y moléculas absorbibles y, sin embargo, otros autores encuentran poco probable la hidrólisis de los taninos en rumen (Makkar *et al.,* 1995). Además, es necesario considerar otros efectos de los compuestos fenólicos de la dieta en relación con su capacidad antioxidante, tales como su capacidad para aumentar los niveles de enzimas antioxidantes endógenas en tejidos animales (Anila y Vijayalakshmi, 2003). En este sentido, ha sido demostrado que los compuestos fenólicos pueden interaccionar con antioxidantes endógenos presentes en los tejidos animales, así como con antioxidantes exógenos, y puede ser difícil distinguir sus efectos directos e indirectos en la resistencia de la carne a daños oxidativos.

En relación con la interacción entre los metabolitos polifenólicos y otros antioxidantes exógenos en los tejidos animales, algunos estudios en ratas (Gladine *et al.,* 2007a; 2007b), pollos (Goñi *et al.,* 2007), vacas (Gobert *et al.,* 2010) y ovejas (Gladine *et al.,* 2007c) han observado que, al alimentar a los animales con dietas enriquecidas con una mezcla de vitamina E y polifenoles, tiene lugar una reducción de la oxidación lipídica del hígado, del plasma y/o de la carne y en algún caso se han observado mayores niveles de vitamina E en los tejidos, superando los niveles encontrados cuando la vitamina E se administra sola. Los polifenoles aportados con la dieta pueden ejercer una acción antioxidante indirecta y sinérgica con la vitamina E, así como participar en el reciclado y la protección de la vitamina E frente a la oxidación (Deckert *et al.,* 2002).

Calidad microbiológica

La degradación microbiana provoca en la carne decoloraciones, olores y sabores desagradables, defectos en la textura, exudados limosos, producción de gas y alteraciones en el pH (Jayasena y Jo, 2013), llegando a causar intoxicaciones alimenticias, que hacen al producto no apto para el consumo (Ercolini *et al.,* 2006).

La oxidación lipídica de la carne aumenta a lo largo de su almacenamiento y beneficia su deterioro microbiano, debido a que los factores de oxidación y contaminación microbiana están interrelacionados. Si bien la fuente principal de energía para el crecimiento de las bacterias alterantes de carne fresca es la glucosa, una vez que esta se agota, los microorganismos pueden utilizar metabolitos procedentes de la oxidación en los componentes de la carne, como lípidos y proteínas, favoreciendo así su crecimiento. A su vez, el deterioro microbiano también provoca una mayor exposición de las estructuras celulares a las ROS, por lo que se incrementa la oxidación. Por otro lado, la CRA y por tanto el volumen de extracto liberado a lo largo del almacenamiento pueden considerarse también indicadores de la carga bacteriana del producto, ya que existe una relación inversa entre el grado de alteración de la carne y el volumen de extracto liberado (Jay *et al.,* 1966).

Entre las diferentes técnicas de conservación de la carne frente al deterioro microbiano, el empleo de sustancias naturales alternativas con propiedades antimicrobianas que sustituyan a los aditivos sintéticos y puedan mejorar la calidad y la vida útil de la carne producida presenta un gran interés. Entre estas sustancias con efecto antimicrobiano, destacan los compuestos fenólicos, cuyos grupos hidroxilo son vitales para su actividad. En este sentido, diversos autores han estudiado el efecto de la inclusión, en dietas de ganado ovino, de polifenoles de diferentes orígenes, como flavonoides (Andrés *et al.,* 2013), productos derivados del romero (Nieto *et al.,* 2010b; Serrano *et al.,* 2014) o del tomillo (Nieto *et al.*, 2010a), observando un retraso en el deterioro microbiano de la carne, manifestado por reducciones en los recuentos de bacterias viables, mohos psicotrofos y/o levaduras a lo largo del almacenamiento. De este modo se demuestra el poder antimicrobiano de estos compuestos, que pasan del sistema circulatorio de los animales a depositarse en músculo y ejercer su efecto a lo largo de todo el almacenamiento (Nieto *et al.,* 2010b).

2.5. Calidad de leche

2.5.1. Concepto de calidad y parámetros que definen la calidad de la leche

La calidad de la leche se define por sus características físico-químicas, higio-sanitarias y organolépticas.

La composición nutricional de la leche es uno de los parámetros más empleados para establecer su calidad. Dentro de sus componentes, las materias nitrogenadas y la grasa presentan gran interés, ya que determinan en gran medida el rendimiento quesero de la leche. El punto crioscópico y la acidez son otros parámetros físico-químicos importantes para establecer la calidad de la leche.

La calidad higiénica de la leche resulta de gran importancia también, ya que además de afectar al proceso tecnológico de producción de queso y a su calidad final, puede repercutir sobre la salud del consumidor. En este sentido, los parámetros indicadores del estado higiénico de la leche son el recuento bacteriano, el recuento de células somáticas y la presencia de inhibidores o sustancias extrañas.

La producción y la calidad de la leche pueden verse modificadas por factores extrínsecos al animal, o del medio ambiente, a través de prácticas de manejo y a través de la alimentación.

2.5.2. Efecto de los compuestos fenólicos sobre la calidad de la leche

En ovejas lecheras, al igual que en otros rumiantes, la alimentación es el principal determinante de la producción y calidad de la leche. En este sentido, la producción y la calidad de la leche puede verse afectada por la ingestión de los polifenoles presentes en los alimentos que consumen habitualmente los rumiantes (plantas, residuos de la industria alimentaria, etc.).

Entre los compuestos fenólicos, los taninos condensados pueden ejercer efectos beneficiosos sobre la producción y la composición de la leche. Vasta *et al.* (2008) han indicado que la inclusión de taninos en dietas de rumiantes incrementa la producción de leche, de proteína y de lactosa debido a su efecto de protección de la degradación proteica en el rumen y al incremento de aminoácidos esenciales, especialmente metionina y lisina, para su absorción a nivel intestinal. Este efecto ha sido observado en múltiples trabajos,

tanto en ganado vacuno (Blauwiekel *et al.,* 1997; Woodward *et al.,* 2002), como ovino (Molle *et al.,* 2003). Asimismo, una mayor disponibilidad de aminoácidos ha sido asociada a una mayor síntesis de glucosa y por tanto de lactosa en la leche. En ocasiones, también se han observado reducciones en la concentración de grasa pero atribuibles más a un efecto de dilución simple al aumentar las concentraciones de lactosa y proteína que a un efecto directo de los taninos sobre la síntesis de grasa de la leche.

También han sido señalados efectos beneficiosos o no perjudiciales de otros compuestos fenólicos sobre los rendimientos lecheros. Chiofalo *et al.* (2012) al incluir extracto de romero en dietas de ovejas, observaron de forma lineal mayor producción lechera y producción diaria de grasa, de proteína, de caseína y de lactosa al aumentar la dosis de extracto. Ben Khedim (2014) al incluir mejorana (*Thymus mastichina*), rica en compuestos fenólicos, en dietas de ovejas no encontraron efecto sobre la producción lechera, pero sí observaron una reducción en el nivel de grasa y de sólidos totales, y un incremento en la proporción de proteína y de caseína. Los resultados son muy variables, de tal modo que en algunos casos no han sido señalados efectos sobre la producción y composición de la leche al añadir este tipo de compuestos en dietas de vacas (Ferlay *et al.,* 2010; Hristov *et al.,* 2013), de ovejas (Toral *et al.,* 2013) o de cabras (Jordán *et al.,* 2010).

El perfil de ácidos grasos de la leche puede ser modificado mediante estrategias de alimentación que permitan interferir en el metabolismo de los lípidos a nivel ruminal e incrementar los niveles en leche de algunos ácidos grasos con interés desde el punto de vista de la salud humana. Recientemente ha sido sugerido que la suplementación de dietas de rumiantes con compuestos fenólicos, como taninos condensados y otros polifenoles, podría modificar el metabolismo ruminal y mejorar el perfil lipídico de la grasa láctea, debido a que, tal y como ha sido señalado en apartados anteriores, los taninos alteran la flora ruminal y pueden inhibir el último paso de la BH ruminal, impidiendo la reducción del VA a C18:0 y aumentar los contenidos de PUFA, especialmente de VA y RA en la leche (Vasta *et al.,* 2008; 2009a) gracias a su posterior aporte a la glándula mamaria y a la actividad de la enzima Δ^9-desaturasa. Este efecto ha sido observado al incluir compuestos fenólicos de distintas procedencias en dietas de vacas (Ferlay *et al.,* 2010), ovejas (Ben Khedim, 2014) y cabras (Heidarian-Miri *et al.,* 2013). En contraposición a lo anterior, diversos estudios *in vivo* han puesto en duda dicho efecto inhibitorio de los taninos sobre el proceso de BH ruminal, así como su acción sobre el nivel de RA en la grasa láctea. En este sentido Benchaar y Chouinard (2009) señalaron que la adición de 0,67% de taninos condensados en dietas de vacas lecheras no fue suficiente para alterar el proceso de BH ruminal y modificar el perfil de la grasa de la leche. Tampoco Toral *et al.* (2013) evidenciaron efectos significativos

sobre la concentración de los principales grupos de ácidos grasos de la leche en general, ni sobre el enriquecimiento en VA y RA en particular, al añadir extractos de taninos a una dieta para ovejas en lactación suplementada con aceite de girasol.

Por otra parte, ha sido señalada una transferencia de los polifenoles de la dieta a la leche (O'Connel y Fox, 2001; Jordán *et al.,* 2010; Chiofalo *et al.,* 2012). De este modo, Abbeddou *et al.* (2011) y Ben Khedim (2014) observaron una mayor actividad antirradicalaria y/o capacidad antioxidante en leche de ovejas suplementadas con distintas fuentes de polifenoles en relación a un grupo control.

2.6. El orujo de uva

2.6.1. Consideraciones generales

El vino es una de las bebidas alcohólicas más populares del mundo, producida y consumida a lo largo de la historia por razones culturales, económicas, sociales, religiosas, y más recientemente, por motivos de salud.

España con 943.000 hectáreas, es el país con mayor extensión de viñedo del mundo (14%) y el tercer productor de vino mundial con 3,2 millones de toneladas al año (12% de la producción global) (FAOSTAT, 2012). Castilla-La Mancha es la principal Comunidad Autónoma española productora de vino, donde se genera casi el 50% de la producción nacional. Alrededor del 6% de la producción española de vino se genera en Castilla y León, donde se elaboran 1,9 millones de hectolitros anuales.

La materia prima necesaria para la elaboración del vino es la uva (*Vitis vinifera* L.), cuyos racimos se componen de raspón y de granos o bayas. A la bodega llegan ambos y cada uno de ellos puede transmitir al vino determinadas características. Los granos de uva, a su vez, están constituidos por hollejos o pieles, pulpa y semillas.

La vinificación es el conjunto de operaciones realizadas en el proceso de elaboración de los vinos a partir de la uva. Este proceso varía en función del vino que se pretende obtener. La Figura 2.4 esquematiza el proceso de elaboración del vino tinto.

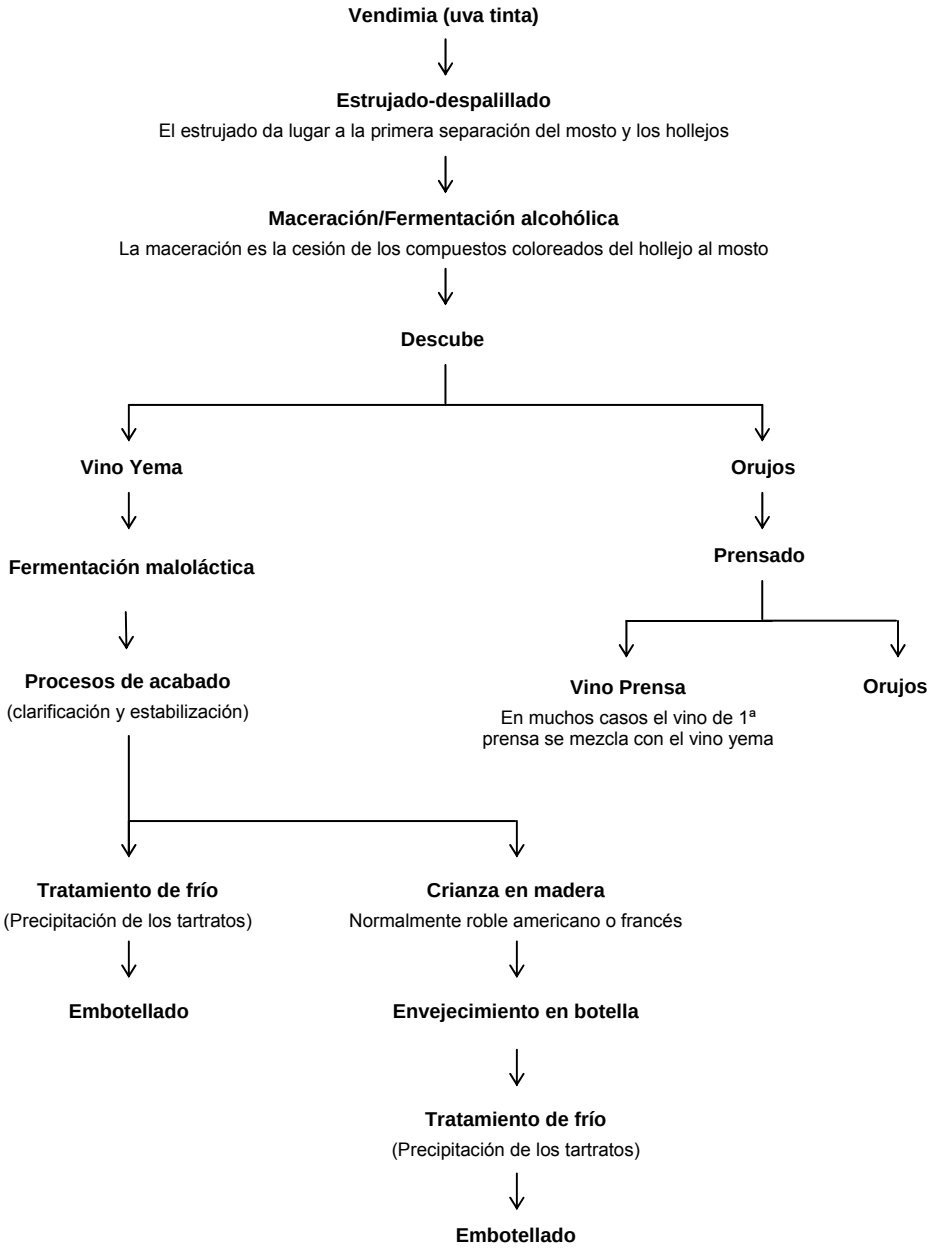


Figura 2.4. Proceso de vinificación en tinto

Tanto en la viticultura como en la industria del vino se producen grandes cantidades de subproductos, siendo los más importantes los raspones o escobajos, el orujo integral y las lías. Los raspones o escobajos son la estructura leñosa del racimo que se recoge durante el despalillado y constituyen el principal subproducto de la viticultura. El orujo integral se

produce en el aplastamiento y prensado de la uva, y está constituido por una mezcla de escobajo, pulpa y semillas en proporciones variables (25, 55 y 20%, como media, respectivamente) y se generan al año 750.000 t en España. Las lías son las células de levadura producidas durante la fermentación alcohólica, depositadas en el fondo de los tanques de fermentación, mezcladas con sales tartáricas y bacterias (FEDNA, 2010).

2.6.2. Valor nutritivo del orujo de uva

Los productos normalmente comercializados para alimentación animal son el orujo integral y sus componentes por separado (la pulpa y hollejo y la granilla o semilla desengrasada).

El orujo consiste en orujo integral del que se ha separado el escobajo. Representa aproximadamente el 18-20% del peso de la uva procesada para la elaboración del vino (Llobera y Cañellas, 2007; Spanghero *et al.,* 2009) y su valor energético depende en gran parte de la eficacia del proceso de separación del escobajo (FEDNA, 2010). La pulpa y el hollejo resulta de separar las semillas del orujo. La granilla está constituida por las semillas separadas en el proceso anterior, y éstas están compuestas por un tegumento o cubierta muy lignificado y por un albumen rico en lípidos. Este aceite tiene un alto valor comercial y, por ello, se suele extraer con disolventes orgánicos. El producto resultante se denomina granilla desengrasada (1-2% extracto etéreo) y contiene, aproximadamente, un 50% de fibra bruta. A veces, bien por razones comerciales, o bien por imperativo legal, se elimina una parte de esta fibra, la más lignificada, dando lugar a un producto de mayor concentración proteica y con un contenido en fibra bruta inferior al 35% (FEDNA, 2010).

El valor nutritivo de este tipo de residuos es bajo. Además, actualmente existe un problema de falta de tipificación que dificulta su valoración nutritiva. Por ello, se estima que sólo un 3% de la producción nacional se destina a la alimentación animal. La Tabla 2.6 muestra la composición química media de los subproductos de la industria del vino.

El orujo de uva se caracteriza por un elevado contenido en componentes de la pared celular. Una parte importante de la fibra neutro detergente (FND) corresponde a lignina ácido detergente (LAD), y las proporciones de hemicelulosa y celulosa son relativamente bajas (6-8 y 16-19%, respectivamente) (FEDNA, 2010). La mayor parte de los componentes fibrosos se encuentran en la semilla, por lo que su concentración aumenta a

medida que se incrementa el porcentaje de semillas en el orujo, ya que la fracción correspondiente a hollejos y pulpa es más digestible.

Tabla 2.6. Composición química de los subproductos de la industria del vino empleados en alimentación animal (% MS).

Subproducto	MO	PB	EE	FB	FND	FAD	PB-FAD	LAD	Azúc.	Referencia
Orujo integral de uva	94,0	9,40	5,20	-	56,8	47,6	-	20,0	-	Abarghuei *et al.,* 2010
	89,3	12,6	-	-	50,2	31,2	-	19,4	-	Alipour y Rouzbehan, 2007
	92,6	6,40	1,01	-	25,9	25,5	-	-	-	Besharati y Taghizadeh, 2009
	94,9	9,60	4,70	-	40,0	32,8	-	-	-	Balasan *et al.,* 2011
	93,8	12,4	5,90	25,6	40,7	31,1	-	32,5	15,8	Baumgärtel *et al.,* 2007
	93,7	12,2	8,20	35,4	62,2	55,0	-	34,3	3,30	FEDNA, 2010
	91,0	14,9	5,10	35,4	64,6	56,2	-	29,5	-	Malossini *et al.,* 1993
	94,3	17,3	3,70	22,8	59,5	52,5	-	-	-	Mirzaei-Aghasaghali *et al.,* 2011
	85,8	9,70	5,20	-	59,8	51,2	4,50	37,9	-	Molina-Alcaide *et al.,* 2008
	92,8	13,1	-	-	50,4	-	-	-	-	Pirmohammadi *et al.,* 2007
	90,0	13,0	-	36,0	-	-	-	-	-	Valizadeh y Sobhanirad, 2009
	92,7	11,2	7,10	56,2	-	-	-	-	5,70	Yi *et al.,* 2009
	92,9	10,6	6,10	25,0	54,8	50,5	-	42,0	-	Zalikarenab *et al.,* 2007
Residuo de uva ensilado	91,4	20,6	10,5	-	58,4	43,1	-	10,1	-	Santos *et al*., 2014
Concentrado de orujo de uva	9,80	13,9	1,00	15,1	-	-	-	-	-	Brenes *et al.,* 2008;
Hollejos de uva	88,3	12,0	4,70	-	41,3	36,0	-	-	-	Balasan *et al.,* 2011
	94,6	9,50	3,60	-	-	-	-	-	-	Deng *et al.,* 2011
	92,6	13,9	6,30	33,3	60,2	53,3	-	32,6	4,80	FEDNA, 2010
	90,6	10,5	8,30	-	39,2	36,4	8,30	22,6	-	Spanghero *et al.,* 2009
Semilla de uva entera	97,2	9,30	6,30	55,2	49,2	-	-	-	-	Balasan *et al*., 2011
	97,3	9,30	10,9	-	53,9	-	-	41,0	-	Correddu *et al.,* 2015a
	96,8	10,1	12,1	45,1	73,0	62,1	-	46,7	1,20	FEDNA, 2010
	95,5	12,3	11,9	-	52,2	49,2	6,80	40,8	-	Spanghero *et al.,* 2009
Harina de extracción de granilla de uva	95,8	12,2	1,20	52,5	78,8	69,2	-	52,4	1,10	FEDNA, 2010
Lías	76,2	16,5	1,50	-	24,5	12,7	3,10	7,80	-	Molina-Alcaide *et al*., (2008)

MO: materia orgánica; PB: proteína bruta; EE: extracto etéreo; FB: fibra bruta; FND: fibra ácido detergente; FAD: fibra ácido detergente; PB-FAD: proteína ligada a la FAD; LAD: lignina ácido detergente; Azúc.: azúcares

El contenido en proteína bruta (PB) de los subproductos de uva es del orden del 10%, siendo ligeramente superior en el hollejo que en la granilla. Sin embargo, de acuerdo con

FEDNA (2010), su utilización digestiva es baja en todas las especies, debido a su elevada proporción de proteína ligada a la pared celular, y a la presencia de taninos. Aunque la PB es menor que en otros alimentos, las lías de vino pueden representar una fuente de proteína para rumiantes, al igual que la harina de girasol o algunas semillas de leguminosas.

El contenido en azúcares en este tipo de subproductos es generalmente bajo, y los mayores valores se encuentran en orujos no fermentados, como es el caso del vino blanco (Baumgärtel *et al.,* 2007).

Las semillas de uva, y en menor grado el orujo integral, tienen un apreciable contenido en extracto etéreo (EE) (11 y 6%, respectivamente). En el perfil de ácidos grasos predominan los poliinsaturados, por lo que un interesante potencial de estos subproductos de bodega en alimentación animal es referido a la presencia de algunos ácidos grasos insaturados como el ácido linoleico (C18:2) y el ácido oleico (C18:1) (Tabla 2.7).

Tabla 2.7. Perfil de ácidos grasos de los subproductos de bodega (% ácidos grasos totales).

	Orujo integral de uva		Residuo de uva ensilado	Hollejo de uva	Harina de extracción de granilla de uva	Semilla de uva entera	
	FEDNA, 2010	Yi *et al.,* 2009	Santos *et al.,* 2014	FEDNA, 2010	FEDNA, 2010	Correddu *et al.,* 2015a	FEDNA, 2010
C14:0	-	0,32	-	-	-	-	-
C16:0	7,50	12,7	12,5	7,50	8,00	8,50	8,00
C16:1	-	0,68	-	-	-	-	-
C18:0	4,00	4,69	4,40	4,00	4,00	4,90	4,00
C18:1	19,0	14,2	19,2	19,0	19,0	9,60	19,0
C18:2	67,5	60,4	61,2	67,5	67,0	74,0	67,0
C18:3	-	2,25	14,1	-	-	0,30	-
C>=20	-	3,37	-	-	-	-	-

El porcentaje de cenizas es superior en los hollejos que en las semillas (6,8 *vs.* 3,2) (FEDNA, 2010). Los hollejos y semillas son pobres en fósforo, sodio, cloro y magnesio. El fósforo, además, es de muy baja disponibilidad. Algunas partidas pueden contener niveles elevados de Cu (hasta 150 mg kg^{-1}) según las condiciones de cultivo y el tipo de vino producido (Spanghero *et al.,* 2009; FEDNA, 2010).

De forma general, aunque los trabajos realizados son escasos, los subproductos de bodega, presentan una baja digestibilidad (Tabla 2.8), pero superior a la de la paja de

cereales y similar a la de los subproductos de oliva. Diversos autores han determinado la digestibilidad *in vitro* de los nutrientes del orujo de uva, señalando valores de digestibilidad de la MS de 32-41% (Pirmohammadi *et al.,* 2007; Molina-Alcaide *et al.,* 2008; Balasan *et al.,* 2011). También ha sido determinado su digestibilidad *in vivo* (Baumgärtel *et al.,* 2007; Zalikarenab *et al.,* 2007) mostrando valores de digestibilidad de la MS de entre el 32-58%. La digestibilidad de la proteína es baja y, puede explicarse por la presencia de taninos y porque además, gran parte de la PB se asocia con la fracción de FAD.

Tabla 2.8. Valores de digestibilidad (%) en rumiantes de los subproductos de bodega determinados *in vitro* e *in vivo.*

	MS	MO	PB	EE	FB	FND	FAD	Referencia
Digestibilidad *In vitro*								
Orujo integral de uva	41,0	-	-	-	-	36,4	-	Balasan *et al*., 2011
	32,5	-	52,0	-	-	-	-	Molina-Alcaide *et al.,* 2008
	34,5	23,0	-	-	-	-	-	Pirmohammadi *et al*., 2007
Orujo de uva ensilado	28,5	18,2	-	-	-	-	-	Pirmohammadi *et al.,* 2007
Hollejos de uva	77,6	-	-	-	-	39,8	-	Balasan *et al*., 2011
Semillas de uva entera	73,9	-	-	-	-	38,8	-	Balasan *et al.,* 2011
Lías	82,0	-	71,5	-	-	-	-	Molina-Alcaide *et al.,* 2008
Digestibilidad *In vivo*								
Orujo integral de vino tinto	33,0	32,0	30,0	81,0	6,00	15,0	0,00	Baumgärtel *et al*., 2007
	31,6	28,3	8,61	-	-	14,9	-	Zalikarenab *et al.,* 2007
Orujo integral de vino blanco	58,0	56,0	8,00	70,0	19,0	21,0	7,00	Baumgärtel *et al.,* 2007
	39,0	34,3	34,3	-	-	22,4	-	Zalikarenab *et al*., 2007

Ha sido observada una reducción en la digestibilidad de la dieta de ovejas al incluir orujo de uva en la misma, lo cual, es probablemente debido a la presencia de taninos o altos contenidos en lignina del orujo, ya que, tal y como fue explicado en apartados anteriores, los taninos son conocidos por formar complejos, especialmente con la PB y la fibra, por inhibir la acción de ciertas enzimas en el rumen y por proteger las partículas de su degradación por los microorganismos (Baumgärtel *et al.,* 2007; Zalikarenab *et al.,* 2007).

En relación con la degradabilidad ruminal del orujo de uva, Molina-Alcaide *et al.* (2008) y Pirmohammadi *et al.* (2007) obtuvieron en ovejas valores relativamente bajos de degradabilidad efectiva *in situ* de la MS del orujo de uva (33 y 36% respectivamente), lo cual podría deberse a su elevado contenido fibroso (Van Soest *et al.,* 1994). Sin embargo, Besharati y Taghizadeh (2009) obtuvieron valores más altos (64%). Molina-Alcaide *et al.*

(2008) señalaron valores de 45% degradabilidad efectiva *in situ* de la PB de orujo de uva en ovejas.

El valor total de energía del orujo de uva es bajo, sin embargo, dependiendo de los detalles técnicos de la elaboración del vino y de la variedad de uva de origen, existen marcadas diferencias. El contenido en energía metabolizable (EM) del orujo de uva indicado en la bibliografía oscila entre 4,2 y 7,1 MJ kg^{-1} MS (INRA, 2007; Baumgärtel *et al.* 2007). Spanghero *et al.* (2009) y Balasan *et al.* (2011) han señalado valores de EM en pulpa-hollejos y semillas de 5,8-12,7 y 6,2-12,1 MJ kg^{-1} MS, respectivamente.

2.6.3. Compuestos fenólicos del orujo de uva

Las uvas presentan un alto contenido en metabolitos secundarios, y por lo tanto, en los subproductos generados en la elaboración del vino también están presentes, ya que aunque durante el proceso de vinificación parte de ellos son transmitidos al vino, una alta proporción permanece en los residuos que se generan (Alonso *et al.,* 2002; Spanghero *et al.,* 2009).

Los compuestos fenólicos se encuentran irregularmente distribuidos en la uva, donde cada una de sus partes presenta una composición más o menos específica. Los fenoles sencillos o no flavonoides, que agrupan a los ácidos fenólicos y a los estilbenos (entre los que destaca el resveratrol), están ubicados principalmente en el hollejo y pulpa, y su concentración varía a lo largo del desarrollo de la baya. Los flavonoides constituyen una gran parte de los fenoles totales (85% aproximadamente) en las uvas. Dentro del grupo de los flavonoides, en las uvas destaca la presencia de diversos grupos de compuestos como los flavonoles, los flavanoles y los antocianos.

Los flavonoles son pigmentos amarillos presentes únicamente en las pieles de uva tinta y blanca. Dentro del grupo de los flavanoles destacan los taninos catequéticos o taninos condensados, siendo la semilla la fuente principal de estos compuestos, aunque también se han detectado en la pulpa. Los antocianos se encuentran en uvas tintas, siendo la malvidina el más común y responsable del color magenta, mientras que los colores violeta y azul son generalmente debidos a la delfinidina (Leighton y Urquiaga, 2000; Ribéreau-Gayon *et al.,* 2003). La Tabla 2.9 muestra el contenido medio en compuestos fenólicos mayoritarios en los subproductos derivados de la industria del vino y la Tabla 2.10 el perfil de compuestos fenólicos detallado del orujo de uva.

Tabla 2.9. Composición fenólica de los subproductos derivados de la elaboración del vino (g kg^{-1} MS).

	Polifenoles extraíbles	Taninos condensados	Polifenoles hidrolizables	Antocianos	Referencia
Orujo integral de uva	70,5	79,0	-	-	Abarghuei *et al.,* 2010
	22,7	15,6	-	-	Alipour y Rouzbehan, 2007
	55,0	36,5	-	-	Baumgärtel *et al.,* 2007
	67,0	52,3	-	-	Besharati y Taghizadeh, 2009
	26,3	223	-	-	Llobera y Cañellas, 2007
	-	147	-	-	Molina-Alcaide *et al*., 2008
	41,9	22,3	-	9,80	Negro *et al*., 2003
	23,6	18,6	-	-	Pirmohammadi *et al.,* 2007
	4,00	-	-	2,40*	Yi *et al.,* 2009
	22,6	17,8	-	-	Zalikarenab *et al*., 2007
Hollejo de uva	16,6	13,0	-	0,80	Deng *et al.,* 2011
	1,40	-	-	0,80	Katalinić *et al.,* 2010
	33,3	8,60	-	19,2	Negro *et al.,* 2003
	45,0	-	-	28,0*	Spanghero *et al.,* 2009
Semilla de uva	85,8	64,1	-	0,00	Negro *et al.,* 2003
	68,3	-	-	62,3*	Spanghero *et al.,* 2009
Concentrado de orujo de uva	48,7	151	26,0	-	Brenes *et al*., 2008
Lías	-	97,7	-	-	Molina-Alcaide *et al*., 2008

*(mg Kg^{-1} MS)

El contenido en compuestos fenólicos del orujo de uva es muy variable y depende del tipo de uva (blanca o roja) (Spanghero *et al.,* 2009) y del tejido considerado (piel, pulpa o semillas). La variabilidad en los niveles de compuestos fenólicos entre las diferentes fracciones de orujo (semillas o pulpa) es probablemente debida a la variedad de uva, a los factores que afectan al desarrollo del fruto, como son el suelo, las condiciones de ubicación geográfica y el clima, así como la etapa de madurez (Ojeda *et al.,* 2002). Por otra parte, los procesos de elaboración y de producción del vino (extracción, fraccionamiento, secado) también afectan a la variabilidad existente entre los tipos de orujos blancos y tintos (Muñoz *et al.,* 2004; Baumgärtel *et al.,* 2007) y hace que los resultados de composición fenólica no puedan extrapolarse entre orujos de distintas procedencias.

Tabla 2.10. Perfil de compuestos fenólicos del orujo de uva (mg kg^{-1} MS).

Compuesto fenólico	Orujo de uva tinta [1]	Orujo de uva blanca [2]
Compuestos no flavonoides		
- Ácidos fenólicos		
. Ácidos cinámicos		
Ácido caftárico	-	158
Ácido cafeico	2,57	-
Ácido p-cumárico	10,4	-
Ácido *cis*-cutárico	-	11,2
Ácido *trans*-cutárico	-	32,3
Ácido ferúlico	3,88	-
Ácido fertárico	-	5,40
. Ácidos benzoicos		-
Ácido gálico	40,7	49,3
Ácido siríngico	279	-
- Estilbenos		
. Polydatin	1,98	-
. Piceatannol	6,25	-
. *Trans* resveratrol	5,79	-
Compuestos flavonoides		
- Flavonoles		
. Quercetin-3-rutinósido	-	30,0
. Quercetin-3-glucurónido	-	293
. Quercetin-3-galactósido	-	37,7
. Quercetin-3-glucósido	18,1	304
. Quercetin pentósido	-	3,02
. Quercetina	89,7	-
. Kaempferol-3-galactósido	-	20,5
. Kaempferol-3-glucurónido	-	4,68
. Kaempferol-3-glucósido	-	82,5
. Isorhamnetin-3-glucósido	-	9,32
. Isorhamnetin-3- glucurónido	-	6,84
- Flavanoles		
. (+) - Catequina	545	1,31*
. (–) - Epicatequina	284	404
. (–) - Galocatequina	-	-
. (–) - Epigalocatequina	-	-
. (–) - Epigalocatequina galato	-	-
. Procianidinas	-	3,35*
- Antocianidinas o antocianos		
. Delfinidín 3-O-glucóscido	738	-
. Cianidín 3-O-glucóscido	140	-
. Petunidín 3-O-glucóscido	1,11*	-
. Peonidín 3-O-glucóscido	396	-
. Malvidín 3-O-glucóscido	4,29*	-
. Delfinidín 3-O-acetilglucóscido	168	-
. Petunidín 3-O-acetilglucóscido	229	-
. Peonidín 3-O-acetilglucóscido	306	-
. Malvidín 3-O-acetilglucóscido	647	-
. Cianidín 3-O-cumaroilglucóscido	304	-
. Petunidín 3-O-cumaroilglucóscido	399	-
. Peonidín 3-O-cumaroilglucóscido	298	-
. Malvidín 3-O-cumaroilglucóscido	2,07*	-

[1] Elaborado a partir de Antoniolli *et al.* (2015); [2] Elaborado a partir de Jara-Palacios *et al.* (2014).
* (g Kg^{-1} MS)

2.6.4. Empleo de orujo de uva en alimentación de rumiantes

El orujo de uva presenta un bajo valor nutritivo, debido a su escaso valor energético y a su alto contenido en fibra lignificada (Alipour y Rouzbehan, 2007; Baumgärtel *et al.,* 2007). Por este motivo, el orujo de uva se ha incluido en dietas para rumiantes cuando se alimentan cerca del nivel de mantenimiento o en situaciones en las que no son necesarias altas tasas de crecimiento o de producción de leche (FEDNA, 2010).

Además del valor nutritivo que presenta el orujo de uva en sí mismo, los compuestos fenólicos que contiene, especialmente los taninos condensados (Makris *et al.,* 2007), pueden interferir en la utilización nutritiva de los alimentos y afectar a los parámetros productivos. Así, se han observado descensos en la degradabilidad y en la digestibilidad de los alimentos al incluir taninos condensados de orujo de uva en raciones animales a diferentes niveles (Alipour y Rouzbehan 2010; Abarghuei *et al.,* 2010), habiendo sido señalada una reducción de la degradación de proteína en rumen, a medida que se incrementa la concentración de taninos.

Los efectos sobre los rendimientos productivos al incluir orujo de uva en raciones de corderos son muy variados. Algunos autores (Bahrami *et al.,* 2010) han indicado que la suplementación con orujo de uva al 5 y 10% mejora el crecimiento y la digestibilidad de los alimentos. En el lado opuesto, Malossini *et al.* (1993) al incluir orujo de uva a niveles del 30 y del 60% en raciones de corderos observaron un incremento de la ingestión diaria por kilogramo de peso metabólico unido a una menor ganancia media diaria de los animales, y a un incremento del índice de conversión del alimento, probablemente debido a su bajo valor nutritivo. Sin embargo, en otros casos no se han observado efectos al incluir este tipo de subproductos en dietas de corderos. En este sentido, Pétriz-Celaya *et al.* (2010) al añadir 10 y 30% de orujo de uva en raciones de corderos observaron un ligero incremento en la ingestión, sin que el crecimiento y los parámetros productivos se viesen afectados. Tampoco Jerónimo *et al.* (2010) encontró diferencias sobre la ingestión, crecimiento y parámetros productivos de corderos cuyas dietas fueron suplementadas con 25 g kg^{-1} MS de extracto de granilla de uva.

Tal y como fue explicado en los apartados anteriores, los compuestos fenólicos pueden interferir sobre los parámetros que definen la calidad carne, y por tanto el orujo de uva es susceptible de actuar sobre ellos.

En primer lugar, la incorporación de orujo de uva en dietas de rumiantes puede interferir sobre el perfil lipídico de la carne debido a la presencia de componentes activos de

naturaleza fenólica, como los taninos, que pueden afectar al proceso de BH ruminal, aunque en muchos casos los resultados encontrados son contradictorios. En este sentido, Jerónimo *et al.* (2012) al incluir 25 g kg^{-1} MS de extracto de granilla de uva en dietas de corderos observó un incremento de los PUFA n6 de cadena larga de la grasa intramuscular de los corderos respecto al tratamiento control. Sin embargo, estos mismos autores en un trabajo previo (Jerónimo *et al.,* 2010) no observaron efecto sobre la BH de corderos.

En segundo lugar, los compuestos fenólicos presentes en el orujo de uva pueden interferir retrasando la oxidación lipídica y proteica de la carne, ya que el orujo de uva ha mostrado ejercer actividad antiradicalaria (Llobera y Cañellas, 2007; Yi *et al.,* 2009) siendo los métodos utilizados para su valoración muy diversos. En este sentido, tanto en modelos *in vitro* como *in vivo* se ha comprobado que los extractos de piel y de semilla de uva presentan una alta actividad antioxidante debido, entre otros compuestos fenólicos, a las proantocianidinas o taninos condensados, los cuales presentan capacidad de captar radicales libres y de quelar iones metálicos, que son fuertes promotores de la peroxidación lipídica, además de ejercer sinergia con otros antioxidantes. Algunos autores han indicado la existencia de una correlación lineal entre la capacidad antioxidante y el contenido total de compuestos fenólicos del vino y sus subproductos (Alonso *et al.,* 2002).

La actividad antioxidante de aditivos procedentes de la uva ha sido comprobada al añadirlos sobre carne picada de diferentes especies animales (Ahn *et al.,* 2002; Lau y King, 2003; Carpenter *et al.,* 2007). Por ello, recientemente ha crecido el interés por conocer el efecto de su incorporación en dietas animales. Aunque son pocos los trabajos que han investigado *in vivo* el efecto de la inclusión de subproductos derivados de la uva en condiciones prácticas sobre la calidad de la carne, se ha podido comprobar un incremento de la estabilidad oxidativa de la carne, al incluir concentrado de orujo de uva en piensos de pollos (Brenes *et al.,* 2008), llegando incluso en algunos casos a presentar efecto similar a la vitamina E. Gladine *et al.* (2007a; 2007b) confirmaron el efecto protector antioxidante de los polifenoles del extracto de uva en el plasma y el hígado de ratas.

Los trabajos de inclusión de subproductos de uva en alimentación de rumiantes sobre la oxidación lipídica del músculo y de otros parámetros de calidad de la carne, son mucho más reducidos y contradictorios en algunas ocasiones. Los estudios existentes indican que los extractos de hollejos de uva en raciones de ovejas fueron más potentes que otros antioxidantes habituales, como la vitamina E, en inducir la actividad de genes involucrados en las defensas del organismo frente a la oxidación (Sgorlon *et al.,* 2006). Además la inclusión de extracto de semillas y hollejos de uva directamente en el rumen de ovejas ha

mostrado ser efectivo reduciendo la susceptibilidad a la oxidación lipídica del plasma (Gladine *et al.,* 2007c).

En un estudio con corderos, Jerónimo *et al.* (2012) al añadir extracto de granilla de uva en la dieta, observaron una reducción de la oxidación lipídica a partir de 3 días de refrigeración y un pardeamiento inferior en la carne fresca, aunque esta respuesta no se mantuvo a lo largo del almacenamiento. Sin embargo, en otros trabajos no se ha observado una mejora sobre la estabilidad lipídica de la carne al incluir 900 ppm de extracto de vino tinto en dietas de corderos ricas en PUFA n3 (Muíño *et al.,* 2014).

La inclusión de orujo de uva en dietas de rumiantes también puede afectar a los parámetros de producción y de calidad de la leche, debido a su propio valor nutritivo y a los compuestos bioactivos que contiene con resultados dispares entre los trabajos realizados por distintos autores.

Así, diversos trabajos al incluir silo de orujo de uva en dietas de vacas lecheras (Santos *et al.,* 2014) y semilla de uva en dietas de ovejas (Nudda *et al*., 2015) no han encontrado efecto sobre la producción y composición de la leche. Nielsen y Hansen (2004) al incluir orujo de uva en raciones de vacas lecheras tampoco apreciaron efectos en el recuento de células somáticas, ni en la producción de proteína, aunque el porcentaje de proteína fue significativamente superior en la leche de las vacas alimentadas con orujo, probablemente debido a la interferencia de los polifenoles sobre la degradación ruminal. Sin embargo, Moate *et al.* (2014) al añadir orujo en dietas de vacas en forma de pellet o de ensilado observaron un descenso en la producción de grasa de la leche, y además el ensilado de orujo redujo la producción lechera y el contenido proteico.

El perfil lipídico de la grasa de la leche también puede verse afectado por la inclusión de residuos de bodega como consecuencia de la interferencia de los compuestos fenólicos del orujo en el proceso de BH ruminal. En este sentido, Moate *et al.* (2014) al incluir al incluir orujo de uva en dietas de vacas observaron un descenso en el contenido de SFA e incrementos en los niveles de MUFA y PUFA, así como de RA y VA en la leche. Santos *et al.* (2014) también encontró una tendencia a aumentar el nivel de PUFA en la leche a medida que la concentración de orujo se incrementaba.

Por otra parte, han sido encontradas mejoras en el efecto antioxidante de la leche al incluir orujo de uva en raciones de rumiantes. Es este sentido, Santos *et al.* (2014) al incluir silo de orujo de uva en raciones de vacas, observaron un incremento en la actividad antioxidante de la leche sin que la producción se viera afectada, lo que indica una liberación

de compuestos fenólicos del orujo en el rumen, que podría tener efectos beneficiosos para la producción de leche, así como, para el sistema inmunitario (Nudda *et al.,* 2014). Del mismo modo, Correddu *et al.* (2015b) al incluir semilla de uva en raciones de ovejas observó una reducción de la oxidación de los ácidos grasos insaturados de la leche.

Todos estos resultados son indicios de posibles efectos beneficiosos del empleo del orujo de uva en raciones de rumiantes en general y del ganado ovino en particular, y justifica la necesidad de la realización de estudios *in vivo* que clarifiquen el efecto del orujo sobre la calidad de los productos obtenidos.

2.7. Referencias bibliográficas

Abarghuei, M.J., Rouzbehan, Y., Alipour, D. (2010). The influence of the grape pomace on the ruminal parameters of sheep. *Livestock Science 132*, 73–79.

Abbeddou, S., Rischkowsky, B., Richter, E.K., Hess, H.D., Kreuzer, M. (2011). Modification of milk fatty acid composition by feeding forages and agro-industrial byproducts from dry areas to Awassi sheep. *Journal of Dairy Science* 94, 4657–4668.

Acamovic, T., Brooker, J.D. (2005). Biochemistry of plant secondary metabolites and their effects in animals. *Proceedings of the Nutrition Society 64*, 403–412.

Ahn, J., Grün, I., Fernando, L. (2002). Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *Journal of Food Science 67,* 1364–1369.

Albertí, P., Ripoll, G., Casasús, I., Blanco, M., Chapullé, J.L.G., Santamaría, J. (2005). Efecto de la inclusión de antioxidantes en dietas de acabado sobre la calidad de la carne de terneros. *ITEA inforrmación técnica económica agraria 101*, 91–100.

Alfadda, A.A., Sallam, R.M. (2012). Reactive oxygen species in health and disease. *Journal of Biomedicine and Biotechnology,* 1–14.

Alipour, D., Rouzbehan, Y. (2010). Effects of several levels of extracted tannin from grape pomace on intestinal digestibility of soybean meal*. Livestock Science 128*, 87–91.

Alipour, D., Rouzbehan, Y. (2007). Effects of ensiling grape pomace and addition of polyethylene glycol on *in vitro* gas production and microbial biomass yield. *Animal Feed Science and Technology 137*, 138–149.

Allard, J., Kurian, R., Aghdassi, E., Muggli, R., Royall, D. (1997). Lipid peroxidation during n-3 fatty acid and vitamin E supplementation in humans. *Lipids 32*, 535–541.

Alonso, A.M., Guillén, D.A., Barroso, C.G., Puertas, B., García, A. (2002). Determination of antioxidant activity of wine byproducts and its correlation with polyphenolic content. *Journal of Agricultural and Food Chemistry 50*, 5832–5836.

Andrés, S., Huerga, L., Mateo, J., Tejido, M.L., Bodas, R., Morán, L., Prieto, N., Rotolo, L., Giráldez, F.J. (2014b). The effect of quercetin dietary supplementation on meat oxidation processes and texture of fattening lambs. *Meat Science 96*, 806–811.

Andrés, S., Morán, L., Aldai, N., Tejido, M.L., Prieto, N., Bodas, R., Giráldez, F.J. (2014a). Effects of linseed and quercetin added to the diet of fattening lambs on the fatty acid profile and lipid antioxidant status of meat samples. *Meat Science 97*, 156–163.

Andrés, S., Tejido, M., Bodas, R., Morán, L., Prieto, N., Blanco, C., Giráldez, F. (2013). Quercetin dietary supplementation of fattening lambs at 0.2% rate reduces discolouration and microbial growth in meat during refrigerated storage. *Meat Science 93*, 207–212.

Anila, L., Vijayalakshmi, N.R. (2003). Antioxidant action of flavonoids from *Mangifera indica* and *Emblica officinalis* in hypercholesterolemic rats. *Food Chemistry 83*, 569–574.

Antoniolli, A., Fontana, A.R., Piccoli, P., Bottini, R. (2015). Characterization of polyphenols and evaluation of antioxidant capacity in grape pomace of the cv. Malbec. *Food Chemistry 178*, 172–178.

Arnold, R.N., Arp, S.C., Scheller, K.K., Williams, S.N., Schaefer, D.M. (1993). Tissue equilibration and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. *Journal of Animal Scince 71*, 105–118.

Aurosseau, B. (2002). Les radicaux libres dans l'organisme des animaux d'élevage: conséquences sur la reproduction, la physiologie et la qualité de leurs produits. *INRA Productions Animales 15*, 67–82.

Bahrami, Y., Foroozandeh, A.D., Zamani, F., Modarresi, M., Eghbal-Saeid, S., Chekani-Az, S. (2010). Effect of diet with varying levels of dried grape pomace on dry matter digestibility and growth performance of male lambs. *Journal of Animal and Plant Sciences 6*, 605–610.

Bampidis, V.A., Christodoulou, V., Florou-Paneri, P., Christaki, E., Spais, A.B., Chatzopoulou, P.S. (2005). Effect of dietary dried oregano leaves supplementation on performance and carcass characteristics of growing lambs. *Animal Feed Science and Technology 121*, 285–295.

Bañón, S., Méndez, L., Almela, E. (2012). Effects of dietary rosemary extract on lamb spoilage under retail display conditions. *Meat Science 90*, 579–583.

Bas, P., Morand-Fehr, P. (2000). Nutritional factors on fatty acid composition of lamb fat deposits. *Livestock Production Science 64*, 61–79.

Basalan, M., Gungor, T., Owens, F.N., Yalcinkaya, I. (2011). Nutrient content and in vitro digestibility of Turkish grape pomaces. *Animal Feed Science and Technology 16*, 194–198.

Baumgärtel, T., Kluth, H., Epperlein, K., Rodehutscord, M. (2007). A note on digestibility and energy value for sheep of different grape pomace. *Small Ruminant Research 67*, 302–306.

Ben Khedim, M. (2014). Development of traditional processed dairy products which promote cardiovascular health through the inclusion of canola seed and marjoram in ewe's diet. Tesis Doctoral. Universidad de León.

Benchaar, C., Chouinard, P.Y. (2009). Assessment of the potential cinnamaldehyde, condensed tannins and saponins to modify milk fatty acid composition of dairy cows. *Journal of Dairy Science 92*, 3392–3396.

Bento, M.H.L., Acamovic, T., Makkar, H.P.S. (2005). The influence of tannin, pectin and polyethylene glycol on attachment of 15N-labelled rumen microorganism to cellusose. *Animal Feed Science and Technology 122*, 41–57.

Berthelot, V., Bas, P., Schmidely, P. (2010). Utilization of extruded linseed to modify fatty composition of intensively-reared lamb meat: Effect of associated cereals (wheat vs. corn) and linoleic acid content of the diet. *Meat Science 84*, 114–124.

Besharati, M., Taghizadeh, A. (2009). Evaluation of dried grape by-product as a tanniniferous tropical feedstuff. *Animal Feed Science and Technology 152*, 198–203.

Björck, I.M., Nyman, M.E. (1987). In vitro effects os phytic acid and polyphenols on starch digestion and fiber degradation. *Journal of Food Science 52*, 1588–1594.

Bjorneboe, A., Bjorneboe, G.E., Bodd, E., Hagen, B.F., Kveseth, N., Drevon, C.A. (1986). Transport and distribution of alpha-tocopherol in lymph, serum and liver cells in rats. *Biochimica et Biophysica Acta 3*, 305–310.

Blauwiekel, R., Xu, S., Harrison, J.H., Loney, K.A., Riley, R.E., Calhoun, M.C. (1997). Effect of whole cottonseed, gossypol and ruminally protected lysine supplementation on milk yield and composition. *Journal of Dairy Science 80*, 1358–1365.

Bodas, R., Prieto, N., Jordán, M.J., López-Campos, Ó., Giráldez, F.J., Morán, L., Andrés, S. (2012). The liver antioxidant status of fattening lambs is improved by naringin dietary supplementation at 0.15% rates but not meat quality. *Animal 6*, 863–870.

Botterweck, A.A.M., Verhagen, H., Goldbohm, R.A., Kleinjans, J., Van den Brandt, P.A. (2000). Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands Cohort Study. *Food and Chemical Toxicology* 38, 599–605.

Bou, R., Codony, R., Tres, A., Decker, E.A. (2009). Dietary strategies to improve nutritional value, oxidative stability and sensory properties of poultry products*. Critical Reviews in Food Science and Nutrition 8,* 800–822.

Bramley, P.M., Elmadfa, I., Kafatos, A., Kelly, F.J., Manios, Y., Roxborough, H.E., Schuch, W., Sheehy, P.J.A., Wagner, K. (2000). *Vitamin E Journal of the Science of Food and Agriculture 80*, 913–938.

Brenes, A., Viveros, A., Goñi, I., Centeno, C., Sáyago-Ayerdi, S.G., Arija, I., Saura-Calisto, F. (2008). Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. *Poultry Science 87,* 307–316.

Broudiscou, L.P., Cornu, A., Rouzeau, A. (2007). In vitro degradation of 10 mono- and sesquiterpenes of plant origin by caprine rumen micro-organisms. *Journal of the Science of Food and Agriculture 87*, 1653–1658.

Cadenas, E. (1997). Basic mechanisms of antioxidant activity. *BioFactors 6*, 391–397.

Carpenter, R., O'Grady, M.N., O'Callaghan, Y.C., O'Brien, N.M., Kerry, J.P. (2007). Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Meat Science 76*, 604–610.

Carratù, B., Sanzini, E. (2005). Sostanze biologicamente attive presenti negli alimenti di origine vegetale. *Annali Dell Istituto Superiore Di Sanita 41*, 7–16.

Carro, M.D., Saro, C., Mateos, I., Diaz, A., Ranilla, M.J. (2014). Perspectivas y retos de los extractos vegetales como aditivos alimentarios en rumiantes. *Albeitar 179,* 4-6.

Castellini, C., Dal Bosco, A., Bernardini, M., Cyril, H. (1998). Effect of dietary vitamin E on the oxidative stability of raw and cooked rabbit meat. *Meat Science 50*, 153–161.

Castillejos, L., Calsamiglia, S., Ferret, A., Losa, R. (2007). Effects of dose and adaptation time of a specific blend of essential oil compounds on rumen fermentation. *Animal Feed Science and Technology 132*, 186–201.

Castro, T., Manso, T., Mantecón, A.R., Guirao, J., Jimeno, V. (2005). Fatty acid composition and carcass characteristics of growing lambs fed diets containing palm oil supplements. *Meat Science 69*, 757–764.

Chaves, A.V., Stanford, K., Dugan, M.E.R., Gibson, L.L., McAllister, T.A., Van Herk, F., Benchaar, C. (2008). Effects of cinnamaldehyde, garlic and juniper berry essential oils on rumen fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. *Livestock Science 117,* 215–224.

Chihuailaf, R.H., Contreras, P.A., Wittwer, F.G. (2002). Pathogenesis of oxidative stress: consequences and evaluation in animal health. *Veterinaria México 33*, 265–283.

Chiofalo, V., Liotta, L., Fiumanò, R., Benedetta, E., Chiofalo, B. (2012). Influence of dietary supplementation of *Rosmarinus officinalis* L. on performances of dairy ewes organically managed. *Small Ruminant Research 104*, 122–128.

Colomer-Rocher, F. (1988): Estudio de los parámetros que definen los caracteres cuantitativos y cualitativos de las canales bovinas. In: IV Curso Internacional de Producción de Carne y Leche a base de Pastos y Forrajes. pp. 90. CIAM, La Coruña (Spain).

Correddu, F., Nudda, A., Battacone, G., Boe, R., Francesconi, A.H.D., Pulina, G. (2015a). Effects of grape seed supplementation, alone or associated with linseed, on ruminal metabolism in Sarda dairy sheep. *Animal Feed Science and Technology 199*, 61–72.

Correddu, F., Nudda, A., Manca, M.G., Pulina, G., Dalsgaard, T.K. (2015b). Light-induced lipid oxidation in sheep milk: effects of dietary grape seed and linseed, alone or in combination, on milk oxidative stability. *Journal of Agricultural and Food Chemistry 63*, 3980–3986.

Cos, P., Bruyne, T.D., Hermans, N., Apers, S., Berghe, D.V., Vlietinck, A.J. (2003). Proanthocyanidins in health care: current and new trends. *Current Medicinal Chemistry 11*, 1345–1359.

D'Alessandro, A.G., Maiorano, G., Kowaliszyn, B., Loiudice, P., Martemucci, G. (2012). How the nutritional value and consumer acceptability of suckling lambs meat is affected by the maternal feeding system. *Small Ruminant Research 106*, 83–91.

Davidson, P.M., Naidu, A.S. (2000). Phyto-Phenols. In: Natural Food antimicrobial Systens. (A.S. Maidu, ed.). pp. 265–294. CRC Press, Boca Raton, Florida (EEUU).

Davies, K. (1987). Protein damage and degradation by oxygen radicals. I. general aspects. *Journal of Biological Chemistry 262*, 9895–9901.

De Pascual-Teresa, S., Sánchez-Ballesta, M.T. (2008). Anthocyanins: from plant to health. *Phytochemistry Reviews 7*, 281–299.

Deckert, V., Desrumaux, C., Athias, A., Duverneuil, L., Palleau, V., Gambert, P., Masson, D., Lagrost, L. (2002). Prevention of LDL, α-tocopherol consumption, cholesterol oxidation and vascular endothelium dysfunction by polyphenolic compounds from red wine. *Atherosclerosis 165*, 41–50.

Deng, Q., Penner, M.H., Zhao, Y.Y. (2011). Chemical composition of dietary fiber and polyphenols of five different varieties of wine grape pomace skins. *Food Research International 44*, 2712–2720.

Déprez, S., Brezillon, C., Rabot, S., Philippe, C., Mila, I., Lapierre, C., Scalbert, A. (2000). Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. *Journal of Nutrition 130*, 2733–2738.

Descalzo, A.M., Sancho, A.M. (2008). A review of natural antioxidant and their effects on oxidative status, odor and quality of fresh beef produced in Argentina. *Meat Science 79*, 423–436.

Díaz, M.T., Sánchez, M., Martínez, B., Vieira, C., García, M.D. (2005). Valor nutritivo de la carne. Determinación del contenido energético. In: Estandarización de las metodologías para evaluar la calidad del producto (animal vivo, canal, carne y grasa) en los rumiantes. Monografías INIA: Serie Ganadera, nº3. (V. Cañeque, C. Sañudo, eds.). pp. 274–281. Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria. Ministerio de Educación y Ciencia, Madrid (Spain).

Dreosti, I.E. (2000). Antioxidant polyphenols in tea, cocoa, and wine. *Nutrition 16*, 692–694.

El-Agamey, A., Lowe, G.W., McGarvey, D.J., Mortensen, A., Phillip, D.M., Truscott, T.G., Young, A.J. (2004). Carotenoid radical chemistry and antioxidant/prooxidant properties. *Archives of Biochemistry and Biophysics 430*, 37–48.

Emami, A., Nasri, M.H.F., Ganjkhanlou, M., Zali, A., Rashidi, L. (2015). Effects of dietary pomegranate seed pulp on oxidative stability of kid meat. *Meat Science 104*, 14–19.

Ercolini, D., Russo. F., Torrieri, E., Masi, P., Villiani, F. (2006). Changes in the spoilage-related microbiota of beef Turing refrigerated storage under different packaging contitions. *Applied and Environmental Microbiology 72*, 4663–4671.

Esterbauer, H. (1993). Cytotoxicity and genotoxicity of lipid-oxidation products. *American Journal of Clinical Nutrition 57*, 779S–785S.

Falowo, A.B., Fayemi, P.O., Muchenje, V. (2014). Natural antioxidants agains, lipid-protein oxidative deterioration in meat and meat products: A review. *Food Research International 64*, 171–181.

FAOSTAT (2012). http://faostat.fao.org/ [09 de julio de 2015].

Faustman, C., Sun, Q., Mancini, R., Suman, S.P. (2010). Myoglobin and lipid oxidation interactions: mechanistic bases and control. *Meat Science 86*, 86–94.

FEDNA (2010). Tablas de composición y valor nutritivo de los alimentos para la fabricación de piensos compuestos. (C. de Blas, G.G. Mateos, P. García-Rebollar). FEDNA, Madrid (Spain).

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

Frutos, P., Hervás, G., Giráldez, F.J., Fernández, M., Mantecón, A.R. (2000). Digestive utilization of quebracho-treated soya bean meals in sheep. *Journal of Agricultural Science 134*, 101–108.

Frutos, P., Hervás, G., Giráldez, F.J., Mantecón, A.R. (2004). Review. Tannins and ruminant nutrition. *Spanish Journal of Agricultural Research 2*, 191–202.

Gai, F., Gasco, L., Liu, H., Lussiana, C., Brugiapaglia, A., Masoero, G., Zoccarato, I. (2009). Effect of diet chestnut tannin supplementation on meat quality, fatty acid profile and lipid stability in broiler rabbits. *Italian Journal of Animal Science 8*, 787–789.

Giannenas, I.A., Florou-Paneri, P., Botsoglou, N.A., Christaki, E., Spais, A.B. (2005). Effect of supplementing feed with oregano and/or a-tocopheryl acetate on growth of broiler chickens and oxidative stability of meat. *Journal of Animal and Feed Sciences 14*, 521–535.

Gladine, C., Morand, C., Rock, E., Gruffat, D., Bauchart, D., Durand, D. (2007a). The antioxidative effect of plant extracts rich in polyphenols differs between liver and muscle tissues in rats fed n-3 PUFA rich diets. *Animal Feed Science and Technology 139*, 257–272.

Gladine, C., Morand, C., Rock, E., Gruffat, E., Bauchart, D., Durand, D. (2007b). Plant extracts rich in polyphenols (PERP) are efficient antioxidants to prevent lipoperoxidation in plasma lipids from animals fed n-3 PUFA supplemented diets. *Animal Feed Science and Technology 136*, 281–296.

Gladine, C., Rock, E., Morand, C., Bauchart, D., Durand, D. (2007c). Bioavailability and antioxidant capacity of plant extracts rich in polyphenols, given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. *British Journal of Nutrition 98*, 691–701.

Gobert, G., Gruffat, D., Habeanu, M., Parafita, E., Bauchart, D., Durand, D. (2010). Plant extracts combined with vitamin E in PUFA-rich diets of cull cows protect processed beef against lipid oxidation. *Meat Science 85*, 676–683.

Goñi, I., Brenes, A., Centeno, C., Viveros, A., Saura-Calixto, F., Rebolé, A., Arija, I., Estevez, R. (2007). Effect of dietary grape pomace and vitamin E on growth performance, nutrient digestibility, and susceptibility to meat lipid oxidation in chickens*. Poultry Science 86*, 508–516.

Griinari, J.M., Bauman, D.E. (1999). Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In: Advances in Conjugated Linoleic Acid Research. Vol. 1. (M.P. Yurawecz, M.M. Mossoba, J.K.G. Kramer, M.W. Pariza, G.J. Nelson, eds.). pp. 180–200. AOCS Press, Champaign, Illinois (EEUU).

Guardiola, F., Codony, R., Addis, P.B., Rafecas, M., Boatella, J. (1996). Biological effects of oxysterols: Current status. *Food and Chemical Toxicology 34*, 193–211.

Halliwell, B. (1990). How to characterize a biological antioxidant. *Free Radical Research Communications 9*, 1–32.

Hammond, J. (1955). Quality meat production. *Journal of the Yorkshire Agricultural Society 1*, 19–32.

Harfoot, C.G., Hazlewood, G.P. (1997). Lipid metabolism in the rumen. In: The rumen microbial ecosystem. (P.N. Hobson, C.S. Stewart, eds.). pp 382–426. Blackie Academic and Professional, London (UK).

Heidarian Miri, V., Tyagi, A.K., Ebrahimi, S.H., Mohini, M. (2013). Effect of cumin (*Cuminum cyminum*) seed extract on milk fatty acid profile and methane emission in lactating goat. *Small Ruminant Research 113*, 66–72.

Heim, K E., Tagliaferro, A.R., Bobilya, D.J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *Journal of Nutritional Biochemistry 13*, 572–584.

Hervás, G., Frutos, P., Giráldez, F.J., Mantecón, A.R., Álvarez del Pino, M.C. (2003). Effect of different doses of quebracho tannins extracto on rumen fermentation in ewes. *Animal Feed Science and Technology 109*, 65–78.

Honikel, K.O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science 49*, 447–457.

Hou, Z., Lambert, J.D., Chin, K.V., Yang, A.S. (2004). Effects of tea polyphenols on signal transduction pathways related to cancer chemoprevention. *Mutation Research 555*, 3–19.

Hristov, A.N., Lee, C., Cassidy, T., Heyler, K., Tekkipe, J.A., Varga, G.A., Corl, B., Brandt, R.C. (2013). Effect of *Origanum Vulgare* L. leaves on rumen fermentation, production, and milk fatty acid composition in lactating dairy cows. *Journal of Dairy Science 96*, 1189–1202.

INRA, (2007). Alimentation des bovines, ovins et caprins. Besoins des animaux–Valeurs des aliments. Editions Quæ, Paris (France).

Inserra, L., Priolo, A., Biondi, L., Lanza, M., Bognanno, M., Gravador, R., Luciano, G. (2014). Dietary citrus pulp reduces lipid oxidation in lamb meat. *Meat Science 96*, 1489–1493.

Jara-Palacios, M.J., Hernanz, D., González-Manzano, S., Santos-Buelga, C., Escudero-Gilete, M.L., Heredia, F.J. (2014). Detailed phenolic composition of white grape by-products by RRLC/MS and measurement of the antioxidant activity. *Talanta 125*, 51–57.

Jay, J.M. (1966). Beef spoiled beef and aged microbiologically capacity phenomena to and water-holding extract-release volume response. *Applied Microbiology 14*, 492–496.

Jayasena, D.D., Jo, C. (2013). Essential oils as potential antimicrobial agents in meat and meat products: A review. *Trends in Food Science and Technology 34*, 96–108.

Jerónimo, E., Alfaia, C.M.M., Alves, S.P., Dentinho, M.T.P., Prates, J.A.M., Vasta, V., Santos-Silva, J., Bessa, R.J.B. (2012). Effect of dietary grape seed extract and Cistus ladanifer L. in combination with vegetable oil supplementation on lamb meat quality. *Meat Science 92*, 841–847.

Jerónimo, E., Alves, S.P., Dentinho, M.T.P., Martins, S.V., Prates, J.A.M., Vasta, V., Santos-Silva,J., Bessa, R.J.B. (2010) Effect of Grape Seed Extract, *Cistus ladanifer* L., and Vegetable Oil Supplementation on fatty acid composition of abomasal digesta and intramuscular fat of lambs. *Journal of Agricultural and Food Chemistry 58*, 10710–10721.

Jordán, M.J, Castillo, J., Bañón, S., Martínez-Conesa, C., Sotomayor, J.A. (2014) Relevance of the carnosic acid/carnosol ratio for the level of rosemary diterpene transfer and for improving lamb meat antioxidant status. *Food Chemistry 151*, 212–218.

Jordán, M.J., Moñino, M.I., Martinez, C., Lafuente, A., Sotomayor, J.A. (2010). Introduction of distillate rosemary leaves into the diet of the Murciano-Granadina goat: transfer of polyphenolic compounds to goats' milk and the plasma of suckling goat kids. *Journal of Agricultural and Food Chemistry 58*, 8265–8270.

Kandaswami, C., Middleton, E. (1994). Free radical scavenging and antioxidant activity of plant flavonoids. In: International Symposium on Free Radicals in Diagnostic Medicine: A Systems Approach to Laboratory Technology. (D. Armstrong, ed.) pp. 351–376. Springer Science+Business Media, LLC, Buffalo, New York (EEUU).

Kanner, J. (1994). Oxidative processes in meat and meat products: quality implications. *Meat Science 36*, 169–189.

Karami, M., Alimon, A.R., Sazili, A.Q., Goh, Y.M. , Ivan, M. (2011). Effects of dietary antioxidants on the quality, fatty acid profile, and lipid oxidation of *longissimus* muscle in Kacang goat with aging time. *Meat Science 88*, 102–108.

Katalinić, V., Možina, S.S., Skroza, D., Generalić, I., Abramovič, H., Miloš, M., Ljubenkov, I., Piskernik, S., Pezo, I., Terpinc, P., Boban, M. (2010). Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 Vitis vinifera varieties grown in Dalmatia (Croatia). *Food Chemistry 119*, 715–723.

Kepler, C.R., Tove, S.B. (1967). Biohydrogenation of unsaturated fatty acids III. Purification and properties of a linoleate Δ^{12}- cis, Δ^{11}-trans-isomerase from *Butyrivibrio fibrisolvens*. *Journal of Biological Chemistry 242*, 5686–5692.

Krzywicki, K. (1979). Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. *Meat Science 3*, 1–10.

Lanza, M., Bella, M., Priolo, A., Barbagallo, D., Galofaro, V., Landi, C., Pennisi, P. (2006). Lamb meat quality as affected by a natural or artificial milk feeding regime. *Meat Science 73*, 313–318.

Larraín, R.E., Schaefer, D.M., Richards, M.P., Reed, J.D. (2008). Finishing steers with based on corn, high-tannin sorghum or a mix of both: Color and lipid oxidation in beef. *Meat Science 79*, 656–665.

Lau, D.W., King, A.J. (2003). Pre- and post-mortem use of grape seed extract in dark poultry meat to inhibit development of thiobarbituric acid reactive substances. *Journal of Agricultural and Food Chemistry 51*, 1602–1607.

Lauridsen, C., Buckley, D.J., Morrissey, P.A. (1997). Influence of dietary fat and vitamin E supplementation on alpha-tocopherol levels and fatty acid profiles in chicken muscle membranal fractions and on susceptibility to lipid peroxidation. *Meat Science 46*, 9–22.

Lawrie, R.A. (1983). Aspects of the biochemistry of meat. *International Journal of Biochemistry 15,* 233–242.

Lee, J., Lee, H.K., Kim, C.Y., Choe, C.M., You, T.W., Seong, G.J. (2005) Purified high-dose anthocyanoside oligomer administration improves nocturnal vision and clinical symptoms in myopia subjects. *British Journal of Nutrition 93*, 895–899.

Leighton, F., Urquiaga, I. (2000). Polifenoles del vino y salud humana. *Antioxidantes y calidad de vida 7*, 5–13.

Leopoldini, M., Russo, N., Toscano, M. (2011). The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chemistry 125*, 288–306.

Lesiow, T., Ockerman, H.W. (1998). Functional and sensory attributes of normal pH in *Semimembranosus* and *Longissimus dorsi* muscles depending on time of cutting and ageing. In: Proceedings 44th International Congress of Meat Science and Technology. (A. Diestre, J.M. Monfort, eds.). pp 484–485. Institute for Food and Agricultural Research and Technology (IRTA), Barcelona (Spain).

Liu, Q., Scheller, K.K., Arp, S.C., Schaefer, D.M., Frigg, M. (1996). Color coordinates for assessment of dietary vitamin E effects on color stability. *Journal of Animal Science 74*, 106–116.

Llobera, A., Cañellas, J. (2007). Dietary fiber content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): Pomace and stem. *Food Chemistry 101*, 659–666.

López-Bote, C.J., Daza, A., Soares, M., Berges, E. (2001). Dose-response effect of dietary vitamin E concentration on meat quality characteristics in light-weight lambs. *Animal Science 73*, 451–458.

López-Bucio, J., Nieto-Jacobo, M.F., Ramírez-Rodríguez, V., Herrera-Estrella, L. (2000). Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. *Plant Science 160*, 1–13.

Luciano, G., Monahan, F.J., Vasta, V., Biondi, L., Lanza, M., Priolo, A. (2009). Dietary tannins improve lamb meat colour stability. *Meat Science 81,* 120–125.

Luciano, G., Vasta, V., Monahan, F.J., López-Andrés, P., Biondi, L., Lanza, M., Priolo, A. (2011). Antioxidant status, colour stability and myoglobin resistance to oxidation of *Longissimus dorsi* muscle from lambs fed a tannin-containing diet. *Food Chemistry 124*, 1036–1042.

Lund, M.N., Heinonen, M., Baron, C.P., Estévez, M. (2011). Protein oxidation in muscle foods: A review. *Molecular Nutrition and Food Research 55*, 83–95.

Lund, M.N., Lametsch, R., Hviid, M.S., Jensen, O.N., Skibsted, L.H. (2007). Highoxygen packaging atmosphere influences protein oxidation and tenderness of porcine *longissimus dorsi* during chill storage. *Meat Science 77,* 295–303.

Makkar, H.P.S., Becker, K., Abel, H.J., Szegletti, C. (1995). Degradation of condensed tannins by rumen microbes exposed to quebracho tannins (QT) in rumen simulation technique (RUSITEC) and effects of QT on fermentative processes in the RUSITEC. *Journal of the Science of Food and Agriculture 69*, 495–500.

Makris, D.P., Boskou, G., Andrikopoulos, N.K. (2007). Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. *Journal of Food Composition and Analysis 20*, 125–132.

Malossini, F., Pinosa, M., Piasentier, E., Bovolenta, S. (1993). Grape marc and maize cobs in heavy lamb diets. *Annales De Zootechnie 42*, 315–328

Manach, C., Scalbert, A., Morand, C., Rémésy, C., Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition 79*, 727–747.

Mancini, R., Hunt, M.C. (2005). Current research in meat color. *Meat Science 71,* 100–121.

Manso, T., Bodas, R., Castro, T., Jimeno, V., Mantecon, A.R. (2009). Animal performance and fatty acid composition of lambs fed with different vegetable oils. *Meat Science 83*, 511–516.

McAllister, T.A., Martínez, T., Bae, H.D., Muir, A.D., Yanke, L.J., Gones, G.A. (2005). Characterization of condensed tannins purified from legume forages: Chromophore production, protein precipitation and inhibitory effects on cellulose digestion. *Journal of Chemical Ecology 31*, 2049–2068.

McKenna, D.R., Mies, P.D., Baird, B.E., Pfeiffer, K.D., Ellebracht, J.W., Savell, J.W. (2005). Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. *Meat Science 70*, 665–682.

McSweeney, C.S., Palmer, B., McNeill, D.M., Krause, D.O. (2001). Microbial interactions with tannins: nutritional consequences for ruminants. *Animal Feed Science and Technology 91*, 83–93.

Metin-Donma, M., Donma, O. (2005) Phytonutrient and children: the other side of the medallion *Food Research International 38*, 681–92

Middleton, E., Kandaswami, C., Theoharides, T.C. (2000). The effect of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological Reviews 52,* 673–751.

Min, B.R., Barry, T.N., Attwood, G.T., McNabb, W.C. (2003). The effect of condensed tannins on the nutrition and health of ruminants feed fresh temperate forages. A review. *Animal Feed Science and Technology 106*, 3–19.

Mirzaei-Aghsaghali, A., Maheri-Sis, N., Mansouri, H., Razeghi, M.E., Telli, A.S., Aghajanzadeh-Golshani, A. (2011). Estimation of the nutritive value of grape pomace for ruminant using gas production technique. *African Journal of Biotechnology 10*, 6246–6250.

Moate, P.J., Williams, S.R.O., Torok, V.A., Hannah, M.C., Ribaux, B.E., Tavendale, M.H., Eckard, R.J., Jacobs, J.L., Auldist, M.J., Wales, W.J. (2014). Grape marc reduces methane emissions when fed to dairy cows. *Journal of Dairy Science 97,* 5073–5087.

Molina-Alcaide, E., Moumen, A., Martín-García, I. (2008). By-products from viticulture and the wine industry: potential as sources of nutrients for ruminants. *Journal of the Science of Food and Agriculture 88*, 597–604.

Molle, G., Decandia, M., Fois, N., Ligios, S., Cabiddu, A., Sitzia, M. (2003). The performance of Mediterranean dairy sheep given access to sulla (*Hedysarum coronarium* L.) and annual ryegrass (*Lolium rigidum* Gaudin) pastures in different time proportions. *Small Ruminant Research 49*, 319–328.

Moloney, A.P., Kennedy, C., Noci, F., Monahan, F.J., Kerry, J.P. (2012). Lipid and colour stability of M. longissimus muscle from lambs fed camelina or linseed as oils or seeds. *Meat Science 92*, 1–7.

Moñino, M.I., Martínez, C., Sotomayor, J.A., Lafuente, A., Jordán, M.J. (2008). Polyphenolic transmission to Segureño lamb meat from ewes dietary supplemented with the distillate from rosemary (*Rosmarinus officinalis*) leaves. *Journal of Agricultural and Food Chemistry 56,* 3363–3367.

Morán, L., Andrés, S., Bodas, R., Prieto, N., Giráldez, F.J. (2012b). Meat texture and antioxidant status are improved when carnosic acid is included in the diet of fattening lambs. *Meat Science 91*, 430–434.

Morán, L., Rodríguez-Calleja, J.M., Bodas, R., Prieto, N., Giráldez, F.J., Andrés, S. (2012a). Carnosic acid dietary supplementation at 0.12% rates slows down meat discoloration in gluteus medius of fattening lambs. *Meat Science 90*, 789–795.

Morrissey, P.A., Sheehy, P.J.A., Galvin, K., Kerry, J.P., Buckley, D.J. (1998). Lipid stability in meat and meat products. *Meat Science 49*, S73–S86.

Muíño, I., Apeleo, E., De la Fuente, J., Pérez-Santaescolástica, C., Rivas-Cañedo, A., Pérez., C., Díaz, M.T., Cañeque, V., Lauzurica, S. (2014). Effect of dietary supplementation with red wine extract or vitamin E, in combination with linseed and fish oil, on lamb meat quality. *Meat Science 98*,116–123.

Mukai, K., Sawada, K., Kohno, Y., Terao, J. (1993). Kinetic study of the prooxidant effect of tocopherol. Hydrogen abstraction from lipid hydroperoxides by tocopheroxyls in solution. *Lipids 28*, 747–752.

Muñoz, O., Sepúlveda, M., Schwartz, M. (2004). Effects of enzymatic treatment on anthocyanic pigments from grapes skin from chilean wine. *Food Chemistry 87*, 487–490.

Murphy, T.A., Loerch, S.C., McClure, K.E., Solomon, M.B. (1994). Effects of grain or pasture finishing systems on carcass composition and tissue accretion rates of lambs. *Journal of Animal Science 72*, 3138–3144.

Nardone, A., Valfrè, F. (1999). Effects of changing production methods on quality of meat, milk and eggs. *Livestock Production Science 59*, 165–182.

Negro, C., Tommasi, L., Miceli, A. (2003). Phenolic compounds and antioxidant activity from red grape marc extracts. *Bioresource Technology 87*, 41–44.

Nielsen, B., Hansen, H. (2004). Effect of grape pomace rich in flavonoids and antioxidants on production parameters in dairy production. *Journal of Animal and Feed Science 13*, 535–538.

Nieto, G., Bañón, S., Garrido, M.D. (2011). Effect of supplementing ewes' diet with thyme (*Thymus zygis* ssp. *gracilis*) leaves on the lipid oxidation of cooked lamb meat. Food Chemistry 125, 1147–1152.

Nieto, G. (2013). Incorporation of by-products of rosemary and thyme in the diet of ewes: effect on the fatty acid profile of lamb. *European Food Research and Technology 236*, 379–389.

Nieto, G., Bañón, S., Garrido, M.D. (2012). Incorporation of thyme leaves in the diet of pregnant and lactating ewes: Effect on the fatty acid profile of lamb. *Small Ruminant Research 105*, 140–147.

Nieto, G., Díaz, P., Bañón, S., Garrido, M.D. (2010a). Effect on lamb meat quality of including thyme (*Thymus zygis* ssp. gracilis) leaves in ewes' diet. *Meat Science 85*, 82–88.

Nieto, G., Díaz, P., Bañón, S., Garrido, M.D. (2010b). Dietary administration of ewe diets with a distillate from rosemary leaves (*Rosmarinus officinalis* L.): Influence on lamb meat quality. *Meat Science 84*, 23–29.

Norouzian, M.A., Ghiasi, S.E. (2012). Carcass performance and meat mineral content in Balouchi lamb fed pistachio by-products. *Meat Science 92*,157–159.

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

O'Keefe, M., Hood, D.E. (1982). Biochemical factors influencing metmyoglobin formation in beef from muscles of differing colour stability. *Meat Science 7,* 209–228.

O'Connell, J.E., Fox, P.F. (2001). Significance and applications of phenolic compounds in the production and quality of milk and dairy products: A review. *International Dairy Journal 11*, 103–120.

Ojeda, H., Andary, C., Creaba, E., Carbonneau, A., Deloire, A. (2002). Influence of pre- and post-veraison water deficit on síntesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* var. Shiraz. *American Journal of Enology and Viticulture 53*, 261–267.

Pétriz-Celaya, Y., Calderón-Cortés, J.F., Pérez, C., Montaño, M.F., Plascencia, A. (2010). Influence of substitution of alfalfa hay for unfermented dried grape pomace on performance and carcass characteristics of growing sheep*. American Society of Animal Science*, *Proceedings, Western Section 61*, 308–311.

Piñeiro, Z., Palma, M., Barroso, C.G. (2006). Determination of trans-resveratrol in grapes by pressurized liquid extraction and fast high performance liquid chromatography. *Journal of Chromatography A 1110,* 61–65.

Pirmohammadi, R., Hamidi, O., Mohsenpur, A. (2007). Effects of polyethylene glycol (PEG) addition on chemical composition, degradability and digestibility og white grape ponace. *Journal of Animal and Veterinary Advances 6*, 1135–1139.

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

Prescott, J., Young, O., O'Neil, L. (2001). The impact of variations in flavour compounds on meat acceptability: a comparation of Japanese and New Zealand consumers. *Food Quality and Preference 12*, 257–264

Priolo, A., Bella, M., Lanza, M., Galofaro, V., Biondi, L., Barbagallo, D., Ben Salem, H., Pennisi, P. (2005). Carcass and meat quality of lambs fed fresh sulla (*Hedysarum coronarium* L.) with or without polyethylene glycol or concentrate. *Small Ruminant Research 59*, 281–288.

Priolo, A., Vasta, V. (2007). Effect of tannin-containing diets on small ruminant meat quality*. Italian Journal of Animal Science 6*, 527–530.

Priolo, A., Vasta, V., Fasone, V., Lanza, C.M., Scerra, M., Biondi, L., Bella, M., Whittington, F.M. (2009). Meat odour and flavour and indoles concentration in ruminal fluid and adipose tissue of lambs fed green herbage or concentrates with or without tannins. *Animal 3*, 454–460.

Priolo, A., Waghorn, G.C., Lanza, M., Biondi, L., Pennisi, P. (2000). Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: Effects on lamb growth performance and meat quality. *Journal of Animal Science 78,* 810–816.

Quiñones, M., Miguel, M., Aleixandre, A. (2013). Beneficial effects of polyphenols on cardiovascular disease. *Pharmacological Research 68*, 125–131.

Reineccius, G. (1994). Flavor and aroma chemistry. In: Quality attributes and their measurement in meat, poultry and fish products. Advances in Meat Research Series, Volumen 9. (A.M. Pearson, T.R. Dutson, eds.). pp. 185-201. Blackie Academic and Professional, London (UK).

Ribéreau-Gayon, J., Peynaud, E., Ribereau-Gayon, P., Sudraud, P. (2003). Tratado de enología. Ciencias y técnicas del vino. Tomo II. Hemisferio Sur, Mundi-Prensa, Madrid (Spain).

Rietjens, I.M., Boersma, M.G., Haan, L.D., Spenkelink, B., Awad, H.M., Cnubben, N.H., van Zandena, van der Woudea, H., Gerrit, M.A., Koemana, J.H. (2002). The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. *Environmental Toxicology and Pharmacology* 11, 321–333.

Ripoll, G., Joy, M., Muñoz, F. (2011). Use of dietary vitamin E and selenium (Se) to increase the shelf life of modified atmosphere packaged light lamb meat. *Meat Science 87*, 88–93.

Robbins, K., Jensen, J., Ryan, K.J., Homco-Ryan, C., Mckeith, F.K., Brewer, M.S. (2003). Dietary vitamin E supplementation effects on the color and sensory characteristics of enhanced beef steaks. *Meat Science 64*, 279–285.

Rodríguez, A.B. (2005). Alternativas a los sistemas actuales de alimentación en el cebo intensive de corderos: Efecto de la supresión de la paja de la ración y la utilización de cereal en grano sobre la ingestión, el crecimiento y las características de la canal y de la carne. Tesis Doctoral. Universidad de León.

Rowe, L.J., Maddock, K., Lonergan, S., Huff-Lonergan, E. (2004). Oxidative environments decrease tenderization of beef steaks through inactivation of μ-calpain. *Journal of Animal Science 82*, 3254–3266.

Sang, S., Cheng, X., Stark, R.E., Rose, R.T., Yang, C.S., Ho, C.T. (2002). Chemical studies on antioxidant mechanism of tea catechins: Analyses of radical reaction products of catechin and epicatechin with 2,2-Diphenyl-1-picrylhydrazyl. *Bioorganic and Medicinal Chemistry 10*, 2233–2237.

Santos, N.W., Santos, G.T.D., Silva-Kazama, D.C., Grande, P.A., Pintro, P.M., de Marchi, F.E., Jobim, C.C., Petit, H.V. (2014). Production, composition and antioxidants in milk of dairy cows fed diets containing soybean oil and grape residue silage. *Livestock Science 159*, 37–45.

Scerra, V., Caparra, P., Fotia, F., Lanza, M., Priolo, A. (2001). Citrus pulp and wheat straw silage as an ingredient in lamb diets: effects on growth and carcass and meat quality. *Small Ruminant Research 40*, 51–56.

Schreurs, N.M., Tavendale, M.H., Lane, G.A., Barry, T.N., McNabb, W.C., Cummings, T., Fraser, K., Lopez-Villalbos, N. (2007). The effect of supplementation of a white clover or perennial ryegrass diet with grape seed extract on indole and skatole metabolism and the sensory characteristics of lamb. *Journal of the Science of Food and Agriculture 87*, 1030–1041.

Sen, A.R., Santra, A., Karim, S.A. (2004). Carcass yield, composition and meat quality attributes of sheep and goat under semiarid conditions. *Meat Science 66*, 757–763

Serrano, R., Jordán, M.J., Bañón, S. (2014). Use of dietary rosemary extract in ewe and lamb to extend the shelf life of raw and cooked meat. *Small Ruminant Research 116*, 144–152.

Sgorlon, S., Stradaioli, G., Zanin, D., Stefanon B. (2006). Biochemical and molecular responses to antioxidant supplementation in sheep. *Small Ruminant Research 64*, 143–151

Shingfield, K.J., Bernard, L., Leroux, C., Chilliard, Y. (2010). Role of trans fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. *Animal 4*, 1140–1166.

Simitzis, P.E., Deligeorgis, S.G., Bizelis, J.A., Dardamani, A., Theodosiou, I., Fegeros, K. (2008). Effect of dietary oregano oil supplementation on lamb meat characteristics. *Meat Science 79,* 217–223.

Simitzis, P.E., Ilias-Dimopoulos, V., Charismiadou, M.A., Biniari, E.E., Deligeorgis, S.G. (2013). The effects of dietary hesperidin supplementation on lamb performance and meat characteristics. *Animal Science Journal 84*, 136–143.

Simopoulos, A.P. (1999). Essential fatty acids in health and chronic disease. *The American Journal of Clinical Nutrition 70*, 560S–569S.

Spanghero, M., Salem, A.Z.M., Robinsond, P.H. (2009). Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces. *Animal Feed Science and Technology 152*, 243–255.

Staerfl, S.M., Soliva, C.R., Leiber, F., Kreuzer, M. (2011). Fatty acid profile and oxidative stability of the perirenal fat of bulls fattened on grass silage and maize silage supplemented with tannins, garlic, maca and lupines. *Meat Science 89*, 98–104.

Szczesniak, A.S. (1963). Classification of textural characteristics. *Journal of Food Science 29*, 385–389.

Toral, P.G., Hervás, G., Belenguer, A., Bichi, E., Frutos, P. (2013). Effect of the inclusion of quebracho tannins in a diet rich in linoleic acid on milk fatty acid composition in dairy ewes. *Journal of Dairy Science 96,* 431–439.

Tucker, J.M., Townsend, D.M. (2005). Alpha-tocopherol: roles in prevention and therapy of human disease. *Biomedicine and Pharmacotherapy 59*, 380–387.

Urquiaga, I., Leighton, F. (2000). Plant polyphenol antioxidants and oxidavite stress. *Biological Research 33*, 55–64.

Ursini, F., Maiorino, M., Morazzoni, P., Roveri, A., Pifferi, G. (1994). A novel antioxidant flavonoid (Idb-1031) affecting molecular mechanisms of cellular activation. *Free Radical Biology and Medicine 16*, 547–553.

Valizadeh, R., Sobhanirad, S. (2009). The potential of agro-industrial by-products as feed sources for livestock in Khorasan Razavi province of Iran. *Journal of Animal and Veterinary Advances 8*, 2375–2379.

Van Soest, P.J. (1994). Nutritional ecology of the ruminant. Cornell University Press, Ithaca, New York (EEUU).

Vasta, V., Aouadi, D., Brogna, D.M.R., Scerra, M., Luciano, G., Priolo, A., Ben Salem, H. (2013). Effect of the dietary supplementation of essential oils from rosemaryand artemisia on muscle fatty acids and volatile compound profiles in Barbarine lambs. *Meat Science 95*, 235–241

Vasta, V., Luciano, G. (2011). The effects of dietary comsuption of plants secondary compounds on small ruminals' products quality. *Small Ruminan Research 101*, 150–159.

Vasta, V., Mele, M., Makkar, H.P.S., Priolo, A. (2009a). Ruminal biohydrogenation as affected by tannins in vitro. *British Journal of Nutrition 102,* 82–92.

Vasta, V., Mele, M., Serra, A., Scerra, M., Luciano, G., Lanza, M., Priolo, A. (2009b). Metabolic fate of fatty acids involved in ruminal biohydrogenation in sheep fed concentrate or herbage with or without tannins. *Journal of Animal Science 87*, 2674–2684

Vasta, V., Nudda, A., Cannas, A., Lanza, M., Priolo, A. (2008). Alternative feed resources and their effects on the quality of meat and milk from small ruminants. Review. *Animal Feed Science and Technology 147*, 223–246.

Vasta, V., Pennisi, P., Lanza, M., Barbagallo, D., Bella, M., Priolo, A. (2007). Intramuscular fatty acid composition of lambs given a tanniniferous diet with or without polyethylene glycol supplementation. *Meat Science 76*, 739–745.

Vertuani, S., Angusti., A., Manfredini, S. (2004). The antioxidants and pro-antioxidants network: an overview. *Current Pharmaceutical Design 10*, 1677–1694.

Vieira, C., Fernández-Diez, A., Mateo, J., Bodas, R., Soto, S., Manso, T. (2012). Effects of addition of different vegetable oils to lactating dairy ewes' diet on meat quality characteristics of suckling lambs reared on the ewes' milk. *Meat Science 91*, 277–283.

Woodward, S.L., Waghorn, G.C., Lassey, K.R., Laboyre, P. (2002). Does feeding sulla (*Hedysarum coronarium*) reduce methane emissions from dairy cows? *Proceedings of the New Zealand Society of Animal Production 62*, 227–230.

Yang, C.S., Landau, J.M., Huang, M.T., Newmark, H.L. (2001). Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annual Review of Nutrition 21*, 381–406.

Yi, C., Shi, J., Kramer, J., Xue, S., Jiang, Y., Zhang, M., Ma, Y., Pohorly, J. (2009). Fatty acid composition and phenolic antioxidants of winwmaking pomace powder. *Food Chemistry 114*, 570–576.

Zalikarenab, L., Pirmohammadi, R., Teimuriyansari, A. (2007). Chemical composition and digestibility of dried white and red grape pomace for ruminants. *Journal of Animal and Veterinary Advances 6*, 1107–1111.

Zembayashi, M., Lunt, D.K., Smith, S.B. (1999). Dietary tea reduces the iron content of beef. *Meat Science 53*, 221–226.

Zhang, W., Xiao, S., Samaraweera, H., Lee, E.J., Ahn, D.U. (2010). Improving functional value of meat products. *Meat Science 86*, 15–31.

Zhou, K., Yin, J.J., Yu, L.L. (2006). ESR determination of the reactions between selected phenolic acids and free radicals or transition metals. *Food Chemistry 95*, 446–457.

3. Objetivos y planteamiento experimental

En muchos procesos agroindustriales como la elaboración de vinos se generan numerosos subproductos y residuos que no se aprovechan suficientemente y en muchos casos su valor es parcialmente desconocido. Las industrias no encuentran en ellos más que un problema para su eliminación final y una complicación desde el punto de vista ambiental. Por ello, su empleo en alimentación de rumiantes en general, y alimentación ovina en particular, no sólo podría ser económicamente viable, sino también ambientalmente favorable, contribuyendo a la disminución del impacto ambiental derivado del acúmulo de dichos subproductos.

El orujo de uva presenta un valor nutritivo medio o bajo y además, cuenta con la presencia de factores antinutritivos como los taninos condensados, que en dosis altas, pueden interferir en la utilización digestiva de los nutrientes. Sin embargo, el interés de la incorporación de este subproducto en alimentación ovina, podría radicar, más que en su valor nutritivo, en su contenido en metabolitos secundarios, como polifenoles, con importantes propiedades antioxidantes, antimicrobianas y bioactivas.

Aunque el efecto dosis-respuesta de los compuestos fenólicos, así como su mecanismo de acción no están completamente esclarecidos, su inclusión en dietas de pequeños rumiantes parece ser una estrategia prometedora para la mejora de la calidad de los productos, y como alternativa natural a los antioxidantes de origen sintético habitualmente empleados en alimentación animal.

Teniendo en cuenta todo lo anteriormente expuesto, y considerando que en los últimos años ha cobrado especial interés el enriquecimiento de los productos animales en ácidos grasos más insaturados y beneficiosos para la salud humana, con el consiguiente incremento de su susceptibilidad a la oxidación, el desarrollo de estrategias nutricionales basadas en la incorporación de subproductos naturales, como el orujo de vino, que minimicen este proceso, podría contribuir a mejorar el valor nutricional y tecnológico de los productos del ganado ovino en general y, por tanto, la rentabilidad de las explotaciones de ganado ovino, las cuales representan una enorme importancia en Castilla y León (España).

El objetivo general de esta tesis fue estudiar los efectos de la incorporación de orujo de uva procedente de la elaboración de vino tinto, en las raciones de ganado ovino sobre los rendimientos productivos, la calidad de la canal y de la carne de lechazos y corderos (ácidos grasos, estabilidad oxidativa y vida útil de la carne).

Para lograr este objetivo general se plantearon los siguientes objetivos específicos:

1. Conocer el valor nutritivo del orujo de uva de vino tinto mediante el estudio de su composición química, con especial referencia al perfil lipídico y contenido en compuestos fenólicos, así como su digestibilidad *in vitro*, cinética de degradación y actividad ruminal en ganado ovino.

2. Analizar el efecto de la inclusión de diferentes niveles de orujo de uva de vino tinto en relación con el antioxidante habitualmente utilizado (vitamina E), en la dieta de ovejas de raza Churra en inicio de lactación sobre los rendimientos productivos y la composición y el perfil de ácidos grasos de la leche, así como sobre las características de la canal y de la carne, el perfil de ácidos grasos y la vida útil de la carne de los lechazos producidos.

3. Evaluar el efecto de la inclusión de orujo de uva de vino tinto, en relación con antioxidantes comerciales como el extracto de granilla de uva y vitamina E en el pienso de cebo de corderos de raza Merina sobre los rendimientos productivos, características de la canal y de la carne, el perfil de ácidos grasos y la vida útil de la carne producida.

4. Integrar la información obtenida en las pruebas experimentales y elaborar conclusiones que permitan realizar recomendaciones concretas para optimizar los sistemas de alimentación del ganado ovino en relación con el empleo del orujo de uva, y la mejora de la calidad de sus productos desde el punto de vista nutricional y tecnológico.

Para poder alcanzar los objetivos establecidos se diseñaron tres pruebas experimentales.

La primera prueba experimental se diseñó para estudiar la composición de orujo de uva de vino tinto procedente de distintas bodegas de Castilla y León, así como su digestibilidad *in vitro*, degradación y actividad ruminal en ovejas de raza Churra no gestantes ni lactantes, provistas de una cánula ruminal.

En la segunda prueba experimental se evaluó el efecto de la incorporación de dos niveles de orujo de uva y vitamina E en raciones de ovejas en inicio de lactación sobre la producción, composición y el perfil de ácidos grasos de la leche, y sobre los rendimientos productivos de los lechazos, así como sobre las características de la canal y de la carne, especialmente sobre el perfil de ácidos grasos de la grasa intramuscular y la vida útil de la carne de los lechazos.

Por último, en la tercera prueba experimental se estudió el efecto de la inclusión en la ración de corderos en crecimiento de orujo de uva, extracto de granilla de uva y vitamina E,

sobre los rendimientos productivos, las características de la canal y de la carne, el perfil de ácidos grasos de la grasa intramuscular y la vida útil de la carne de los corderos.

Finalmente, se incluye una discusión general que integra los resultados de las tres pruebas experimentales donde se compara el efecto del orujo de uva en las distintas fases productivas del ganado ovino.

Aunque ya ha sido señalado en la introducción de esta tesis doctoral, es preciso señalar que la tesis se estructura por apartados redactados en inglés, de acuerdo con las pruebas experimentales realizadas, y que, conscientes de que en algún caso pueda resultar coincidente, la metodología de cada prueba se incluye en el capítulo correspondiente con el objetivo de facilitar la lectura.

4. Prueba experimental I

Evaluation of grape pomace from red wine by-product as feed for sheep

4.1. Introduction

Grapes are one of the major fruit crops and about 80% of the harvest is used by the winemaking industry. Winemaking has great social and economic importance in several countries, especially in the Mediterranean area. Spain, with nearly a million hectares for the cultivation of grapes, is the country with the largest area of vineyards in the world, and it is the third largest wine producer in the world (FAOSTAT, 2012). Castilla y León is a Spanish region closely connected with the culture of vine and wine, where several Designations of Origin and other indicators of quality have been recognized for various types of wine (white, rosé and red) from different varieties of white and red grapes. However, the production of red wine is the most abundant in the region.

The wine industry generates large quantities of by-products and waste. Grape pomace, which accounts for approximately 20% of the total volume of grapes used for wine production, is the residue of the harvested grapes after the grape juice has been obtained by pressing. Its composition depends on the wine being made and consists mainly of peel, pulp and seeds, since grapes are generally destalked as one of the initial processes in winemaking. Red grape pomace also undergoes a fermentation-maceration process in which some substances from the grapes, especially polyphenols, are transferred to the wine, although a high proportion still remains in the wine by-products (Alonso *et al.*, 2002). The phenolic compound content depends on the type of grape (white or red) and the part of tissue considered (skin and pulp or seeds). The variability in the levels of grape phenolic compounds between the type and location of grape cultivation and among grape fractions (pulp or seeds) is likely to be due to the biological variability of the grape material (grape variety, factors that affect berry development such as soil, geographical location, climate) and the stage of maturity (Ojeda *et al.*, 2002). Moreover, the winemaking process from which they are derived (level of pressing, maceration time, fermentation contact time, temperature and alcohol levels) also affects the variability between types of grape pomace (Baumgärtel *et al.*, 2007).

These by-products generate a serious environmental and economic problem in relation to their storage, processing and disposal. Therefore any sustainable strategy for grape cultivation and wine production must take into account the reuse and evaluation of these by-products. Traditional uses of grape pomace have largely been restricted to obtaining ethanol for distilleries and to land applications as fertilizers, but these activities represent a high economic cost for the wine industry.

Nowadays, the profitability of farming is mainly limited by the availability of food, the high price of raw materials at certain times of the year and the low price obtained for the products. In this regard, the use of grape pomace could make it possible to utilize this waste, reducing feeding costs and possibly achieving improvements in the quality of the products obtained, taking into account an environmental point of view, with the need to recycle waste material which is costly to dispose of. It is estimated that only 3% of Spanish grape pomace production is used for animal feed, and so far its main use has been in ruminant maintenance feed (FEDNA, 2010) owing to its low nutritional value for ruminants (Alipour and Rouzbehan, 2007; Baumgärtel *et al.*, 2007).

According to some authors, a major limitation of the use of grape pomace as ruminant feed is the presence of a high level of lignified fibre, and of secondary compounds, including phenolics such as tannins and anthocyanins (Makris *et al.*, 2007), which can have potentially negative effects on rumen nutrient utilization, especially the seed fraction rather than the pulp. In contrast, it has been reported that tannins may also improve rumen metabolism, for example by increasing the protein supply to the small intestine by decreasing ruminal degradability, and decreasing methanogenesis (Patra and Saxena, 2009). Furthermore, several studies have shown that phenolic compounds possess many properties, such as antioxidant capacity, which could have beneficial effects on animal product quality (Vasta and Luciano, 2011). In addition, the fatty acids of grape pomace have been examined and the presence of high amounts of linoleic and oleic acids reported in winery wastes (Yi *et al.*, 2009) may also have beneficial effects on meat and milk fatty acid profiles, with the possibility of obtaining more unsaturated ruminant products, promoting health and preventing disease.

Evaluation and chemical and phenolic characterization of red grape pomace might offer the possibility of expanding the number of available local feedstuffs, which is essential to formulate adequate rations from the point of view of nutrition and economic development. The chemical composition and nutritional properties of grape by-products vary with the method of wine production, type of grape and relative ratio of seeds and pulp plus skin in the pomace (Baumgärtel *el al.,* 2007), which sometimes makes it difficult to extrapolate the

results obtained by other authors. Therefore, analysis of chemical composition, digestibility and ruminal degradability of seeds and pulp of red grape pomace obtained from winery by-products is a prior necessity in order to evaluate its potential as ruminant feed.

The objective of the present work was to study the pulp and seeds of grape pomace derived from red wine produced in Castilla y León, by analysing their chemical composition and *in vitro* digestibility and the *in sacco* degradability of their nutrients in sheep.

4.2. Material and methods

4.2.1. Grape pomace samples and chemical composition

Six wineries of red wine in Castilla y León (Spain) belonging to the most important Designations of Origin in this area were selected, and representative fresh grape pomace samples (*Vitis vinifera* sp.) were collected. Samples were dried in a forced air oven at 50 °C until constant weight. Each dried sample was manually sieved to separate seed from the pulp plus skin fraction (hereafter referred to as 'pulp') and each fraction was weighed to evaluate the proportion of each one. The samples were ground to pass a 1-mm screen and they were frozen at −20 °C until chemical analysis.

The chemical composition of the grape pomace (dry matter, DM; organic matter, OM; ether extract, EE, and crude protein, CP) was determined by standard methods (AOAC, 2012). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) analyses were performed according to Van Soest *et al.* (1991), using an ANKOM^{200} fibre analyser (Ankom Technology Corporation). Hemicellulose was estimated by difference as NDF–ADF, and cellulose was calculated as ADF–ADL. Neutral detergent-soluble carbohydrates (NDSC) were estimated by difference as OM–EE–CP–NDF. Acid detergent insoluble crude protein (ADICP) was determined by Kjeldahl analysis of ADF residues and transformed into CP by multiplying by 6.25.

Lipid extraction from grape pomace (seed and pulp fractions) was performed according to Bligh and Dyer (1959). Lipid extract was methylated following the method of Morrison and Smith (1964), and the methyl esters were quantified with a gas chromatograph (Agilent 6890N Network System, PA, USA) equipped with a flame ionization detector and auto sampler, and a Hewlett Packard Innovax column (30 m x 0.32 mm x 0.25 µm, Hewlett Packard, Avondale, PA, USA). The fatty acid (FA) amount was expressed as percentage of total fatty acids.

For determination of phenolic compounds, samples of grape pomace fractions (seeds and pulp) were extracted by shaking at room temperature with methanol–water (50:50 vol/vol, 50 mL g^{-1} of sample during 60 min) and acetone–water (70:30 vol/vol, 50 mL g^{-1} of sample during 60 min). After centrifugation (15 min, 3,000 x g), supernatants were combined and used to measure the extractable polyphenols (EP), hydrolysable polyphenols (HP), condensed tannins (CT) and total anthocyanins (TA) by colorimetric methods using a spectrometer (Perkin-Elmer Lambda 25, UV/VIS Spectrometer). EP were determined by the Folin–Ciocalteu procedure (Ribérau-Gayon, 1970), using gallic acid as standard at 760 nm. To determine the HP content, the methanol–acetone–water extracts were hydrolysed by a methanol–H_2SO_4 treatment (90:10 vol/vol at 85 °C for 20 h) following the method of Hartzfeld *et al.* (2002), and then phenolic content was determined in the hydrolysates by the Folin–Ciocalteu procedure (Ribérau-Gayon, 1970). CT were analysed by means of a HCl–butanol (5:95 vol/vol, 100 °C for 1.5 h) treatment of the methanol–acetone–water extracts (modified method of Reed *et al.*, 1982). CT content was calculated from the absorbance at 550 nm (A1), by subtracting the absorbance of the extract treated with HCl–butanol without heating (A2). According to Bate-Smith (1981), and following the method described by Ribéreau-Gayon and Stonestreet (1966), the CT concentration was calculated by the expression: CT (g L^{-1}) = (A1 – A2) x 2.378. TA were determined according to Ribéreau-Gayon and Stonestreet (1965) in extract from selected samples, using the bisulfite bleaching method.

Phenolic acids (cinnamics: caffeic acid, chlorogenic acid, p-coumaric acid, sinapic acid, and ferulic acid; benzoics: gallic acid, gentisic acid, ellagic acid, syringic acid, vanillic acid and protocatechuic acid), stilbenes (*trans*-resveratrol), flavonols (quercetin, kaempferol and myricetin) and flavanols (catechin and epicatechin) had been quantified according to the methods of the Del Álamo *et al.* (2004a) and Gallego *et al.* (2011). Seed and pulp compounds extraction from the previous methanol-acetone-water extracts was carried out by 200 mg cartridges Waters Oasis HLB (Milford, MA, USA).The analyses were performed using a Hewlett-Packard 1100 (LC-DAD) system (Avondale, PA, USA). The separation of phenolic compounds was performed using a Hypersil ODS column (200 mm x 4.6 mm i.d., particle size, 0.45 µm) from Sugelabor (Madrid, Spain). The chromatographic conditions included the following: 10 µL injection volume; solvent A consisted of water:acetic acid (98:2), solvent B consisted of acetic acid:acetonitrile:water (2:20:78) and solvent C acetonitrile. The gradient profile was: 0 min A:B:C (100:0:0); at 0–55 min A:B:C (30:70:0); at 55–56 min A:B:C (0:0:100); and at 56–60 min A:B:C (0:0:100), all at a flow rate of 1 mL min^{-1}. The system was equilibrated using the starting conditions for 10 min prior to injection of the next sample. Detection was performed at 3 wavelengths: 280 nm, 254 nm and 340 nm and quantification was performed by external standard method.

Anthocyanins (delphinidin 3-O-glucoside, cyanidin 3-O-glucoside, petunidin 3-O-glucoside, peonidin 3-O-glucoside, malvidin 3-O-glucoside) and other anthocyanin derivatives were determined from the previous methanol-acetone-water seed and pulp extracts following the method proposed by Del Álamo *et al.* (2004b). The analyses were performed using a Hewlett-Packard 1100 (LC-DAD) system (Avondale, PA, USA), equipped with Hypersorb Prontosil ODS column (250 mm x 4.6 mm i.d., particle size 0.45 µm) from Sugelabor (Madrid, Spain). The chromatographic conditions of anthocyanic compounds included the following: 30 µL injection volume; solvent A was formic acid and water (2:98), solvent B was methanol, formic acid and water (45:10:45), and solvent C was methanol. It had followed the following gradient: 0 min A:B:C (75:25:0); at 0–25 min A:B:C (40:60:0); at 25–45 min A:B:C (0:60:40), at 45–52 min A:B:C (0:0:100), at 52–60 A:B:C (75:25:0) for 5 min, all at a flow rate of 1 mL min^{-1}. Calibration and quantification of anthocyanins were performed at 528 nm by the external standard method.

4.2.2. Degradation kinetics and ruminal fermentation

Eight healthy, non-pregnant, non-lactating Churra ewes (62.7 ± 5.81 kg), each fitted with a permanent rumen cannula (i.d. 35 mm), were used. The animals were housed in individual pens and had free access to water. They were fed at maintenance level (45 g DM kg $LBW^{-0.75}$), in two meals at 8:00 and 17:00 h, with a total mixed ration (TMR) consisting of lucerne hay and concentrate in a ratio of 50:50. The ewes (4 animals per group) were assigned randomly on the basis of live body weight to two dietary treatments (Table 4.1): a control treatment (CTRL, without grape pomace), and a grape pomace treatment (GP-7.5, 7.5% of grape pomace from red wine production, DM basis). In this experiment grape pomace was supplied in fresh form. The sheep were adapted to the diet for 10 days. Daily intake was recorded and there were no orts. The chemical composition of the feeds (OM, CP, EE, NDF, ADF) was determined by the standard methods previously described for grape pomace. All management and experimental procedures were conducted by trained personnel in strict accordance with the recommendations of the European Council Directive 2010/63/EU for the protection of animals used for scientific purposes. The experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Valladolid (Spain). The fistulated sheep remained in good health and welfare throughout the experimental period.

Table 4.1. Ingredients and chemical composition of the experimental ewe diets.

	Treatments[1]	
	CTRL	GP-7.5
Ingredients (g kg^{-1} DM)		
Lucerne hay	499	462
Barley	202	187
Oats	202	187
Soybean meal	78.3	72.5
Vitamin-mineral premix	16.4	15.1
Sodium bicarbonate	2.20	2.00
Grape pomace	-	75.0
Composition (g kg^{-1} DM)		
Dry matter (DM)	899	797
Organic matter	918	919
Neutral detergent fibre	361	362
Acid detergent fibre	238	244
Crude protein	184	179
Ether extract	28.6	31.9

[1] Treatments: CTRL, without grape pomace; GP-7.5, 7.5% of grape pomace from red wine, DM basis.

Ruminal degradability of DM, OM and CP of grape pomace fractions (seeds and pulp) was measured *in sacco* using the nylon bag technique described by (Ørskov *et al.*, 1980). Approximately 4-g samples, ground to pass through a 2-mm screen, were weighed into nylon bags (70 mm × 130 mm, 50 µm pore size). Two bags per sample and incubation time were incubated in the rumen of each of the eight ewes prior to the morning feeding and incubated for periods of 0, 3, 6, 12, 24, 48 and 72 h. At the end of the incubation period the nylon bags were rinsed under cold tap water, and then stored at −20 °C for at least 24 h to remove any microbial cells adhering to the particles, then the bags were washed with cold water in a washing machine for 20 min. Zero time disappearance was obtained by washing unincubated bags in a similar way. Finally, the bags were dried for 48 h at 60 °C. Bag residues were ground (1-mm screen) and chemical composition was determined by standard methods (AOAC, 2012). DM, OM and CP degradation were calculated according to the exponential model of Ørskov and McDonald (1979): $Y = a + b\ (1 - e^{(-ct)})$. The effective degradability (ED) was calculated as $ED = a + [bc/(c + k)]$, where a is the soluble fraction, b is the potentially degradable insoluble fraction, c is the degradation rate of b, and k is the fractional outflow rate. The value of k was taken as 0.02, 0.06 and 0.08 h^{-1}.

On days 7 and 9 of the experimental period, rumen content was manually extracted through the cannula of all sheep before first feeding (0 h) and at 1, 3, 6 and 9 h after feeding. Rumen fluid was strained through four layers of cheesecloth, and its pH value was measured immediately. Five mL of rumen fluid was acidified with the same volume of 0.2 N HCl for ammonia-N determination. In addition, 0.8 mL of rumen liquid was added to 0.5 mL of deproteinizing solution (10% of metaphosphoric acid and 0.06% of crotonic acid; w/v) for volatile fatty acids (VFA) analyses. All samples were stored at −20 °C until analyses were undertaken. Ammonia-N concentration in rumen samples was determined by a modified colorimetric method (Wheatherburn, 1967). VFA were determined in centrifuged samples (10,000 x g for 20 min) by gas chromatography as described by Carro *et al.* (1992).

4.2.3. *In vitro* true digestibility

In vitro true digestibility of seed and pulp samples from red grape pomace was determined by the procedure of Tilley and Terry (1963) using a Daisy^{II} incubator according to the methodology described by Ankom Technology Corporation. The rumen inoculum was withdrawn from four Churra sheep fitted with a permanent rumen cannula and assigned to the CTRL treatment. Rumen fluid was transferred into pre-warmed (39 °C) thermal bottles and squeezed through four layers of cheesecloth under anaerobic conditions. The inoculum was a mixture (4:1 vol/vol) of rumen liquor and buffer solution. Samples were ground to pass through a 1-mm screen. Portions of 0.5 ± 0.05 g were weighed into F57 filter bags (Ankom Technology), which were then sealed with a heat sealer. Three bags per feedstuff were used in each assay. The bags were incubated with the inoculum in the Daisy^{II} incubator for 48 h at 39 ± 1 °C under permanent agitation. After incubation the bags were removed and rinsed thoroughly with cold tap water until the water was clear. Afterwards, the bags were placed in the ANKOM^{200} fibre analyser for determination of NDF.

4.2.4. Ewe plasma lipid peroxidation

On days 12 and 14 of the experimental period, ewes were blood sampled by jugular venepuncture at 0, 3, 6 and 9 h after feeding in the morning. The blood samples collected in heparin tubes were immediately placed in ice and centrifuged at 1,600 x g for 10 min at 4 °C. Then plasma was separated and stored at −80 °C. Lipid peroxidation was analysed in the plasma samples using the thiobarbituric acid-reactive substances (TBARS) Assay Kit

provided by Cayman Chemical (MI, USA), according to the manufacturer's instructions. TBA values were expressed as μM of malonaldehyde (MDA).

4.2.5. Statistical analyses

Data of the chemical composition of grape pomace fractions, *in vitro* true digestibility, ruminal pH, ammonia-N, VFA and plasma TBARS were subjected to analysis of variance using the general linear model (GLM) procedure of the SAS 9.2. package $Y_i = \mu + T_i + \varepsilon_i$; where Y is the dependent variable; μ the overall mean; T_i the fixed effect of dietary treatment (two levels, CTRL and GP-7.5) and ε_i the error. The LSD test was used to assess the significance between treatment means where the effect was significant.

The values for disappearance of grape pomace fractions (DM, OM and CP) with time were fitted to the exponential model of Ørskov and McDonald (1979): $Y = a + b\ (1 - e^{(-ct)})$ by non-linear regression using the NLIN procedure of the SAS package. Degradability parameters were analysed using the SAS GLM procedures according to a factorial model which considered the effect of the samples (seeds and pulp) (S), the effect of the dietary treatment (CTRL and GP-7.5) (T) and their interaction (S × T): $y = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$, where μ is the overall mean; α is the fixed effect of samples, β is the fixed effect of treatment and ε the error.

For all statistical procedures the statistical significance of differences was defined as $P < 0.05$ and trends as $P < 0.10$.

4.3. Results and discussion

4.3.1. Primary compounds and fatty acid composition

Dry matter content of whole grape pomace averaged 411 g kg^{-1}, which is close to the range obtained in other works (Molina-Alcaide *et al.*, 2008; Spanghero *et al.*, 2009) and is higher than the values reported by Balasan *et al.* (2011) and Baumgärtel *et al.* (2007) for pomace from red grapes. The pressure applied during the processing stage and the moment of collection in the winery, which is the time elapsed since pressing, affect evaporation losses and could explain differences in moisture content of the grape pomace reported in the literature. The proportion of seeds and pulp plus skin of the whole grape pomace depends

largely on the processing or separation procedures employed and the variety and maturity of the grapes at harvest in each type of wine. In this study, the proportion of seeds in the grape pomace averaged 472 g kg^{-1} DM. These data are also in the range obtained by Spanghero *et al.* (2009) for grape pomace of red wines of other varieties (Italian and Californian), therefore the type of red wine produced did not seem to affect the proportion.

The chemical composition (OM, NDF, ADF, ADL, cellulose, hemicellulose, CP, EE, ADICP) and *in vitro* true digestibility of seeds and pulp from the grape pomace studied is given in Table 4.2, and Table 4.3 shows chemical composition of whole grape pomace calculated from seed and pulp fractions. Whole grape pomace is of a lignocellulosic nature, with high NDF (376 g kg^{-1} DM), ADF (317 g kg^{-1} DM) and lignin contents (207 g kg^{-1} DM), which reveals its low energy content and low digestibility. This was corroborated in the present study, since the lower level of NDF (243 *vs.* 523), ADF (193 *vs.* 454) and much lower level of LAD (74.7 *vs.* 353) content in the pulp fraction compared with the seed fraction are correlated with the higher *in vitro* true digestibility ($P < 0.05$) in pulp compared with seeds (0.82 *vs.* 0.51, respectively), supporting the view that digestibility of grape pomace is mainly due to non-structural carbohydrates. In this study, the NDF, ADF and LAD contents of the seeds are in line with results reported by Spanghero *et al.* (2009) and Balasan *et al.* (2011), but the pulp showed lower values in cell wall components. This finding reveals differences between grape varieties, cultivation conditions and the winemaking process of each wine, and thus differences in the potential of these by-products to be used as conventional feed in ruminants.

Lignocellulosic products of this type generally have a low CP ratio. According to our results, the protein content of whole grape products is around 12.2% (Table 4.3) being lower ($P < 0.05$) in seeds than in pulp (10.4 and 13.8%, respectively, in the present study). However, its digestive use is very low in all animal species owing to the high proportion of protein bound to the cell wall and the presence of tannins. In the present study, the ratio of CP bound to fibre (ADICP) was 14.2 and 25.1% for seeds and pulp, respectively, being significantly higher ($P < 0.05$) in pulp.

The ether extract of whole grape pomace (63.9 g kg^{-1} DM) (Table 4.3) was within the range found in other studies (Yi *et al.*, 2009). Grape pomace pulp has a lower content of EE than seeds (31.7 *vs.* 99.0 g kg^{-1} DM, $P < 0.05$). However, much of the fat in the pulp is likely to be digested, since it will not be protected by the seed coat (Spanghero *et al.*, 2009).

Table 4.2. Chemical composition and *in vitro* true digestibility of seeds and pulp from grape pomace from red wine.

	Seeds		Pulp		RSD[1]	*P*. value
	Mean	Range	Mean	Range		
Chemical composition (g kg^{-1} DM)						
Organic matter	927	(902–936)	811	(780–845)	21.6	<0.001
Neutral detergent fibre	523	(468–549)	243	(181–321)	47.2	<0.001
Acid detergent fibre	454	(409–475)	193	(156–257)	32.8	<0.001
Acid detergent lignin	353	(313–375)	74.7	(47.3–119)	24.77	<0.001
Hemicellulose [2]	68.9	(59.5–77.7)	50.0	(17.2–83.4)	21.72	0.163
Cellulose [3]	101	(87.6–113)	118	(105–138)	12.1	0.033
Neutral detergent-soluble carbohydrates[4]	201	(155–236)	399	(339–459)	18.4	<0.001
Crude protein	104	(96.1–115)	138	(108–149)	14.6	0.003
Acid detergent insoluble crude protein	14.4	(12.5–16.2)	33.9	(28.0–46.8)	5.36	<0.001
Ether extract	99.0	(87.5–111)	31.7	(25.0–39.0)	6.65	<0.001
Fatty acid composition (% of identified fatty acids)						
C8:0	-	-	0.12	(0.10–0.20)	0.029	<0.001
C10:0	-	-	0.52	(0.40–0.60)	0.053	<0.001
C12:0	0.02	(0.00–0.06)	0.84	(0.61–0.96)	0.136	<0.001
C14:0	0.10	(0.10–0.10)	0.74	(0.50–1.20)	0.183	<0.001
C14:1	-	-	0.12	(0.10–0.20)	0.029	<0.001
C15:0	-	-	0.20	(0.20–0.20)	0.000	<0.001
C16:0	8.07	(7.50–8.40)	19.3	(17.9–21.9)	1.136	<0.001
C16:1	0.20	(0.10–0.30)	1.70	(1.10–2.30)	0.363	<0.001
C17:0	0.10	(0.10–0.10)	0.23	(0.20–0.30)	0.037	<0.001
C17:1	0.12	(0.10–0.20)	0.10	(0.10–0.40)	0.113	0.804
C18:0	3.97	(3.60–4.20)	5.62	(5.20–6.00)	0.257	<0.001
C18:1	17.2	(16.1–18.3)	12.7	(11.0–14.3)	1.20	<0.001
C18:2	69.0	(67.3–70.8)	40.2	(34.3–42.7)	2.46	<0.001
C18:3	0.52	(0.50–0.60)	12.5	(10.6–15.0)	1.10	<0.001
C20:0	0.20	(0.20–0.20)	1.38	(1.20–1.60)	0.130	<0.001
C20:1	0.32	(0.30–0.40)	0.72	(0.20–2.10)	0.569	0.251
C20:2	0.07	(0.00–0.10)	0.18	(0.10–0.20)	0.047	0.002
C20:3	-	-	0.30	(0.30–0.30)	0.000	<0.001
C20:4	-	-	0.13	(0.10–0.20)	0.037	<0.001
C22:0	0.10	(0.10–0.10)	1.57	(1.30–1.80)	0.139	<0.001
C22:1	-	-	0.05	(0.00–0.10)	0.039	0.049
C23:0	-	-	0.30	(0.20–0.40)	0.044	<0.001
C24:0	-	-	0.63	(0.50–0.80)	0.073	<0.001
Ratios [5]						
SFA	12.6	(12.0–12.8)	31.4	(29.4–35.5)	1.76	<0.001
MUFA	17.8	(16.0–19.2)	15.3	(12.7–17.5)	1.61	0.023
PUFA	69.6	(68.0–71.7)	53.3	(46.9–56.2)	2.64	<0.001
PUFA:SFA	5.55	(5.23–5.94)	1.71	(1.32–1.89)	0.253	<0.001
Phenolic compounds (g kg^{-1} DM)						
Extractable polyphenols	55.0	(49.1–58.4)	32.1	(26.4–38.3)	3.73	<0.001
Hydrolysable polyphenols	9.18	(8.90–9.56)	8.11	(7.00–8.53)	0.447	0.002
Condensed tannins	93.5	(69.2–116)	19.4	(14.1–26.7)	14.97	<0.001
Total anthocyanins	0.28	(0.09–0.41)	7.63	(3.80–9.35)	1.406	<0.001
In vitro true digestibility	0.51	(0.50–0.56)	0.82	(0.81–0.90)	0.011	0.001

[1] RSD: residual standard deviation.

[2] Hemicellulose: NDF–ADF; [3] Cellulose: ADF–ADL; [4] Neutral detergent-soluble carbohydrates (NDSC): OM–EE–CP–NDF.

[5] SFA (saturated fatty acids) = C8:0 + C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C23:0 + C24:0; MUFA (monounsaturated fatty acids) = C14:1 + C16:1 + C17:1 + C18:1 + C20:1 + C22:1; PUFA (polyunsaturated fatty acids) = C18:2 + C18:3 + C20:2 + C20:3 + C20:4.

Table 4.3. Chemical composition of whole grape pomace from red wine calculated from seed and pulp fractions.

	Whole grape pomace from red wine	
	Mean	Range
Dry matter (DM, g kg^{-1} fresh grape pomace)	411	(240–637)
Seeds (g kg^{-1} DM)	472	(406–564)
Composition of DM (g kg^{-1} DM)		
Organic matter	866	(851–884)
Neutral detergent fibre	376	(316–414)
Acid detergent fibre	317	(281–343)
Acid detergent lignin	207	(163–232)
Hemicellulose [1]	58.3	(34.4–79.9)
Cellulose [2]	110	(95.3–123)
Neutral detergent-soluble carbohydrates[3]	305	(241–369)
Crude protein	122	(104–134)
Acid detergent insoluble crude protein	24.9	(19.2–32.4)
Ether extract	63.9	(56.4–73.3)
Fatty acid composition (% of identified fatty acids)		
C8:0	0.03	(0.02–0.04)
C10:0	0.13	(0.09–0.15)
C12:0	0.23	(0.14–0.29)
C14:0	0.28	(0.17–0.37)
C14:1	0.03	(0.01–0.06)
C15:0	0.05	(0.03–0.07)
C16:0	11.1	(9.89–12.3)
C16:1	0.62	(0.37–0.92)
C17:0	0.14	(0.11–0.17)
C17:1	0.11	(0.08–0.16)
C18:0	4.41	(3.98–4.72)
C18:1	16.0	(14.6–17.3)
C18:2	61.3	(58.6–63.3)
C18:3	3.69	(2.65–4.94)
C20:0	0.52	(0.36–0.68)
C20:1	0.40	(0.26–0.68)
C20:2	0.10	(0.04–0.13)
C20:3	0.08	(0.04–0.11)
C20:4	0.04	(0.01–0.07)
C22:0	0.50	(0.29–0.66)
C22:1	0.01	(0.00–0.04)
C23:0	0.08	(0.03–0.12)
C24:0	0.17	(0.09–0.24)
Ratios[4]		
SFA	65.2	(63.4–67.2)
MUFA	17.2	(15.7–18.4)
PUFA	17.6	(15.5–19.8)
PUFA:SFA	3.37	(3.22–4.26)
Phenolic compounds (g kg^{-1} DM)		
Extractable polyphenols	42.8	(37.3–49.6)
Hydrolysable polyphenols	8.64	(7.82–9.11)
Condensed tannins	54.6	(37.9–63.9)
Anthocyanins	4.10	(2.29–5.04)

[1] Hemicellulose: NDF–ADF; [2] Cellulose: ADF–ADL; [3] Neutral detergent-soluble carbohydrates (NDSC): OM–EE–CP–NDF.

[4] SFA (saturated fatty acids) = C8:0 + C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C23:0 + C24:0; MUFA (monounsaturated fatty acids) = C14:1 + C16:1 + C17:1 + C18:1 + C20:1 + C22:1; PUFA (polyunsaturated fatty acids) = C18:2 + C18:3 + C20:2 + C20:3 + C20:4.

Yi *et al.* (2009) found in whole grape pomace from red wine average values of 21.2% saturated fatty acids (SFA), 14.4% monounsaturated fatty acids (MUFA) and 62.7% polyunsaturated fatty acids (PUFA). These data are close to the fatty acid profile of whole red grape pomace (17.6% SFA, 17.2% MUFA and 65.2% PUFA) from the sum of pulp and seed fatty acid content, taking into account each proportion in the present study. The fatty acid composition of whole grape pomace has been reported by Yi *et al.* (2009), but the composition of the tissue part (seeds and pulp plus skin) has not been accurately characterized. As shown in Table 4.2, the lipid profile of seeds was less ($P < 0.05$) saturated than that of pulp (12.6 *vs.* 31.4%), therefore seeds had a higher level of polyunsaturated fatty acids than pulp (69.6 *vs.* 53.3%, $P < 0.05$). Seed and pulp FA results are in agreement with the data provided by FEDNA, (2010). The major seed fatty acids were linoleic acid (C18:2; 69.0%), oleic acid (C18:1; 17.2%), palmitic acid (C16:0; 8.07%) and stearic acid (C18:0; 3.97%). The sum of these four fatty acids exceeded 98% of the total fat of the seeds. In the pulp FA profile the major fatty acids were C18:2 (40.2%), C16:0 (19.3%), C18:1 (12.7%), α-linolenic acid (C18:3; 12.5%) and C18:0 (5.62%). The sum of these five fatty acids accounted for over 90% of total fat from the pulp. As a result of FA composition of seeds and pulp, the major fatty acids in whole grape pomace (Table 4.3) were C18:2 (61.3%), C18:1 (16.0%), C16:0 (11.1%), C18:0 (4.41%) and C18:3 (3.69%). The high PUFA concentration, especially the high percentage of linoleic acid, found in the seed (69.0%) and pulp fractions (40.2%) may suggest potential value for dietary inclusion with the objective of providing substrate for the production of bioactive fatty acids in the rumen. Because most seeds are not broken open during eating or rumination, fatty acids of pulp could be more useful to provide substrate to increase the level of vaccenic and rumenic fatty acids in ruminant products.

4.3.2. Phenolic compounds

One aspect that presents great interest in grape pomace is its content of phenolic compounds, since numerous beneficial properties, such as antioxidant power, have been attributed to these compounds (González-Paramás *et al.*, 2004).

Table 4.2 presents the average values of the phenolic compound contents (EP, HP, CT and TA) of seeds and pulp of grape pomace derived from the production of red wine. Table 4.3 shows the average values of the phenolic compounds of whole grape pomace calculated from seed and pulp fractions Total phenolic levels in whole grape pomace were comparable

to reported values obtained by Besharati and Taghizadeh (2009). EP content was much higher ($P < 0.05$) in seeds than in pulp (55.0 *vs.* 32.1 g kg^{-1} DM respectively), which is in accordance with Spanghero *et al.* (2009). HP content was similar in pulp and seeds, although slightly higher ($P < 0.05$) in seeds (9.18 *vs.* 8.11 g kg^{-1} DM respectively). As for condensed tannin content, some authors have indicated that grape by-products, especially their seeds, present high levels, which could have potentially negative effects on ruminal fermentation. In contrast, the fraction which includes the skin and pulp has a lower level of fibre, with less lignin, and an overall reduction in tannin levels (Makris *et al.*, 2007). This statement is in agreement with the present results, since CT content was higher in seeds than in pulp (93.5 *vs.*19.4 g kg^{-1} DM). These values are lower than those obtained by Molina-Alcaide *et al.* (2008). However, it should be noted that the samples analysed by that author came from two different wineries and the author did not specify the percentage of pulp and seeds of the samples, or the type of wine produced. Anthocyanin contents were much lower ($P < 0.05$) in seeds than in pulp (0.28 *vs.* 7.63 g kg^{-1} DM respectively). The reason for these results is that grapes owe their colour to this pigment, which is mostly in their pulp and skins, colouring wine deep red or purple (Baumgärtel *et al.*, 2007).

The detailed profile of the seed and pulp phenolic compounds is given in Table 4.4. Table 4.5 shows detailed profile phenolic compounds of whole grape pomace calculated from seed and pulp fractions The total content of phenolic acids was lower ($P < 0.05$) in seeds than in pulp (34.7 *vs.* 122 mg kg^{-1} MS), syringic acid, vanillic acid and gallic acid being the majority phenolic acids. *Trans* resveratrol stilbene was only detected in pulp (1.08 mg kg^{-1} MS). The content of total flavonols was not different between seeds and pulp ($P > 0.05$), but there was a notable difference in the content of quercetin flavonol (3.62 *vs.* 1.10 g kg^{-1} MS in seeds and pulp, respectively). As for total flavanols, the most abundant phenolics, the content was higher in seeds than in pulp (12.3 *vs.* 6.80 g kg^{-1} MS), because the catechin concentration was significantly higher ($P < 0.05$) in seeds. The main anthocyanins were anthocyanin derivatives, malvidin 3-O glucoside and petunidin 3-O glucoside, which is in agreement with Antoniolli *et al.* (2015), and they were only detected in the pulp fraction.

All phenolic compounds may be potential sources of antioxidant polyphenols (Alonso *et al.*, 2002), which can prevent animal tissue damage, mainly the unsaturated lipids in membranes due to reactive oxygen metabolites. An interesting prospect of grape pomace utilization and the use of its fractions (seeds or pulp) as a feedstuff for ruminants comes from the possibility of enriching animal products with substances that have health benefits for consumers. These differences in secondary compounds between seeds and pulp and the lower fibre level, with less lignin, found in pulp suggest that if the seeds could be separated

from the pulp and skin fraction the latter might have potential value for dietary inclusion with the objective of improving the health status of animals and might also be an important source of polyphenols, such as *trans* resveratrol stilbene and anthocyanins, with a powerful biological activity which includes antioxidative activities.

Table 4.4. Concentration of phenolic compounds in seeds and pulp from grape pomace from red wine (mg kg^{-1} DM).

	Seeds		Pulp		RSD[1]	*P. value*
	Mean	Range	Mean	Range		
Non-flavonoids						
- Phenolic acids						
. Cinnamic acids						
Caffeic acid	-	-	-	-	-	-
Chlorogenic acid	-	-	6.75	(0.02–18.3)	5.330	0.053
p-Coumaric acid	-	-	-	-	-	-
Sinapic acid	-	-	-	-	-	-
Ferulic acid	-	-	-	-	-	-
. Benzoic acids						
Gallic acid	4.94	(1.88–10.2)	23.5	(5.87–69.2)	16.61	0.081
Gentisic acid	-	-	-	-	-	-
Ellagic acid	0.48	(0.06–1.14)	2.61	(0.06–6.33)	2.098	0.109
Syringic acid	16.8	(8.19–25.8)	54.7	(45.2–66.9)	7.93	<0.001
Vanillic acid	0.24	(0.00–0.60)	30.0	(19.3–41.0)	6.90	<0.001
Protocatechuic acid	12.3	(0.60–44.9)	4.45	(1.90–9.94)	12.358	0.299
Total phenolic acids	34.7	(25.9–64.1)	122	(82.5–206)	33.20	0.001
- Stilbene						
. *Trans* resveratrol	-	-	1.08	(0.00–3.82)	1.130	0.130
Flavonoids						
- Flavonols						
. Quercetin *	3.62	(0.37–8.74)	1.10	(0.09–4.00)	2.973	0.173
. Kaempferol	0.11	(0.00–0.34)	1.67	(0.00–7.67)	2.131	0.233
. Myricetin	-	-	5.09	(0.00–20.5)	5.499	0.140
Total flavonols *	3.62	(0.41–8.74)	1.11	(0.10–4.00)	2.973	0.174
- Flavanols						
. Catechin *	12.3	(10.2–16.7)	6.79	(4.69–9.32)	2.204	0.002
. Epicatechin	2.18	(1.51–2.66)	9.79	(6.02–11.6)	2.775	0.001
Total flavanols *	12.3	(10.2–16.8)	6.80	(4.70–9.04)	2.202	0.002
- Anthocyanins						
. Delphinidin 3-O- glucoside	-	-	217	(42.0–368)	74.9	0.001
. Cyanidin 3-O-glucoside	-	-	13.2	(3.66–35.2)	8.82	0.027
. Petunidin 3-O-glucoside	-	-	269	(64.3–364)	78.8	<0.001
. Peonidin 3-O-glucoside	0.34	(0.00–1.16)	60.0	(33.8–115)	24.89	<0.001
. Malvidin 3-O-glucoside	13.2	(3.77–25.8)	977	(334–1356)	256.4	<0.001
. ∑ Anthocyanin derivatives *	-	-	3.36	(0.58–6.47)	1.409	0.002
Total anthocyanins *	0.01	(0.004–0.027)	4.90	(1.05–8.03)	1.695	0.001

* g kg^{-1} DM.

[1] RSD: residual standard deviation.

Table 4.5. Concentration of phenolic compounds in whole grape pomace from red wine calculated from seed and pulp fractions (mg kg^{-1} DM).

	Whole grape pomace from red wine	
	Mean	Range
Non-flavonoids		
- Phenolic acids		
. Cinnamic acids		
Caffeic acid	-	-
Chlorogenic acid	3.26	(0.01–7.99)
p-Coumaric acid	-	-
Sinapic acid	-	-
Ferulic acid	-	-
. Benzoic acids		
Gallic acid	13.7	(7.20–31.7)
Gentisic acid	-	-
Ellagic acid	1.68	(0.36–3.63)
Syringic acid	36.9	(29.2–42.1)
Vanillic acid	15.6	(11.2–21.7)
Protocatechuic acid	7.46	(1.24–19.3)
Total phenolic acids	78.6	(59.4–104)
- Stilbene		
. *Trans* resveratrol	0.63	(0.00–2.27)
Flavonoids		
- Flavonols		
. Quercetin *	2.15	(0.67–3.82)
. Kaempferol	0.96	(0.00–4.11)
. Myricetin	2.79	(0.00–11.0)
Total flavonols *	2.15	(0.68–3.82)
- Flavanols		
. Catechin *	9.32	(7.77–11.5)
. Epicatechin	6.14	(4.19–9.30)
Total flavanols *	9.32	(7.77–11.5)
- Anthocyanins		
. Delphinidin 3-O- glucoside	114	(25.0–209)
. Cyanidin 3-O-glucoside	6.62	(0.91–15.3)
. Petunidin 3-O-glucoside	140	(38.2–207)
. Peonidin 3-O-glucoside	31.0	(15.3–50.2)
. Malvidin 3-O-glucoside	510	(200–716)
. ∑ Anthocyanin derivatives *	1.72	(0.34–3.02)
Total anthocyanins *	2.52	(0.62–3.77)

* g kg^{-1} DM.

4.3.3. Degradation kinetics and rumen fermentation

Table 4.6 presents the rumen degradation parameters for DM, OM and CP of seeds and pulp of grape pomace derived from red wine production. The present values are within the

range obtained by Besharati and Taghizadeh (2009) for dried grape by-product, and close to the results reported by Molina-Alcaide *et al.* (2008) for other types of grape pomace.

Table 4.6. Effect of experimental ewe diets on ruminal degradation profiles of seeds and pulp from grape pomace from red wine.

	Treatments[1]				RSD[2]	*P. value*[3]		
	CTRL		GP-7.5					
	Seeds	Pulp	Seeds	Pulp		S	T	S × T
Dry matter								
a	0.20	0.40	0.19	0.38	0.005	<0.001	0.024	0.280
b	0.20	0.30	0.21	0.30	0.046	0.079	0.900	0.815
c (h^{-1})	0.06	0.01	0.07	0.03	0.010	0.001	0.299	0.700
PD	0.40	0.70	0.41	0.68	0.042	<0.001	0.852	0.695
ED 0.02	0.35	0.50	0.36	0.51	0.008	<0.001	0.284	1.000
ED 0.06	0.30	0.45	0.31	0.45	0.011	<0.001	0.685	0.887
ED 0.08	0.29	0.44	0.29	0.44	0.011	<0.001	0.821	0.828
Organic matter								
a	0.19	0.35	0.18	0.32	0.007	<0.001	0.038	0.277
b	0.20	0.32	0.22	0.25	0.030	0.041	0.426	0.175
c (h^{-1})	0.07	0.01	0.07	0.04	0.010	0.003	0.225	0.387
PD	0.39	0.67	0.40	0.57	0.025	<0.001	0.136	0.071
ED 0.02	0.34	0.46	0.35	0.47	0.009	<0.001	0.294	0.906
ED 0.06	0.29	0.40	0.30	0.41	0.012	<0.001	0.527	0.836
ED 0.08	0.28	0.39	0.28	0.40	0.012	<0.001	0.667	0.911
Crude protein								
a	0.37	0.32	0.25	0.27	0.082	0.862	0.311	0.659
b	0.43	0.48	0.59	0.33	0.086	0.235	0.948	0.106
c (h^{-1})	0.11	0.01	0.14	0.05	0.020	0.001	0.129	0.723
PD	0.80	0.70	0.84	0.60	0.058	0.017	0.587	0.267
ED 0.02	0.74	0.36	0.76	0.49	0.053	<0.001	0.189	0.346
ED 0.06	0.66	0.38	0.66	0.41	0.017	<0.001	0.399	0.470
ED 0.08	0.63	0.37	0.62	0.39	0.022	<0.001	0.720	0.562

[1] Treatments: CTRL, without grape pomace; GP-7.5, 7.5% of grape pomace from red wine, DM basis.

[2] RSD: residual standard deviation.

[3] Probability of significant effects due to sample, seeds or pulp (S), dietary treatment (T) and interaction effects (S × T).

a, soluble fraction; *b*, potentially degradable insoluble fraction; *c*, rate of degradation of *b* fraction; *PD*, potential degradability; *ED*, effective degradability = $a + [bc/(c + k)]$, where k = 0.02; 0.06 and 0.08 h^{-1}.

The soluble fraction (*a*) and the potentially degradable insoluble fraction (*b*) were higher ($P < 0.05$) and the degradation rate of *b* (*c*) of DM and OM was lower ($P < 0.05$) in pulp than in seeds. Consequently the highest potential degradability (*PD*) and effective degradability

(*ED*) values ($P < 0.05$) were found in pulp, probably owing to the lower lignified fibre content and possible presence of rapidly fermentable substrates in pulp compared with seed (Van Soest, 1994). With regard to the CP degradability parameters, they did not present statistically significant ($P > 0.05$) differences between samples. However, *PD* and *ED* were significantly higher ($P < 0.05$) in seeds than in pulp. This result is probably attributable to the higher CP bound to ADF content in the pulp fraction, as previously mentioned, since that protein presents high lignification. The crude protein *ED* value of whole grape pomace calculated from the values reported for seeds and pulp was 0.56.

The experimental sheep diet (CTRL *vs.* GP-7.5) had minor effects on ruminal degradation parameters of DM, OM and CP and only affected the immediately degradable fraction of DM and OM, showing higher ($P < 0.05$) values in the CTRL group than in the GP-7.5 treatment. No differences were found ($P > 0.05$) in protein degradation due to the dietary treatment. However, our results did not seem to contradict the general idea that secondary compounds, such as tannins, decrease the rate of protein degradation in the rumen (Patra and Saxena, 2009), because a numerical reduction ($P < 0.05$) of the fractional rate of degradation of CP was found in the GP-7.5 diets. The small differences in the present study may be due to the low level of grape pomace added to the diet, or an adaptation of the rumen microorganisms to tannins from grape pomace in animals receiving the GP-7.5 treatment, since several ruminal microorganisms have been identified that can tolerate relatively high concentrations of both hydrolysable tannins and CT (Jones *et al.*, 1994), and they could produce an improvement in degradability efficiency. Similarly, O'Donovan and Brooker (2001) indicated that proteolytic bacteria, which are initially sensitive to tannins, can, after a short period of adaptation, respond by modifying their metabolism. This is only one example of how ruminal bacteria with proteolytic and cellulolytic activity can maintain their function when tannin levels are not too high (Jones *et al.*, 1994).

Figure 4.1 represents the evolution of pH and ammonia-N after feeding in ruminal fluid from sheep belonging to the two experimental treatments (CTRL and GP-7.5). The ruminal pH values for both diets were within the normal range and followed the typical evolution, dropping after feeding, showing the lowest pH values 3 h after eating. Mean values of pH corresponding to the CTRL diet were lower ($P < 0.05$) than for the GP-7.5 diet (6.35 *vs.* 6.48). Changes in rumen pH are primarily determined by fermentation products, VFA and lactic acid, derived from carbohydrate fermentation, which were higher in CTRL ewes, as detailed later.

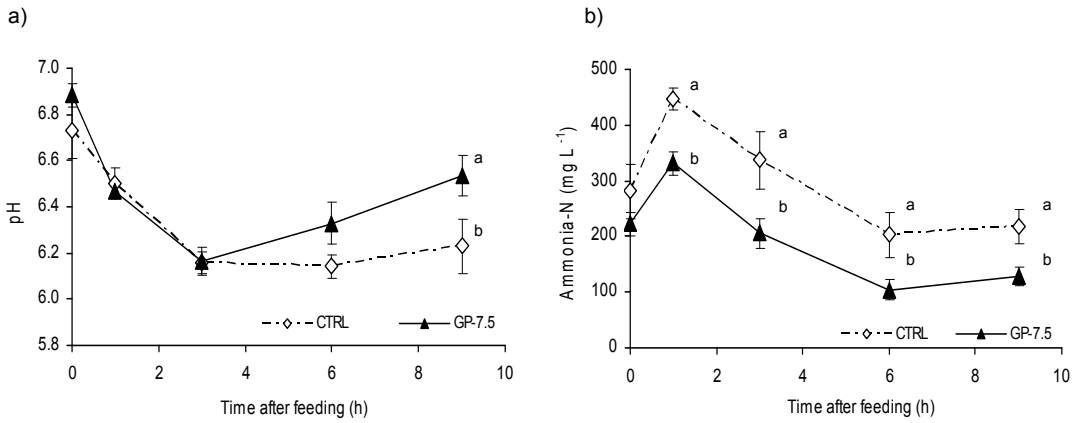


Figure 4.1. Effect of experimental ewe diets (CTRL, without grape pomace; GP-7.5, 7.5% of grape pomace from red wine, DM basis) on ruminal pH (a) and ammonia-N (b) concentration at different times after feeding. Different small letters mean significant differences ($P < 0.05$) between treatments within time. The error bars represent standard error.

Ammonia-N concentration followed the expected evolution, increasing after feeding, reaching the highest level 1 h after the meal and decreasing later. The ammonia-N concentration in ruminal liquid was significantly higher ($P < 0.05$) in sheep that had not consumed grape pomace in the diet. The energy:protein ratio was similar in the two experimental diets (1.10 *vs.* 1.12 for CTRL and GP-7.5, respectively), and the calculated value of non-degradable protein in rumen did not present great differences between treatments (74.0 *vs.* 72.9% of CP, for CTRL and GP-7.5, respectively). Therefore, the lower ruminal ammonia-N content in GP-7.5 animals can probably be attributed to the presence of a high proportion of CP bound to the ADF of grape pomace, and to the presence of phenolic compounds, such as tannins, in the diet of the animals that were supplemented with grape residue in their ration. These results are in accordance with other authors (El-Waziry *et al.*, 2005; Besharati and Taghizadeh, 2009; Abarghuei *et al.*, 2010). The reduction in ammonia-N concentration (−33%) suggests that addition of grape pomace, rich in tannins and other phenolics, could help to reduce proteolysis, degradation of peptides and deamination of amino acids, with a subsequent increase in amino acid flow to the small intestine, as has been reported when low or moderate levels of tannins are included in ruminant diets (Frutos *et al.*, 2004). These effects on nutrition could be reflected in animal performance. In spite of this, the disappearance of DM, OM and CP did not seem to be negatively affected by the reduced ruminal ammonia concentration, probably because many rumen bacteria use not only ammonia-N but also peptides or amino acids (Fernández *et al.*, 2009). Moreover,

protein degradation does not necessarily require deamination; therefore the lack of differences in CP degradability of grape pomace samples was not contrary to the decreased ruminal ammonia concentration data when ewes were fed with 7.5% of grape pomace in the diet.

Mean values of ruminal VFA concentrations are presented in Figure 4.2. The major VFA (acetic acid, butyric acid and propionic acid), and therefore total VFA, of both experimental treatments followed the same evolution as ammonia-N, which inversely coincides with the evolution of pH: when the pH is lower, the ammonia-N and VFA concentration is higher. The experimental treatment (CTRL *vs.* GP-7.5) affected ($P < 0.05$) total VFA concentration and molar proportions of acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid in the rumen, with samples from animals assigned to the GP-7.5 group showing lower values. Besharati and Taghizadeh (2009), in accordance with our results, found a higher ($P < 0.05$) VFA concentration in a control treatment compared with animals consuming the basal diet, replacing alfalfa with different amounts of dried grape by-products. As there were not many differences in estimated non-structural carbohydrate between diets (205 and 190 g kg^{-1} DM for starch and 32.0 and 31.9 g kg^{-1} for sugars in CTRL and GP-7.5, respectively), VFA variations can almost certainly be attributed to the high lignified fibre and phenolic content of grape pomace. Some authors have shown a reduction in the total VFA proportion in the presence of tannins (Priolo *et al.*, 2000; Frutos *et al.*, 2004; Tiemann *et al.*, 2008), which could be due to the capacity of tannins to bind to fibre portions of the feed (McSweeney *et al.*, 2001) or to their inhibitory effect on microbial activity (Jones *et al.*, 1994). There were no significant differences in acetate:propionate ratio between the two experimental treatments for any time, so effects on animal performance and product quality are not expected.

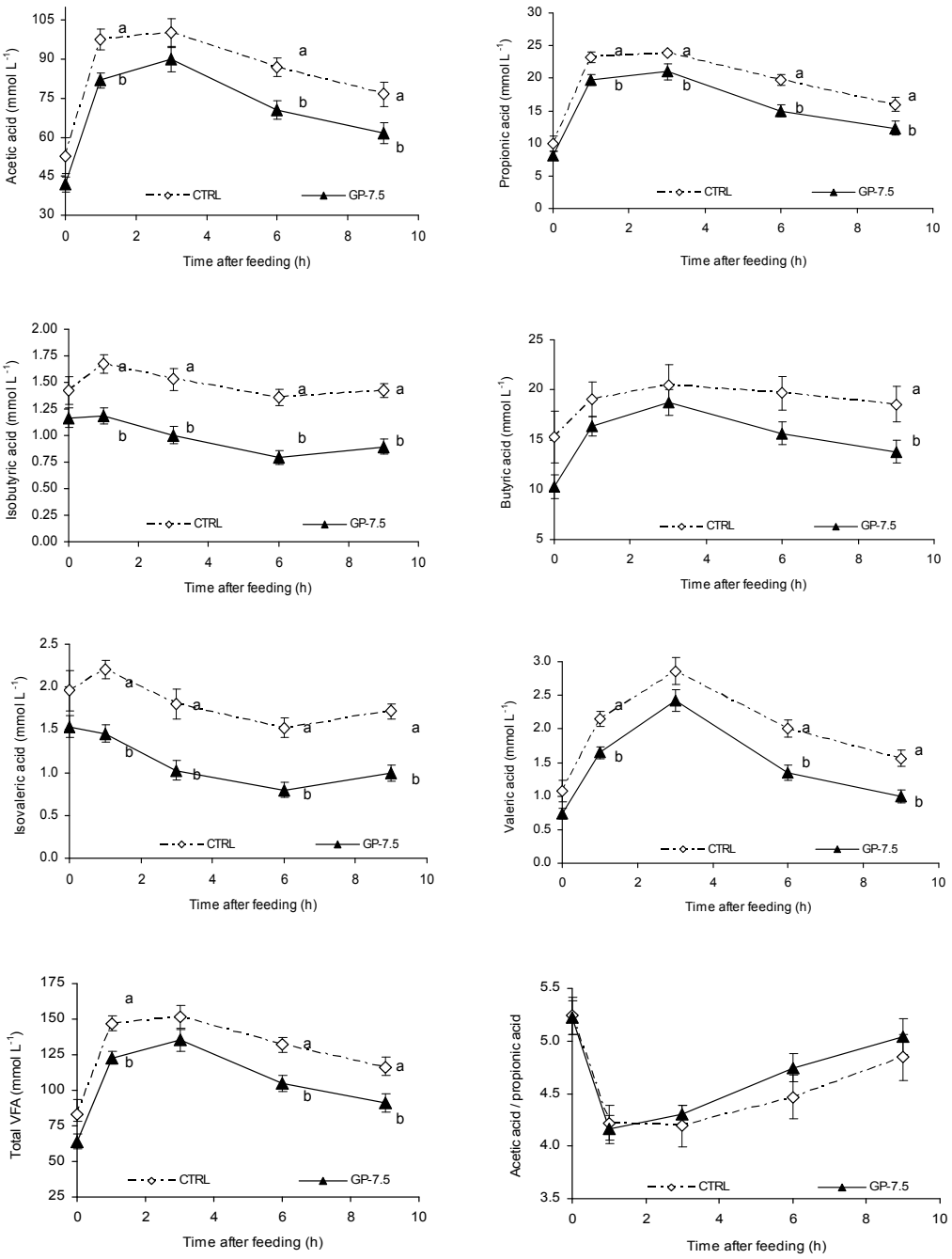


Figure 4.2. Effect of experimental ewe diets (CTRL, without grape pomace; GP-7.5, 7.5% of grape pomace from red wine, DM basis) on volatile fatty acid concentrations at different times after feeding. Different small letters mean significant differences ($P < 0.05$) between treatments within time. The error bars represent standard error.

4.3.4. Ewe plasma oxidation

The effect of the experimental ewe diets on plasma MDA at different times after feeding is shown in Figure 4.3.

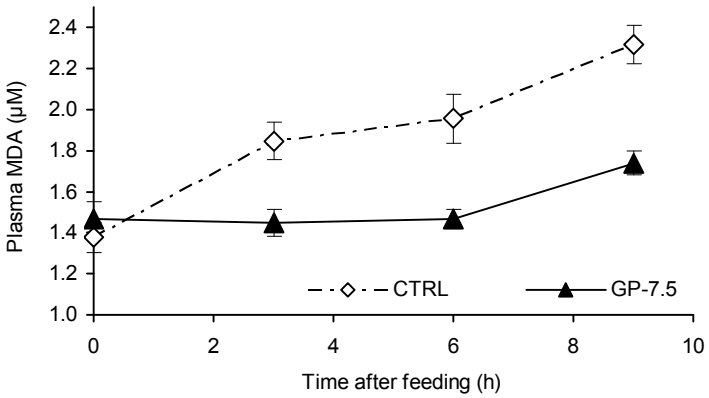


Figure 4.3. Effect of experimental ewe diets (CTRL, without grape pomace; GP-7.5, 7.5% of grape pomace from red wine, DM basis) on plasma MDA (μM) at different times after feeding. The error bars represent standard error.

There were no significant differences ($P > 0.05$) between dietary treatments for any time. Grape pomace inclusion in the diet did not significantly affect plasma TBARS ($P > 0.05$), which is in agreement with Sgorlon *et al.* (2006), who did not report differences in lamb plasma due to supplementation with 5.5 g day^{-1} of grape skin extract. In spite of the lack of significant differences, lower numerical MDA values were found when grape pomace was included in the ewe diets. The decrease in plasma TBARS values as a consequence of grape pomace supplementation of the ewe diets accounted for 22%, 25% and 25% for 3, 6 and 9 h after feeding compared with CTRL. In this regard, Gladine *et al.* (2007) reported that the inclusion of 10% DM of grape seed and peel extract directly into the rumen of sheep improved the antioxidant status and reduced the susceptibility to lipid oxidation of plasma measured by an oxidation induced assay. That statement has been corroborated by Bodas *et al.* (2011), Casamassima *et al.* (2012) and Salinas-Ríos *et al.* (2015), since they found lower plasma TBARS values when they supplemented sheep diets with phenolic compounds. The decrease in the blood concentration of MDA can probably be attributed both to direct action (trapping of free radicals by polyphenols owing to antioxidant activity during the propagation of oxidative chain reactions) and to blocking of the initiation phase of

oxidation by inhibition of pro-oxidant enzymes responsible for the production of free radicals (Casamassima *et al.*, 2012). These results suggest that grape pomace may be a potential source of antioxidants which could be transferred directly or after metabolic transformation by rumen microbes to ruminant products. *In vivo* trials are necessary to test the effect of the inclusion of this winery by-product in practical diets for ruminants on meat and milk quality.

4.4. Conclusions

Chemical composition, *in vitro* true digestibility and ruminal degradability results indicate that grape pomace from red wine is of limited nutritive value. The chemical composition of grape pomace is variable, depending on the proportion of seeds and pulp plus skin. The results clearly indicate that pulp plus skin had the benefit of lower lignified fibre, higher digestibility and higher content of some polyphenols, with a more powerful biological activity than seeds. The interest of this by-product in sheep feeding could be related to its antioxidant power, evidenced as lower numerical sheep plasma MDA values, and its PUFA content, which provides substrate for the production of bioactive fatty acids in the rumen.

4.5. References

Abarghuei, M.J., Rouzbehan, Y., Alipour, D. (2010). The influence of the grape pomace on the ruminal parameters of sheep. *Livestock Science 132*, 73–79

Alipour, D., Rouzbehan, Y. (2007). Effects of ensiling grape pomace and addition of polyethylene glycol on *in vitro* gas production and microbial biomass yield. *Animal Feed Science and Technology 137*, 138–149.

Alonso, A.M., Guillén, D.A., Barroso, C.G., Puertas, B., García, A. (2002). Determination of antioxidant activity of wine byproducts and its correlation with polyphenolic content. *Journal of Agricultural and Food Chemistry 50*, 5832–5836.

Antoniolli, A., Fontana, A.R., Piccoli, P., Bottini, R. (2015). Characterization of polyphenols and evaluation of antioxidant capacity in grape pomace of the cv. Malbec. *Food Chemistry 178*, 172–178.

AOAC (2012). Official Methods of Analysis of AOAC International, 19th Edition.

Basalan, M., Gungor, T., Owens, F.N., Yalcinkaya, I. (2011). Nutrient content and in vitro digestibility of Turkish grape pomaces. *Animal Feed Science and Technology 16*, 194–198.

Bate-Smith, E.C. (1981). Astringent tannins of the leaves of Geranium species. *Phytochemistry 20*, 211–216.

Baumgärtel, T., Kluth, H., Epperlein, K., Rodehutscord, M. (2007). A note on digestibility and energy value for sheep of different grape pomace. *Small Ruminant Research 67*, 302–306.

Besharati, M., Taghizadeh, A. (2009). Evaluation of dried grape by-product as a tanniniferous tropical feedstuff. *Animal Feed Science and Technology 152*, 198–203.

Bligh, E.G., Dyer, W.J. (1959). A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology 37*, 911–917.

Bodas, R., Prieto, N., López-Campos, O., Giráldez, F.J., Andrés, S. (2011). Naringin and vitamin E influence the oxidative stability and lipid profile of plasma in lambs fed fish oil. *Research in Veterinary Science 91*, 98–102.

Carro, M.D., Lebzien, P., Rohr, K. (1992). Influence of yeast culture on the in vitro fermentation (Rusitec) of diets containing variable portions of concentrates. *Animal Feed Science and Technology 37*, 209–220.

Casamassima, D., Palazzo, M., Martemucci, G., Vizzarri, F., Corino, C. (2012). Effects of verbascoside on plasma oxidative status and blood and milk production parameters during the peripartum period in Lacaune ewes. *Small Ruminant Research 105*, 1–8.

Del Álamo, M., Nevares, I., García, S. (2004a). Influence of different aging systems and oak woods on aged wine color and anthocyanin composition. *European Food Research and Technology 219*, 124–132.

Del Álamo, M., Casado, L., Hernández, V., Jiménez, J.J. (2004b). Determination of free molecular phenolics and catechins in wine by solid phase extraction on polymeric cartridges and liquid chromatography with diode array detection. *Journal of Chromatography A 1049*, 97–105.

El-Waziry, A.M., Nasser, M.E.A., Sallam, S.M.A. (2005). Processing methods of soybean meal: 1-effect of roasting and tannic acid treated-soybean meal on gas production and rumen fermentation in vitro. *Journal of Applied Sciences Research 1*, 313–320.

FAOSTAT (2012). http://faostat3.fao.org [15 de julio de 2015].

FEDNA (2010). Tablas de composición y valor nutritivo de los alimentos para la fabricación de piensos compuestos. (C. de Blas, G.G. Mateos, P. García-Rebollar). FEDNA, Madrid (Spain).

Fernández, B., Bodas, R., López-Campos, O., Andrés, S., Mantecón, A.R., Giráldez F.J. (2009). Vinasse added to dried sugar beet pulp: preference rate, voluntary intake, and digestive utilization in sheep. *Journal of Animal Science 87*, 2055–2063.

Frutos, P., Hervás, G., Giráldez, F.J., Mantecón, A.R. (2004). Review. Tannins and ruminant nutrition. *Spanish Journal of Agricultural Research 2*, 191–202.

Gallego, L., Nevares, I., Fernández, J.A., Del Álamo, M. (2011). Determination of low-molecular mass phenols in red wines: The influence of chips, staves and micro-oxygenation aging tank. *Food Science and Technology International 17*, 429–438.

Gladine, C., Rock, E., Morand, C., Bauchart, D., Durand, D. (2007). Bioavailability and antioxidant capacity of plant extracts rich in polyphenols, given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. *British Journal of Nutrition 98*, 691–701.

González-Paramás, A.M., Esteban-Ruano, S., Santos-Buelga, C., De Pascual-Teresa, S., Rivas-Gonzalo, J.C. (2004). Flavanol content and antioxidant activity in winery products. *Journal of Agricultural and Food Chemistry 52,* 234–238.

Hartzfeld, P.W., Forkner, R., Hunter, M.D., Hagerman, A.E. (2002). Determination of hydrolyzable tannins (gallotannins and ellagitannins) after reaction with potassium iodate. *Journal of Agricultural and Food Chemistry 50*, 1785–1790.

Jones, G.A., McAllister, T.A., Cheng, K.J., Muir, A.D. (1994). Effect of sainfoin (*Onobrychris viciifolia* Scop.) condensed tannins on growth and proteolysis by 4 strains of rumen bacteria. *Applied and Environmental Microbiology 60*, 1374–1378.

Makris, D.P., Boskou, G., Andrikopoulos, N.K. (2007). Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. *Journal of Food Composition and Analysis 20*, 125–132.

McSweeney, C.S., Palmer, B., McNeill, D.M., Krause, D.O. (2001). Microbial interactions with tannins: nutritional consequences for ruminants. *Animal Feed Science and Technology 91*, 83–93.

Molina-Alcaide, E., Moumen, A., Martín-García, I. (2008). By-products from viticulture and the wine industry: potential as sources of nutrients for ruminants. *Journal of the Science of Food and Agriculture 88*, 597-604.

Morrison, W.R., Smith, L.M. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *Journal of Lipid Research* 5*,* 600–608.

O'Donovan, L., Brooker, J.D. (2001). Effect of hydrolysable and condensed tannins on growth, morphology and metabolism of *Streptococcus gallolyticus* (*S. caprinus*) and *Streptococcus bovis*. *Microbiology 147*, 1025–1033.

Ojeda, H., Andary, C., Creaba, E., Carbonneau, A., Deloire, A. (2002). Influence of pre- and post-veraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* var. Shiraz. *American Journal of Enology and Viticulture 53*, 261–267.

Ørskov, E.R., Hovell, F.D.DeB., Mould, F. (1980). The use of the nylon bag technique for the evaluation of feedstuffs. *Tropical Animal Health and Production 5*, 195–213.

Ørskov, E.R., McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agricultural 92*, 499–503.

Patra, A.K., Saxena, J. (2009). A review of the effect and mode of action of saponins on microbial population and fermentation in the rumen and ruminant production. *Nutrition Research Reviews 22*, 204–219.

Priolo, A., Waghorn, G.C., Lanza, M., Biondi, L., Pennisi, P. (2000). Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: Effects on lamb growth performance and meat quality. *Journal of Animal Science 78,* 810–816.

Reed, J.D., Mcdowell, R.E., Van Soest, P.J., Horvath, P.J. (1982). Condensed tannins: A factor limiting the use of cassava forage. *Journal of the Science of Food and Agriculture 33*, 213–220.

Ribérau-Gayon, J. (1970). Le dosage des composés phénoliques totaux dans les vins rouges. *Chimique Analytique 52,* 627–631.

Ribérau-Gayon, J., Stonestreet, E. (1965). Le dosage des anthocyanes dans le vin rouge. *Bulletin de la Société Chimique de France 9*, 2649–2652.

Ribérau-Gayon, J., Stonestreet, E. (1966). Dosage des tanins du vin rouge et détermination de leur structure. *Chimie Analitique 48*, 188–196.

Salinas-Ríos, T., Ortega-Cerrilla, M.E., Sachez-Torres-Esqueda, M.T., Hernandez-Bautista, J., Díaz-Cruz, A., Figueroa-Velasco, J.L., Guinzberg-Perrusquía, R., Cordero-Mora, J.L. (2015). Productive performance and oxidative status of sheep fed diets supplemented with coffee pulp. *Small Ruminant Research 123*, 17–21.

Sgorlon, S., Stradaioli, G., Zanin, D., Stefanon B. (2006). Biochemical and molecular responses to antioxidant supplementation in sheep. *Small Ruminant Research 64,* 143–151

Spanghero, M., Salem, A.Z.M., Robinsond, P.H. (2009). Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces. *Animal Feed Science and Technology 152*, 243–255.

Tiemann, T.T., Ávila, P., Ramírez, G., Lascano, C.E., Kreuzer, M., Hess, H.D. (2008). In vitro ruminal fermentation of tanniniferous tropical plants: plant-specific tannin effects and counteracting efficiency of PEG. *Animal Feed science and Technology 146*, 222–241.

Tilley, J.M.A., Terry, R.A. (1963). A two-stage technique for the *in vitro* digestion of forage crops. *Journal of the British Grassland Society 18*, 104–111.

Van Soest, P.J. (1994). Nutritional ecology of the ruminant. Cornell University Press, Ithaca, New York (EEUU).

Van Soest, P.J., Robertson, J.B., Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science 74*, 3583–3597.

Vasta, V., Luciano, G. (2011). The effects of dietary consumption of plants secondary compounds on small ruminals' products quality. *Small Ruminan Research 101*, 150–159.

Weatherburn, M.W. (1967). Phenol-hypochlorite reaction for determination of ammonia. *Analytical Chemistry 39*, 971–974.

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

5. Prueba experimental II

Effects of grape pomace supplementation of lactating ewe diets on animal performance, milk composition, carcass and meat quality of suckling lambs

5.1. Introduction

In the last few decades, the scientific community has focused on obtaining increases in levels of polyunsaturated fatty acids (PUFA) and other functional fatty acids, such as CLA or PUFA n-3, in ruminant products. However, the increase in unsaturated fatty acids (UNSFA) in meat and milk makes them more susceptible to lipid oxidation. One of the most common methods for reducing oxidation in animal products is the use of various dietary synthetic antioxidants in the diet. However, the effect of synthetic compounds on consumer health is still controversial. The use of natural additives to improve the oxidative stability of animal products is of increasing interest owing to consumer demand for natural products and their willingness to pay a price premium for natural foods.

A number of plant secondary compounds, such as phenolic compounds, possess antioxidant properties, and therefore their use in animal feeding could be promoted (Vasta and Luciano, 2011). Indeed, polyphenols have demonstrated their ability to prevent meat lipid oxidation when they are incorporated in diets of fattening lambs (Karami *et al.*, 2011; Andrés *et al.*, 2014) or lactating ewes (Nieto *et al.*, 2010a; Santos *et al.*, 2014) or used as milk replacers for suckling lambs (Morán *et al.*, 2014). Also, numerous studies have provided evidence for the efficacy of polyphenols as antimicrobial agents, being able to alter bacterial cell membranes and microbial enzymatic metabolism with high antibiotic activity (Rota *et al.*, 2008), which could impact positively on meat shelf life. On the other hand, it has been indicated that polyphenols can induce an effect on PUFA rumen biohydrogenation (BH) and consequently on milk and meat fatty acid profiles (Vasta *et al.*, 2009a).

Grape pomace is a by-product resulting from the winery industry that is costly to dispose of. Owing to incomplete extraction during the winemaking process, seeds and skins of crushed grapes are very rich in phenolic compounds (Yi *et al.*, 2009) and therefore could play an important role as antioxidants. Furthermore, because of its low cost and high 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 regions because it is being a traditionally consumed food in that area. The high quality of this meat is related to its tenderness, juiciness and palatability (Gorraiz *et al.*, 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.

A transfer of phenolic compounds from diets to milk of cows (O'Connell and Fox, 2001), ewes (Chiofalo *et al.*, 2012) and goats (Jordán *et al.*, 2010) has been reported, and Santos *et al.* (2014) indicated that grape residue silage improved antioxidant activity in milk. Therefore the phenolic compounds and antioxidant activity of milk could be transferred to suckling lamb muscle and exert beneficial effects on their meat quality. Some studies have been carried out on the effects of including wine extracts in growing lamb diets (Jerónimo *et al.*, 2010; Muíño *et al.*, 2014). However, no studies have investigated the effects of the inclusion of whole grape pomace in lactating ewe diets on milk composition and suckling lamb performance, meat quality and meat shelf life.

The aim of this study was to determine the effect of dietary inclusion of different doses of grape pomace mixed with linseed oil in lactating ewe diets on animal performance, milk composition and quality and shelf life of meat of suckling lambs during storage in retail sale conditions after it had been packaged under modified atmosphere. To compare the results obtained, a negative control group (animals without antioxidants in the diet) and a positive control group (animals supplemented with vitamin E) were used. Vitamin E was used because it is one of the antioxidants most frequently employed in animal nutrition.

5.2. Material and methods

5.2.1. 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, all gave birth 3–4 days before starting the experiment. 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, 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 for 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 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% of grape pomace from red wine production, DM basis), and GP-10 (10% of grape pomace from red wine production, DM basis). In this experiment, the grape pomace was supplied in fresh form. The ingredients and chemical composition of the experimental diets are given in Table 5.1.

Table 5.1. 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 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}; 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 identified fatty acids): C16:0, 11.1; C18:0, 4.41; C18:1, 16.0; C18:2, 61.3; C18:3, 3.69.

The chemical composition of the TMR was determined by 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) and the method of Van Soest *et al.* (1991). TMR was supplied twice a day and fresh water was always available. The ewes were fed individually during the whole experimental period and each intake was recorded. The amounts of diet offered and refusals were weighed daily for each ewe, and samples were collected for subsequent analyses.

At the beginning and the end of the experimental period the ewes were weighed and body condition score was assessed to the nearest 0.25 score using the Russel *et al.* (1969) technique, which employs a 0 to 5 score. All the ewes were blood sampled by jugular venipuncture in duplicate in the third week of lactation two hours after first feeding to determine the extent of lipid oxidation in plasma.

5.2.2. Milk sampling and composition

Milk yield and composition were recorded weekly during the first month of lactation. The animals were milked once a day in a 2 × 24 low-line Casse system milking parlour with twelve milking units and two milkers during the entire experimental period. The milking machine (Alfa-Laval Iberia, S.A., Madrid, Spain) was set to provide 180 pulsations per minute in a 50:50 ratio with a vacuum level of 36 kPa.

Once a week, individual ewe milk production was recorded and samples were taken in milk collection jars. For this purpose, milk production was recorded by the oxytocin technique: in the morning, before milking, each ewe was injected with 0.35 cc of oxytocin (Oxiton, Laboratorios Ovejeros, S.A., Spain) and then immediately milked. Ewes were returned to their paddock for six hours while the lambs were confined, and after that were milked again for milk sampling. One sub-sample of milk was kept at 4 °C until analysed for fat, protein, lactose and total solids, in accordance with International Dairy Federation recommendations (IDF, 2000), using a MilkoScan-400 analyser (Foss Electric, Hillerød, Denmark). In the second and fourth weeks, another sub-sample was stored at –80 °C for subsequent fatty acid (FA) analysis.

Milk fat separation was carried out using the method proposed by Luna *et al.* (2005). Separated lipids were stored in amber vials, blanketed with a stream of N_2, and stored at

–20 °C until analysis. Fatty acid methyl esters (FAME) were prepared by base-catalysed methanolysis of glycerides with KOH in methanol (ISO-IDF, 2002). The methyl esters were quantified by gas chromatography using an Agilent Technologies 6890 (PA, USA) gas chromatograph (GC) equipped with a flame ionization detector (FID). Separation was carried out on an HP-88 capillary column (100 m × 0.25 mm, i.d., 0.20 mm film thickness, Agilent Technologies, PA, USA). The GC conditions were as follows: injector and detector temperatures were 240 °C and 300 °C, respectively, and the helium flow ratio was 3 mL min^{-1}. An initial oven temperature of 170 °C was held for 24 min, followed by a rise to 220 °C at a rate of 7.5 °C min^{-1} and a subsequent increase of 10 °C min^{-1} to 230 °C (held for 5 min). Fatty acid methyl esters were identified by comparison with standards run previously alone or together with samples. Fatty acid methyl esters were expressed as percentage of total methyl ester content.

5.2.3 Ewe plasma lipid peroxidation

Ewe blood samples were collected in a Vacutainer containing heparin and were immediately placed in iced water and centrifuged at 1,600 x g for 10 min at 4 °C. Then the plasma was separated and stored at –80 °C until required for antioxidant analyses. The extent of lipid oxidation in plasma was assessed by measuring thiobarbituric acid-reactive substances (TBARS), using the TBARS Assay Kit provided by Cayman Chemical (MI, USA), according to the manufacturer's instructions. TBA values were expressed as μM of malonaldehyde (MDA).

5.2.4. Slaughter and carcass measurements

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 a commercial EU-licensed abattoir and slaughtered. The lambs were stunned and slaughtered by section of the jugular vein in the neck. After slaughter, the skin and all internal organs were removed and the carcasses were immediately weighed (hot carcass weight, HCW) and transferred to a cooler at 4 °C. After 24 hours, the carcasses were weighed again (cold carcass weight, CCW) and the dressing percentage was calculated as the ratio of CCW to slaughter live weight. Kidney knob fat was removed from the carcass and weighed as fatness measurement.

5.2.5. Meat 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 this 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 (0° is red; 90° is yellow), was calculated as arctangent (*b*/a**), and the chroma (*C**), a measure of colour intensity (0 is dull; 60 is vivid), was computed as $(a^{*2} + b^{*2})^{1/2}$.

The *longissimus thoracis et lumborum* (LTL) muscle was excised from both sides of the carcasses and used to measure muscle colorimetric parameters and to carry out meat analysis.

Meat chemical composition was predicted using a NIRS method. A representative sample of ground LTL muscle was placed in the ring cups of the NIRS machine, avoiding air bubbles. Subsequently, each meat sample was scanned 32 times over the range (400–2500 nm) using a benchtop XDS NIR Rapid Content Analyser (Foss, Hillerød, Denmark), and spectra were averaged by the equipment software. Two meat samples per animal were scanned using two different cells, increasing the area of muscle scanned and reducing the sampling error. Calibration and validation of the NIRS data were performed using WinISI software for fresh meat.

Meat fat extraction from the LTL was performed using the method of Bligh and Dyer (1959), and fatty acid methyl esters were prepared using the Morrison and Smith (1964) technique. The methyl esters were quantified as previously described for milk (section 5.2.2).

Meat water holding capacity (WHC) was determined as cooking losses using the method described by Honikel (1998). Samples of LTL, weighing 150 g approx., 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 measurement of cooking losses, 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 for all the evaluated strips.

A triangle test was conducted according to ISO 4120 (2004) with the objective of studying whether dietary antioxidant incorporation produces perceptible sensory differences in comparison with the typical organoleptic lamb quality, represented by the control group. Each panellist was offered three samples simultaneously, two corresponding to the same diet and the third to a different one, and they were asked to taste the samples and identify the odd sample. The comparisons were performed on meat samples of lambs fed different diets (*i.e.* CTRL *vs.* VIT-E; CTRL *vs.* GP-5; CTRL *vs.* GP-10; VIT-E *vs.* GP-5; VIT-*E vs.* GP-10 and GP-5 *vs.* GP-10). Sensory assessment was performed six assessors selected and trained in accordance with the International Standard method for selection, training and monitoring of assessors (UNE-ISO 13300-2, 2008). The assessors performed each comparison in triplicate, thus, 18 replies were completed for comparison. The taste panel performed the trial under controlled conditions, in a booth with a red light to mask colour differences. The sensory analysis was performed as follows: frozen vacuum-packaged LTL muscle samples were thawed for 24 h at 4 °C. The upper and lower ends were cut off at approximately 2 cm below and 2 cm above the respective end points. The central part was then divided into slices (2 cm thick). The slices were broiled on a double-sided griddle preheated at 220 °C until they reached a core temperature of 70 °C, following the guidelines for cooking procedures. After cooking, each slice was wrapped in aluminium foil, codified, distributed in the booths in groups of three in accordance with the triangle test, and kept warm until the time of assessment (15 min). Water and unsalted bread were provided to cleanse the palate of residual flavour notes between samples.

5.2.6. Meat shelf life analysis

Sample preparation

After slicing, LTL chops (about 3 cm thick) from each carcass were placed in trays and randomly assigned to different storage periods (0, 3, 7, 10 and 14 days). Then the trays were flushed with the selected gas mixture (80:20% / O_2:CO_2), closed by heat-sealing with a packer (TECNOVAC mod: Linvac 400) with a high barrier film (with an oxygen transmission rate of 1.8 cm^3/m^2/24 h/bar at 20 °C and 65% RH, supplied by Fibosa Packaging S.L., Tordera, Spain). The trays were placed randomly in a cabinet illuminated with white fluorescent light (620 lux) at 4 ± 1 °C, simulating retail display conditions for storage. The trays were rotated daily to minimize light intensity differences and possible temperature variations. On each sampling day, the corresponding trays were removed for subsequent analysis. Half of the trays to be analysed at each sampling point within each treatment were used for carrying out microbial analyses and the other half were used for the other measurements.

Microbiological analysis

For microbiological assays, after opening the pack, 10 g was taken aseptically from each tray and homogenised with 90 mL of tryptone water (Scharlau, Spain) 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 tryptone 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 of the samples that were performed were: total viable counts (TVC) determined on 3M Petrifilm Aerobic Count Plate (Bioser, Barcelona, Spain) incubated at 30 °C for 72 h; enterobacteria on 3M Petrifilm Enterobacteriaceae Count Plate (Bioser, Barcelona, Spain) incubated at 42 °C for 24 h; *Escherichia coli* on 3M Petrifilm Selective E.coli Count Plate (3M, Spain) incubated at 42 °C for 24 h; lactic acid bacteria (LAB) on MRS Agar (Scharlau, Spain) incubated at 30 °C for 72 h; Pseudomonads on Pseudomonas Agar (Oxoid, Spain) supplemented with Cetrimide, Fucidine and Cephaloridine (CFC, Oxoid, Spain) and incubated at 30 °C for 48 h, and *Brochothrix thermosphacta* on STAA Agar (Oxoid, Spain) supplemented with STAA selective supplement (Oxoid, Spain) and 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). The detection limit of the above techniques was 1 log cfu g^{-1} except for pseudomonads, for which the limit was 2 log cfu g^{-1}. When counts were above 7 log cfu g^{-1} the product was considered unsuitable for consumption (ICMSF, 1986).

Instrumental colour measurement

Surface instrumental colour of LTL muscle slices was measured during storage after opening the packages as detailed in section 5.2.5. The measurements were performed on each slice of muscle three times.

The oxidation state of myoglobin was also measured indirectly in LTL muscle by spectrophotometry during storage for each time. Haem pigment percentages were estimated according to Krzywicki (1979) from 400 to 740 nm. Metmyoglobin (MMb) was calculated as: MMb = (1.395 – a_1), where: $a_1 = \left[\left(D_R^{572} - D_R^{730}\right) / \left(D_R^{525} - D_R^{730}\right)\right]$; D_R = (–log R).

Lipid oxidation analysis

The extent of lipid oxidation in LTL muscle during storage was assessed by measuring thiobarbituric acid-reactive substances (TBARS). TBARS were determined in meat samples after measuring colour, according to the method described by Maraschiello *et al.* (1999). TBA values were expressed as micrograms of malonaldehyde per gram of meat.

Sensory evaluation

Sensory analysis was carried out on raw meat. For each sampling day, samples were evaluated for appearance, display of muscle oxidation, presence of off-odours and overall rating of the sample by a panel of six people selected and trained in accordance with the International Standard method for selection, training and monitoring of assessors (UNE-EN ISO 8586:2014). The taste panel performed the trial under controlled conditions in booths, at 22 °C.

The meat's appearance was assessed in unopened trays, using a structured scale with numerical scores 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 had been removed, the panellists were asked to score odour by sniffing, using a 5-point scale (1, no off-odours; 2, slight off-odours; 3, small 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).

5.2.7. Statistical analysis

Data regarding ewe dry matter intake, milk yield and composition and meat shelf life were analysed by repeated measures using the MIXED procedure of the SAS 9.2. package, according to the model $Y_{ijk} = \mu + T_i + D_k + T_iD_k + B_j + \varepsilon_{ijk}$; 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_iD_k the interaction (T × D), B_j the block effect and ε_{ijk} the residual error.

Ewe weight and body condition data, milk FA profile data, plasma data and lamb carcass and meat data were subjected to analysis of variance using the general linear model (GLM) procedure of the SAS 9.2. package, 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 the SAS 9.2. package was used to calculate the correlation coefficients of the FA content in milk and suckling lamb meat. Additionally, a binomial distribution was used for the results of the sensory analysis (triangle test).

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.

For all statistical procedures the statistical significance of differences was defined as $P < 0.05$ and trends as $P < 0.10$.

5.3. Results and discussion

5.3.1. Milk yield and composition

Compared with the control treatment, the VIT-E, GP-5 and GP-10 diets did not negatively affect ewe dry matter intake (Table 5.2). These results agree with previous studies on dairy cows (Belibasakis *et al.*, 1996; Moate *et al.*, 2014) and ewes (Correddu *et al.*, 2015), supporting the idea that the inclusion of grape residue, with or without linseed oil, in the ruminant diet does not depress food intake. Milk yield was not modified by the various treatments, and the period of sampling did not have a significant influence on animal performance (Table 5.2).

Table 5.2. Effect of experimental ewe diets and duration of supplementation on dry matter intake and milk yield and composition.

	Treatments[1]				SED[2]	*P. value*[3]		
	CTRL	VIT-E	GP-5	GP-10		T	D	T × D
Dry matter intake (g d^{-1})	2439	2441	2512	2495	38.3	0.448	0.683	0.896
Yield (g d^{-1})								
Milk	2557	2449	2291	2397	164.1	0.427	0.679	0.946
Fat	148	154	140	151	13.7	0.756	0.457	0.572
Protein	109	108	99.2	104	6.69	0.436	0.930	0.945
Lactose	135	126	116	122	9.0	0.208	0.724	0.893
Total solids	415	410	377	399	28.2	0.514	0.771	0.901
Composition (%)								
Protein	4.33	4.44	4.40	4.39	0.078	0.512	0.171	0.939
Fat	5.92	6.13	6.29	6.42	0.371	0.540	0.106	0.167
Lactose	5.24[a]	5.13[ab]	5.02[b]	5.09[b]	0.073	0.027	0.203	0.333
Total solids	16.4	16.6	16.6	16.8	0.34	0.650	0.107	0.167

[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 experimental dietary treatment (T), sampling day (D) and their interaction (T × D).

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

Although the presence of grape pomace in the diet decreased ($P < 0.01$) lactose concentration, fat and protein in milk were not affected (Table 5.2). These results confirm previous research on this issue. For instance, in dairy cows the replacement of maize silage with ensiled grape marc in the diet had no effect on milk production and milk fat and protein concentration (Belibasakis *et al.*, 1996), and no detrimental effects of the dietary inclusion of

grape-residue silage on animal performance were observed (Santos *et al.*, 2014). In dairy ewes it was reported that grape seed in the diet did not reduce milk yield or yields of milk constituents when compared with a control diet (Nudda *et al.*, 2015). Sheep body weight and body condition were not affected ($P > 0.05$) by the conditions of the experiment (Table 5.3), so changes in milk composition and FA profile would only be due to the effect of the diets.

Table 5.3. Effect of experimental ewe diets on live weight and body condition score of ewes.

	Treatments[1]				RSD[2]	*P. value*
	CTRL	VIT-E	GP-5	GP-10		
Initial live weight (kg)	58.3	58.2	62.8	60.4	6.81	0.330
Final live weight (kg)	58.8	60.0	61.7	60.6	7.06	0.798
Initial body condition	2.38	2.38	2.52	2.48	0.388	0.726
Final body condition	2.27	2.20	2.30	2.39	0.454	0.823

[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.

The composition of milk fat FA with the various treatments is shown in Table 5.4. The inclusion of vitamin E did not modify total PUFA content, but it produced an increase in the total content of saturated fatty acids (SFA) ($P < 0.05$) at the expense of monounsaturated fatty acids (MUFA). In contrast, grape pomace supplementation did not affect the percentages of total SFA, MUFA, and PUFA (Table 5.4). This behaviour was similar to that observed by Santos *et al.* (2014) when a similar proportion of grape residue silage was included in dairy cow diets.

The impact of GP-5 and GP-10 diets on the individual SFA contents was variable. The supplementation with grape pomace did not modify the percentages of C4:0, C6:0, C12:0, C14:0, C16:0 and C18:0 in milk fat, whereas a significant diminishing ($P < 0.05$) of C8:0 and C10:0 was found (Table 5.4). This decrease is probably grape pomace dose-dependent, because only these FA percentages were significantly different with the GP-10 treatment. Feeding with high proportions of grape marc also lowered C8:0 and C10:0 in dairy cows (Moate *et al.*, 2014). Those authors attributed this result principally to the presence in the grape residue of a great amount of lignin, which is not fermented in the rumen. C8:0 and C10:0 FA are almost exclusively produced *de novo* in the mammary gland, and their synthesized amount has been shown to be related to the dietary intake of fermentable

carbohydrate (Moate *et al.*, 2008). The lack of changes in the levels of these C8:0, C10:0, and C12:0 with the VIT-E diet (Table 5.4) would support this argument.

Table 5.4. Effect of experimental ewe diets on milk fatty acid composition (% of identified fatty acids).

	Treatments[1]				RSD[2]	*P. value*
	CTRL	VIT-E	GP-5	GP-10		
C4:0	4.46	4.73	4.46	4.64	0.299	0.129
C6:0	3.58^{ab}	3.83^{b}	3.48^{a}	3.41^{a}	0.290	0.016
C8:0	3.20^{ac}	3.41^{c}	3.05^{ab}	2.83^{b}	0.384	0.013
C10:0	8.25^{ab}	8.73^{b}	8.10^{ab}	7.17^{a}	1.203	0.044
C12:0	4.17	4.30	4.05	3.63	0.639	0.123
C14:0	8.45	8.80	8.87	8.39	0.764	0.407
C15:0	0.79	0.82	0.78	0.79	0.101	0.799
C16:0	19.4	20.4	20.5	20.5	1.42	0.218
C16:1	0.50	0.45	0.49	0.49	0.084	0.547
C17:0	0.59	0.51	0.54	0.52	0.082	0.116
C18:0	10.8	10.3	10.4	10.7	1.49	0.856
cis-9 C18:1	16.6	14.1	15.6	15.8	2.70	0.216
trans-11 C18:1 (VA)	3.17	3.49	3.58	3.93	1.065	0.466
cis-9 cis-12 C18:2 n6	1.85^{a}	1.83^{a}	1.89^{a}	2.04^{b}	0.165	0.034
cis-9 trans-11 C18:2 (RA)	1.11	1.21	1.26	1.41	0.377	0.374
C18:3 n3	1.09	1.18	1.01	0.96	0.213	0.124
C20:0	0.18	0.18	0.18	0.19	0.016	0.370
C20:4 n6	0.13	0.13	0.14	0.15	0.0244	0.233
C20:5 n3 (EPA)	0.06	0.06	0.06	0.06	0.010	0.378
C22:4 n6	0.01	0.01	0.01	0.01	0.003	0.452
C22:5 n3 (DPA)	0.12	0.12	0.12	0.11	0.021	0.904
C22:6 n3 (DHA)	0.04	0.04	0.04	0.04	0.010	0.883
Ratios[3]						
SFA	63.8^{ab}	66.0^{a}	64.4^{ab}	62.8^{b}	2.42	0.041
MUFA	20.3	18.0	19.7	20.2	2.31	0.118
PUFA	4.72	4.91	4.87	5.12	0.606	0.543
PUFA:SFA	0.07	0.07	0.08	0.08	0.010	0.363
n3	1.31	1.41	1.23	1.17	0.228	0.143
n6	1.99	1.98	2.04	2.20	0.172	0.023
n6:n3	1.56^{a}	1.44^{a}	1.67^{a}	1.94^{b}	0.262	0.001

[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] SFA (saturated fatty acids) = C4:0 + C6:0 + C8:0 + C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0; MUFA: (monounsaturated fatty acids) = C16:1 + *cis-9* C18:1 + *trans-11* C18:1; PUFA: (polyunsaturated fatty acids) = *cis-9 cis-12* C18:2 n6 + *cis-9 trans-11* C18:2 + C18:3 n3 + C20:4 n6 + C20:5 n3 + C22:4 n6 + C22:5 n3 + C22:6 n3; n3 = C18:3 n3 + C20:5 n3 + C22:5 n3 + C22:6 n3; n6 = *cis-9 cis-12* C18:2 n6 + C20:4 n6 + C22:4 n6.

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

Oleic acid (*cis-9* C18:1) was the most abundant MUFA (about 15% of total FAME) in all treatments (Table 5.4). No significant differences were found between control, vitamin E and grape pomace diets for *cis-9* C18:1. These results can be justified by the origin of this FA in milk. Part of milk fat oleic acid comes from the diet, but endogenous synthesis in the mammary gland via Δ^9-desaturase using C18:0 as substrate is the major source of *cis-9* C18:1, accounting for more than 60% of its content in ewe milk fat (Bichi *et al.*, 2012). As can be calculated from the results shown in Table 5.4, Δ^9-desaturase activities were not affected by the treatments and hence increases in oleic acid by this route should not be expected. Nor is it likely that oleic acid would increase as a result of the diet, because the presence of this MUFA in grape pomace is low (Table 5.1).

The remarkable levels of *trans-11* C18:1 (vaccenic acid, VA) in the milk fats analysed (more than 3% of total FAME) are attributable to the presence of linseed oil in all the diets, because VA is a very important intermediate of α-linolenic (C18:3 n3) acid BH (Loor *et al.*, 2004; 2005). The incorporation of a source of linoleic acid such as grape pomace (Table 5.1) could also potentially enhance the VA content in milk, as has previously been demonstrated in dairy ewes and cows (Tsiplakou and Zervas, 2008; Moate *et al.*, 2014). In fact, VA increased in the GP-5 and GP-10 treatments in comparison with the control diet, but the increases were not statistically significant (Table 5.4), as occurred in ruminal fluid from sheep fed linseed oil when grape seed was incorporated in the diet (Correddu *et al.*, 2015). The explanation for this probably lies in the dose of grape residues. The proportion of grape marc in the studies of Tsiplakou and Zervas (2008) and Moate *et al.* (2014) was higher than 20% on a DM basis, whereas in the present research (Table 5.1) as well as in the study by Correddu *et al.* (2015) it did not exceed 10%.

Table 5.4 shows the *cis-9 cis-12* C18:2 n6 contents in milk fat with the various treatments assayed. The presence of vitamin E (VIT-E) did not significantly modify the linoleic acid content. In contrast, the incorporation of grape pomace in the diet could increase the percentage of this FA in milk fat. This increase is dose-dependent, because significant modifications were only found with 10% of this supplement in the diet (Table 5.4). Because its origin is exclusively exogenous, this increase is attributable to the presence of linoleic acid in the grape pomace (Table 5.1). In this regard, Moate *et al.* (2014) succeeded in tripling the linoleic acid content in milk when more than 25% (on a DM basis) of the diet was based on ensiled grape marc.

The content of *cis-9 trans-11* C18:2 (rumenic acid, RA), the most important CLA isomer, showed no statistically significant effect due to the experimental diets (Table 5.4). RA in ewe

milk fat is mostly produced in the mammary gland by Δ^9-desaturase from VA formed in the rumen (Bichi *et al.*, 2012). It is also a direct intermediate in rumen BH of linoleic acid, and a portion of it is absorbed to provide the remainder of the RA in milk fat. Thus the GP-5 and GP-10 diets, which contain a high proportion of linoleic acid (Table 5.1), should lead to an increase in the levels of RA in the rumen and consequently in the milk. In fact, in previous research (Tsiplakou and Zervas, 2008; Moate *et al.*, 2014) it was reported that the inclusion of a variety of winery industry residues in ruminant feeding could be a good way of multiplying the levels of RA in milk fat. The higher presence of grape marc in those studies (Tsiplakou and Zervas, 2008; Moate *et al.*, 2014) in comparison with the diets assayed in the present research (Table 5.1) could justify this discrepancy. Moreover, the addition of grape seed to linseed-supplemented diets at similar levels to those reported in the present research hardly modified the RA content in the ruminal fluid of dairy ewes (Correddu *et al.*, 2015), as occurred with VA. Therefore, to produce drastic changes in the metabolism of dietary FA in the rumen of dairy ewes an intake of higher proportions of grape pomace would be required.

The α-linolenic acid contents in all the samples analysed can be regarded as high (around 1%) (Table 5.4) in comparison with the usual values of ruminant milks (Shingfield *et al.*, 2013; Nudda *et al.*, 2014). This is a consequence of the supplementation of all the diets with linseed oil, a substrate very rich in this FA (Table 5.1), and is due to the fact that the molecules of this PUFA can escape from the BH process in the rumen. Although not significantly, the α-linolenic acid content increased in the VIT-E treatment ($P > 0.05$), supporting the view that vitamin E can play a part as an antioxidant in ewe diets. In contrast, the presence of grape pomace in the diets did not modify the percentage of this n3 FA in milk fat.

Other PUFA with more than 18 carbon atoms were detected in milk fats but in very low amounts (Table 5.4). The contents of the remaining n3 PUFA (C20:5, C22:5 and C22:6) reported in this study did not vary with the presence of vitamin E or the inclusion of different levels of grape pomace in the diets. The n6 to n3 ratio was significantly affected by feeding ($P < 0.001$). However, the n6:n3 values in all the treatments may be considered very favourable from the human health point of view because they are clearly below 4, which is the recommended maximum value in dietary fat (Simopoulos, 2008).

5.3.2. Ewe plasma oxidation

Plasma ewe MDA concentrations were 2.26, 1.74, 2.01 and 1.73 μM (RSD = 0.934) for CTRL, VIT-E, GP-5 and GP-10, respectively, and there were no significant differences ($P > 0.05$) between dietary treatments. In spite of the lack of significant differences, lower numerical MDA values were found when vitamin E or grape pomace was included in the ewe diets. Compared with the CTRL treatment, the decrease in TBARS value accounted for 23%, 11% and 23% for VIT-E, GP-5 and GP-10 respectively. This is in line with Bodas *et al.* (2011), who found that lambs consuming diets supplemented with vitamin E or phenolic compounds showed lower plasma TBARS. Also Gladine *et al.* (2007) reported that the inclusion of 10% DM of grape seed and peel extract directly into the rumen of sheep improved the antioxidant status and reduced the susceptibility to lipid oxidation of plasma measured by an oxidation induced assay. The decrease in the blood concentration of MDA can be attributed both to direct action (trapping of free radicals by polyphenols due to antioxidant activity during propagation of oxidative chain reactions) and to blocking of the initiation phase of oxidation by inhibition of pro-oxidant enzymes responsible for the production of free radicals (Casamassima *et al.*, 2012).

5.3.3. Suckling lamb performance and carcass traits

Suckling lamb performance and carcass traits are shown in Table 5.5. No differences attributable to any experimental treatment were observed for suckling lamb performance ($P > 0.05$). As there were no differences in ADG and live weights, it was expected that 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; a lack of differences in milk yield and composition would explain the lack of effect on lamb performance (Manso *et al.*, 2011). Similar results to those in this study were reported in suckling lambs from ewes supplemented with different kinds of oils (Manso *et al.*, 2011; Gallardo *et al.*, 2014), in suckling lambs from ewes supplemented with linseed oil plus vitamin E (Gallardo *et al.*, 2015), and in lambs from ewes supplemented with thyme (Nieto *et al.*, 2011).

Table 5.5. Effect of experimental ewe diets on growth performance and carcass traits of suckling lambs.

	Treatments[1]				RSD[2]	*P. value*
	CTRL	VIT-E	GP-5	GP-10		
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[a]	27.6[a]	24.6[b]	28.6[a]	4.66	0.096
Carcass traits						
Hot carcass weight (kg)	6.41	6.33	6.27	6.12	0.438	0.281
Cold carcass weight (kg)	6.23	6.14	6.09	5.91	0.418	0.160
Chilling losses (%)	2.75	3.00	2.97	3.45	1.053	0.284
Carcass yield (%)	53.1	53.4	52.7	52.3	1.99	0.426
Kidney knob fat (g)	229	229	211	255	61.7	0.270

[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.

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

5.3.4. Meat quality

Meat pH, colour, meat shear force, cooking losses and chemical composition as well as fat colour parameters are shown in Table 5.6.

Muscular pH did not present significant differences ($P > 0.05$) between experimental treatments (Table 5.6). These results are in agreement with the lack of differences found in meat pH by other authors employing vitamin E or phenolic compounds in milk replacers for suckling lambs (Morán *et al.*, 2014), and in ewe diets (Nieto *et al.*, 2012; Nieto, 2013). Our result reflects a regular trend of the *post mortem* glycolysis in muscle, and, according to Inserra *et al.* (2014), these results are 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.

Colour is a highly variable parameter which affects consumer decisions concerning the purchase of red meat (Morrissey *et al.*, 1994). As Table 5.6 shows, 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. It is not easy to offer a plausible explanation for the effect observed, given

that there are also no contrasting results from suckling lamb studies in which phenolic compounds were supplemented in diets for ewes. Several authors have suggested that vitamin E supplementation and 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; Priolo *et al.*, 2000; Luciano *et al.*, 2009). However, these changes in meat and fat colour associated with oxidation processes are generally not apparent for 24 h after slaughter.

Table 5.6. 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 (%)	24.1^{a}	18.9^{b}	21.4^{c}	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

[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.

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

Some studies (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 vitamin E or grape pomace were within acceptable values and no statistical differences were found between treatments ($P > 0.05$) (Table 5.6). A significant reduction in shear force has been reported in muscles with high ultimate pH (Devine *et al.*, 1993) and with higher water holding capacity (Morán *et al.*, 2012). Our results showed meat pH below 6, and no differences between treatments were found in ultimate pH, which corresponded to the lack of differences in shear force for muscle. At the same time, these results (Table 5.6.) do not disagree with the lack of significant differences in meat water holding capacity. It has also been reported that toughening of meat is related to the oxidation of myofibrillar proteins (Estevez, 2011). Consequently, the numerically lower Warner-Bratzler shear force values in suckling lambs from vitamin E or grape pomace groups could reflect the transfer of some antioxidant substances from milk to suckling lamb meat.

Meat oxidation reduces WHC between muscle myofibrils, which increases juice loss from the meat and, as a result, meat lightness (Huff-Lonergan and Lonergan, 2005). It has been reported that the use of antioxidants might improve WHC, avoiding the loss of membrane integrity and protein cross-link with oxidative processes. In accordance with that statement, WHC measured by cooking losses (Table 5.6) was higher in lambs fed with vitamin E and grape pomace ($P < 0.05$) compared with the control group. 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. However, in a further study Morán *et al.* (2014) did not report statistical differences ($P > 0.05$) in meat WHC from suckling lambs fed with milk replacer enriched with vitamin E or carnosic acid. This discrepancy could be explained as being due to the different doses of antioxidants in the diets or to the cooking method used, which provides high variability in this parameter (Morán *et al.*, 2014).

Chemical composition data corresponding to the suckling lamb LTL samples are summarized in Table 5.6 and are within the range obtained by other authors for suckling lamb meat (Manso *et al.*, 2011). As expected, the chemical composition of muscle was not affected ($P > 0.05$) by the ewe dietary treatment. These results are due to the lack of differences in milk yield and composition, since the lambs were fed exclusively with maternal milk. Morán *et al.* (2014) 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. Nieto (2013) also found no differences in muscle lamb chemical composition when thyme and rosemary were included in ewe diets. Those results contrast with others reported by Nieto *et al.* (2012), who found a lower percentage of protein and higher percentage of fat in meat of lambs whose mothers' diets were supplemented with thyme leaves characterized by a high content of secondary compounds.

The fatty acid composition of LTL muscle from each dietary treatment is reported in Table 5.7.

Table 5.7. 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		
C10:0	0.32^{a}	0.40^{bc}	0.44^{b}	0.36^{ac}	0.105	0.008
C12:0	0.62	0.66	0.66	0.57	0.174	0.428
C14:0	6.41	6.94	6.96	6.54	1.077	0.342
C16:0	23.1	23.9	24.0	23.8	1.64	0.374
C16:1	2.27	2.29	2.24	2.39	0.250	0.339
C17:0	0.84^{a}	0.80^{a}	0.80^{a}	0.73^{b}	0.081	0.003
C17:1	0.49^{a}	0.42^{b}	0.42^{b}	0.40^{b}	0.058	<0.001
C18:0	13.6	13.9	13.6	13.2	1.09	0.338
cis-9 C18:1	35.3^{a}	32.5^{b}	31.5^{b}	32.2^{b}	2.88	0.002
trans-11 C18:1 (VA)	3.63^{a}	4.03^{b}	4.41^{b}	4.55^{b}	0.815	0.009
cis-9 cis-12 C18:2 n6	6.06	6.13	6.55	6.78	1.093	0.193
cis-9 trans-11 C18:2 (RA)	1.18^{a}	1.37^{ac}	1.52^{bc}	1.71^{b}	0.361	<0.001
C18:3 n3	1.32	1.62	1.51	1.38	0.352	0.068
C20:0	0.16^{a}	0.19^{b}	0.19^{b}	0.19^{b}	0.026	0.003
C20:1	0.08	0.08	0.09	0.08	0.011	0.055
C20:4 n6	2.53	2.32	2.47	2.55	0.711	0.779
C20:5 n3 (EPA)	0.64	0.74	0.75	0.71	0.221	0.503
C22:4 n6	0.17	0.16	0.18	0.18	0.049	0.442
C22:5 n3 (DPA)	0.94	0.99	1.03	1.05	0.270	0.661
C22:6 n3 (DHA)	0.65	0.65	0.70	0.70	0.237	0.840
Ratios[3]						
SFA	44.98	46.69	46.61	45.32	2.631	0.145
MUFA	41.5^{a}	39.3^{b}	38.7^{b}	39.6^{b}	2.42	0.007
PUFA	13.5	14.0	14.7	15.1	2.66	0.322
PUFA:SFA	0.30	0.30	0.32	0.33	0.071	0.525
n3	3.54	4.00	3.98	3.83	0.961	0.470
n6	8.76	8.60	9.19	9.51	1.787	0.462
n6:n3	2.51^{a}	2.22^{b}	2.36^{ab}	2.53^{a}	0.335	0.033

[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] SFA (saturated fatty acids) = C10:0 + C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0; MUFA: (monounsaturated fatty acids) = C16:1 + C17:1 + *cis-9* C18:1 + *trans-11* C18:1 + C20:1; PUFA: (polyunsaturated fatty acids) = *cis-9 cis-12* C18:2 n6+ *cis-9 trans-11* C18:2 + C18:3 n3 + C20:4 n6 + C20:5 n3 + C22:4 n6 + C22:5 n3 + C22:6 n3; n3 = C18:3 n3+ C20:5 n3 + C22:5 n3 + C22:6 n3; n6 = *cis-9 cis-12* C18:2 n6 + C20:4 n6 + C22:4 n6.

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

The differences observed in intramuscular fat reflected those found in their mothers' milk. The relationship between ewe diets and the fatty acid profile of meat from their suckling lambs has already been described (Manso *et al.*, 2011). Despite changes due to the treatments administered, the FA composition values observed in this study are within the range of those reported earlier by Gallardo *et al.* (2015) for suckling lambs supplemented with linseed oil.

The values of most fatty acids observed in our trial were within the reference ranges obtained in similar breeding and production systems (Manso *et al.*, 2011). The SFA and *cis*-MUFA constituted the majority of the FA, as is expected in lamb meat, oleic acid (*cis-9* C18:1) being the most abundant fatty acid, followed by palmitic acid (C16:0) and stearic acid (C18:0).

Table 5.8. shows correlation coefficients between milk and intramuscular and fatty acids. Despite the presence of significant correlations between fatty acids from the same source, there was almost no correlation between milk and intramuscular fatty acids. According to Manso *et al.* (2011), muscle is less likely to change its FA composition because of the high phospholipid fraction in cell membranes of intramuscular fat. In fact, we observed little relationship between the main fatty acids.

Studies on the effects of antioxidants, such as vitamin E or polyphenols, in ewe diets on the intramuscular fatty acid composition of suckling lambs are scarce. In general terms, incorporation of vitamin E and grape pomace into the ewes' diet had minimal effects on intramuscular SFA, PUFA, PUFA:SFA and n3 fatty acid contents of suckling lamb meat. Similar results have been observed previously in suckling lambs when ewes were fed linseed oil plus vitamin E (Gallardo *et al.*, 2015); muscle MUFA content was lower ($P < 0.05$) when vitamin E and grape pomace were incorporated in the ewes' diet compared with CTRL animals.

It should be highlighted that meat from grape pomace lambs showed higher ($P < 0.05$) VA and RA contents compared with CTRL. These results are related to the fact that, although the increases were not statistically significant, VA increased in milk from ewes of the GP-5 and GP-10 treatments compared with the control diet. In fact, there was a positive correlation ($P < 0.05$) between RA and VA from milk and intramuscular fat (Table 5.8).

Table 5.8. Correlation coefficients between milk and intramuscular fatty acids composition.

	Milk							Intramuscular					
	VA	C18:1	C18:2	RA	RA+VA	C18:3	PUFA	VA	C18:1	C18:2	RA	RA+VA	C18:3
Milk													
VA													
C18:1	-0.52***												
C18:2	0.20	0.11											
RA	0.86***	-0.56***	0.14										
RA + VA	0.99***	-0.55***	0.19	0.92***									
C18:3	0.22	-0.36*	0.22	0.21	0.22								
PUFA	0.75***	-0.50**	0.50**	0.83***	0.79***	0.63***							
Intramuscular													
VA	0.41**	-0.13	0.22	0.36*	0.41**	0.02	0.35*						
C18:1	-0.29†	0.37*	-0.03	-0.25	-0.29†	-0.04	-0.22	-0.70***					
C18:2	0.20	-0.11	0.25	0.17	0.18	0.17	0.28†	0.25	-0.32*				
RA	0.40*	-0.17	0.23	0.41**	0.41**	-0.08	0.33*	0.99***	-0.58***	0.22			
RA + VA	0.41**	-0.14	0.23	0.38*	0.42**	-0.01	0.35*	0.41**	-0.67***	0.24	0.96***		
C18:3	-0.11	0.03	0.27†	-0.04	-0.09	0.27	0.17	0.24	-0.53***	0.38*	0.19	0.23	
PUFA	0.20	-0.15	0.19	0.21	0.20	0.22	0.32*	0.29†	0.40*	0.97***	0.24	0.28†	0.48**

VA, vaccenic acid (*trans-11* C18:1); C18:1, *cis-9* C18:1; C18:2, *cis-9 cis-12* C18:2 n6; RA, rumenic acid (*cis-9 trans-11* C18:2); C18:3, C18:3 n3; PUFA, polyunsaturated fatty acids.

†, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

The increase in VA and RA meat levels in grape pomace treatments could be attributed to several causes. In the first place, it could be due to the high VA and RA contents in milk from ewes of the grape pomace treatments. Secondly, it could be due to the transmission of the dietary phenolic compounds to the milk, since several authors have reported this occurrence (Moñino *et al.*, 2008; Jordán *et al.*, 2010; Nieto, 2013). Some studies have reported higher endogenous synthesis of CLA when phenolic compounds are included in the diet, since this fatty acid can be generated in animal tissues through VA conversion by the action of Δ^9-desaturase enzyme (Min *et al.*, 2003; Vasta *et al.*, 2009b; Rana *et al.*, 2012).

With regard to n3 fatty acids, in spite of lack of statistical differences ($P > 0.05$), docosahexaenoic acid (DHA, C:22:6 n3) and docosapentaenoic acid (DPA, C22:5 n3) increased in grape pomace treatments when they were compared with the CTRL and VIT-E groups. This is in accordance with Muíño *et al.* (2014), who showed that meat obtained from fattening lambs supplemented with red wine extract or vitamin E had numerically higher values of very long chain n3 fatty acids than a non-antioxidant-supplemented group. Morán *et al.* (2013), in fattening lamb diets supplemented with another polyphenol (carnosic acid), also found a higher DHA content ($P < 0.10$). According to those authors, this finding could be interesting because lamb meat is very low in highly unsaturated fatty acid, but no explanation for these results has been found in the literature.

Despite all existing knowledge, it is not yet possible to come to an unequivocal conclusion about the effect of phenolic compounds on meat fatty acid composition (Vasta and Luciano, 2011). The increases in the VA and CLA contents in the grape pomace group are interesting because their nutritional value in the human diet is well recognized as beneficial and increased consumption is recommended.

With regard to the meat sensory results, according to the significance table used to analyse the results of triangle tests, for statistical significance at the 5% level the correct number of replies needed to be 12. Results of the triangle test (Table 5.9) suggest that none of the treatments identified was different. These results are in agreement with Priolo *et al.* (1998), who reported that trained panellists were unable to distinguish meats from fattening lambs fed a diet with carob pulp (rich in condensed tannins) from meat of lambs fed a control diet, and Chaves *et al.* (2008) did not find differences in sensory meat attributes when different polyphenol sources were included in lamb diets. Also, Muíño *et al.* (2014) did not find significant differences in lamb meat when vitamin E or red wine extract were included in diets.

Table 5.9. Effect of experimental ewe diets on sensory evaluation by triangle test of suckling lamb meat.

Compared treatments[1]	Number of comparison tests	D	
CTRL *vs.* VIT-E	18	7	(ns)
CTRL *vs.* GP-5	18	11	(ns)
CTRL *vs.* GP-10	18	5	(ns)
VIT-E *vs.* GP-5	18	7	(ns)
VIT-E *vs.* GP-10	18	3	(ns)
GP-5 *vs.* GP-10	18	10	(ns)

[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.

D: number of comparison test that were able to distinguish the meats.

ns, non-significant effect ($P < 0.05$).

5.3.5. Meat shelf life

Microbial results

Table 5.10 summarizes the results of the microbial analysis of LTL muscle slices packaged under a gas mixture (80:20% / O_2:CO_2) during refrigerated storage, from suckling lambs assigned to the various experimental ewe dietary treatments.

Shelf life of meat is highly influenced by the initial bacterial load; thus high numbers of microorganisms in meat before storage shorten the shelf life since the microorganism limit will be achieved more rapidly (Blix and Borch, 2002). As usual, no differences between treatments were observed initially, which is in line with other studies on lamb meat before storage. As expected, all the microbial populations increased significantly during refrigerated storage ($P < 0.05$). Modified atmosphere packaging in a high concentration of oxygen allows the growth of aerobic microorganisms, although CO_2 controls the growth of bacteria associated with meat spoilage (Buys *et al.*, 1994).

Total viable counts (TVC) and enterobacteria, considered as hygiene indicators, in general, began to increase significantly ($P < 0.05$) from 10 and 14 days of storage, respectively. TVC were not different ($P > 0.05$) between experimental treatments for any time. However, enterobacteria presented significantly lower ($P < 0.05$) values in meat from the VIT-E and GP-5 treatments than the values from the CTRL and GP-10 groups at the end of storage (days 10 and 14). *Escherichia coli* levels remained stable throughout storage and there were no statistical differences ($P > 0.05$) between experimental treatments. The average value was significantly higher ($P < 0.05$) in the CTRL treatment than in the other

experimental groups (2.15 *vs.* 1.72, 1.50 and 1.42; CTRL *vs.* VIT-E, GP-5 and GP-10, respectively).

Table 5.10. 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}	^{A}1.00^{b}	^{A}1.60^{ab}	0.267	0.039	0.230	0.199
	3	1.78	1.60	^{AB}1.96	^{A}1.60				
	7	2.00^{ab}	1.60^{a}	^{B}2.57^{b}	^{A}1.60^{a}				
	10	3.01^{a}	1.69^{b}	^{AB}1.60^{b}	^{AB}1.92^{ab}				
	14	2.97^{a}	1.97^{ab}	^{A}1.30^{b}	^{B}3.02^{a}				
Escherichia coli	0	2.40^{a}	1.60^{ab}	1.30^{b}	1.30^{b}	0.230	0.021	0.958	0.807
	3	1.89	1.60	1.30	1.60				
	7	1.99	1.60	1.99	1.30				
	10	2.23	1.49	1.60	1.60				
	14	2.25	2.28	1.30	1.30				
Lactic acid bacteria	0	2.74	^{A}2.04	^{A}1.96	^{A}1.98	0.424	0.496	<0.001	0.837
	3	2.43	^{A}2.30	^{A}2.12	^{AB}2.62				
	7	2.93	^{A}2.57	^{AB}3.40	^{AB}2.79				
	10	2.77	^{AB}3.08	^{AB}3.86	^{B}4.16				
	14	4.18	^{B}4.56	^{B}4.80	^{C}6.07				
Pseudomonads	0	2.00	^{A}2.00	2.00	2.00	0.245	0.435	0.641	0.463
	3	2.30	^{A}2.00	2.00	2.00				
	7	2.00	^{A}2.30	2.30	2.30				
	10	2.00	^{A}2.00	2.00	2.00				
	14	2.00^{a}	^{B}3.62^{b}	2.00^{a}	1.80^{a}				
Brochothrix thermosphacta	0	2.52	2.00	^{A}2.00	2.00	0.247	0.180	0.418	0.882
	3	2.30	2.00	^{A}2.00	2.00				
	7	2.66^{ab}	2.30^{ab}	^{B}3.24^{b}	1.80^{a}				
	10	2.30	2.00	^{A}2.00	2.00				
	14	2.48	2.30	^{AB}2.78	2.00				

[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).

[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 ($P < 0.05$).

With regard to lactic acid bacteria, which behave as facultative anaerobes and are able to grow under relatively high concentrations of CO_2, no statistical differences were detected between treatments from 0 to 14 days of storage. The behaviour of *Pseudomonas* spp. was similar in all groups, beginning to increase in the VIT-E group from day 10 to the end of storage, when the highest values were reached. *Brochothrix thermosphacta* remained stable during storage, and counts were higher in the GP-5 treatment compared with the other groups at 7 days.

Several studies (Parfene *et al.*, 2013) have reported that microbial counts could be affected by the fatty acid profile of meat. In our previous work, no important differences in meat fatty acid composition were found between treatments.

Our results seem to indicate that vitamin E was effective ($P < 0.05$) in preventing enterobacteria development from day 10. α-Tocopherol acetate is used in sheep feeding to stabilize lamb meat fat and colour, although no antibacterial effects have been reported previously in fattening lambs (Lauzurica *et al.*, 2005). Therefore its antibacterial effects might be related to its antioxidant capacity. Grape pomace at the level of 5% (GP-5) was also effective ($P < 0.05$) in preventing enterobacteria growth from day 10. These results should be attributed to the presence of polyphenols, identified as antibacterial compounds, which were transmitted to the ewes' milk and then consumed by the lambs. Several works have indicated a transfer of phenolic compounds from cow (O'Connel and Fox, 2001), ewe (Chiofalo *et al.*, 2012) and goat diets (Jordán *et al.*, 2010) to milk. In this regard, some studies have shown that supplementation of ewe diets with rosemary (Nieto *et al.*, 2010a; Serrano *et al.*, 2014) and thyme (Nieto *et al.*, 2010b), rich in phenolic compounds, reduced microbial populations responsible for lamb meat spoilage during storage owing to the accumulation of these compounds in the muscles during the life of the animals. In contrast, Morán *et al.* (2012) did not find any effect due to the inclusion of rosemary polyphenols in fattening lamb diets on microbial spoilage. With regard to the grape pomace antibacterial effect, Reddy *et al.* (2013) reported that the addition of grape seed extract to mutton slices significantly reduced total psychrophilic and coliform counts in meat during refrigerated storage; therefore, grape by-products could exert a protective effect against microbiological spoilage. Grape pomace was not effective in preventing microbial development at the 10% level of incorporation. In any case, most of these studies lack crucial information for assessing dose effects, particularly concerning the degree of degradation of active polyphenol compounds in the feed given to lambs.

Finally, taking into account that shelf life is defined as the time in days to reach mean values at the limit of 7 log cfu g^{-1} of TVC (ICMSF, 1986), the microbiological shelf life of the meat from any of the experimental treatments did not reach that level, so the shelf life of the lamb meat under high O_2:CO_2 modified atmosphere packaging was not limited by microbial spoilage.

Colour coordinates and metmyoglobin percentage

Results of the colour measurements and metmyoglobin haem pigment percentage are shown in Figure 5.1. Meat colour has been reported as being the most important factor when consumers assess meat quality, since they relate colour to freshness. In general, storage time significantly affects the evolution of meat colour parameters, because, as many authors have stated (Faustman and Cassens, 1990), there is a strong relationship between lipid oxidation and myoglobin oxidation, and the consequent accumulation of MMb, which is responsible for meat browning. Modified atmosphere with a high proportion of oxygen improves meat colour owing to oxymyoglobin formation, which maintains the desirable bright red colour of meat. However, many authors have proved that an increase in oxygen level is useful for colour stability, but oxygen promotes many deteriorative reactions (fat oxidation and microbiology failure) during meat storage.

The behaviour of lightness (L^*) remained stable during the first days of storage for all treatments. However, VIT-E and GP-5 started to decrease from day 10 to the end of the trial, presenting lower ($P < 0.05$) L^* average values at 14 days compared with CTRL and GP-10. In this regard, studies (Nieto *et al.*, 2010b) have observed lower L^* lamb meat values when phenols (from thyme) were included in dams' diets. However, grape pomace inclusion at the level of 10% did not affect the L^* index ($P > 0.05$) compared with the CTRL group. Various studies with ewes (Nieto *et al.*, 2010a) did not find statistically significant differences in the L^* lamb meat coordinate when dams were supplemented with polyphenol-rich substances (rosemary). Redness (a^* values) is one of the most important colour parameters for evaluating meat oxidation, since consumers prefer fresh, red-coloured meat. Myoglobin oxidation, with consequent meat browning over time of storage, is generally associated with a decrease in a^* values (Bodas *et al.*, 2012). However, in the present trial the redness (a^*) values increased during storage. In contrast to the results found by Gallardo *et al.* (2015), who reported higher a^* in meat from suckling lambs whose mothers' diets were supplemented with vitamin E, no differences were found in our work as a result of vitamin E

supplementation in ewes' diets. However, a higher *a** value was observed at the end of storage as a result of incorporation of grape pomace at 5%, which is consistent with the results of Nieto *et al.* (2010a), who reported a positive effect on redness in meat of lambs from dams fed with a phenol-rich source. Yellowness values (*b**) remained stable during the first days of storage. From day 10 onwards, an increase ($P < 0.05$) in *b** was observed in the VIT-E and GP-5 treatments, with values that were higher than CTRL and GP-10 at the end of storage. Our current results are in agreement with Karami *et al.* (2011), who reported higher *b** values in vitamin E supplemented growing lambs, but, in contrast, Gallardo *et al.* (2015), reported a lower *b** coordinate in suckling lamb meat from dietary vitamin E supplemented ewes. Lower *b** values were reported by Nieto *et al.* (2010a) in meat of lambs from ewes fed different polyphenol sources. In our work, the different behaviour of GP-5 and GP-10 may be due to the effect of different doses of polyphenol sources. Likewise, the intensity of the red colour (chroma, *C**) increased at the end of the trial, indicating a very vivid colour (greater *C** values). The inclusion of vitamin E and grape pomace at the level of 5% resulted in higher *C** values ($P < 0.05$) at day 14. These results could be related to lower meat decolouration induced by polyphenols. However, in our study no differences were found between GP-10 and CTRL at the end of storage. With regard to *H**, which has been related to the visual appraisal of meat discolouration (Luciano *et al.*, 2011), although slight differences were observed between groups, our data showed that *H** remained almost stable throughout the trial for all treatments.

The lack of a clear evolution of colorimetric parameters could be explained by several causes, such as the greater variability of the colour coordinates, the doses of antioxidants employed, the time of feeding, the oxidative susceptibility of the meat, the sampling technique or the thickness of the slices. However, we can say that antioxidants contributed to delaying meat discolouration, since the MMb haem pigment percentage was clearly influenced by the presence of vitamin E and grape pomace (Figure 5.1). The accumulation of MMb during storage is responsible for meat browning, and inclusion of vitamin E and grape pomace in the ewes' diets inhibited MMb formation ($P < 0.05$) from day 10, related to their antioxidant properties, avoiding reaching a 40% level (Greene and Cumuze, 1971), which has been reported to be a level that causes rejection. Our results confirm those found by Gallardo *et al.* (2015), who observed a suckling lamb meat colour stabilization through ewes' dietary vitamin E. According to Descalzo *et al.* (2007), vitamin E is able to extend meat oxidative stability by improving the overall muscle antioxidant status, by lowering the formation of some oxidation markers and, in turn, by extending meat colour stability. The effect of vitamin E on meat colour is not completely understood, but it has been speculated that it is principally due to its ability to prevent oxidation of myoglobin and/or oxymyoglobin to

metmyoglobin and thus meat discoloration (Morrissey *et al.*, 1998). These results might be related to the lower sheep plasma oxidation reported in animals assigned to the VIT-E treatment (section 5.3.2).

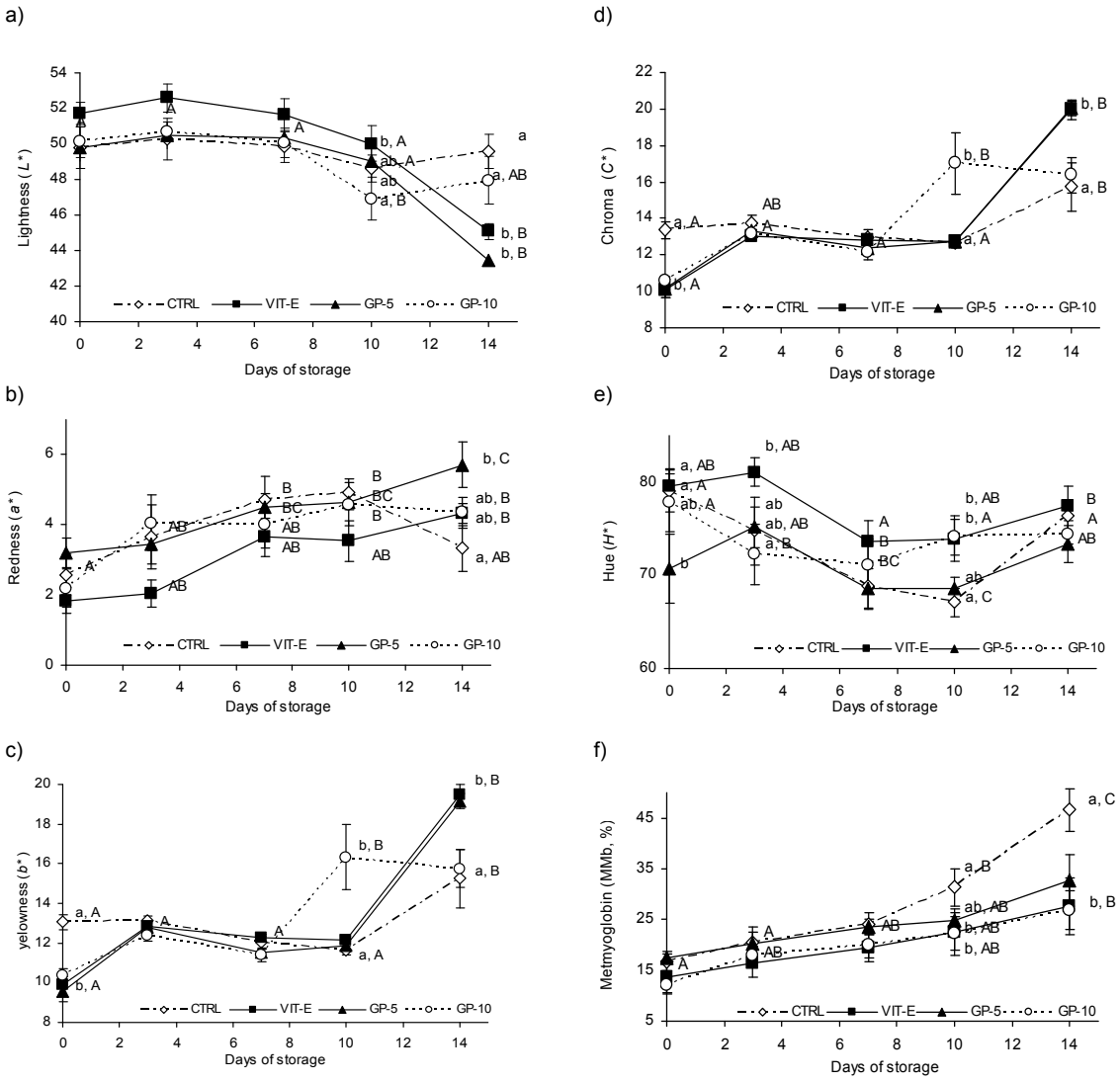


Figure 5.1. Effect of experimental ewe diets (CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of TMR, DM basis; GP-5, 5% of grape pomace from red wine, DM basis; GP-10, 10% of grape pomace from red wine, DM basis) and storage time on evolution of colour parameters: lightness (a), redness (b), yellowness (c), chroma (d), hue (e) and metmyoglobin haem pigment (f) in m. *longissimus thoracis et lumborum* from suckling lambs stored at 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.

It is interesting to note that the effect of grape pomace supplementation (GP-5 and GP10) was close to that of vitamin E, probably owing to the transfer of dietary phenolic compounds from milk to suckling lamb muscle, which could have contributed to the protective effect of these substances against meat discolouration, preventing MMb formation. Similar results in meat discolouration were obtained by Nieto *et al.* (2010a), probably as a result of decreased myoglobin oxidation by the presence of phenolics. The effect of these phenolic-rich extracts on haeminic pigment oxidation has been explained previously by Samman *et al.* (2001), who related this effect to the fact that they are iron chelating agents, promoting inhibition of iron absorption and thus either lower red blood cell counts or low haemoglobin levels (Bodas *et al.*, 2012). The lower MMb meat oxidation in grape pomace treatment animals may be linked to the lower ewe plasma oxidation levels observed (section 5.3.2).

Lipid oxidation

Lipid oxidation results from production of free radicals, which may lead to oxidation of meat pigments and generation of rancid odours and flavours (Faustman and Cassens, 1990). As expected, meat lipid oxidation increased strongly ($P < 0.05$) with storage time (Figure 5.2), reflecting the reduction of meat capacity to resist against lipid oxidation during storage under retail display conditions. Therefore the high MDA values observed are probably the result of the oxidizing conditions during storage (high O_2:CO_2 atmosphere and intense lighting), since lipid oxidation in meat packed in high-oxygen modified atmosphere can be encouraged by the high O_2 level. The limiting threshold for oxidized meat acceptability varies according to the animal and the study. According to Soldatou *et al.* (2009), rancidity can be detected in lamb meat when TBARS values are higher than 4.4 μg MDA g^{-1} meat.

As expected, initially TBARS concentrations were not significantly different in the four experimental treatments, ranking from 0.04 to 0.70 μg MDA g^{-1} muscle. However, the oxidative processes in muscle throughout the storage, reflected in TBARS values, were significantly affected by dietary treatment ($P < 0.05$).

The dietary administration of antioxidants can be an interesting strategy as, for instance, supplementing animal diets with vitamin E has been extensively shown to effectively enhance the resistance of meat to oxidative deterioration (Faustman *et al.*, 1998). Vitamin E is the primary lipid-soluble antioxidant in biological systems and it breaks the chain of lipid

oxidation in cell membranes (Buckley *et al.*, 1995). In the present study, vitamin E supplementation in the diet was effective in preventing muscle MDA formation during storage in modified atmosphere packing, since significantly lower (P < 0.05) TBARS values were found from day 10 onward with respect to CTRL treatment. These findings are consistent with those of other authors in meat from Churra breed suckling lambs also fed only on maternal milk, employing α-tocopherol acetate in ewe diets (Gallardo *et al.*, 2015), and in lambs fed with milk replacers (Morán *et al.*, 2014). Those authors reported that dietary vitamin E powerfully inhibited MDA formation in lamb meat, especially during longer retail display periods. It is worth mentioning that the improvement in lamb meat TBARS from vitamin E supplemented ewes is consistent with the lower numerical value observed in ewe plasma TBARS reported in these animals (section 5.3.2).

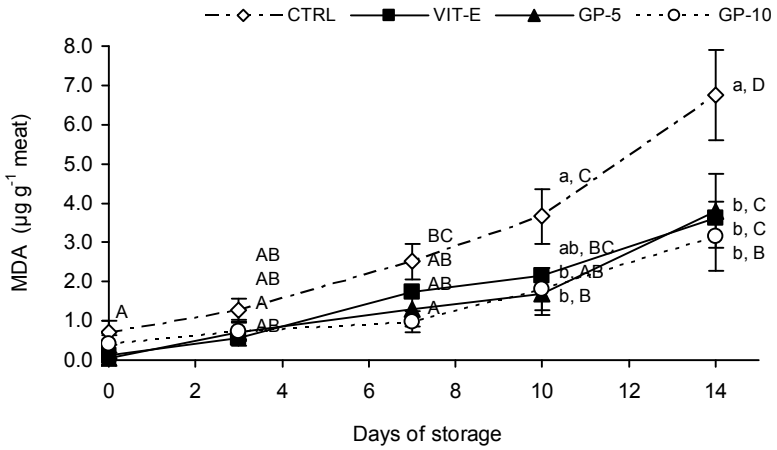


Figure 5.2. Effect of experimental ewe diets (CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of TMR, DM basis; GP-5, 5% of grape pomace from red wine, DM basis; GP-10, 10% of grape pomace from red wine, DM basis) and storage time on evolution of TBARS (µg g^{-1} meat) in m. *longissimus 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.

In relation to the inclusion of dietary phenolic compounds, although several studies (Moñino *et al.*, 2008; Luciano *et al.*, 2011) have shown that these compounds favoured the antioxidant stability of meat, their mechanisms of action have not been completely established. However, it has been reported that many plant secondary compounds, such as phenolic compounds, have the ability to attenuate oxidative damage of tissue, since they

may interfere with the propagation reaction, besides inhibiting the enzymatic systems involved in initiation reactions (You *et al.*, 1999). Phenolic compounds contain conjugated ring structures and hydroxyl groups which 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 in tissues (Luciano *et al.*, 2009). 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 *et al.*, 2004). Hydrolysis of polymers into compounds with a low degree of polymerization or monomers would make their absorption possible. Nevertheless, the effect of dietary polyphenols on meat oxidative stability may be indirect, through interaction between phenols and other antioxidant compounds or pro-oxidant compounds present in meat.

The antioxidant effect of phenolic compounds is confirmed by our results, since lower TBARS values ($P < 0.05$) were observed from day 10 in grape pomace treatments (GP-5 and GP-10) (Figure 5.2), showing that grape pomace inclusion at the two levels employed was effective in preventing lipid peroxidation compared with CTRL. As previously reported, several works have indicated transfer of grape pomace phenolic compounds from animal diets to milk. 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 reason for the reduction in MDA formation when grape pomace was incorporated in the ewes' diets. Previous studies on suckling lambs have demonstrated improved lipid oxidation of the meat during storage when different polyphenol sources were included in diets of lactating ewes (Nieto *et al.*, 2010a; 2010b; Serrano *et al.*, 2014), or included in milk replacers for suckling lambs (Morán *et al.*, 2014). As in the case of vitamin E, the improvement in lamb meat TBARS from grape pomace treatments could be connected with the lower numerical value of ewe plasma TBARS reported in grape pomace animals (section 5.3.2).

From our results, inclusion of vitamin E and of grape pomace have similar effects on delaying meat lipid oxidation, which seems to confirm 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.

Sensory evaluation

Figure 5.3 shows the results of the sensory evaluation of the LTL muscle slices packaged under a gas mixture (80:20% / O_2:CO_2). As expected, no differences were found between treatments (P > 0.05) in the recently cut meat (day 0), all of the samples having a score of 1.

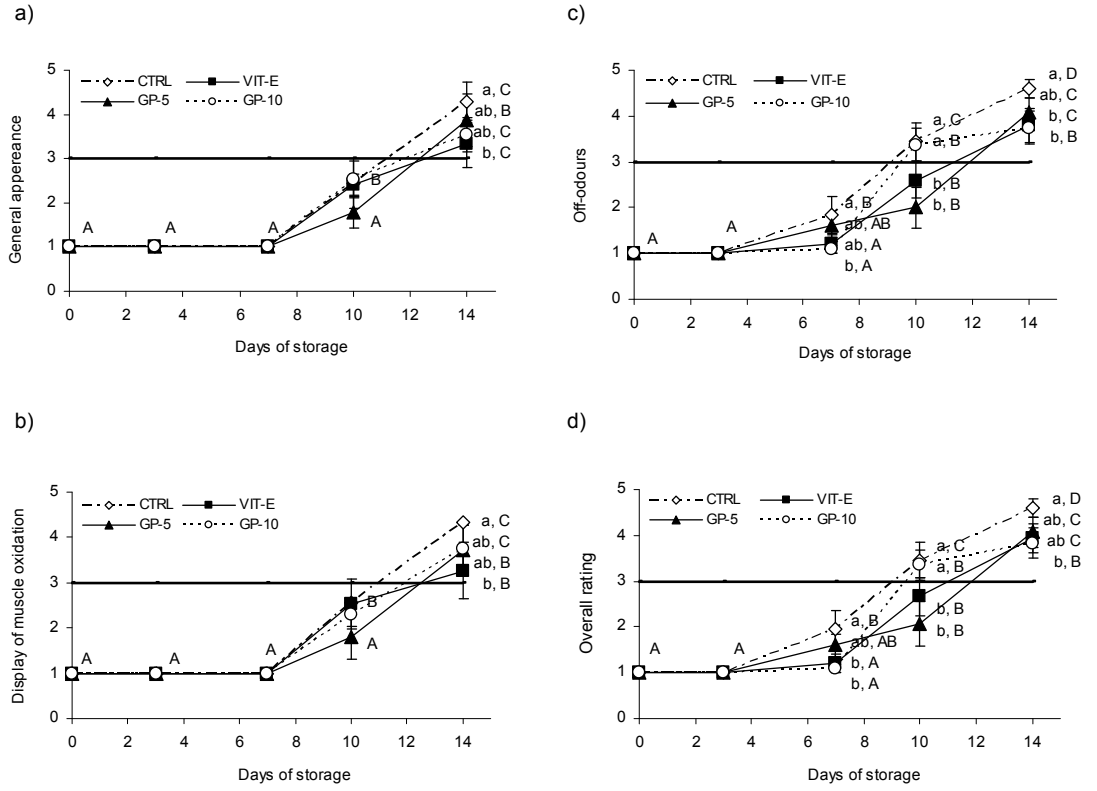


Figure 5.3. Effect of experimental ewe diets (CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of TMR, DM basis; GP-5, 5% of grape pomace from red wine, DM basis; GP-10, 10% of grape pomace from red wine, DM basis) and storage time on general appearance (a), display of muscle oxidation (b), 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.

In general, sensory quality decreased gradually during the whole period of storage, presenting the highest scores at the end of the experimental period. Meat sensory spoilage is associated with oxidizing phenomena, such as lean browning, fat darkening, exudation, loss of metallic blood odour and increasing rancid odour and flavour. The perception of discoloration of lamb meat increased gradually during storage in all groups, but the behaviour differed between treatments. At the 14-day sampling point, VIT-E showed lower scores in these sensory parameters ($P < 0.05$) than CTRL, although it reached scores above 3 at this sampling point. These results are consistent with the relative MMb percentage observed. However, the low MMb values in grape pomace groups were not reflected in the display of muscle oxidation.

Similarly, the presence of off-odour increased gradually and significantly during storage in all groups. Nevertheless, from 10 days of display, VIT-E and GP-5 showed values that were significantly lower ($P < 0.05$), showing scores below 3, which was established as the threshold for rejection. Although all treatments showed values above 3 at the end of storage, the GP-10 off-odour score was lower ($P < 0.05$) than that of CTRL. These results are probably due to the lower TBARS in lamb meat from these experimental treatments, since lipid oxidation is linked to rancid odours.

The overall rating of the samples reflects the changes in the other sensory attributes studied. Thus, according to the panellists, CTRL samples reached values above 3 at 10 days, while VIT-E and grape pomace treatments reached that level at day 14.

VIT-E was effective ($P < 0.05$) in preventing sensory spoilage and improving colour stability in the last days of storage compared with CTRL. This statement agrees with Wulf *et al.* (1995) and 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 a non-supplemented meat.

Grape pomace was as effective as VIT-E at the end of storage, showing lower score values in off-odours and overall rating compared with CTRL. The presence of polyphenols in milk could be the cause of these results. Some studies have indicated an improvement in certain sensory attributes related to meat colour and odour under display meat conditions when polyphenols were included in dams' diets (Nieto *et al.*, 2010a; 2010 b; Serrano *et al.*, 2014).

5.4. Conclusions

Whole dried grape pomace at inclusion levels of 5% and 10% of TMR can be included in lactating ewe diets without adverse effects on milk yield and composition, animal performance, carcass characteristics and meat quality of their suckling lambs when compared with vitamin E and with a control diet. Grape pomace and vitamin E improved the water holding capacity of the meat. The presence in the diet of grape pomace at the doses assayed did not substantially modify the milk fat FA profile. However, grape pomace increases some FA intermediates of the biohydrogenation process, such as RA and VA, with potential beneficial effects on human health. Furthermore, grape pomace was as effective as vitamin E in preventing meat deterioration, showing a lower metmyoglobin haem pigment percentage and lower meat TBARS values, which could be connected with lower numerical values in ewe plasma TBARS. Meat from grape pomace treatments showed better scores in sensory perception from day 10 of storage under retail display conditions compared with non-supplemented ewes. Consequently, grape pomace constitutes an inexpensive source of polyphenols that can be used in early lactating ewe diets while reducing the environmental impact of waste disposal in the wine industry.

5.5. References

Andrés, S., Huerga, L., Mateo, J., Tejido, M.L., Bodas, R., Morán, L., Prieto, N., Rotolo, L., Giráldez, F.J. (2014). The effect of quercetin dietary supplementation on meat oxidation processes and texture of fattening lambs. *Meat Science 96*, 806–811.

AOAC (2012). Official Methods of Analysis of AOAC International, 19th Edition.

Belibasakis, N.G., Progia, E., Papaioannou, A., Skulidis, G. (1996). Effects of ensiled wet grape marc on milk production, milk composition and blood components of dairy cows. *Czech Journal of Animal Science 41*, 307–310.

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

Bligh, E.G., Dyer, W.J. (1959). A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology 37*, 911–917.

Blixt, Y., Borch, E. (2002). Comparison of shelf life of vacuum packed pork and beef. *Meat Science 60*, 371–378.

Bodas, R., Prieto, N., Jordán, M.J., López-Campos, O., Giráldez, F.J., Morán, L., Andrés, S. (2012). The liver antioxidant status of fattening lambs is improved by naringin dietary supplementation at 0.15% rates but not meat quality. *Animal 6,* 863–870.

Bodas, R., Prieto, N., López-Campos, O., Giráldez, F.J., Andrés, S. (2011). Naringin and vitamin E influence the oxidative stability and lipid profile of plasma in lambs fed fish oil. *Research in Veterinary Science* 91, 98–102.

Buckley, D.J., Morrissey, P.A., Gray, J.I. (1995). Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *Journal of Animal Science 73*, 3122–3130.

Buys, E.M., Krüger, J., Nortjé, G.L. (1994). Centralised bulk pre-packaging of fresh pork retail cuts in various gas atmospheres. *Meat Science 36*, 293–308.

Casamassima, D., Palazzo, M., Martemucci, G., Vizzarri, F., Corino, C. (2012). Effects of verbascoside on plasma oxidative status and blood and milk production parameters during the peripartum period in Lacaune ewes. *Small Ruminant Research 105*, 1–8.

Chaves, A.V., Stanford, K., Dugan, M.E.R., Gibson, L.L., McAllister, T.A., Van Herk, F., Benchaar, C. (2008). Effects of cinnamaldehyde, garlic and juniper berry essential oils on rumen fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. *Livestock Science 117*, 215–224.

Chiofalo, V., Liotta, L., Fiumanò, R., Benedetta, E., Chiofalo, B. (2012). Influence of dietary supplementation of *Rosmarinus officinalis* L. on performances of dairy ewes organically managed. *Small Ruminant Research 104*, 122–128.

CIE (1986). Colorimetry, 15.2. (2nd ed). CIE Publications, Vienna (Austria).

Correddu, F., Nudda, A., Battacone, G., Boe, R., Francesconi, A.H.D., Pulina, G. (2015). Effects of grape seed supplementation, alone or associated with linseed, on ruminal metabolism in Sarda dairy sheep. *Animal Feed Science and Technology 199*, 61–72.

Decker, E.A. (1995). The role of phenolics, conjugated linoleic acid, carnosine, and pyroloquinoline quinone as nonessential dietary antioxidants. *Nutrition Reviews 53*, 49–58.

Descalzo, A.M., Rossetti, L., Grigioni, G., Irurueta, M., Sancho, A.M., Carrete, J., Pensel, N.A. (2007). Antioxidant status and odour profile in fresh beef from pasture or grain fed cattle. *Meat Science 75*, 299–307.

Devine, C.E., Graafhuis, P.H., Muir, P.D., Chrystall, B.B. (1993). The effect of growth rate and ultimate pH on meat quality of lambs. *Meat Science 35*, 63–77.

Estévez, M. (2011). Protein carbonyls in meat systems: A review. *Meat Science 89*, 259–279.

Faustman, C., Cassens, R.G. (1990). The biochemical basis for discoloration in fresh meat: A review. *Journal of Muscle Foods 1*, 217–243.

Faustman, C., Chan, W.K.M., Schaefer, D.M., Havens, A. (1998). Beef color update: the role for vitamin E. *Journal of Animal Science 76*, 1019–1026.

Gallardo, B., Gómez-Cortés, P., Mantecón, A.R., Juárez, M., Manso, T., De la Fuente, M.A. (2014) Effects of olive and fish oil Ca soaps in ewe diets on milk fat and muscle and subcutaneous tissue fatty-acid profiles of suckling lambs. *Animal 8*, 1178–1190.

Gallardo, B., Manca, M.G., Mantecón, A.R., Nudda, A., Manso, T. (2015). Effects of linseed oil and natural or synthetic vitamin E supplementation in lactating ewes' diets on meat fatty acid profile and lipid oxidation from their milk fed lambs. *Meat Science 102,* 79–89.

Gladine, C., Rock, E., Morand, C., Bauchart, D., Durand, D. (2007). Bioavailability and antioxidant capacity of plant extracts rich in polyphenols, given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. *British Journal of Nutrition* 98, 691–701.

Gorraiz, C., Beriain, M.J., Chasco, J., Iraizoz, M. (2000). Descriptive analysis of meat from young ruminants in Mediterranean systems. *Journal of Sensory Studies 15,* 137–150.

Greene, B.E., Cumuze, T.H. (1981). Relationship between TBA-numbers and inexperienced panelists' assessments of oxidized flavor in cooked beef. *Journal of Food Science 47*, 52–54.

Honikel, K.O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science 49,* 447–457.

Huff-Lonergan, E., Lonergan, S.M. (2005).Mechanisms of water holding capacity of meat: The role of *postmortem* biochemical and structural changes. *Meat Science 71*, 194–204.

ICMSF (1986). Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Scientific Applications, Vol. 2. Toronto: University of Toronto Press, Toronto (Canada).

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

Inserra, L., Priolo, A., Biondi, L., Lanza, M., Bognanno, M., Gravador, R., Luciano, G. (2014). Dietary citrus pulp reduces lipid oxidation in lamb meat. *Meat Science 96*, 1489–1493.

ISO 4120 (2004). Sensory analysis-methodology–Triangle test. International Standard ISO 4120: 2004(E).

ISO-IDF (2002). Milk Fat-Preparation of Fatty Acid Methyl Esters. International Standard ISO 15884-IDF 182:2002.

Jerónimo, E., Alves, S.P., Dentinho, M.T.P., Martins, S.V., Prates, J.A.M., Vasta, V., Santos-Silva, J., Bessa, R.J.B. (2010). Effect of grape seed extract, *Cistus ladanifer* L., and vegetable oil supplementation on fatty acid composition of abomasal digesta and intramuscular fat of lambs. *Journal of Agricultural and Food Chemistry 58*, 10710–10721.

Jordán, M.J., Moñino, M.I., Martínez, C., Lafuente, A., Sotomayor, J.A. (2010). Introduction of distillate rosemary leaves into the diet of the Murciano-Granadina goat: Transfer of polyphenolic compounds to goats' milk and the plasma of suckling goat kids. *Journal of Agricultural and Food Chemistry 58*, 8265–8270.

Karami, M., Alimon, A.R., Goh, Y.M. (2011). Effect of vitamin E, Andrographis paniculata and turmeric as dietary antioxidant supplementation on lipid and color stability of goat meat. *Small Ruminant Research 97*, 67–71.

Krzywicki, K. (1979). Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. *Meat Science 3*, 1–10.

Lauzurica, S., De la Fuente, J., Díaz, M.T., Álvarez, I., Pérez, C., Cañeque, V. (2005). Effect of dietary supplementation of vitamin E on characteristics of lamb meat packed under modified atmosphere. *Meat Science 70*, 639–646.

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

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

Luciano, G., Monahan, F.J., Vasta, V., Biondi, L., Lanza, M., Priolo, A. (2009). Dietary tannins improve lamb meat colour stability. *Meat Science 81*, 120–125.

Luciano, G., Vasta, V., Monahan, F.J., López-Andrés, P., Biondi, L., Lanza, M., Priolo, A. (2011). Antioxidant status, colour stability and myoglobin resistance to oxidation of *Longissimus dorsi* muscle from lambs fed a tannin-containing diet. *Food Chemistry 124*, 1036–1042.

Luna, P., Juárez, M., De la Fuente, M.A. (2005). Validation of a rapid milk fat separation method to determine the fatty acid profile by gas chromatography. *Journal of Dairy Science 88,* 3377–3381.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition 79,* 727–747.

Manso, T., Bodas, R., Vieira, C., Mantecón, A.R., Castro, T. (2011) Feeding vegetable oils to lactating ewes modifies the fatty acid profile of suckling lambs *Animal 5*, 1659–667

Maraschiello, C., Sarraga, C., García-Regueiro, J.A. (1999). Glutathione peroxidase activity, TBARS, and α-tocopherol in meat from chickens fed different diets. *Journal of Agriculture and Food Chemistry 47*, 867–872.

Min, B.R., Barry, T.N., Attwood, G.T., McNabb, W.C. (2003). The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Animal Feed Science Technology 106,* 3–19.

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

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

Moniño, I., Martínez, C., Sotomayor, J.A., Lafuente, A., Jordan, M.J. (2008) Polyphenolic transmission to Segureño lamb meat from ewes' diet supplemented with the distillate from rosemary (*Rosmarinus officinalis*) leaves. *Journal of Agricultural and Food Chemistry 56,* 3363–3367.

Morán, L., Andrés, S., Mateo, J., Blanco, C., Soto, S., Giráldez, F.J. (2014). Effect of dietary carnosic acid on meat quality from suckling lambs. *Small Ruminant Research 121*, 314–319.

Morán, L., Giráldez, F.J., Panseri, S., Aldai, N., Jordán, M.J., Chiesa, L.M., Andrés, S. (2013). Effect of dietary carnosic acid on the fatty acid profile and flavour stability of meat from fattening lambs. *Food Chemistry 138*, 2407–2414.

Morán, L., Rodríguez-Calleja, J.M., Bodas, R., Prieto, N., Giráldez, F.J., Andrés, S. (2012). Carnosic acid dietary supplementation at 0.12% rates slows down meat discoloration in *gluteus medius* of fattening lambs. *Meat Science 90*, 789–795.

Morrison, W.R., Smith, L.M. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *Journal of Lipid Research* 5*,* 600–608.

Morrissey, P.A., Buckley, D.J., Sheehy, P.J.A., Monahan, F.J. (1994). Vitamin E and meat quality. *Proceedings of the Nutrition Society 53*, 289–295.

Morrissey, P.A., Sheehy, P.I.A., Galvin, K., Kerry, J.P., Buckley, D.J. (1998). Lipid stability in meat and meat products. *Meat Science 49,* (Supplement 1), S73–S86.

Muíño, I., Apeleo, E., De la Fuente, J., Pérez-Santaescolástica, C., Rivas-Cañedo, A., Pérez., C., Díaz, M.T., Cañeque, V., Lauzurica, S. (2014). Effect of dietary supplementation with red wine extract or vitamin E, in combination with linseed and fish oil, on lamb meat quality. *Meat Science 98*, 116–123.

Nieto, G. (2013). Incorporation of by-products of rosemary and thyme in the diet of ewes: effect on the fatty acid profile of lamb. *European Food Research and Technology 236*, 379–389.

Nieto, G., Bañón, S., Garrido, M.D. (2011). Effect of supplementing ewes' diet with thyme (*Thymus zygis* ssp. *gracilis*) leaves on the lipid oxidation of cooked lamb meat. *Food Chemistry 125*, 1147–1152.

Nieto, G., Bañón, S., Garrido, M.D. (2012). Incorporation of thyme leaves in the diet of pregnant and lactating ewes: Effect on the fatty acid profile of lamb. *Small Ruminant Research 105*, 140–147.

Nieto, G., Díaz, P., Bañón, S., Garrido, M.D. (2010a). Dietary administration of ewe diets with a distillate from rosemary leaves (*Rosmarinus officinali*s L.): influence on lamb meat quality. *Meat Science 84*, 23–29.

Nieto, G., Díaz, P., Bañón, S., Garrido, M.D. (2010b). Effect on lamb meat quality of including thyme (*Thymus zygis* ssp. *gracilis*) leaves in ewes' diet. *Meat Science 85*, 82–88.

Nudda, A., Battacone, G., Boaventura-Neto, O., Cannas, A., Francesconi, A.H.D., Atzori, A.S., Pulina, G. (2014). Feeding strategies to design the fatty acid profile of sheep milk and cheese. *Revista Brasileira de Zootecnia 43,* 445–456.

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

O'Connell, J.E., Fox, P.F. (2001). Significance and applications of phenolic compounds in the production and quality of milk and dairy products: A review. *International Dairy Journal 11*, 103–120.

Parfene, G., Horincar, V., Tyagi, A.K., Malik, A., Bahrim, G. (2013). Production of medium chain saturated fatty acids with enhanced antimicrobial activity from crude coconut fat by solid state cultivation of *Yarrowia lipolytica*. *Food Chemistry 136*, 1345–1349

Priolo, A., Lanza, M., Biondi, L., Pappalardo, D., Young, O.A. (1998). Effect of partially replacing dietary barley with 20% carob pulp on post-weaning growth, and carcass and meat characteristics of Comisana lambs. *Meat Science 50*, 355–363.

Priolo, A., Waghorn, G.C., Lanza, M., Biondi, L., Pennisi, P. (2000). Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: Effects on lamb growth performance and meat quality. *Journal of Animal Science 78*, 810–816.

Rana, M.S., Tyagi, A., Hossain, S.A., Tyagi, A.K. (2012). Effect of tanniniferous *Terminalia chebula* extract on rumen biohydrogenation, Δ9-desaturase activity, CLA content and fatty acid composition in *longissimus dorsi* muscle of kids. *Meat Science 90*, 558–563.

Reddy, B.G.V., Sen, A.R., Nair P.N., Reddy, K.S., Reddy, K.K., Kondaiah, N. (2013). Effects of grape seed extract on the oxidative and microbial stability of restructured mutton slices. *Meat Science 95*, 288–294.

Rota, M.C., Herrera, A., Martínez, R.M., Sotomayor, J.A., Jordán, M.J. (2008). Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oil. *Food Control 19,* 681–687.

Russel, A.J.F., Doney, J.M., Gunn, R.G. (1969). Subjective assessment of fat in live sheep. *Journal of Agricultural Science 72*, 157–454.

Samman, S., Sandstrom, B., Toft, M.B., Bukhave, K., Jensen, M., Sorensen, S.S., Hansen, M. (2001). Green tea or rosemary extract added to foods reduces nonheme-iron absorption. *The American Journal of Clinical Nutrition 73*, 607–612.

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

Serrano, R., Jordán, M.J., Bañón, S. (2014). Use of dietary rosemary extract in ewe and lamb to extend the shelf life of raw and cooked meat. *Small Ruminant Research 116*, 144–152.

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

Simopoulos, A.P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine 233,* 674–688.

Soldatou, N., Nerantzaki, A., Kontominas, M.G., Savvaidis, I.N. (2009). Physicochemical and microbiological changes of “Souvlaki” - A Greek delicacy lamb meat product: Evaluation of shelf-life using microbial, colour and lipid oxidation parameters. *Food Chemistry 113*, 36–42.

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

UNE-ISO 13300-2 (2008). Sensory analysis. General guidance for the staff of a sensory evaluation laboratory. Part 2: recruitment and training of panel leaders.

Van Soest, P.J., Robertson, J.B., Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science 74*, 3583–3597.

Vasta, V., Luciano, G. (2011) The effects of dietary consumption of plants secondary compounds on small ruminants’ products quality. *Small Ruminant Research 101,* 150–159.

Vasta, V., Mele, M., Makkar, H.P.S., Priolo, A. (2009a). Ruminal biohydrogenation as affected by tannins in vitro. *British Journal of Nutrition 102*, 82–92.

Vasta, V., Priolo, A., Scerra, M., Hallett, K.G., Wood, J.D., Doran, O. (2009b). Δ^9 desaturase protein expression and fatty acid composition of *longissimus dorsi* muscle in lambs fed green herbage or concentrate with or without added tannins. *Meat Science 82*, 357–364.

Wulf, D.M., Morgan, J.B., Sanders, S.K., Tatum, J.D., Smith, G.C., Williams, S. (1995). Effects of dietary supplementation of vitamin E on storage and caselife properties of lamb retail cuts. *Journal of Animal Science 73,* 399–405.

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

You, K.M., Jong, H.G., Kim, H.P. (1999). Inhibition of cyclooxygenase/lipoxygenase from human platelets by polyhydroxylated/methoxylated flavonoids isolated from medicinal plants. *Archives Pharmacology Research 2*2, 18–24.

6. Prueba experimental III

Effects of grape pomace supplementation of growing lamb diets on animal performance, carcass and meat quality

6.1. Introduction

Grape pomace, consisting of seeds, skin and pulp, is the main residue left in the wine industry and accounts for approximately 20% of the total volume of grapes used for wine production. This material is under-exploited, and the possibility of using this winery by-product as feed in ruminant diets could be adopted as a strategy to reduce feeding costs and also to cope with the need to recycle waste material which is costly to dispose of.

Grape pomace is seldom used as a feedstuff owing to its low nutritive value for ruminants. Major limitations of the use of grape pomace as a ruminant feed are its high fibre and lignin content, especially in seeds, and its low digestibility (Spanghero *et al*., 2009). However, grape pomace could offer an added value based on its high content of phenolic compounds owing to incomplete extraction during the winemaking process. Grape pomace is rich in a wide range of polyphenols mainly composed of flavonoids, including anthocyanins, flavonols and flavanols such as proanthocyanidins or condensed tannins (CT). CT may have adverse or beneficial nutritional effects on ruminants, depending on their chemical structure and dietary concentration. Tannins may limit nutrient utilization in ruminants and may also increase the protein supply to the small intestine by decreasing ruminal degradability and thereby benefit animal performance (Frutos *et al*., 2004).

Several studies in ruminants have shown that the dietary fatty acid (FA) profile may be reflected in the FA profile of the meat. In this regard, the presence of high amounts of linoleic and oleic acids reported in winery wastes (Yi *et al*., 2009) may also have beneficial effects on meat fatty acid profile, with the hypothesis of obtaining a more unsaturated meat. Some studies have suggested that polyphenols could be a useful strategy to improve the nutritional value of ruminant fat. *In vitro* and *in vivo* studies have shown that CT are effective in the reduction of dietary polyunsaturated fatty acid (PUFA) ruminal biohydrogenation (Vasta *et al*., 2009). Therefore the inclusion of phenolic compounds in the diets may favourably modify the fatty acid profile of lamb meat to the benefit of consumer health.

Lipid peroxidation has been implicated in the deterioration of physiological functions that include growth and reproduction, as well as immunity. Furthermore, it has an impact on the nutritional value of meat, causing spoilage of meat products during processing and storage. The digestibility and the water-holding capacity (WHC) of muscle proteins and the *post mortem* tenderization of meat are believed to be affected by oxidative reactions (Estévez, 2011). Therefore, control of this process is important for the meat industry.

Due to changes in shopping and consumption habits, producers try to extend the shelf life of meat by storage practices such as modified atmosphere packaging. Shelf life is conditioned by oxidative processes, which are brought about by temperature, oxygen exposure, light and microbial growth. It has been reported that optimum colour stability in red meat is obtained by using gas mixtures containing high concentrations of oxygen together with low proportions of carbon dioxide, which exhibits antimicrobial activity and therefore restricts the growth of aerobic spoilage bacteria (Jeremiah, 2001). A gas composition of 20 to 30% and 70 to 80% (O_2:CO_2) is generally used for meat packaging. Meat quality is increasingly important in animal production in order to meet consumer demands, with colour and flavour being among the most relevant attributes. The gas O_2 is responsible for the desirable bright red colour (oxymyoglobin) at the time of purchase. However, the presence of high oxygen concentrations in packages may enhance lipid oxidation in meat (Fernandes *et al.,* 2014). Besides microbial development, one of the main reasons for the deterioration of meat products during processing, storage and retail display is lipid and myoglobin oxidation, which generates products that are undesirable from a sensory point of view, making the meat unfit for consumption. Therefore, control of this process is essential to preserve the quality and shelf life of the product (Falowo *et al.,* 2014). This objective has been approached in several studies by the exogenous addition of antioxidants, or by adopting feeding systems that can improve the antioxidant status of muscle. Indeed, synthetic additives have been widely used in animal nutrition and the food industry in order to preserve meat, but they have been questioned because of their toxicity, pathogenicity and carcinogenic effects on humans and animals (Hayes *et al.,* 2010). The increasingly demanding consumer preference for natural products and health benefits has intensified the search for alternative methods to retard lipid oxidation in foods, such as the use of natural antioxidants, which could be a suitable alternative in animal feedstuffs, thus avoiding any further manipulation of the meat. Studies have shown that phenolic compounds have the capacity to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions. This statement has been corroborated by several authors who included various flavonoid groups in lamb diets (Andrés *et al*., 2014a), showing satisfactory results with regard to meat peroxidation stability. Winemaking by-products have been associated

with effective antioxidant activity in beef (Ahn *et al.,* 2002), lamb meat (Jerónimo *et al.,* 2012) and sheep plasma (Gladine *et al.,* 2007), so they could be an alternative to synthetic antioxidant additives.

No studies have investigated the effects of the inclusion of the whole dried grape pomace in lamb diets on animal performance, carcass and meat quality and meat self life. Therefore, the aim of the present study was to investigate animal performance, meat quality (chemical composition, fatty acid composition, water-holding capacity, texture and sensory assessment) and the shelf life of lamb meat during storage in retail sale conditions after it had been packaged under modified atmosphere, when grape pomace from red wine was included in the diet of lambs. Vitamin E was included in another group as a positive control because it is one of the antioxidants most frequently used in animal nutrition, and grape seed extract was a commercially available natural extract from grape seed, rich in polyphenols.

6.2. Material and methods

6.2.1. Animals and experimental design

Forty-eight weaned male Merino lambs (initial age 8–9 weeks) housed in individual pens were assigned randomly on the basis of live body weight (LBW, 14.3 ± 2.05 kg) to four homogeneous dietary treatments (12 lambs per treatment): control (CTRL, without grape pomace), vitamin E (VIT-E, 500 mg of vitamin E per kg of concentrate), grape seed extract (GSE, 50 mg of grape seed extract per kg of concentrate, GRAPE-AOX, Cargill Animal Nutrition Spain) and grape pomace (GP-5, 5% of dry grape pomace from red wine production, as fed). All concentrates were formulated so as to be isonitrogenous and isoenergetic in terms of net energy (UFV, feed unit for maintenance and meat production), except GP-5 concentrate, which displayed the same energy:protein ratio as the other groups. Table 6.1 shows the ingredients and composition of the experimental concentrates.

After 10 days of adaptation to the experimental diets, the lambs were allowed *ad libitum* access to experimental concentrates, barley straw (DM 956 g kg^{-1}, ash 42.1 g kg^{-1}, NDF 798 g kg^{-1} DM, ADF 460 g kg^{-1} DM and CP 28.0 g kg^{-1} DM) and fresh drinking water. Feed was provided once daily at 9:00 h. Feed intakes and orts were recorded daily, and samples were collected for subsequent analyses.

Table 6.1. Ingredients and chemical composition of experimental concentrates.

	Treatments[1]			
	CTRL	VIT-E	GSE	GP-5
Ingredients (g kg^{-1})				
Barley	740	740	740	701
Soya	200	200	200	189
Molasses	30.0	30.0	30.0	30.0
Vitamin-mineral premix	30.0	30.0	30.0	30.0
Grape seed extract (mg kg^{-1}) [2]	-	-	50.0	-
Grape pomace [3]	-	-	-	50.0
Vitamin E (mg kg^{-1})	50.0	500	50.0	50.0
Chemical composition (g kg^{-1} DM)				
Dry matter (DM)	982	985	980	876
Organic Matter	932	926	919	931
Neutral detergent fibre	154	157	153	172
Acid detergent fibre	56.6	61.1	61.8	81.1
Crude protein	189	189	187	187
Ether extract	43.9	43.8	44.6	46.6
UFV [4] (kcal kg^{-1} DM)	1.15	1.15	1.15	1.10
PDI [5]:UFV	9.80	9.80	9.80	9.80

[1] Treatments: CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of concentrate; GSE, 50 mg of grape seed extract per kg of concentrate; GP-5, 5% of dry grape pomace from red wine, as fed.

[2] Grape seed extract composition (g kg^{-1} DM): extractable polyphenols, 330; condensed tannins, 413; anthocyanins, 4.40.

[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 identified fatty acids): C16:0, 11.1; C18:0, 4.41; C18:1, 16.0; C18:2, 61.3; C18:3, 3.69.

[4] UFV = feed unit for maintenance and meat production (FEDNA, 2010).

[5] PDI = protein truly digestible in the small intestine (FEDNA, 2010).

The chemical composition of experimental concentrates (dry matter, DM; ash; crude protein, CP and ether extract, EE) and orts were determined by standard methods (AOAC, 2012). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) analyses were performed by the sequential procedure using an ANKOM^{200} fibre analyser (Ankom Technology Corporation), by the method of Van Soest *et al.* (1991).

All handling practices followed the recommendations of the European Council Directive 2010/63/EU for the protection of animals used for scientific purposes, and all of the animals were able to see and hear other lambs. The experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Valladolid (Spain).

6.2.2. Slaughter and carcass measurements

Lambs were weighed weekly and were slaughtered when they reached 27 kg LBW, by stunning and exsanguination from the jugular vein; they were eviscerated and skinned. The hot carcasses were chilled at 4 °C for 24 h in a cooling room and weighed again, and chilling losses were calculated as the difference between hot (HCW) and cold carcass weight (CCW) expressed as a proportion of the initial HCW. Killing-out percentage was calculated as CCW expressed as percentage of slaughter body weight. Subcutaneous fat thickness (mm), conformation and fatness score were determined. Conformation was assessed according to a EUROP ranking scale using a 15-point scale, from 1 (the worst conformation) to 15 (the best conformation). Fatness score was measured on a 12-point scale, from 1 (the lowest fatness grade) to 12 (the highest fatness grade).

6.2.3. Meat measurements

At 24 h *post mortem*, the pH value of *longissimus thoracis* muscle was measured at the 6^{th} rib site with a pHmeter, equipped with penetrating electrode and temperature probe. At this 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 (0° is red; 90° is yellow), was calculated as arctangent (b^*/a^*), and the chroma (C^*), a measure of colour intensity (0 is dull; 60 is vivid), was computed as $(a^{*2} + b^{*2})^{1/2}$.

The *longissimus thoracis et lumborum* (LTL) muscle was excised from both sides of the carcasses and used to measure muscle colorimetric parameters and to carry out the various analyses.

Meat chemical composition was predicted using a NIRS method. A representative sample of ground LTL muscle was placed in the ring cups of the NIRS machine, avoiding air bubbles. Subsequently, each meat sample was scanned 32 times over the range (400–2500 nm) using a benchtop XDS NIR Rapid Content Analyser (Foss, Hillerød, Denmark), and spectra were averaged by the equipment software. Two meat samples per animal were scanned using two different cells, increasing the area of muscle scanned and reducing the

sampling error. Calibration and validation of the NIRS data were performed using WinISI software for fresh meat.

Meat fat extraction from the LTL was performed using the method of Bligh and Dyer (1959), and fatty acid methyl esters were prepared using the Morrison and Smith (1964) technique. The methyl esters were quantified by gas chromatography using an Agilent Technologies 6890 (PA, USA) gas chromatograph (GC) equipped with a flame ionization detector (FID). Separation was carried out on an HP-88 capillary column (100 m × 0.25 mm, i.d., 0.20 mm film thickness, Agilent Technologies, USA). The GC conditions were as follows: injector and detector temperatures were 240 °C and 300 °C, respectively, and the helium flow ratio was 3 mL min^{-1}. An initial oven temperature of 170 °C was held for 24 min, followed by a rise to 220 °C at a rate of 7.5 °C min^{-1} and a subsequent increase of 10 °C min^{-1} to 230 °C (held for 5 min). Fatty acid methyl esters were identified by comparison with standards run previously alone or together with samples. Fatty acid methyl esters were expressed as percentage of total methyl ester content.

Meat water holding capacity (WHC) of the meat was determined as cooking losses using the method described by Honikel (1998). Samples of LTL, weighing 150 g approx., were thawed overnight at cooler temperature (4 °C). Cooking losses were determined after cooking the sample 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, EEUU) 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 measurement of cooking losses, 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 for all the evaluated strips.

A triangle test was conducted according to ISO 4120 (2004) with the objective of studying whether dietary antioxidant incorporation produces perceptible sensory differences in comparison with the typical organoleptic lamb quality, represented by the control group.

Each panellist was offered three samples simultaneously, two corresponding to the same diet and the third to a different one, and they were asked to taste the samples and identify the odd sample. The comparisons were performed on meat samples of lambs fed different diets *(i.e.* CTRL *vs.* VIT-E; CTRL *vs.* GSE; CTRL *vs.* GP-5; VIT-E *vs.* GSE; VIT-E *vs.* GP-5 and GSE *vs.* GP-5). Sensory assessment was performed by six assessors selected and trained in accordance with the International Standard method for selection, training and monitoring of assessors (UNE-ISO 13300-2, 2008). The assessors performed each comparison in triplicate, thus, 18 replies were completed for comparison. The taste panel performed the trial under controlled conditions, in a booth with a red light to mask colour differences. The sensory analysis was performed as follows: frozen vacuum-packaged LTL muscle samples were thawed for 24 h at 4 °C. The upper and lower ends were cut off at approximately 2 cm below and 2 cm above the respective end points. The central part was then divided into slices (2 cm thick). The slices were broiled on a double-sided griddle preheated at 220 °C until they reached a core temperature of 70 °C, following the guidelines for cooking procedures. After cooking, each slice was wrapped in aluminium foil, codified, distributed in the booths in groups of three in accordance with the triangle test, and kept warm until the time of assessment (15 min). Water and unsalted bread were provided to cleanse the palate of residual flavour notes between samples.

6.2.4. Meat shelf life analysis

Sample preparation

After slicing, LTL chops (about 3 cm thick) from each carcass were placed in trays and were randomly assigned to different storage periods (0, 4, 7, 11 and 14 days). Then the trays were flushed with the selected gas mixture (80:20% / O_2:CO_2), closed by heat-sealing with a packer (TECNOVAC mod: Linvac 400) with a high barrier film (with an oxygen transmission rate of 1.8 cm^3/m^2/24 h/bar at 20 °C and 65% RH, supplied by Fibosa Packaging S.L., Tordera, Spain). The trays were placed randomly in a cabinet illuminated with white fluorescent light (620 lux) at 4 ± 1 °C, simulating retail display conditions for storage. The trays were rotated daily to minimize light intensity differences and possible temperature variations. On each sampling day, the corresponding trays were removed for subsequent analysis. Half of the trays to be analysed at each sampling point in each treatment were used for carrying out microbial analyses and the other half were used for the other measurements.

Microbiological analysis

For microbiological assays, after opening the pack 10 g was taken aseptically from each tray and homogenised with 90 mL of tryptone water (Scharlau, Spain) 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 tryptone 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 of the samples that were performed 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; *Pseudomonas* spp. on Pseudomonas Agar (Oxoid, Spain) supplemented with Cetrimide, Fucidine and Cephaloridine (CFC, Oxoid, Spain) incubated at 30 °C for 48 h; lactic acid bacteria (LAB) on MRS Agar (Scharlau, Spain) incubated at 30 °C for 72 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). The detection limit of the above techniques was 1 log cfu g^{-1} except for pseudomonads, for which the limit was 2 log cfu g^{-1}.

Instrumental colour measurements

Surface instrumental colour of LTL muscle slices was measured during the storage after opening the packages as detailed in 6.2.3 section. The measurements were performed on each slice of muscle three times.

The oxidation state of myoglobin was also measured indirectly in LTL muscle by spectrophotometry during storage for each time. Haem pigment percentages were estimated according to Krzywicki (1979) from 400 to 740 nm. Metmyoglobin (MMb) was calculated as: MMb = (1,395 – a_1), where: $a_1 = \left[\left(D_R^{572} - D_R^{730}\right) / \left(D_R^{525} - D_R^{730}\right)\right]$; D_R = (–log R).

Lipid oxidation analysis

The extent of lipid oxidation in LTL muscle during storage was assessed by measuring thiobarbituric acid-reactive substances (TBARS). TBARS were determined in meat samples

according to the method described by Maraschiello *et al.* (1999). TBA values were expressed as micrograms of malonaldehyde (MDA) per gram of meat.

Sensory evaluation

Sensory analysis was carried out on raw meat. For each sampling day, samples were evaluated for appearance, display of muscle oxidation, presence of off-odours and overall rating of the sample by a panel of six people selected and trained in accordance with the International Standard method for selection, training and monitoring of assessors (UNE-EN ISO 8586, 2014). The taste panel performed the trial under controlled conditions in booths, at 22 °C.

The meat's appearance was assessed in unopened trays, using a structured scale with numerical scores 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 had been removed, the panellists were asked to score odour by sniffing, using a 5-point scale (1, no off-odours; 2, slight off-odours; 3, small off-odours but not spoiled; 4, clearly recognizable off-odours and 5, extremely strong off-odour). Overall rating was also evaluated using a 5-point scale (1, excellent; 2, good; 3, acceptable; 4, fair; 5, unacceptable).

6.2.5. 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 performance, carcass and meat data were subjected to analysis of variance using the general linear model (GLM) procedure of the SAS 9.2. package, 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, GSE and GP-5) and ε_i the error. The LSD test was used to assess the significance between treatment means where the effect was

significant. Additionally, a binomial distribution was used for the results of the sensory analysis (triangle test).

Shelf life data were analysed by repeated measures using the MIXED procedure of the SAS 9.2. package, according to the model $Y_{ijk} = \mu + T_i + D_k + T_iD_k + B_j + \varepsilon_{ijk,}$ where Y_{ijk} is the response variable, μ the overall mean, T_i the dietary treatment (T) effect (CTRL, VIT-E, GSE and GP-5), D_k the sampling day (D), T_iD_k the interaction (T × D), B_j the block effect, and ε_{ijk} the residual error.

For all statistical procedures the statistical significance of differences was defined as $P < 0.05$ and trends as $P < 0.10$.

6.3. Results and discussion

6.3.1. Animal performance and carcass traits

In accordance with the experimental design, initial and final LBW were similar ($P > 0.05$) among experimental groups. As can be seen in Table 6.2, no significant differences ($P > 0.05$) in concentrate and straw dry matter intake (DMI), average daily gain (ADG) and feed conversion were observed between the different experimental treatments. Vitamin E did not affect yield parameters, which is in agreement with other authors (Lauzurica *et al*., 2005) who supplemented growing lamb diets with vitamin E at the same level as was used in this work. The lack of differences in animal performance using grape seed extract and grape pomace is in agreement with other authors using different extracts rich in polyphenols or agro-industrial by-products (Jerónimo *et al*., 2010; Inserra *et al*., 2014).

With regard to the DMI value, the current study showed a numerical difference in favour of GP-5 treatment, resulting in a 4% increase in concentrate DMI. Although grape pomace reduced the energy content of the diet, the lambs reached the same energy intake levels by increasing DM intake. The level of grape pomace used and the fact that all rations displayed the same energy:protein ratio may explain the absence of significant differences with regard to ADG. In some instances, feeds containing high concentrations of CT are not palatable. However, in this study no apparent palatability problems with dried red wine grape pomace were observed. Most researchers believe that the consumption of plant species with high condensed tannin contents (> 50 g kg^{-1} DM) significantly reduces voluntary feed intake, and

could have a clear negative effect on productivity, while medium or low consumption (< 50 g kg^{-1} DM), as employed in the current study, seems not to affect it (Frutos *et al*., 2004).

Table 6.2. Effect of experimental diets on lamb performance and carcass traits.

	Treatments[1]				RSD[2]	*P. value*
	CTRL	VIT-E	GSE	GP-5		
Dry matter intake (g $animal^{-1}$ day^{-1})						
Concentrate	758	751	776	787	80.7	0.685
Barley straw	31.9	28.7	33.4	31.0	9.80	0.682
Total	790	779	810	818	78.4	0.615
Initial live weight (kg)	13.6	14.5	14.3	14.9	2.03	0.391
Final live weight (kg)	27.0	27.1	27.2	27.1	0.65	0.898
Average daily gain (g $animal^{-1}$ day^{-1})	267	268	290	279	47.6	0.592
Days to reach 27 kg	50.5	47.7	46.2	43.6	10.73	0.466
Feed conversion (kg feed DM kg^{-1} gain)	2.90	2.84	2.71	2.88	0.375	0.582
Ruminal pH	5.99	6.07	5.83	6.31	0.548	0.277
Carcass traits						
Hot carcass weight (kg)	12.8	13.0	13.2	12.7	0.55	0.228
Cold carcass weight (kg)	12.4	12.7	12.6	12.4	0.49	0.414
Chilling losses (%)	2.56	2.44	3.45	2.47	1.911	0.498
Carcass yield (%)	46.0	46.7	46.5	45.7	1.69	0.491
Carcass conformation [3]	8.15	7.92	8.00	7.91	0.548	0.661
Fatness score [4]	4.69	4.67	4.85	4.64	1.072	0.963
Kidney knob and channel fat depots (g)	205	253	265	230	74.5	0.198
Subcutaneous fat thickness (mm)	2.50	3.14	2.96	2.48	1.067	0.353

[1] Treatments: CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of concentrate; GSE, 50 mg of grape seed extract per kg of concentrate; GP-5, 5% of dry grape pomace from red wine, as fed.

[2] RSD: residual standard deviation.

[3] Conformation score: 1 = the worst conformation, 15 = the best conformation.

[4] Fatness score: 1 = the lowest fatness grade, 12 = the highest fatness grade.

Moderate concentrations of CT may improve the digestive utilization of feed, mainly as a result of a reduction in protein degradation in the rumen and a subsequent increase in amino acid flow to the small intestine (Frutos *et al*., 2004). Salem *et al*. (2011) indicated better synchronization between energy and protein release in the rumen in the presence of some chemical constituents of plant extracts. These effects on nutrition are usually reflected in ADG. Various factors may be responsible for the lack of a clear effect of GSE and GP-5 on ADG. Higher levels of inclusion of grape seed extract and grape pomace in animal diets and a longer fattening period might be necessary to find any effect on lamb performance.

With regard to carcass characteristics, as shown in Table 6.2, no changes ($P > 0.05$) were attributed to addition of vitamin E, grape seed extract or grape pomace. As there were no differences in DMI and animal performance, it was expected that carcass performance would not be affected. These results are in agreement with Wulf *et al*. (1995), who found no differences in carcass traits between lambs supplemented with the same level of vitamin E as used in the present study and with a higher level. Pétriz-Celaya *et al*. (2010) did not find differences in lamb performance and carcass characteristics as a result of including 10, 20 and 30% of dried grape pomace in the diet, nor did other studies employing various natural substances rich in phenols (Inserra *et al*., 2014). Carcass weight and classification scores for conformation and fatness in carcasses from all dietary treatments were within the normal range for EU lamb production.

6.3.2. Meat quality

Muscular pH did not present significant differences ($P > 0.05$) between experimental treatments (Table 6.3). These results are in agreement with other authors, who did not find an effect on muscle pH when vitamin E (Lauzurica *et al*., 2005) or various polyphenol sources (Morán *et al*., 2012a) were included in lamb diets, and they reflect a regular trend of *post mortem* glycolysis in muscle. According to Inserra *et al*. (2014), these results are supported by the fact that the dietary treatments did not affect either the main performance parameters of the lambs (DMI and ADG), which may have an impact on the pool of muscle glycogen, or the colour of muscle between treatments (Table 6.3). Very little information is available about the effects of grape pomace on meat quality. In any case, the effect of grape pomace in lamb diets on muscle pH did not reflect the inadequate nutrient availability and darker meat that was reported by Priolo *et al*. (2000) when different sources of tannins were used in lamb diets.

Colour is the single most important sensory attribute affecting consumer purchasing decisions with regard to red meat, because consumers associate a red colour with freshness. As for meat and fat colour parameters, changes were not expected, since they are usually associated with differences in pigment deposition or ultimate pH, and no differences were observed for these parameters. Several authors have suggested that supplementation with vitamin E, tannins or other polyphenols can reduce meat colour degradation over time during storage owing to its ability to prevent oxidation of myoglobin (Priolo *et al*., 2000; Gallardo *et al*., 2015). These changes in meat and fat colour associated

with oxidation processes are not apparent for 24 h after slaughter and could explain the lack of differences (P > 0.05) due to vitamin E, grape seed extract and grape pomace supplementation. Some authors have observed that the inclusion of various phenol-rich extracts or CT produces lighter meat because they are iron-chelating agents promoting lower blood haemoglobin concentration and probably lower myoglobin concentration before slaughter. Our results do not support that suggestion and are in agreement with Francisco *et al*. (2015), who did not find differences in meat colour parameters (*L**, *a**, *b**, *C** and *H**) at day 0 of storage when a scrub *(Cistus ladanifer)* rich in CT was included in lamb diets.

Table 6.3. Effect of experimental lamb diets on pH, colour, texture, cooking losses and chemical composition of meat.

	Treatments[1]				RSD[2]	*P. value*
	CTRL	VIT-E	GSE	GP-5		
Meat pH 24 h post-slaughter *L. thoracis R. abdominis* colour	5.61	5.61	5.69	5.65	0.105	0.190
*L**	50.8	51.5	50.3	54.1	4.34	0.174
*a**	9.38	9.17	9.08	8.83	1.757	0.895
*b**	3.02	2.83	2.27	3.64	2.281	0.539
*H**	16.6	16.1	13.6	23.2	14.203	0.415
*C**	10.1	9.72	9.54	10.17	1.610	0.757
L. thoracis et lumborum colour						
*L**	43.6	41.0	42.2	42.2	2.20	0.352
*a**	7.60	8.17	7.20	6.96	1.074	0.338
*b**	11.7	14.0	14.6	14.6	3.67	0.567
*H**	14.0	16.4	16.4	16.3	3.10	0.532
*C**	57.0	57.9	62.6	63.4	7.89	0.487
Subcutaneous fat colour						
*L**	69.2	67.5	68.3	67.5	3.57	0.619
*a**	4.23	4.47	4.53	4.85	1.386	0.740
*b**	10.0[a]	9.31[a]	9.57[a]	7.99[b]	1.791	0.053
*H**	66.9	64.2	64.9	57.9	14.20	0.415
*C**	10.9	10.4	10.6	9.95	1.311	0.329
Warner-Bratzler shear force (kgF cm^{-2})	4.47	3.61	4.13	3.43	0.989	0.218
Cooking losses (%)	19.9[a]	14.0[b]	17.9[a]	20.6[a]	3.22	0.003
Chemical composition						
Moisture (%)	75.7	75.6	75.7	76.2	0.58	0.110
Fat (% DM)	10.9	10.9	10.5	9.5	1.61	0.149
Protein (% DM)	86.4	87.1	87.3	87.7	2.22	0.575

[1] Treatments: CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of concentrate; GSE, 50 mg of grape seed extract per kg of concentrate; GP-5, 5% of dry grape pomace from red wine, as fed.

[2] RSD: residual standard deviation

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

Meat shear force measurements in lambs fed vitamin E, grape seed extract or grape pomace were within acceptable values and no statistical differences were found between treatments ($P > 0.05$) (Table 6.3). These results are in disagreement with some studies that observed lower shear force values in meat from lambs supplemented with vitamin E or polyphenols compared with control animals, which was associated with the protection exerted by antioxidants against endogenous proteases (Morán *et al*., 2012b). A significant reduction in shear force has been reported in muscles with high ultimate pH. Our results showed ultimate pH below 6 and no differences between treatments were found in ultimate pH, which corresponded to the lack of differences in muscle shear force.

Meat oxidation reduces the WHC between muscle myofibrils, which increases juice loss from the meat and, as a result, meat lightness. It has been reported that the use of antioxidants might improve WHC, avoiding loss of membrane integrity and protein cross-link with oxidative processes. That statement is in agreement with the present results, since WHC measured by cooking losses (Table 6.3) was higher ($P < 0.05$) in lambs fed vitamin E than the other dietary treatments. It is in agreement with Morán *et al*. (2012a), who found higher meat WHC when vitamin E was included in lamb diets. GSE and GP-5 did not exert a significant effect ($P > 0.05$) on WHC compared with CTRL, which is in agreement with other authors who included various polyphenol sources in lamb diets (Priolo *et al*., 2000; Andrés *et al*., 2014a). However, Morán *et al*. (2012a) found higher meat WHC when carnosic acid (natural polyphenol) was included in lamb diets. This discrepancy could be due to the different doses of antioxidants in the diets or to the cooking method used, which provides high variability in this parameter.

As can be seen in Table 6.3, the chemical composition of LTL meat samples is within the range obtained by other authors (Manso *et al*., 2009) and was not affected significantly ($P > 0.05$) by the dietary treatments. Other studies have shown that the inclusion of vitamin E (Morán *et al*., 2012a) or plants rich in polyphenols (Priolo *et al*., 2000) did not significantly affect protein, fat and moisture values of lamb meat. However, our results showed that the numerical value of fat content from the GP-5 group was lower than that of the other groups. This result is in agreement with Vasta *et al*. (2007), who observed lower ether extract content when lambs received tannins in their diets compared with a control group.

The fatty acid composition of LTL muscle from each dietary treatment is reported in Table 6.4. The values of most fatty acids observed in our trial were within the reference ranges obtained in similar breed and production systems (Manso *et al*., 2009). The saturated fatty acids (SFA) and *cis*-MUFA (monounsaturated fatty acids) made up the majority of the

fatty acids, as is expected in lamb meat, with oleic acid (*cis-9* C18:1) being the most abundant FA, followed by palmitic acid (C16:0) and stearic acid (C18:0).

Table 6.4. Effect of experimental lamb diets on intramuscular fatty acid composition (% of identified fatty acids).

	Treatments[1]				RSD[2]	*P. value*
	CTRL	VIT-E	GSE	GP-5		
C10:0	0.24	0.16	0.18	0.17	0.145	0.497
C12:0	0.23	0.24	0.25	0.26	0.076	0.759
C14:0	3.28	3.08	3.32	3.13	0.568	0.681
C16:0	23.4	23.0	23.2	22.6	1.05	0.292
C16:1	2.35	2.30	2.39	2.17	0.276	0.258
C17:0	1.28	1.36	1.31	1.16	0.296	0.438
C17:1	0.73	0.81	0.80	0.69	0.163	0.222
C18:0	14.6	14.0	14.4	15.2	1.53	0.309
cis-9 C18:1	37.4	37.5	38.0	36.1	2.11	0.193
trans-11 C18:1 (VA)	1.96	1.99	2.04	2.00	0.248	0.867
cis-9 cis-12 C18:2 n6	9.5	10.0	9.1	10.7	1.91	0.198
cis-9 trans-11 C18:2 (RA)	0.40	0.40	0.40	0.43	0.084	0.771
C18:3 n3	0.55	0.60	0.57	0.59	0.089	0.448
C20:0	0.09	0.08	0.08	0.09	0.011	0.124
C20:1	0.10	0.09	0.09	0.09	0.015	0.554
C20:4 n6	2.74	2.92	2.72	3.18	0.703	0.370
C20:5 n3 (EPA)	0.30	0.25	0.25	0.27	0.155	0.817
C22:4 n6	0.22	0.23	0.22	0.26	0.065	0.341
C22:5 n3 (DPA)	0.53	0.58	0.55	0.65	0.135	0.179
C22:6 n3 (DHA)	0.20	0.26	0.23	0.25	0.079	0.145
Ratios[3]						
SFA	43.1	41.9	42.7	42.6	1.75	0.418
MUFA	42.5	42.7	43.3	41.1	2.23	0.112
PUFA	14.4[a]	15.3[ab]	13.5[a]	16.4[b]	2.60	0.068
PUFA:SFA	0.34	0.37	0.32	0.39	0.070	0.102
n3	1.57	1.69	1.60	1.76	0.348	0.525
n6	12.5	13.2	12.1	14.2	2.53	0.202
n6:n3	8.19	7.90	7.59	8.14	1.299	0.639

[1] Treatments: CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of concentrate; GSE, 50 mg of grape seed extract per kg of concentrate; GP-5, 5% of dry grape pomace from red wine, as fed.

[2] RSD: residual standard deviation

[3] SFA (saturated fatty acids) = C10:0 + C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0; MUFA: (monounsaturated fatty acids) = C16:1 + C17:1 + *cis-9* C18:1 + *trans-11* C18:1 + C20:1; PUFA: (polyunsaturated fatty acids) = *cis-9 cis-12* C18:2 n6 + *cis-9 trans-11* C18:2 + C18:3 n3 + C20:4 n6 + C20:5 n3 + C22:4 n6 + C22:5 n3 + C22:6 n3; n3 = C18:3 n3+ C20:5 n3+ C22:5 n3+ C22:6 n3; n6 = *cis-9 cis-12* C18:2 n6 + C20:4 n6 + C22:4 n6.

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

In general terms, the incorporation of vitamin E, grape seed extract and grape pomace in the lamb diet had minimal effects on the SFA, PUFA and n3 fatty acid contents of intramuscular meat.

In contrast, there are reports of beneficial effects of vitamin E on meat FA composition, reducing SFA and promoting enrichment in PUFA and n3 fatty acids of intramuscular fat, probably owing to its protective effect against PUFA peroxidation. Indeed, the effect of antioxidant sources on fatty acid profile was more evident when unsaturated sources of fatty acids, such as flaxseed, were used in diets (Andrés *et al*., 2014b; Francisco *et al*., 2015). In the present experiment, no additional unsaturated fats were used in the diets. The available concentration of antioxidants within the muscle to delay oxidation in regular meat may have been sufficient in the control group, and this might explain the lack of significant differences in fatty acids between treatments.

GSE and GP-5 did not show statistically significant differences ($P > 0.05$) in meat FA profile when they were compared with the other groups, which is in agreement with Muíño *et al*. (2014), who reported that the inclusion of red wine extract did not significantly modify the meat FA profile compared with a control group. In contrast, the use of plants or extracts rich in polyphenols seems to reduce the concentration of SFA and improve the nutritional profile of the meat (Andrés *et al*., 2014b).

It should be noted that meat from GP-5 lambs tended ($P < 0.10$) to increase the PUFA content in comparison with CTRL and GSE treatments. This could be attributed to the high concentration of *cis-9 cis-12* C18:2 in grape pomace. Our results showed that the numerical value of LTL muscle fat content from the GP-5 group was lower than that of the other groups. Therefore, differences in PUFA content in the GP-5 treatment could be ascribed mostly to differences in intramuscular fat content, with the resulting variation in the triglyceride-to-phospholipid ratio, rather than to effects on microbes involved in rumen biohydrogenation (BH) or to a higher protective effect of CT or polyphenols of grape pomace against PUFA peroxidation.

With regard to n3 fatty acids, in spite of lack of statistical differences ($P > 0.05$), docosahexaenoic acid (DHA, C22:6 n3) and docosapentaenoic acid (DPA, C22:5 n3) increased in VIT-E, GSE and GP-5 treatments when they were compared with the CTRL group. This is in agreement with Muíño *et al*. (2014), who showed that meat from lambs supplemented with red wine extract or vitamin E had numerically higher values of very-long-chain n3 fatty acids than a non-antioxidant-supplemented group. Morán *et al*. (2013), in lamb

diets supplemented with another polyphenol (carnosic acid), also found a higher concentration of DHA ($P < 0.10$) in intramuscular fat. According to those authors, this finding could be interesting because lamb meat is very low in highly unsaturated fatty acid, however no explanation for these results has been found in the literature.

With regard to the effects of the treatments on rumenic acid (RA, *cis-9 trans-11* C18:2), the main health-promoting conjugated linoleic acid (CLA) isomer, GP-5 presented the greatest values in comparison with the other treatments, although the differences were not statistically significant ($P > 0.05$). As has been reported previously, grape pomace contains oil characterized by a high content of linoleic acid. This could explain our results as it is known that linoleic acid enhances the accumulation and ruminal outflow of vaccenic acid (VA, *trans-11* C18:1) and RA.

A wide range of plant extracts rich in tannins and other phenolic compounds has been identified with antibacterial and rumen-modulating properties modifying the extent of BH of dietary PUFA. In this respect, the ability of phenolic compounds to increase CLA in lamb meat is highly controversial, probably because of differences in the type and dosage rate employed. Some studies have reported that some polyphenols inhibit the last step of BH and are an efficient way of facilitating a higher rumen output of PUFA, CLA and VA (Vasta *et al*., 2009). Furthermore, some other studies have reported a higher endogenous synthesis of CLA, since this fatty acid can also be generated in animal tissues through VA conversion by the action of Δ^9-desaturase enzyme (Vasta *et al*., 2009).

Despite all the knowledge that exists, it is not yet possible to come to an unequivocal conclusion about the effect of phenolic compounds on meat FA composition. The increase in PUFA and CLA contents in the GP-5 group is interesting, because their nutritional value in the human diet is well recognized as beneficial and increased consumption is recommended. However, the low level of inclusion of grape seed extract and grape pomace in the animal diets and the short fattening period (47 days) could explain the lack of a clear effect of grape seed extract or grape pomace on the fatty acid composition of lamb meat.

With regard to meat sensory results, according to the significance table used to analyse the results of triangle tests, for statistical significance at the 5% level the number of correct replies needed to be 12. Results of the triangle test (Table 6.5) show that, in comparison with the control group, the only treatment identified as different was GSE. This group was significantly differentiated ($P < 0.05$) when it was compared with any of the other groups ($P < 0.05$; a total of 12 of the 18 replies correctly identified the different samples). Tasters also

found significant differences between GP-5 and VIT-E treatment ($P < 0.05$; in 12 of the 18 comparisons the odd sample was correctly identified). Priolo *et al*. (1998) reported that trained panellists were unable to distinguish between meat from lambs fed a diet with carob pulp (rich in CT) and meat of lambs fed a control diet, and Chaves *et al*. (2008) did not find differences in sensory meat attributes when various polyphenol sources were included in lamb diets. Also, Muíño *et al*. (2014) did not find significant differences in lamb meat after slaughter when vitamin E or red wine extract were included in diets. In contrast with these results, Priolo *et al*. (2009) and Schreurs *et al*. (2007) reported that lamb meat flavour and odour are affected by CT when the diets are supplemented with 10 % of quebracho or supplied with 33 g day^{-1} of grape seed extract as liquid supplement, respectively. In those works, the dietary CT reduced the typical sheep meat odour and flavour. In this respect, some studies have indicated that dietary tannin supplementation reduces ruminal biosynthesis of skatole and its accumulation in meat (Priolo *et al*., 2009), providing more favourable lamb meat odour values.

Table 6.5. Effect of experimental lamb diets on sensory evaluation by triangle test of meat.

Compared treatments[1]	Number of comparison tests	D	
CTRL *vs.* VIT-E	18	7	(ns)
CTRL *vs.* GSE	18	12	(*)
CTRL *vs.* GP-5	18	3	(ns)
VIT-E *vs.* GSE	18	12	(*)
VIT-E *vs.* GP-5	18	12	(*)
GSE *vs.* GP-5	18	12	(*)

[1] Treatments: CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of concentrate; GSE, 50 mg of grape seed extract per kg of concentrate; GP-5, 5% of dry grape pomace from red wine, as fed.

D, number of comparison test that were able to distinguish the meats.

ns, non significant effect ($P > 0.05$); *, ($P < 0.05$).

6.3.3. Meat shelf life

Microbial results

Table 6.6 summarizes the results of the microbial analysis of LTL muscle slices packaged under a gas mixture (80:20% / O_2:CO_2) during refrigerated storage, from lambs belonging to the various experimental dietary treatments.

Table 6.6. Effect of experimental lamb diets and storage time on microbial counts (log cfu g^{-1}) on m. *longissimus thoracis et lumborum* from lambs during refrigerated storage at 2 ºC.

Microorganisms	Days	Treatments[1]				SED[2]	*P. value*[3]		
		CTRL	VIT-E	GSE	GP-5		T	D	T × D
Total viable counts	0	$^{A}2.48$	$^{A}2.68$	$^{A}2.12$	$^{A}2.44$	0.328	0.006	<0.001	0.043
	4	$^{A}2.26$	$^{A}2.25$	$^{AB}3.00$	$^{AB}2.28$				
	7	$^{B}3.89$	$^{AB}3.51$	$^{B}3.92$	$^{B}3.48$				
	11	$^{C}5.69^{a}$	$^{B}4.18^{b}$	$^{C}6.24^{a}$	$^{C}6.14^{a}$				
	14	$^{C}6.08^{a}$	$^{B}4.43^{b}$	$^{D}8.03^{c}$	$^{D}7.89^{c}$				
Enterobacteria	0	$^{A}1.00$	1.00	$^{A}1.00$	$^{A}1.35$	0.352	0.006	<0.001	0.568
	4	$^{A}1.00$	1.00	$^{AB}2.16$	$^{A}1.00$				
	7	$^{AB}2.12$	1.00	$^{AB}2.32$	$^{AB}1.75$				
	11	$^{B}3.40^{ab}$	1.85^{a}	$^{BC}3.50^{b}$	$^{B}3.24^{ab}$				
	14	$^{B}3.70^{a}$	1.57^{b}	$^{C}4.51^{a}$	$^{B}3.08^{ab}$				
Lactic acid bacteria	0	$^{AB}2.20$	1.97	$^{A}1.92$	$^{A}2.15$	0.535	0.052	0.001	0.242
	4	$^{A}1.60$	1,56	$^{A}2.05$	$^{A}1.60$				
	7	$^{AB}3.62$	2.27	$^{A}2.39$	$^{AB}3.41$				
	11	$^{AB}3.64$	2.36	$^{AB}4.52$	$^{A}2.52$				
	14	$^{B}4.30^{a}$	1.60^{b}	$^{B}6.71^{a}$	$^{B}5.29^{a}$				
Pseudomonads	0	$^{A}2.00$	$^{A}2.00$	$^{A}2.00$	$^{A}2.00$	0.349	<0.001	<0.001	0.026
	4	$^{A}2.00$	$^{A}2.00$	$^{A}2.00$	$^{A}2.00$				
	7	$^{B}3.65$	$^{AB}2.73$	$^{B}3.80$	$^{B}3.66$				
	11	$^{BC}5.01^{a}$	$^{AB}2.67^{b}$	$^{C}5.79^{a}$	$^{C}5.71^{a}$				
	14	$^{C}5.91^{a}$	$^{B}3.85^{b}$	$^{D}7.90^{c}$	$^{D}7.95^{c}$				
Brochothrix thermosphacta	0	1.00	1.00	$^{A}1.00$	1,00	0.161	0.524	0.192	0.673
	4	1.00	1.00	$^{A}1.00$	1.00				
	7	1.00	1.00	$^{A}1.00$	1.00				
	11	1.00^{a}	1.42^{ab}	$^{B}2.06^{b}$	1.00^{a}				
	14	1.00	1.00	$^{A}1.00$	1.00				

[1] Treatments: CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of concentrate; GSE, 50 mg of grape seed extract per kg of concentrate; GP-5, 5% of dry grape pomace from red wine, as fed.

[2] SED: standard error of the difference.

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

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

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

The initial bacterial load is important for determining the shelf life of meat. A high number of microorganisms in meat before storage shortens the shelf life, since the microorganism limit will be achieved more rapidly (Berruga *et al.,* 2005). In our work, no differences between treatments were observed initially in the lamb meat before storage. As has been widely reported, atmospheres containing 20% CO_2 control the growth of bacteria, but modified atmosphere packaging with a high concentration of oxygen allows the growth of aerobic microorganisms in refrigerated storage conditions, associated with meat spoilage (Buys *et*

al., 2000). Therefore, as expected, all the microbial populations increased significantly during refrigerated storage ($P < 0.05$). However, the evolution of the microbial groups studied varied according to the treatment. When counts were above 7 log cfu g^{-1} the product was considered unsuitable for consumption, according to the limit for total microbial count for cuts of meat established by the International Commission on Microbiological Specifications for Foods (ICMSF, 1986), since higher microbial loads lead to sensory loss due to off-flavours, off-odours and slime. This level has previously been suggested as being indicative of bacterial spoilage. Counts of TVC remained stable for all the dietary treatments from the beginning to the seventh day of storage, when they began to increase significantly ($P < 0.05$). However, while the counts in meat from the CTRL, GSE and GP-5 groups continued increasing until the end of storage, VIT-E values remained constant from day 7 onwards. As a result, counts of this microbial group presented significantly lower ($P < 0.05$) average values in meat from VIT-E treatment than average values from the CTRL, GSE and GP-5 groups from 11 days of storage. Furthermore, the VIT-E treatment did not reach counts above the limit of 7 log cfu g^{-1}, while the other treatments showed counts exceeding this limit at 14 days of storage. Enterobacteria counts, generally considered a good hygiene indicator, began to increase significantly ($P < 0.05$) after 11 days of storage. From this sampling point, meat from the VIT-E treatment presented significantly lower ($P < 0.05$) average values than the other treatments. The final values of the VIT-E treatment in both microbial groups (TVC and enterobacteria) were much lower than those reported by Berruga *et al.* (2005), who obtained counts similar to those observed in our study for the CTRL, GSE and GP-5 treatments. With regard to lactic acid bacteria, which behave as facultative anaerobes and are able to grow under relatively high concentrations of CO_2, no statistical differences were detected between treatments from 0 to 11 days of storage. At day 14, VIT-E showed lower ($P < 0.05$) values than the other treatments. The behaviour of *Pseudomonas* spp. was similar in all groups, beginning to increase from day 7 to the end of storage, when the highest values were reached. However, from day 11 the VIT-E treatment showed lower values than the others ($P < 0.05$).

Our results seem to indicate that vitamin E was effective ($P < 0.05$) in preventing microbial development. In contrast, Lauzurica *et al.* (2005), using vitamin E in lamb diets, reported that dietary vitamin E supplementation did not affect microbial growth in meat packaged under modified atmosphere. Similarly, although most studies on the effect of dietary vitamin E observe an improvement in pigment and lipid stability, no important effects of vitamin E on microbial growth during storage have been reported (Ripoll *et al.,* 2011). Since we have not found evidence in the literature of the presence of specific antibacterial

compounds resulting from vitamin E dietary treatment, we can only hypothesize that these antibacterial effects might be related to its antioxidant capacity.

It is interesting to note that no statistical differences ($P > 0.05$) in microbial results were observed between the polyphenol treatments (GSE and GP-5) and the CTRL group. These results are in agreement with Morán *et al.* (2012a), who did not find that inclusion of rosemary polyphenols in lamb diets had an effect on microbial spoilage. In contrast to our results, Rota *et al.* (2008) provided evidence for the efficacy of polyphenols as antimicrobial agents, capable of altering bacterial cell membranes and microbial enzymatic metabolism with high antibiotic activity. In this regard, several studies have shown that dietary supplementation with rosemary (Ortuño *et al.,* 2014) or quercetin flavonoid (Andrés *et al.,* 2013) reduced microbial populations responsible for meat spoilage during storage owing to the accumulation of these compounds in the muscles during the life of animals (Raccach, 1984). In this regard, Reddy *et al.* (2013) reported that the addition of grape seed extract to mutton slices significantly reduced total psychrophilic and coliform counts in meat during refrigerated storage; therefore, grape by-products could exert a protective effect against microbiological spoilage. In any case, most of these studies lack crucial information for assessing dose effects, particularly concerning the degree of degradation of active polyphenol compounds in the feed given to lambs, which could explain our results. Given that the cut-off point for microbiological shelf life was set at a bacterial count of 7 log cfu g^{-1}, the microbiological shelf life of meat from the VIT-E group would be longer than that of meat from the polyphenol treatments (GSE and GP-5) and CTRL.

Colour coordinates and metmyoglobin percentage

Results of the colour measurements and metmyoglobin haem pigment percentage are shown in Figure 6.1. In general, storage time significantly affected the evolution of meat colour parameters. Modified atmosphere with a high proportion of oxygen enhances meat colour owing to the formation of oxymyoglobin, which maintains the desirable bright red colour of meat and which appears in approximately the upper 5 mm of the meat cut. Thus high O_2 levels prolong the colour of the meat before metmyoglobin becomes visible on the surface, which is responsible for meat browning (Fernandes *et al.,* 2014).

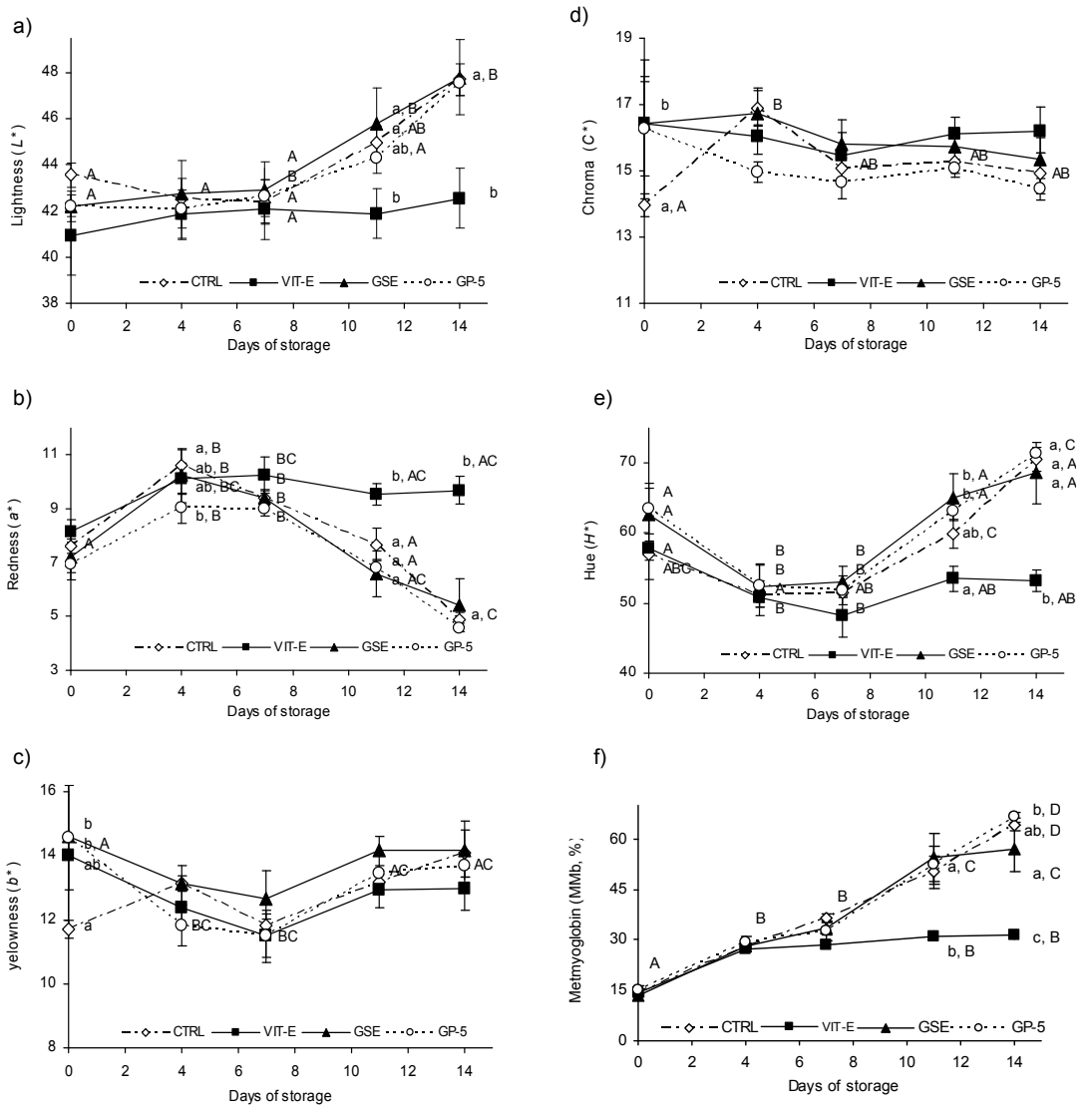


Figure 6.1. Effect of experimental lamb diets (CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of concentrate; GSE, 50 mg of grape seed extract per kg of concentrate; GP-5, 5% of dry grape pomace from red wine, as fed) and storage time on evolution of colour parameters: lightness (a), redness (b), yellowness (c), chroma (d), hue (e) and metmyoglobin haem pigment (f) on m. *longissimus thoracis et lumborum* 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.

The behaviour of the colour coordinates was fairly similar for all treatments during the first days of storage. In this regard, lightness (L^*), yellowness (b^*) and hue angle (H^*)

remained nearly stable during the first 7 days, while the redness parameter (*a**) increased during the first 4 days of storage and then decreased. Although the intensity of the red colour (chroma, *C**) remained stable throughout the trial, it reached higher numerical values between days 0 and 4 of storage, following a similar tendency to *a**. These results agree with similar studies regarding the evolution of colour in lamb meat during retail storage under modified atmosphere packaging (Bodas *et al.,* 2012; Fernandes *et al.,* 2014) as time of storage progresses.

From day 7 onwards, lightness (*L**) generally increased with time of storage. With regard to the dietary treatment effects, lower *L** average values were obtained in the VIT-E treatment throughout the complete storage period when compared with CTRL, GSE and GP-5 (41.86 vs. 44.23, 44.29 and 43.76, respectively), but the difference was only statistically different ($P < 0.05$) from 11 days of storage. Lamb colour stabilization through dietary vitamin E has also been reported in other studies with lambs (Karami *et al.,* 2011; Ripoll *et al.,* 2011). Vitamin E is able to improve the overall muscle antioxidant status by lowering the formation of some oxidation markers and consequently extending meat colour stability (Descalzo *et al.,* 2007). The stabilization and improvement of meat colour by vitamin E supplementation is not completely understood, but it has been speculated that it is principally due to its ability to prevent the oxidation of myoglobin and/or oxymyoglobin to metmyoglobin and thus meat discoloration (Morrissey *et al.,* 1998). Some authors have observed that dietary inclusion of phenolic compounds produces lighter meat because they are iron-chelating agents promoting a lower blood haemoglobin concentration and probably lower myoglobin concentration before slaughter (Samman *et al.,* 2001). Our results do not support that suggestion and are in agreement with various authors (Bodas *et al.,* 2012; Andrés *et al.,* 2013) who did not find statistically significant differences in *L** in lamb meat supplemented with polyphenol-rich substances (naringin and quercetin, respectively).

Redness (*a** value) is one of the most important colour parameters for evaluating meat oxidation, since consumers prefer fresh, red-coloured meat. In the present trial, lambs fed VIT-E showed higher overall *a** values ($P < 0.05$) than lambs fed CTRL, GSE and GP-5 (9.55 vs. 8.03, 7.77 and 7.27, respectively), but the difference was only significant at 11 and 14 days of storage, similarly to what was found by Lauzurica *et al.* (2005) when they included vitamin E in lamb diets. Our results contrast with Karami *et al.* (2011) and Atay *et al.* (2009), who did not report differences in *a** index when lambs received vitamin E supplementation compared with a control group. The GSE and GP-5 supplementation did not affect the *a** value, which is in agreement with several authors (Karami *et al.,* 2011; Andrés *et al.,* 2013), who did not find statistically significant differences in *a** in lamb and

goat meat supplemented with different natural sources of polyphenols compared with a control group. However, other studies (Luciano *et al.,* 2009; Ortuño *et al.,* 2014) have reported lower decreases in redness in meat from lambs fed a phenol-rich source compared with meat from lambs fed a control diet, probably as a result of decreased myoglobin oxidation by the phenolic presence. No effect of dietary treatment ($P > 0.05$) on yellowness (b^*) was observed, which is in line with what was reported by Atay *et al.* (2009), who did not find differences in b^* when they included vitamin E in lamb diets compared with a control group. In contrast, Karami *et al.* (2011) reported higher b^* values in vitamin E fed goats compared with a control group. Like Bodas *et al.* (2012), we did not observe b^* differences resulting from the addition of dietary phenolic compounds. However, the present results contrast with other studies (Luciano *et al.,* 2009; Andrés *et al.,* 2013), which reported lower b^* values during storage in meat of lambs fed with different polyphenol sources compared with a control group.

There were no significant differences in chroma ($P > 0.05$) among the groups, which is in agreement with Andrés *et al.* (2013) in meat of lambs fed with polyphenols. However, these results contrast with other authors (Ortuño *et al.,* 2014) who recorded higher C^* values in meat from animals supplemented with polyphenols in their diets, related to lower meat discoloration. Hue angle (H^*) provides a more realistic view of meat browning than individual colour coordinates (Luciano *et al.,* 2009). In this regard, Ripoll *et al.* (2008) stated that human evaluators are not able to appreciate individual L^*, a^*, b^* coordinates, but they are able to understand real colour (hue); thus an increase in H^* values over time is considered a good descriptor of meat browning as it correlates well with the visual appraisal of meat discoloration and with the accumulation of MMb on the meat surface (Luciano *et al.,* 2011). Our data showed that the administration of vitamin E reduced ($P < 0.05$) the rate of increase in H^* values over time compared with the other diets from day 11; therefore, VIT-E seems to delay meat discoloration. Our results are in agreement with Ripoll *et al.* (2011), who found lower H^* values in lambs fed with vitamin E compared with a control group. However, the present results contrast with Karami *et al.* (2011), who found higher H^* in meat from goats supplemented with vitamin E. Our results are also in accordance with previous works that showed that colour of meat was not affected by dietary phenol sources (O'Grady *et al.,* 2008). The lack of differences ($P > 0.05$) in H^* index between the GSE and GP-5 groups compared with CTRL could be explained by the greater variability values. This response contrasts with results reported by other authors (Luciano *et al.,* 2011; Jerónimo *et al.,* 2012; Morán *et al.,* 2012a) employing polyphenol-rich plants, who found lower H^* values in meat from lambs fed a diet supplemented with these phenolic substances, owing to the protective effect of these substances against meat discoloration, preventing MMb formation.

The results of the colorimetric parameters, particularly *H** values, are consistent with the percentages of MMb observed in the VIT-E treatment, since lower percentages of MMb were observed ($P < 0.05$) in this group after 11 days of storage, when all the treatments except VIT-E reached values above the limit of 40% that has been reported to be a level that causes rejection. Similar results were observed by Lauzurica *et al.* (2005), who reported a delay in MMb formation when they included vitamin E in lamb diets. With regard to the polyphenol treatments, it should be noted that, despite showing percentages above the limit of acceptance, GSE presented lower values than GP-5 at 14 days of storage, while CTRL values were intermediate. In this regard, Luciano *et al.* (2011) reported that consumption of tannins could improve the colour stability of lamb meat, since meat from lambs fed with a polyphenol source presented lower increases in MMb percentage compared with meat from animals fed with a control diet.

Lipid oxidation

Initial TBARS concentrations were not significantly different between the four experimental treatments, ranging from 0.03 to 0.09 μg MDA g^{-1} muscle. As expected, meat lipid oxidation increased strongly ($P < 0.05$) with storage time (Figure 6.2), reaching high MDA values, probably as a result of the oxidizing conditions during storage (high O_2:CO_2 atmosphere and intense lighting). The limiting threshold for oxidized meat acceptability varies according to the animal and the study. In lambs, a TBA value of 4.4 mg MDA kg^{-1} meat was taken to mark the initiation of lipid oxidation/rancidity by Soldatou *et al.* (2009). Dietary administration of antioxidants could be an interesting strategy because, for instance, supplementing animal diets with vitamin E has been extensively shown to effectively enhance the resistance of meat to oxidative deterioration. Vitamin E is the primary lipid-soluble antioxidant in biological systems and breaks the chain of lipid oxidation in cell membranes (Buckley *et al.,* 1995). In the present study, vitamin E supplementation in the diet was effective in preventing muscle MDA formation during storage in modified atmosphere packaging, since significantly lower ($P < 0.05$) TBARS values were found from day 7 onward with respect to the other treatments. These findings are consistent with results found by other authors in sheep dietary studies on α-tocopheryl acetate (Lauzurica *et al.,* 2005; Ripoll *et al.,* 2011; Muíño *et al.,* 2014), who reported that dietary vitamin E inhibited MDA formation powerfully in lamb meat, especially during longer retail display periods.

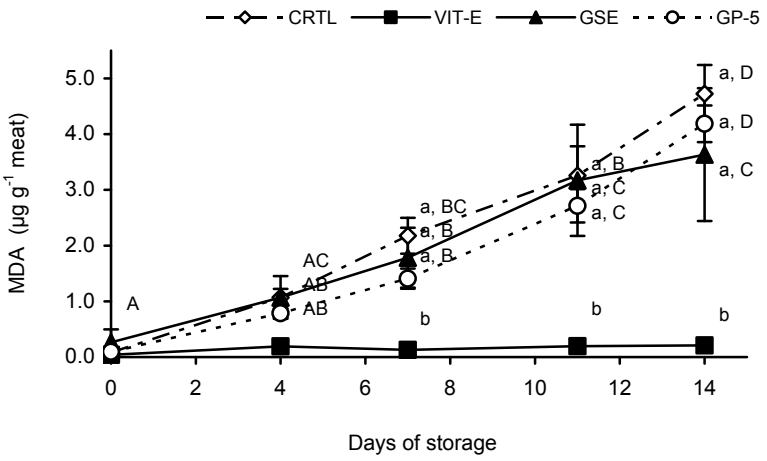


Figure 6.2. Effect of experimental lamb diets (CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of concentrate; GSE, 50 mg of grape seed extract per kg of concentrate; GP-5, 5% of dry grape pomace from red wine, as fed) and storage time on evolution of TBARS (µg g^{-1} meat) on m. *longissimus thoracis et lumborum* 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.

On the other hand, a number of secondary compounds in plants, such as phenolic compounds and essential oils, possess antioxidant properties, and therefore their use as natural antioxidants in animal feeding could be promoted. In this regard, several studies have indicated that polyphenols in diets of small ruminants, such as rosemary or its derivatives (Morán *et al.,* 2012a; Ortuño *et al.,* 2014), or other phenol-rich substances (Karami *et al.,* 2011; Andrés *et al.,* 2014a) clearly delayed lipid oxidation of meat in retail display conditions, reducing MDA formation. In the current study, as seen in Figure 6.2, lower numerical values of TBARS, although not significant ($P > 0.05$), were found in the GSE and GP-5 groups from day 7 of storage compared with CTRL, achieving an MDA percentage improvement of 18.3% and 35.3% at day 7, 2.8% and 16.6% at day 11, and 23.3% and 11.4% at day 14, for the GSE and GP-5 treatments, respectively, with respect to CTRL, which could be attributed to the phenolic content of the GSE and GP-5 diets. The lack of significant differences in our study could be due to the doses employed, the time of feeding and the meat oxidative susceptibility. Indeed, Jerónimo *et al.* (2012) indicated that the inclusion of different polyphenol sources in lamb diet, including grape seed extract, reduced meat lipid oxidation during storage. In that study the diets were supplemented with vegetable oil, making the meat more prone to oxidation.

It is interesting to note that, in spite of the lack of significant differences in oxidation stability between VIT-E and wine by-products (GSE and GP-5) at the end of storage, the meat from antioxidant treatments (VIT-E, GSE and GP-5) did not reach the rancidity threshold established (4.4 μg MDA g^{-1} meat), but this level was exceeded by the CTRL treatment. This finding could be an indication of the antioxidant effects of wine by-product additives.

This effect of natural antioxidants, in particular phenolic substances, has been attributed to their ability to attenuate oxidative damage of a tissue indirectly by enhancing the natural defences of the cell and/or directly by scavenging the free radical species or through activation of antioxidant enzymes, combating disorders generated by phytochemical reactive oxygen species (ROS) (Du *et al.,* 2010). Although several studies (Moñino *et al.,* 2008; Luciano *et al.,* 2011) have shown that their dietary inclusion favoured the antioxidant stability of meat, their mechanisms of action remain to be established. Direct antioxidant activity of dietary polyphenols would imply their absorption through the gastrointestinal tract and their transfer to tissues (Luciano *et al.,* 2009). 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 *et al.,* 2004). Hydrolysis of polymers into compounds with a low degree of polymerization or monomers would make their absorption possible. However, the effect of dietary polyphenols on meat oxidative stability may be indirect, through interaction between phenols and other antioxidant compounds or pro-oxidant compounds present in meat.

Sensory evaluation

Figure 6.3 shows the results of sensory evaluation of the LTL muscle slices packaged under a gas mixture (80:20% / O_2:CO_2) during refrigerated storage. As expected, no differences were found between treatments ($P > 0.05$) in the recently cut meat (day 0), all of the samples having a score of 1. In general, sensory quality decreased gradually during the whole period of storage and reached the highest scores at the end of the experimental period. However, the changes in deterioration scores varied between dietary treatments. It should be noted that for all attributes the mean score of 3 was considered as the borderline of consumer acceptability, following the guidelines of Land and Shepherd (1988).

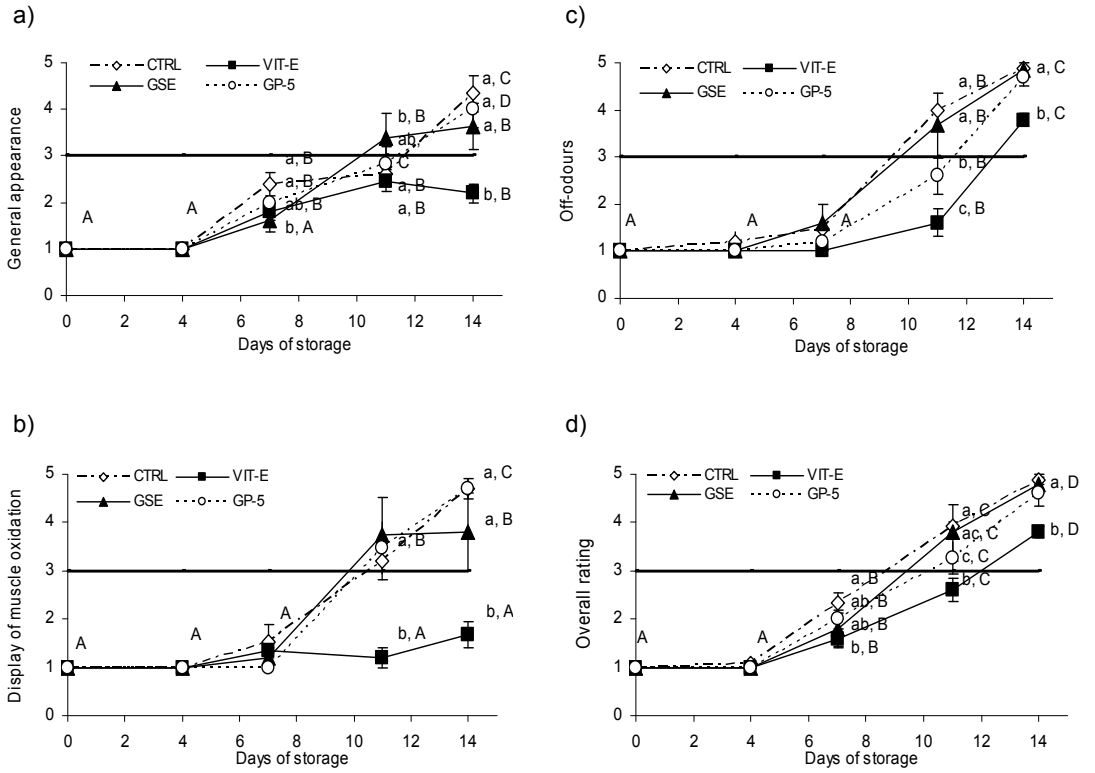


Figure 6.3. Effect of experimental lamb diets (CTRL, without grape pomace; VIT-E, 500 mg of vitamin E per kg of concentrate; GSE, 50 mg of grape seed extract per kg of concentrate; GP-5, 5% of dry grape pomace from red wine, as fed) and storage time on general appearance (a), display of muscle oxidation (b), off-odours (c) and overall rating (d) evaluated by a panel of trained members on m. *longissimus thoracis et lumborum* 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.

At the day 7 sampling point, GSE showed lower general appearance scores than CTRL and GP-5, VIT-E scores being intermediate and not different. However, this potential antioxidant effect of GSE disappeared at the end of storage, when only lower scores of VIT-E were detected ($P < 0.05$). From day 11 onwards the perception of discoloration was lower in VIT-E than in the other groups, which had already reached scores above 3 at this sampling point. These results are consistent with the relative metmyoglobin percentage observed, since at that sampling point the values reached for all groups except VIT-E were higher than 40%, the limit established by several works as acceptable.

Similarly, the presence of off-odour increased gradually and significantly during storage in all groups. However, after 11 days of display VIT-E showed significantly lower values ($P <$

0.05) than those of CTRL and GSE, with GP-5 having intermediate values. It is interesting to note that at that point only VIT-E and GP-5 showed scores below 3, which was established as the threshold for rejection. Although VIT-E had the lowest values at the end of storage, all treatments presented values above this limit.

The overall rating of the samples reflects the changes in the other sensory attributes studied. Thus, according to the panellists, all the samples except meat slices from the VIT-E group reached values above 3 at 11 days of storage (3.93, 3.80 and 3.27 for the CRTL, GSE and GP-5 groups, respectively, and 2.60 for the VIT-E treatment).

VIT-E dietary treatment was more effective ($P < 0.05$) in preventing sensory spoilage in the last days of storage than the other experimental treatments, coinciding with its favourable effect on preventing meat formation of free radicals (Arnold *et al.,* 1992) and improving colour stability (Faustman and Casens, 1990). This agrees with the findings of Muíño *et al.* (2014), who observed that a trained panel judged the colour, flavour and overall appearance of dietary vitamin E supplemented lamb meat more favourably than non-supplemented meat.

In spite of the slight differences observed at day 7 in general appearance and at day 11 in off-odour and overall rating, where values of GSE and GP-5 were intermediate between CTRL and VIT-E, the inclusion of these polyphenol supplements in the diet did not achieve an extension of the shelf life of lamb meat in comparison with CTRL. As a result, only lamb meat from the VIT-E dietary treatment showed acceptable sensory quality after 11 days of storage in retail conditions. These results are in accordance with other studies (Jerónimo et *al.,* 2012; Muíño *et al.,* 2014), which did not detect effects of inclusion of polyphenols from different sources in lamb diets on sensory meat properties. On the other hand, some studies have observed better values in meat and fat colour when polyphenols are included in lamb diets (Ortuño *et al.,* 2014). Moreover, some authors have also indicated that polyphenol addition in lamb diets reduces off-flavours and rancid-odour perceptions of meat on display, owing to their amelioration of oxidative stability. In this regard, some studies found more favourable lamb meat odour values (Priolo *et al.,* 2009; Ortuño *et al.,* 2014) when lamb diets were supplemented with polyphenols. Given that some positive results in sensory parameters were observed, more research on the use of such wine by-products in sheep feeding might be recommendable.

6.4. Conclusions

Whole dried grape pomace at inclusion levels of 5% of concentrate can be included in lamb diets without adverse effects on animal performance, carcass characteristics and meat quality, when compared with other supplements, such as grape seed extract and vitamin E. Our results show that grape pomace seems to increase the concentration in meat of PUFA and some FA intermediates of the biohydrogenation process, such as RA, with potential beneficial effects on human health. Furthermore, grape pomace did not show adverse effects on the shelf life of meat during storage in retail sale conditions. Although grape seed extract and grape pomace were not as effective as vitamin E in preventing meat deterioration, lambs fed wine by-products showed numerically lower TBARS meat values from day 7 of storage under retail display conditions compared with lambs not supplemented with polyphenols.

6.5. References

Ahn, J., Grün, I., Fernando, L. (2002). Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *Journal of Food Science 67*, 1364–1369.

Andrés, S., Huerga, L., Mateo, J., Tejido, M.L., Bodas, R., Morán, L., Prieto, N., Rotolo, L., Giráldez, F.J. (2014a). The effect of quercetin dietary supplementation on meat oxidation processes and texture of fattening lambs. *Meat Science 96*, 806–811.

Andrés, S., Morán, L., Aldai, N., Tejido, M.L., Prieto, N., Bodas, R., Giráldez, F.J. (2014b). Effects of linseed and quercetin added to the diet of fattening lambs on the fatty acid profile and lipid antioxidant status of meat samples. *Meat Science 97*, 156–163.

Andrés, S., Tejido, M.L., Bodas, R., Morán, L., Prieto, N., Blanco, C., Giráldez, F.J. (2013). Quercetin dietary supplementation of fattening lambs at 0.2% rate reduces discolouration and microbial growth in meat during refrigerated storage. *Meat Science 93*, 207–212.

AOAC (2012). Official Methods of Analysis of AOAC International, 19th Edition.

Arnold, R.N., Scheller, K.K., Arp, S.C., Williams, S.N., Schaefer, D.M. (1992). Effect of long- or shortterm feeding of alpha-tocopheryl acetate to Holstein and crossbred beef steers on performance, carcass characteristics, and beef color stability. *Journal of Animal Sciencie 70*, 3055–3065.

Atay, O., Gokdal, O., Eren, V., Cetiner, S., Yikilmaz, H. (2009). Effects of dietary vitamin E supplementation on fattening performance, carcass characteristics and meat quality traits of Karya male lambs. *Archiv für Tierzucht-Archives of Animal Breeding 52*, 618–626.

Berruga, M.I., Vergara, H., Gallego, L. (2005). Influence of packaging on microbial and lipid oxidation in lamb meat. *Small Ruminant Research 57*, 257–264.

Bligh, E.G., Dyer, W.J. (1959). A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology 37*, 911–917.

Bodas, R., Prieto, N., Jordán, M.J., López-Campos, O., Giráldez, F.J., Morán, L., Andrés, S. (2012). The liver antioxidant status of fattening lambs is improved by naringin dietary supplementation at 0.15% rates but not meat quality. *Animal 6,* 863–870.

Buckley, D.J., Morrissey, P.A., Gray, J.I. (1995). Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *Journal of Animal Science 73*, 3122–3130.

Buys, E.M., Nortjé, G.L., Jooste, P.J., Von Holy, A. (2000). Microbiological shelf life of bulk-packaged *Musculus gluteus medius* supplemented with dietary vitamin E. *Meat Science 55*, 433–441.

Chaves, A.V., Stanford, K., Dugan, M.E.R., Gibson, L.L., McAllister, T.A., Van Herk, F., Benchaar, C. (2008). Effects of cinnamaldehyde, garlic and juniper berry essential oils on rumen fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. *Livestock Science 117*, 215–224.

CIE (1986). Colorimetry, 15.2. (2nd ed), CIE Publications, Vienna (Austria).

Descalzo, A.M., Rossetti, L., Grigioni, G., Irurueta, M., Sancho, A.M., Carrete, J., Pensel., N.A. (2007). Antioxidant status and odour profile in fresh beef from pasture or grain fed cattle. *Meat Science 75*, 299–307.

Du, J., Zeng, Y.,Wang, H., Qian, Y., Li, H., Chen, Q., Chen, W., Cui, J. (2010). CuZnSOD gene expression and its relationship with anti-oxidative capacity and pork quality. *South African Journal of Animal Science 40*, 265–272.

Estévez, M. (2011). Protein carbonyls in meat systems: A review. *Meat Science 89*, 259–279.

Falowo, A.B., Fayemi, P.O., Muchenje, V. (2014). Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: A review. *Food Research International 64*, 171–181.

Faustman, C., Cassens, R.G. (1990). The biochemical basis for discoloration in fresh meat: A review. *Journal of Muscle Foods 1*, 217–243.

FEDNA (2010). Tablas de composición y valor nutritivo de los alimentos para la fabricación de piensos compuestos. (C. de Blas, G.G. Mateos, P. García-Rebollar). FEDNA, Madrid (Spain).

Fernandes, R.P.P., Freire, M.T.A, De Paula, E.S., Kanashiro, A.L, Catunda, F.A., Rosa, A.F., Balieiro, J.C., Trindade, M.A. (2014). Stability of lamb loin stored under refrigeration and packed in different modified atmosphere packaging systems. *Meat Science 96*, 554–561.

Francisco, A., Dentinho, M.T., Alves, S.P., Portugal, P.V., Fernandes, F., Sengo, S., Jerónimo, E., Oliveira, M.A., Costa, P., Sequeira, A., Bessa, R.J.B., Santos-Silva, J. (2015). Growth performance, carcass and meat quality of lambs supplemented with increasing levels of a tanniferous bush (*Cistus ladanifer* L.) and vegetable oils. *Meat Science 100*, 275–282.

Frutos, P., Hervás, G., Giráldez, F.J., Mantecón, A.R. (2004). Review. Tannins and ruminant nutrition. *Spanish Journal of Agricultural Research 2*, 191–202.

Gallardo, B., Manca, M.G., Mantecón, A.R., Nudda, A., Manso, T. (2015). Effects of linseed oil and natural or synthetic vitamin E supplementation in lactating ewes' diets on meat fatty acid profile and lipid oxidation from their milk fed lambs. *Meat Science 102*, 79–89.

Gladine, C., Rock, E., Morand, C., Bauchart, D., Durand, D. (2007). Bioavailability and antioxidant capacity of plant extracts rich in polyphenols, given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. *British Journal of Nutrition 98*, 691–701.

Hayes, J.E., Allen, P., Brunton, N., O'Grady, M.N., Kerry, J.P. (2010). Phenolic composition and in-vitro antioxidant capacity of four commercial phytochemical products: Olive leaf extract (*Olea europaea* L), lutein, sesamol and ellagic acid. *Food Chemistry 126*, 948–955.

Honikel, K.O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science 49,* 447–457.

ICMSF (1986). Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Scientific Applications, Vol. 2. Toronto: University of Toronto Press, Toronto (Canada).

Inserra, L., Priolo, A., Biondi, L., Lanza, M., Bognanno, M., Gravador, R., Luciano, G. (2014). Dietary citrus pulp reduces lipid oxidation in lamb meat. *Meat Science 96*, 1489–1493.

ISO 4120 (2004). Sensory analysis-methodology–Triangle test. International Standard ISO 4120: 2004(E).

Jeremiah, L.E. (2001). Packaging alternatives to deliver fresh meats using short- or long-term distribution. *Food Research International 34*, 749–772.

Jerónimo, E., Alfaia, C.M.M., Alves, S.P., Dentinho, M.T.P., Prates, J.A.M., Vasta, V., Santos-Silva, J., Bessa, R.J.B. (2012). Effect of dietary grape seed extract and *Cistus ladanifer* L. in combination with vegetable oil supplementation on lamb meat quality. *Meat Science 92*, 841–847.

Jerónimo, E., Alves, S.P., Dentinho, M.T.P., Martins, S.V., Prates, J.A.M., Vasta, V., Santos-Silva, J., Bessa, R.J.B. (2010). Effect of grape seed extract, *Cistus ladanifer* L., and vegetable oil supplementation on fatty acid composition of abomasal digesta and intramuscular fat of lambs. *Journal of Agricultural and Food Chemistry 58*, 10710–10721.

Karami, M., Alimon, A.R., Sazili, A.Q., Goh, Y.M., Ivan, M. (2011). Effects of dietary antioxidants on the quality, fatty acid profile, and lipid oxidation of *longissimus* muscle in Kacang goat with aging time. *Meat Science 88*, 102–108.

Krzywicki, K. (1979). Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. *Meat Science 3*, 1–10.

Land, D.G., Shepherd, R. (1988). Scaling and ranking methods. In: Sensory Analysis of Foods (J.R Piggott, ed.). pp. 155–185. Elsevier Applied Science, London (UK)

Lauzurica, S., De la Fuente, J., Díaz, M.T., Álvarez, I., Pérez, C., Cañeque, V. (2005). Effect of dietary supplementation of vitamin E on characteristics of lamb meat packed under modified atmosphere. *Meat Science 70*, 639–646.

Luciano, G., Monahan, F.J., Vasta, V., Biondi, L., Lanza, M., Priolo, A. (2009). Dietary tannins improve lamb meat colour stability. *Meat Science 81*, 120–125.

Luciano, G., Vasta, V., Monahan, F.J., López-Andrés, P., Biondi, L., Lanza, M., Priolo, A. (2011). Antioxidant status, colour stability and myoglobin resistance to oxidation of *Longissimus dorsi* muscle from lambs fed a tannin-containing diet. *Food Chemistry 124*, 1036–1042.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition 79,* 727–747.

Manso, T., Bodas, R., Castro, T., Jimeno, V., Mantecón, A.R. (2009). Animal performance and fatty acid composition of lambs fed with different vegetable oils. *Meat Science 83*, 511–516.

Maraschiello, C., Sarraga, C., García-Regueiro, J.A. (1999). Glutathione peroxidase activity, TBARS, and α-tocopherol in meat from chickens fed different diets. *Journal of Agriculture and Food Chemistry 47*, 867–872.

Moñino, I., Martínez, C., Sotomayor, J.A., Lafuente, A., Jordán, M.J. (2008). Polyphenolic transmission to Segureño lamb meat from ewes' diet supplemented with the distillate from rosemary (*Rosmarinus officinalis*) leaves. *Journal of Agriultural Food Chemistry 56,* 3363–3367.

Morán, L., Andrés, S., Bodas, R., Prieto, N., Giráldez, F.J. (2012b) Meat texture and antioxidant status are improved when carnosic acid is included in the diet of fattening lambs. *Meat Science 91,* 430–434.

Morán, L., Giráldez, F.J., Panseri, S., Aldai, N., Jordán, M.J., Chiesa, L.M., Andrés, S. (2013). Effect of dietary carnosic acid on the fatty acid profile and flavour stability of meat from fattening lambs. *Food Chemistry 138*, 2407–2414.

Morán, L., Rodríguez-Calleja, J.M., Bodas, R., Prieto, N., Giráldez, F.J., Andrés, S. (2012a). Carnosic acid dietary supplementation at 0.12% rates slows down meat discoloration in *gluteus medius* of fattening lambs. *Meat Science 90*, 789–795.

Morrison, W.R., Smith, L.M. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *Journal of Lipid Research* 5*,* 600–608.

Morrissey, P.A., Sheehy, P.I.A., Galvin, K., Kerry, J.P., Buckley, D.J. (1998). Lipid stability in meat and meat products. *Meat Science 49,* S73–S86.

Muíño, I., Apeleo, E., De la Fuente, J., Pérez-Santaescolástica, C., Rivas-Cañedo, A., Pérez., C., Díaz, M.T., Cañeque, V., Lauzurica, S. (2014). Effect of dietary supplementation with red wine extract

or vitamin E, in combination with linseed and fish oil, on lamb meat quality. *Meat Science 98*, 116–123.

O'Grady, M.N., Carpenter, R., Lynch, G.P., O'Brien, N.M., Kerry, J.P. (2008). Addition of grape seed extract and bearberry to porcine diets: Influence on quality attributes of raw and cooked pork. *Meat Science 78*, 438–446.

Ortuño, J., Serrano, R., Jordán, M.J., Bañón, S. (2014). Shelf life of meat from lambs given essential oil-free rosemary extract containing carnosic acid plus carnosol at 200 or 400 mg kg^{-1}. *Meat Science 96,* 1452–1459.

Pétriz-Celaya, Y., Calderón-Cortés, J.F., Pérez, C., Montaño, M.F., Plascencia, A. (2010). Influence of substitution of alfalfa hay for unfermented dried grape pomace on performance and carcass characteristics of growing sheep. *American Society of Animal Science*, *Proceedings, Western Section 61*, 308–311.

Priolo, A., Lanza, M., Biondi, L., Pappalardo, D., Young, O.A. (1998). Effect of partially replacing dietary barley with 20% carob pulp on post-weaning growth, and carcass and meat characteristics of Comisana lambs. *Meat Science 50*, 355–363.

Priolo, A., Vasta, V., Fasone, V., Lanza, C.M., Scerra, M., Biondi, L., Bella, M., Whittington, F.M. (2009). Meat odour and flavour and indoles concentration in ruminal fluid and adipose tissue of lambs fed green herbage or concentrates with or without tannins. *Animal 3*, 454–460.

Priolo, A., Waghorn, G.C., Lanza, M., Biondi, L., Pennisi, P. (2000). Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: Effects on lamb growth performance and meat quality. *Journal of Animal Science 78*, 810–816.

Raccach, M. (1984). The antimicrobial activity of phenolic antioxidants in foods: A review. *Journal of Food Safety 6*, 141–170.

Reddy, B.G.V., Sen, A.R., Nair P.N., Reddy, K.S., Reddy, K.K., Kondaiah, N. (2013). Effects of grape seed extract on the oxidative and microbial stability of restructured mutton slices. *Meat Science 95*, 288–294.

Ripoll, G., Joy, M., Muñoz, F. (2011). Use of dietary vitamin E and selenium (Se) to increase the shelf life of modified atmosphere packaged light lamb meat. *Meat Science 87*, 88–93.

Ripoll, G., Joy, M., Muñoz, F., Albertí, P. (2008). Meat and fat colour as a tool to trace grass-feeding systems in light lamb production. *Meat Science 80*, 239–248.

Rota, M.C., Herrera, A., Martínez, R.M., Sotomayor, J.A., Jordán, M.J. (2008). Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oil. *Food Control 19,* 681–687.

Salem, A.Z.M., Olivares, M., López, S., González-Ronquillo, M., Rojo, R., Camacho, L.M., Cerrillo, S.M.A., Mejía, H.P. (2011). Effect of natural extracts of *Salix babylonica* and *Leucaena leucocephala* on nutrient digestibility and growth performance of lambs. *Animal Feed Science and Technology 170*, 27–34.

Samman, S., Sandstrom, B., Toft, M.B., Bukhave, K., Jensen, M., Sorensen, S.S., Hansen. M. (2001). Green tea or rosemary extract added to foods reduces nonheme-iron absorption. *The American Journal of Clinical Nutrition 73*, 607–612.

Schreurs, N.M., Tavendale, M.H., Lane, G.A., Barry, T.N., McNabb, W.C., Cummings, T., Fraser, K., Lopez-Villalbos, N. (2007). The effect of supplementation of a white clover or perennial ryegrass diet with grape seed extract on indole and skatole metabolism and the sensory characteristics of lamb. *Journal of the Science of Food and Agriculture 87*, 1030–1041.

Soldatou, N., Nerantzaki, A., Kontominas, M.G., Savvaidis, I.N. (2009). Physicochemical and microbiological changes of “Souvlaki” - A Greek delicacy lamb meat product: Evaluation of shelf-life using microbial, colour and lipid oxidation parameters. *Food Chemistry 113*, 36–42.

Spanghero, M., Salem, A.Z.M., Robinsond, P.H. (2009). Chemical composition, including secondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces. *Animal Feed Science and Technology 152*, 243–255.

UNE-EN ISO 8586 (2014). Análisis sensorial. Guía general para la selección, entrenamiento y control de catadores y catadores expertos.

UNE-ISO 13300-2 (2008). Sensory analysis. General guidance for the staff of a sensory evaluation laboratory. Part 2: recruitment and training of panel leaders.

Van Soest, P.J., Robertson, J.B., Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science 74*, 3583–3597.

Vasta, V., Pennisi, P., Lanza, M., Barbagallo, D., Bella, M., Priolo, A. (2007). Intramuscular fatty acid composition of lambs given a tanniniferous diet with or without polyethylene glycol supplementation. *Meat Science 76*, 739–745.

Vasta, V., Priolo, A., Scerra, M., Hallett, K.G., Wood, J.D., Doran, O. (2009). Δ^9 desaturase protein expression and fatty acid composition of *longissimus dorsi* muscle in lambs fed green herbage or concentrate with or without added tannins. *Meat Science 82*, 357–364.

Wulf, D.M., Morgan, J.B., Sanders, S.K., Tatum, J.D., Smith, G.C., Williams, S. (1995). Effects of dietary supplementation of vitamin E on storage and caselife properties of lamb retail cuts. *Journal of Animal Science 73*, 399–405.

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

7. Discusión general

El objetivo general de esta Tesis Doctoral ha sido desarrollar estrategias de alimentación del ganado ovino basadas en la utilización de orujo de uva procedente de la elaboración de vino tinto en las raciones, con el fin de mejorar la calidad de la canal y de la carne de cordero y de lechazo (ácidos grasos, estabilidad oxidativa y vida útil) y como alternativa al empleo de otros antioxidantes comerciales habitualmente utilizados.

Si bien en las distintas pruebas experimentales que constituyen esta Tesis Doctoral, se han analizado y discutido de forma independiente los resultados obtenidos en cada prueba experimental, en el presente apartado se integran de forma resumida y concisa los principales hallazgos obtenidos, así como las posibles relaciones entre ellos.

7.1. Rendimientos productivos

La ingestión de los animales no se vio afectada de forma significativa al incluir en las raciones de ovejas en lactación y en los piensos de corderos en crecimiento orujo de uva procedente de la elaboración de vino tinto, en relación con un tratamiento control (sin orujo) y con un tratamiento con vitamina E (500 mg de vitamina E por kg de MS) (Figura 7.1). El orujo de uva es un alimento con fibra muy lignificada y bajo nivel energético. La ausencia de diferencias en la ingestión probablemente fue debida a que las dietas se formularon para que la relación energía:proteína fuera similar en todos los tratamientos experimentales. Por otra parte, tampoco se observaron problemas de palatabilidad relacionados con la ingestión de taninos condensados derivados de la inclusión de orujo, ya que los niveles de taninos de la dieta permanecieron por debajo del nivel asociado con reducciones en la ingestión voluntaria y efectos negativos sobre la producción (Frutos *et al.*, 2004).

Sin embargo, es destacable que en los dos experimentos realizados la ingestión de MS fue numéricamente superior al incluir orujo en la ración (Figura 7.1). En la prueba con ovejas en lactación fueron señalados incrementos del 3% y del 2,3% al incluir, respectivamente, 5% (GP-5) y 10% (GP-10) de orujo de vino tinto en la ración de las ovejas en comparación con el grupo control. En la prueba experimental realizada con corderos en crecimiento se observó un incremento de un 4% en la ingestión de concentrado al incluir un 5% de orujo (GP-5) respecto al tratamiento control. Estos resultados pueden ser explicados porque el orujo reduce ligeramente el contenido energético de la dieta, y para alcanzar el mismo consumo de energía los animales incrementan su ingestión.

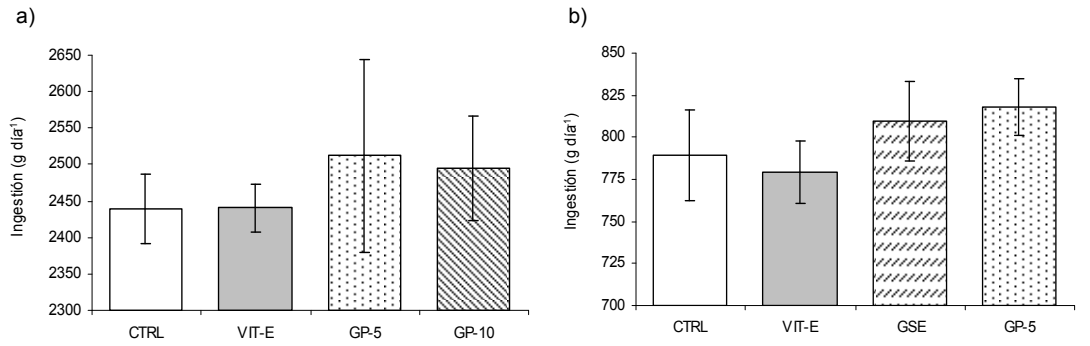


Figura 7.1. Efecto de la inclusión de orujo de uva de vino tinto en la dieta de ovejas en inicio de lactación (a) y de corderos en crecimiento-cebo (b) sobre la ingestión de materia seca (g día^{-1}) (CTRL, sin orujo de uva; VIT-E, 500 mg de vitamina E por kg de MS; GSE, 50 mg de extracto de semilla de uva por kg de MS; GP-5, 5% de orujo de uva; GP-10, 10% de orujo de uva). Las barras de error representan el error estándar.

Tal y como era de esperar, el crecimiento de los corderos y de los corderos lechales (Figura 7.2), y la producción y composición de la leche de las ovejas no se vieron afectados de forma significativa al incluir orujo de uva en las raciones. La ausencia de diferencias en los niveles de ingestión de materia seca entre animales que recibieron orujo de uva en sus raciones, en comparación con el grupo control, podrían explicar estos resultados.

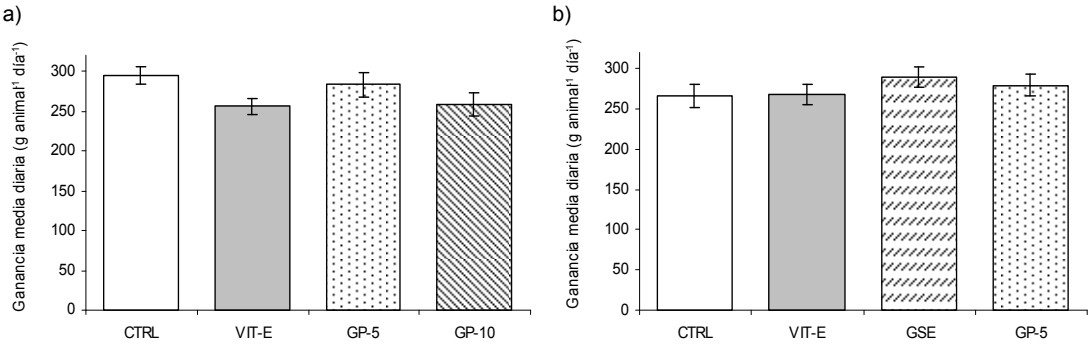


Figura 7.2. Efecto de la inclusión de orujo de uva de vino tinto en la dieta de ovejas en inicio de lactación (a) y de corderos en crecimiento-cebo (b) sobre la ganancia media diaria de peso de los corderos (g animal^{-1} día^{-1}) (CTRL, sin orujo de uva; VIT-E, 500 mg de vitamina E por kg de MS; GSE, 50 mg de extracto de semilla de uva por kg de MS; GP-5, 5% de orujo de uva; GP-10, 10% de orujo de uva). Las barras de error representan el error estándar.

Por otra parte, y en relación con la ingesta de algunos polifenoles presentes en el orujo de uva como los taninos condensados, se ha indicado que cantidades moderadas pueden mejorar la utilización digestiva del alimento como consecuencia de una reducción en la degradación de proteína en el rumen y del consiguiente incremento de aminoácidos disponibles para ser absorbidos en el intestino delgado (Frutos *et al*., 2004). Este efecto sobre la nutrición, podría también haber compensado la diferencia en el aporte de nutrientes y explicar la ausencia de diferencias en los rendimientos productivos al incorporar orujo de uva en el pienso y las raciones.

7.2. Calidad de canal

La adición de orujo de uva de vino tinto en dietas de ovejas en inicio de lactación y en el concentrado de corderos en crecimiento no produjo cambios sobre el rendimiento y las características de la canal. Debido a la ausencia de diferencias en la ingestión y en el crecimiento era de esperar que tampoco fuesen encontradas diferencias en los parámetros que definen la calidad de la canal. Estos resultados han sido corroborados por otros autores en corderos cuyas madres fueron suplementadas con tomillo (rico en polifenoles) (Nieto *et al.,* 2011) y en corderos en crecimiento suplementados con orujo de uva desecado a niveles de inclusión del 10, 20 y el 30% (Pétriz-Celaya *et al*., 2010), así como cuando se incorporan otros residuos de la industria agroalimentaria ricos en sustancias de carácter fenólico (Inserra *et al*., 2014).

7.3. Calidad de carne

El pH muscular de los corderos lechales y de los corderos en crecimiento no se vio afectado por la inclusión de orujo de uva en las dietas de las ovejas y en los corderos en relación con los otros tratamientos experimentales. Estos resultados indican una tendencia normal en la glucolisis *post mortem* en el músculo. El hecho de que la dieta no afectase a los rendimientos productivos de los corderos, que pueden influir en el contenido en glucógeno del músculo (Inserra *et al*., 2014), explica estos resultados. Al no apreciarse efecto sobre el pH muscular, tampoco se produjeron diferencias significativas sobre color del músculo *rectus abdominis* y *longissimus thoracis et lumborum* (LTL) después del sacrificio.

Algunos autores han sugerido que la suplementación con polifenoles reduce la degradación del color durante su almacenamiento debido a su capacidad para prevenir la oxidación de la mioglobina (Priolo *et al*., 2000; Gallardo *et al*., 2015). Sin embargo, estos cambios en el color de la carne y de la grasa asociados con los procesos oxidativos son raramente apreciables a las 24 h después del sacrificio. Los parámetros que definen el color de la carne son muy variables, y nuestros resultados no nos han permitido atribuir un efecto específico de la incorporación de orujo de uva sobre el color de los músculos *rectus abdominis* y LTL de la canal y de la carne de corderos lechales y de corderos en crecimiento.

En relación con la fuerza de corte de la carne, tanto en el experimento con corderos lechales, como en la prueba con corderos en crecimiento, los valores fueron aceptables y no se observaron diferencias significativas entre tratamientos experimentales. Estos resultados contrastan con algunos autores, que han indicado menor fuerza de corte en corderos suplementados con vitamina E o polifenoles en comparación con animales del grupo control debido a la protección que ejercen los antioxidantes frente a las proteasas endógenas (Morán *et al*., 2012a). Además, ha sido indicada una reducción en la fuerza de corte cuando el pH a las 24 h es alto (Devine *et al.,* 1993). Los valores de pH de la carne inferiores a 6 y la ausencia de diferencias entre tratamientos, podría explicar la falta de diferencias en la fuerza de corte del músculo al emplear antioxidantes en las raciones.

El empleo de antioxidantes mejora la capacidad de retención de agua (CRA) de la carne ya que permite mantener la integridad de las membranas y los entrecruzamientos proteicos durante los procesos de oxidación. En las pruebas experimentales que integran este trabajo, las menores pérdidas de agua por cocinado las presentó la carne de los animales pertenecientes al tratamiento con vitamina E en relación con los otros tratamientos, tanto en la prueba experimental con ovejas en lactación como en la prueba con corderos en crecimiento-cebo (Figura 7.3). En el caso de la prueba con corderos lechales, la CRA de la carne mejoró, respecto al tratamiento control, al incluir un 5% y un 10% de orujo de uva en las raciones de las ovejas, lo cual podría ser atribuido al efecto antioxidante del orujo. Sin embargo, el orujo de uva al nivel del 5% del concentrado no mostró efecto sobre la CRA de la carne de corderos en crecimiento respecto al tratamiento control. Las discrepancias obtenidas entre experimentos podrían ser atribuidas al aporte de aceite de linaza en las raciones de las ovejas y a la forma en que se suministró el orujo en las pruebas experimentales (fresco en las raciones de las ovejas o desecado en las raciones de los corderos en crecimiento-cebo).

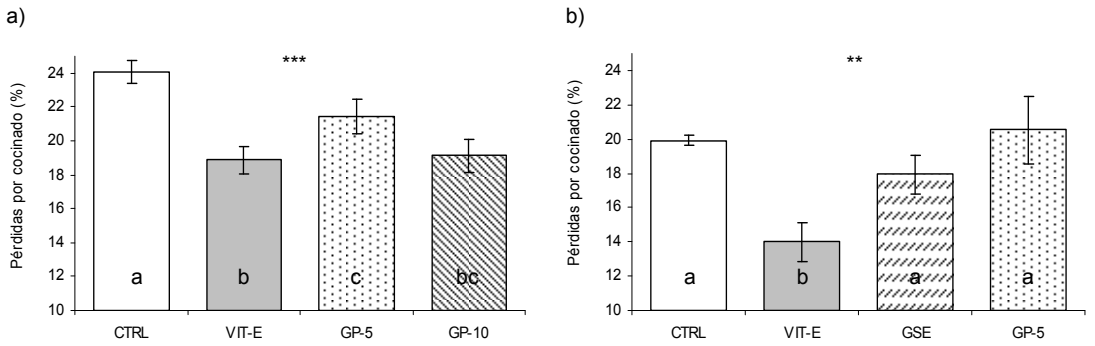


Figura 7.3. Efecto de la inclusión de orujo de uva de vino tinto en la dieta de ovejas en inicio de lactación (a) y de corderos en crecimiento-cebo (b) sobre las pérdidas de agua por cocinado (%) de la carne de los corderos (CTRL, sin orujo de uva; VIT-E, 500 mg de vitamina E por kg de MS; GSE, 50 mg de extracto de semilla de uva por kg de MS; GP-5, 5% de orujo de uva; GP-10, 10% de orujo de uva). Letras distintas indican diferencias significativas entre los tratamientos experimentales (**, $P < 0,01$; ***, $P < 0,001$). Las barras de error representan el error estándar.

La composición química (humedad, grasa y proteína) del músculo LTL de los corderos lechales y de cebo estuvo dentro del rango señalado en trabajos previos (Manso *et al.,* 2011; Morán *et al.,* 2012b) y no se vio afectada por el tratamiento experimental en ninguna de las pruebas realizadas. Debido a que no hubo diferencias en la ingestión y ritmo de crecimiento de los animales, no eran de esperar diferencias en la composición química de la carne de los corderos producidos. Únicamente, nuestros resultados mostraron que los corderos en crecimiento-cebo que recibieron un 5% de orujo (GP-5) en la ración, presentaron un contenido en grasa un 13% inferior que los otros tratamientos. Estos resultados concuerdan con los obtenidos por Vasta *et al*. (2007), al incluir taninos condensados en las dietas de corderos en fase de cebo.

El perfil lipídico de la grasa intramuscular del músculo LTL tanto en la prueba experimental con corderos lechales, como en la prueba con corderos en crecimiento-cebo se mantuvo dentro del rango obtenido por otros autores para razas y sistemas de producción similares (Manso *et al.,* 2011 y Manso *et al.*, 2009, respectivamente). En ambos casos, los ácidos grasos mayoritarios fueron los saturados (SFA) y monoinsaturados (MUFA), siendo el ácido oleico (C18:1) el más abundante, seguido del ácido palmítico (C16:0) y del ácido esteárico (C18:0).

En términos generales, en ambas pruebas experimentales, la incorporación de vitamina E y de orujo de uva en las dieta mostró un efecto poco importante sobre el nivel de SFA,

ácidos grasos poliinsaturados (PUFA), relación PUFA:SFA, PUFA n3 de la grasa intramuscular y relación n6:n3. Resultados similares han sido observados en corderos lechales cuyas madres fueron suplementadas con aceite de linaza y con un contenido en vitamina E superior al de las dietas habituales (Gallardo *et al.,* 2015) y en corderos en crecimiento suplementados con diferentes fuentes de polifenoles en la dieta (Chaves *et al.,* 2008; Morán *et al.,* 2013; Brogna *et al.,* 2014).

Algunos autores han señalado que la vitamina E mejora la composición lipídica del músculo, ya que su efecto protector contra la peroxidación de los PUFA favorece una mayor insaturación y mayor acúmulo de ácidos grasos n3 en la carne. De hecho, el efecto de las fuentes de antioxidantes sobre el perfil de ácidos grasos de la carne es más evidente cuando se han empleado en las dietas fuentes de ácidos grasos insaturados (Juárez *et al.,* 2011; Andrés *et al.,* 2014; Francisco *et al.,* 2015). En nuestro trabajo con corderos en crecimiento no se emplearon grasas en la dieta y la concentración de antioxidantes disponibles dentro del músculo para retrasar la oxidación en la carne podría haber sido suficiente en el grupo de control, lo que podría explicar la ausencia de diferencias significativas en el perfil de ácidos grasos de los distintos tratamientos.

La inclusión de orujo de uva en las dietas de las ovejas y de corderos en crecimiento no dio lugar a diferencias estadísticamente significativas en el perfil lipídico de la grasa intramuscular. Este resultado está de acuerdo con Jerónimo *et al*. (2010) y Muíño *et al.* (2014), quienes no apreciaron diferencias en el perfil lipídico de la carne al incluir extracto de semilla de uva y extracto de vino tinto, respectivamente, en las dietas de corderos en crecimiento. Por otro lado, varios trabajos han indicado que el uso de plantas o extractos ricos en proantocianidinas y otros polifenoles, disminuyen de forma estadísticamente significativa el nivel de SFA y aumentan el contenido en MUFA y PUFA de la carne. Nuestros resultados muestran que la carne de los corderos en crecimiento suplementados con un 5% de orujo tendió a presentar mayor contenido en PUFA que el grupo control. Sin embargo, el mayor porcentaje de PUFA del tratamiento GP-5 podría atribuirse más a las diferencias en el contenido de grasa intramuscular entre tratamientos, con la consiguiente variación en la relación de triglicéridos:fosfolípidos, que a los efectos *per se* de los polifenoles.

Cabe destacar que la carne de los corderos lechales pertenecientes a los tratamientos con orujo de uva presentaron en la grasa intramuscular un contenido significativamente mayor de ácido vacénico (VA, *trans-11* C18:1) y ácido ruménico (RA, *cis-9 trans-11* C18:2) que el tratamiento control. En el caso de los corderos en crecimiento-cebo las diferencias no

fueron significativas pero numéricamente, los niveles de RA y de VA fueron 7,5% y 2% superiores en los corderos que recibieron orujo en su ración que los del grupo control (Figura 7.4). Estos efectos podrían ser atribuidos a varias causas. En primer lugar, el alto contenido en *cis-9 cis-12* C18:2 del orujo de uva, podría servir como precursor para la producción de ácido vacénico y ruménico a nivel ruminal. En segundo lugar, podría ser debido a la transmisión de los compuestos fenólicos de la dieta a la carne (Moñino *et al.,* 2008; Jordán *et al.,* 2010; Nieto, 2013), ya que han sido identificados una amplia gama de extractos vegetales ricos en taninos y otros compuestos fenólicos, a los que se les han atribuido propiedades antibacterianas y capacidad para modificar el proceso de biohidrogenación (BH) de los PUFA de la dieta. En este sentido, algunos estudios han señalado que algunos polifenoles inhiben el último paso de la BH generando mayores niveles de PUFA, CLA y VA en la leche y en la grasa intramuscular (Khiaosa-Ard *et al.,* 2009; Vasta *et al.,* 2009a). Otros estudios han indicado una mayor síntesis endógena de RA al incluir compuestos fenólicos, ya que este ácido graso también puede ser generado en los tejidos de los animales a través de la desaturación del VA por la acción de la enzima Δ^9-desaturasa (Min *et al.,* 2003; Vasta *et al.,* 2009b; Rana *et al.,* 2012).

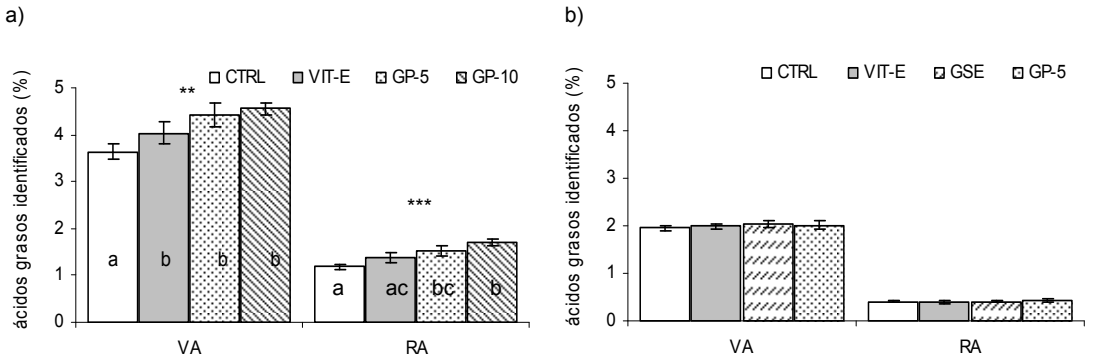


Figura 7.4. Efecto de la inclusión de orujo de uva de vino tinto en la dieta de ovejas en inicio de lactación (a) y de corderos en crecimiento-cebo (b) sobre los niveles ácido vacénico (VA, *trans-11* C18:1), y de ácido ruménico (RA; *cis-9 trans-11* C18:2) del m. *longissimus thoracis et lumborum* (% de ácidos grasos identificados) (CTRL, sin orujo de uva; VIT-E, 500 mg de vitamina E por kg de MS; GSE, 50 mg de extracto de semilla de uva por kg de MS; GP-5, 5% de orujo de uva; GP-10, 10% de orujo de uva). Letras distintas indican diferencias significativas entre los tratamientos experimentales (**, $P < 0,01$; ***, $P < 0,001$). Las barras de error representan el error estándar.

En cuanto a los ácidos grasos PUFA n3, a pesar de la falta de diferencias estadísticas en ambas pruebas experimentales, el ácido docosahexaenoico (DHA, C22:6 n3) y ácido docosapentaenoico (DPA, C22:5 n3) aumentaron en los tratamientos con orujo cuando se compararon con los grupos CTRL y VIT-E. Este resultado está de acuerdo con Muíño *et al.* (2014), que han demostrado que la carne de corderos en crecimiento suplementados con extracto de vino tinto o vitamina E mostraron valores numéricos más altos de ácidos grasos n3 de cadena muy larga que el grupo sin antioxidantes. Morán *et al.* (2013) al suplementar dietas de corderos con ácido carnósico, también encontraron en el músculo mayor contenido de DHA. De acuerdo con estos autores, este hallazgo podría ser interesante, ya que la carne de cordero es muy baja en ácidos grasos altamente insaturados, sin embargo, no se ha encontrado en la bibliografía disponible una explicación para estos resultados.

A pesar de todo el conocimiento existente, no es posible realizar una conclusión inequívoca sobre el efecto de los compuestos fenólicos sobre el perfil de ácidos grasos de la carne. El incremento en el contenido de VA y CLA en los grupos suplementados con orujo de uva en el caso de la prueba con corderos lechales, y del nivel de PUFA en la prueba con corderos en crecimiento, es interesante ya que se ha indicado que estos ácidos grasos pueden tener efectos beneficiosos para la salud humana y redundar en mejoras en el consumo de carne de cordero.

7.4. Vida útil de la carne

Microbiología

No se encontraron diferencias en la población microbiana entre los diferentes tratamientos experimentales tanto en corderos lechales como en corderos en crecimiento-cebo al inicio del almacenamiento, y tal y como se esperaba, los recuentos se fueron incrementando a lo largo del mismo. Sin embargo, la evolución de los diferentes grupos microbianos fue diferente entre tratamientos.

Nuestros resultados parecen indicar que la vitamina E fue efectiva retrasando el desarrollo microbiano. El acetato de α-tocoferol se utiliza en alimentación de ovejas para mejorar la estabilidad de la grasa y del color de la carne de cordero, pero no han sido observados efectos sobre el crecimiento microbiano (Lauzurica *et al.,* 2005). Debido a la ausencia de evidencias en la bibliografía sobre el efecto antibacteriano de la vitamina E, sólo podemos formular la hipótesis de que estos efectos antibacterianos podrían estar

relacionados con su capacidad antioxidante. Aunque no se observaron diferencias estadísticas en los recuentos microbianos entre los tratamientos con orujo y el grupo control, los polifenoles han sido catalogados como compuestos antimicrobianos por su capacidad para alterar las membranas celulares bacterianas y el metabolismo enzimático microbiano (Rota *et al.*, 2008). De hecho, en la prueba con corderos lechales, el tratamiento con 5% de orujo fue efectivo ralentizando el desarrollo de enterobacterias al final del almacenamiento, lo que podría indicar cierta transmisión de compuestos fenólicos desde la dieta a la leche consumida por los corderos lechales y al músculo de los animales (Raccach, 1984), así como un cierto potencial antimicrobiano de los polifenoles del orujo de uva.

Coordenadas de color y porcentaje de metamioglobina

El color de la carne es un parámetro muy complejo que se modifica a lo largo de su almacenamiento, existiendo además una fuerte relación entre la oxidación lipídica y la oxidación de la mioglobina, por consiguiente, cuando se incrementa la oxidación lipídica se ve favorecida la acumulación de metamioglobina (MMb), responsable del pardeamiento de la carne.

Los parámetros colorimétricos fueron muy variables y el efecto de los tratamientos experimentales no fue claro. Sin embargo, podemos decir que los antioxidantes contribuyeron a retrasar la decoloración de la carne, ya que el porcentaje de pigmento metamioglobina se vio claramente influenciado por la presencia de vitamina E y orujo de uva (Figura 7.5).

La incorporación de orujo de uva no fue efectiva en la reducción de MMb de la carne de los corderos en crecimiento-cebo, mientras que en el trabajo realizado con corderos lechales, a los niveles de inclusión del 5 y del 10 %, se redujo la formación de este pigmento a partir del día 10 de almacenamiento en relación con el grupo control de manera similar a la vitamina E. La posible transferencia de compuestos fenólicos de la leche al músculo de los corderos lechales podría explicar nuestros resultados en relación con el efecto del orujo de uva sobre la formación de MMb. Resultados similares han sido obtenidos por otros autores al emplear compuestos fenólicos en la dieta de ovejas (Nieto *et al.,* 2010a) sobre la decoloración de la carne de los corderos y en corderos en crecimiento al incluir taninos condensados en su dieta (Luciano *et al.,* 2011).

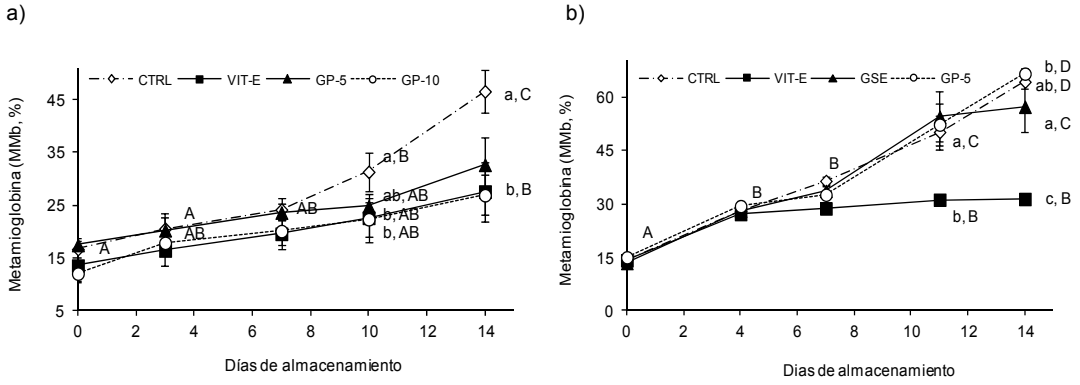


Figura 7.5. Efecto de la inclusión de orujo de uva de vino tinto en la dieta de ovejas en inicio de lactación (a) y de corderos en crecimiento-cebo (b) sobre la formación de metamioglobina (MMb, %) a lo largo del almacenamiento a 2 °C en el m. *longissimus thoracis et lumborum* (CTRL, sin orujo de uva; VIT-E, 500 mg de vitamina E por kg de MS; GSE, 50 mg de extracto de semilla de uva por kg de MS; GP-5, 5% de orujo de uva; GP-10, 10% de orujo de uva). Letras minúsculas distintas indican diferencias significativas entre los tratamientos experimentales ($P < 0,05$) y letras mayúsculas distintas indican diferencias entre tiempos dentro de un mismo tratamiento ($P < 0,05$). Las barras de error representan el error estándar.

Oxidación lipídica

En relación con la inclusión de compuestos fenólicos en la dieta, ha sido descrito que estas sustancias pueden atenuar el daño oxidativo en los tejidos, ya que pueden interferir en las reacciones de propagación e inhibir los sistemas enzimáticos implicados en las reacciones de iniciación (You *et al.,* 1999). En la prueba experimental con corderos lechales, nuestros resultados indican que los niveles de TBARS de la carne a partir del día 10 de almacenamiento en los animales cuyas madres fueron suplementadas con orujo de uva fueron tan bajos como cuando se emplea vitamina E, e inferiores al tratamiento control (Figura 7.6). Estudios previos han observado un descenso en la oxidación lipídica de la carne de cordero al incorporar diferentes fuentes de polifenoles en la dieta de sus madres (Nieto *et al.,* 2010a; 2010b; Serrano *et al.,* 2014), o al incluir polifenoles en lactorreemplazantes para corderos lechales (Morán *et al.,* 2014). Del mismo modo que para la vitamina E, la inclusión de orujo en la dieta de las ovejas podría estar conectado con una reducción en el nivel de MDA en plasma de 11 y 23% para los tratamientos GP-5 y GP-10 respectivamente, en relación al control.

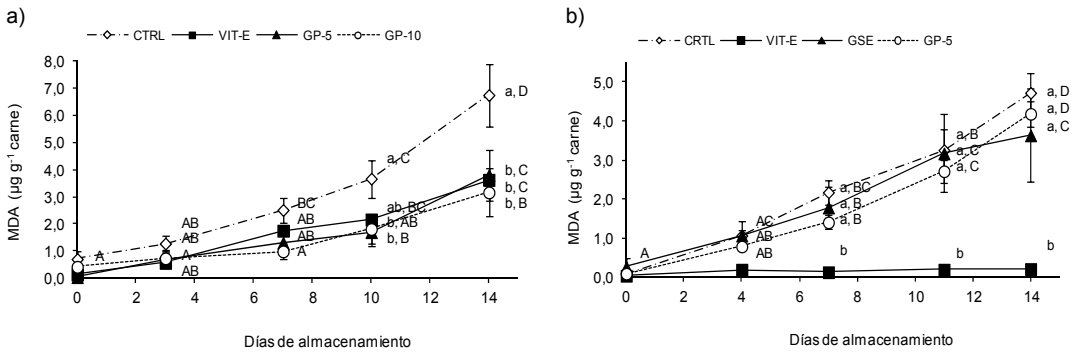


Figura 7.6. Efecto de la inclusión de orujo de uva de vino tinto en la dieta de ovejas en inicio de lactación (a) y de corderos en crecimiento-cebo (b) sobre la evolución de los TBARS (µg g^{-1} carne) a lo largo del almacenamiento a 2 °C en el m. *longissimus thoracis*. *et lumborum* (CTRL, sin orujo de uva; VIT-E, 500 mg de vitamina E por kg de MS; GSE, 50 mg de extracto de semilla de uva por kg de MS; GP-5, 5% de orujo de uva; GP-10, 10% de orujo de uva). Letras minúsculas distintas indican diferencias significativas entre los tratamientos experimentales ($P < 0,05$) y letras mayúsculas distintas indican diferencias entre tiempos dentro de un mismo tratamiento ($P < 0,05$). Las barras de error representan el error estándar.

En la prueba experimental con corderos en crecimiento-cebo no se observaron diferencias significativas respecto al control en el nivel de TBARS del músculo a lo largo de su almacenamiento al incluir orujo de uva en el concentrado de los animales. Sin embargo, sí que se apreciaron descensos numéricos del 35%, 17% y del 11% los días 7, 11 y 14, al incluir 5% de orujo de uva en la ración de los corderos respecto al control (Figura 7.6). En este experimento no se añadieron aceites altamente poliinsaturados a la dieta de los animales, que hubiesen incrementado la susceptibilidad a la oxidación lipídica de la carne, lo que podría explicar la ausencia de diferencias significativas respecto al experimento con lechales. En este sentido, Jerónimo *et al.* (2012) indicaron que la inclusión de extracto de semilla de uva en dietas de corderos en crecimiento redujo la oxidación lipídica de la carne durante su almacenamiento.

Evaluación sensorial

El deterioro sensorial de la carne está asociado con fenómenos de oxidación, tales como el pardeamiento de la superficie muscular, el oscurecimiento de la grasa, la pérdida de jugo y el incremento del olor desagradable, que normalmente se describe como rancio.

En ambas pruebas experimentales, la percepción en la decoloración de la carne aumentó gradualmente durante el almacenamiento en todos los grupos, y en los últimos días de almacenamiento el tratamiento con vitamina E mostró puntuaciones más bajas que el control. Estos resultados coinciden con el menor porcentaje de MMb presentado por la carne de este tratamiento al final del almacenamiento. La percepción de la decoloración de la carne no se vio afectada al incluir orujo de uva en las raciones, a pesar de los valores más bajos de MMb en los grupos con orujo en el caso de la prueba experimental con corderos lechales.

Del mismo modo, la presencia de olores anómalos aumentó gradualmente y de manera significativa durante el almacenamiento en todos los grupos. Sin embargo, al final del almacenamiento, los grupos con vitamina E y con orujo de uva mostraron valores significativamente menores al control. Estos resultados probablemente se deben a los TBARS inferiores en la carne de estos tratamientos, ya que la oxidación de los lípidos está vinculada a olores desagradables.

La valoración global de la carne refleja los cambios señalados en los otros atributos sensoriales estudiados. Teniendo en cuenta que en la escala de 5 puntos utilizada, el valor 3 fue considerado como el límite de aceptación, en el caso de la prueba con corderos lechales, según los panelistas, las muestras CTRL alcanzaron valores por encima de 3 a partir de 10 días, mientras que VIT-E y los tratamientos con orujo de uva llegaron a ese nivel en el día 14. En la prueba con corderos en crecimiento todas las muestras, excepto la carne del grupo con vitamina E, alcanzaron valores por encima de 3 a los 11 días de almacenamiento.

La vitamina E resultó eficaz previniendo el deterioro sensorial en los últimos días de almacenamiento respecto al tratamiento control. En la prueba experimental con corderos lechales, el orujo de uva fue tan eficaz como la vitamina E al final del almacenamiento, en relación con la presencia de olores anómalos y en la valoración global de la carne en comparación con el tratamiento control. La presencia de polifenoles en la leche podría ser la causa de tales resultados. Algunos estudios han indicado una mejora en algunos atributos sensoriales de la carne de cordero cuando se incluyeron polifenoles en la dieta de las ovejas (Nieto *et al.,* 2010a; 2010 b; Serrano *et al.,* 2014). En la prueba experimental con corderos en crecimiento-cebo, al final del almacenamiento el orujo de uva presentó valores intermedios entre el tratamiento con vitamina E y el control.

7.5. Referencias bibliográficas

Andrés, S., Morán, L., Aldai, N., Tejido, M.L., Prieto, N., Bodas, R., Giráldez, F.J. (2014). Effects of linseed and quercetin added to the diet of fattening lambs on the fatty acid profile and lipid antioxidant status of meat samples. *Meat Science 97*, 156–163.

Brogna, D.M.R., Tansawat, R., Cornforth, D., Ward, R., Bella, M., Luciano, G., Priolo, A., Villalba, J. (2014). The quality of meat from sheep treated with tannin- and saponin-based remedies as a natural strategy for parasite control. *Meat Science 96*, 744–749.

Chaves, A.V., Stanford, K., Dugan, M.E.R., Gibson, L.L., McAllister, T.A., Van Herk, F., Benchaar, C. (2008). Effects of cinnamaldehyde, garlic and juniper berry essential oils on rumen fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. *Livestock Science 117*, 215–224.

Devine, C.E., Graafhuis, P.H., Muir, P.D., Chrystall, B.B. (1993). The effect of growth rate and ultimate pH on meat quality of lambs. *Meat Science 35*, 63–77.

Francisco, A., Dentinho, M.T., Alves, S.P., Portugal, P.V., Fernandes, F., Sengo, S., Jerónimo, E., Oliveira, M.A., Costa, P., Sequeira, A., Bessa, R.J.B., Santos-Silva, J. (2015). Growth performance, carcass and meat quality of lambs supplemented with increasing levels of a tanniferous bush (*Cistus ladanifer* L.) and vegetable oils. *Meat Science 100*, 275–282.

Frutos, P., Hervás, G., Giráldez, F.J., Mantecón, A.R. (2004). Review. Tannins and ruminant nutrition. *Spanish Journal of Agricultural Research 2*, 191–202.

Gallardo, B., Manca, M.G., Mantecón, A.R., Nudda, A., Manso, T. (2015). Effects of linseed oil and natural or synthetic vitamin E supplementation in lactating ewes' diets on meat fatty acid profile and lipid oxidation from their milk fed lambs. *Meat Science 102,* 79–89.

Inserra, L., Priolo, A., Biondi, L., Lanza, M., Bognanno, M., Gravador, R., Luciano, G. (2014). Dietary citrus pulp reduces lipid oxidation in lamb meat. *Meat Science 96*, 1489–1493.

Jerónimo, E., Alfaia, C.M.M., Alves, S.P., Dentinho, M.T.P., Prates, J.A.M., Vasta, V., Santos-Silva, J., Bessa, R.J.B. (2012). Effect of dietary grape seed extract and *Cistus ladanifer* L. in combination with vegetable oil supplementation on lamb meat quality. *Meat Science 92*, 841–847.

Jerónimo, E., Alves, S.P., Dentinho, M.T.P., Martins, S.V., Prates, J.A.M., Vasta, V., Santos-Silva, J., Bessa, R.J.B. (2010). Effect of grape seed extract, *Cistus ladanifer* L., and vegetable oil supplementation on fatty acid composition of abomasal digesta and intramuscular fat of lambs. *Journal of Agricultural and Food Chemistry 58*, 10710–10721.

Jordán, M.J., Moñino, M.I., Martinez, C., Lafuente, A., Sotomayor, J.A. (2010). Introduction of distillate rosemary leaves into the diet of the Murciano-Granadina goat: Transfer of polyphenolic compounds to goats' milk and the plasma of suckling goat kids. *Journal of Agricultural and Food Chemistry 58*, 8265–8270.

Juárez, M., Dugan, M.E.R., Aalhus, J.L., Aldai, N., Basarab, J.A., Baron, V.S., McAllister, T.A. (2011). Effects of vitamin E and flaxseed on rumen-derived fatty acid intermediates in beef intramuscular fat. *Meat Science 88*, 434–440.

Khiaosa-Ard, R., Bryner, S.F., Scheeder, M.R.L., Wettstein, H.R., Leiber, F., Kreuzer, M., Soliva, C.R. (2009). Evidence for the inhibition of the terminal step of ruminal α-linolenic biohydrogenation by condensed tannins. *Journal of Dairy Science 92*, 177–188.

Lauzurica, S., De la Fuente, J., Díaz, M.T., Álvarez, I., Pérez, C., Cañeque, V. (2005). Effect of dietary supplementation of vitamin E on characteristics of lamb meat packed under modified atmosphere. *Meat Science 70*, 639–646.

Luciano, G., Vasta, V., Monahan, F.J., López-Andrés, P., Biondi, L., Lanza, M., Priolo, A. (2011). Antioxidant status, colour stability and myoglobin resistance to oxidation of *Longissimus dorsi* muscle from lambs fed a tannin-containing diet. *Food Chemistry 124*, 1036–1042.

Manso, T., Bodas, R., Castro, T., Jimeno, V., Mantecón, A.R. (2009). Animal performance and fatty acid composition of lambs fed with different vegetable oils. *Meat Science 83*, 511–516.

Manso, T., Bodas, R., Vieira, C., Mantecón, A.R., Castro, T. (2011) Feeding vegetable oils to lactating ewes modifies the fatty acid profile of suckling lambs. *Animal 5*, 1659–1667.

Min, B.R., Barry, T.N., Attwood, G.T., McNabb, W.C. (2003). The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Animal Feed Science Technology 106,* 3–19.

Moñino, I., Martínez, C., Sotomayor, J.A., Lafuente, A., Jordán, M.J. (2008). Polyphenolic transmission to Segureño lamb meat from ewes' diet supplemented with the distillate from rosemary (*Rosmarinus officinalis*) leaves. *Journal of Agriultural Food Chemistry 56,* 3363–3367.

Morán, L., Andrés, S., Bodas, R., Prieto, N., Giráldez, F.J. (2012a) Meat texture and antioxidant status are improved when carnosic acid is included in the diet of fattening lambs. *Meat Science 91,* 430–434.

Morán, L., Andrés, S., Mateo, J., Blanco, C., Soto, S., Giráldez, F.J. (2014). Effect of dietary carnosic acid on meat quality from suckling lambs. *Small Ruminant Research 121*, 314–319.

Morán, L., Giráldez, F.J., Panseri, S., Aldai, N., Jordán, M.J., Chiesa, L.M., Andrés, S. (2013). Effect of dietary carnosic acid on the fatty acid profile and flavour stability of meat from fattening lambs. *Food Chemistry 138*, 2407–2414.

Morán, L., Rodríguez-Calleja, J.M., Bodas, R., Prieto, N., Giráldez, F.J., Andrés, S. (2012b). Carnosic acid dietary supplementation at 0.12% rates slows down meat discoloration in *gluteus medius* of fattening lambs. *Meat Science 90*, 789–795.

Muíño, I., Apeleo, E., De la Fuente, J., Pérez-Santaescolástica, C., Rivas-Cañedo, A., Pérez, C., Díaz, M.T., Cañeque, V., Lauzurica, S. (2014). Effect of dietary supplementation with red wine extract or vitamin E, in combination with linseed and fish oil, on lamb meat quality. *Meat Science 98*, 116–123.

Nieto, G. (2013). Incorporation of by-products of rosemary and thyme in the diet of ewes: effect on the fatty acid profile of lamb. *European Food Research and Technology 236*, 379–389.

Nieto, G., Bañón, S., Garrido, M.D. (2011). Effect of supplementing ewes diet with thyme (*Thymus zygis* ssp. *gracilis*) leaves on the lipid oxidation of cooked lamb meat. *Food Chemistry 125*, 1147–1152.

Nieto, G., Díaz, P., Bañón, S., Garrido, M.D. (2010a). Dietary administration of ewe diets with a distillate from rosemary leaves (*Rosmarinus officinali*s L.): influence on lamb meat quality. *Meat Science 84*, 23–29.

Nieto, G., Díaz, P., Bañón, S., Garrido, M.D. (2010b). Effect on lamb meat quality of including thyme (*Thymus zygis* ssp. gracilis) leaves in ewes' diet. *Meat Science 85*, 82–88.

Pétriz-Celaya, Y., Calderón-Cortés, J.F., Pérez, C., Montaño, M.F., Plascencia, A. (2010). Influence of substitution of alfalfa hay for unfermented dried grape pomace on performance and carcass characteristics of growing sheep*. American Society of Animal Science*, *Proceedings, Western Section 61*, 308–311.

Priolo, A., Waghorn, G.C., Lanza, M., Biondi, L., Pennisi, P. (2000). Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: Effects on lamb growth performance and meat quality. *Journal of Animal Science 78*, 810–816.

Raccach, M. (1984). The antimicrobial activity of phenolic antioxidants in foods: A review. *Journal of Food Safety 6*, 141–170.

Rana, M.S., Tyagi, A., Hossain, S.A., Tyagi, A.K. (2012). Effect of tanniniferous *Terminalia chebula* extract on rumen biohydrogenation, Δ9-desaturase activity, CLA content and fatty acid composition in *longissimus dorsi* muscle of kids. *Meat Science 90*, 558–563.

Rota, M.C., Herrera, A., Martínez, R.M., Sotomayor, J.A., Jordán, M.J. (2008). Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oil. *Food Control 19,* 681–687.

Serrano, R., Jordán, M.J., Bañón, S. (2014). Use of dietary rosemary extract in ewe and lamb to extend the shelf life of raw and cooked meat. *Small Ruminant Research 116*, 144–152.

Vasta, V., Mele, M., Makkar, H.P.S., Priolo, A. (2009a). Ruminal biohydrogenation as affected by tannins in vitro. *British Journal of Nutrition 102*, 82–92.

Vasta, V., Pennisi, P., Lanza, M., Barbagallo, D., Bella, M., Priolo, A. (2007). Intramuscular fatty acid composition of lambs given a tanniniferous diet with or without polyethylene glycol supplementation. *Meat Science 76*, 739–745.

Vasta, V., Priolo, A., Scerra, M., Hallett, K.G., Wood, J.D., Doran, O. (2009b). Δ^9 desaturase protein expression and fatty acid composition of *longissimus dorsi* muscle in lambs fed green herbage or concentrate with or without added tannins. *Meat Science 82*, 357–364.

You, K.M., Jong, H.G., Kim, H.P. (1999). Inhibition of cyclooxygenase/lipoxygenase from human platelets by polyhydroxylated/methoxylated flavonoids isolated from medicinal plants. *Archives Pharmacology Research 22*, 18–24.

8. Conclusiones

Conclusiones

Primera. El orujo de uva de vino tinto presenta un valor nutritivo limitado y su composición química es variable en función de su proporción de semillas y de hollejos. Los hollejos presentaron un contenido inferior de fibra lignificada, mayor digestibilidad y mayor contenido de algunos polifenoles con gran actividad biológica que las semillas. El descenso medio de un 24% de la oxidación lipídica del plasma de ovejas alimentadas con orujo de uva y el alto contenido en ácido linoleico del orujo, evidencian su potencial antioxidante y el aporte de sustrato para la producción de ácidos grasos bioactivos a nivel ruminal.

Segunda. El empleo de orujo de uva en las raciones de ovejas en lactación enriquecidas con aceite de linaza, no afectó a la producción y composición de la leche. Aunque al incorporar un 5 y un 10% de orujo de uva en la ración, encontramos aumentos en los niveles de ácido ruménico del 14 y del 27% y en los niveles de ácido vacénico del 13 y del 24%, respectivamente, no hubo diferencias estadísticas en el perfil de ácidos grasos de la leche entre los tratamientos experimentales estudiados.

Tercera. La inclusión de orujo de uva en las raciones de ovejas en inicio de lactación a niveles del 5 y del 10%, no afectó a los rendimientos productivos ni a las características de la canal de los corderos lechales producidos. El orujo de uva mejoró la capacidad de retención de agua y aumentó la concentración de los niveles de ácido ruménico y de ácido vacénico en la carne de los corderos lechales, lo que ha sido relacionado con posibles efectos beneficiosos para la salud humana.

Cuarta. Un 5 y un 10% de orujo de uva en la ración de ovejas en inicio de lactación fue eficaz en prevenir la oxidación de la carne de cordero lechal, ya que los porcentajes de metamioglobina y los valores de TBARS fueron similares a los obtenidos al incluir vitamina E. Además, la carne de los corderos pertenecientes a los tratamientos con orujo de uva, mostraron mejores puntuaciones en la percepción sensorial a partir de 10 días de almacenamiento comparados con los corderos de ovejas no suplementadas.

Quinta. Un 5% de orujo de uva desecado en el concentrado de corderos en crecimiento y cebo en comparación con otros suplementos, como el extracto de semilla de uva y la vitamina E, no afectó a la ingestión, crecimiento e índice de conversión de los animales, ni a las características de la canal y de la carne. Únicamente, el orujo de uva tendió a incrementar la concentración de PUFA en la grasa intramuscular.

Sexta. El orujo de uva podría incluirse en el pienso de corderos en crecimiento al nivel del 5% del concentrado sin que la vida útil de la carne se viera afectada. Aunque el orujo de uva no fue tan eficaz como la vitamina E en la prevención de la oxidación de la carne, dio lugar a reducciones de los valores de TBARS de alrededor del 20% a partir del día 7 de almacenamiento respecto a los corderos no suplementados.

Conclusions

First. Grape pomace from red wine was of a limited nutritive value. The chemical composition of grape pomace was variable depending on their seeds and pulp plus skin proportions. The results clearly indicated that pulp plus skin provided the benefits of a lower lignified fibre, greater digestibility and a higher content of some polyphenols with a power biological activity than seeds. The interest of that by-product in sheep feeding could be related to its antioxidant power evidenced as lower numerical sheep plasma MDA values (24% decrease) and its polyunsaturated fatty acids (PUFA) content, such as linoleic acid, which provide the substrate for the production of bioactive fatty acids in the rumen.

Second. Supplementation with grape pomace of diets of early lactating ewes enriched with linseed oil was not detrimental for animal performance or milk yield. Rumenic acid levels were 14 and 27%, and vaccenic acid levels were 13 and 24% numerically higher, in diets with 5 and 10% of grape pomace respectively. However, there were no statistically significant differences in the milk fat FA profile between experimental groups.

Third. Grape pomace in ewe diets at the levels of 5 and 10%, did not affect suckling lamb performance and carcass characteristics of suckling lambs. Grape pomace improved the water holding capacity of meat and pomace increased the concentration of rumenic acid and vaccenic acid in suckling lamb meat, which has been related to potentially beneficial effects on human health.

Fourth. The inclusion of 5 and 10% of grape pomace in the ewe diets improved suckling lamb meat oxidation, since metmyoglobin and TBARS meat values lambs were similar to vitamin E group. Also, lambs from grape pomace treatments had better scores in sensory perception from day 10 of storage under retail display conditions compared to non supplemented ewes.

Fifth. Dried grape pomace at inclusion levels of 5% of concentrate in fattening lamb diets did not affect dry matter intake, average daily gain and conversion ratio, neither carcass and meat characteristics when compared with other supplements, such as grape seed extract and vitamin E. Only grape pomace tends to increase the PUFA concentration in intramuscular fat.

Sixth. Grape pomace could be included in fattening lamb diets at 5% of concentrate without any adverse effects on the shelf life of meat during storage under retail sale conditions. Although grape pomace were not as effective as vitamin E in preventing meat deterioration, lambs fed grape pomace showed reductions in TBARS values (of about 20%) from day 7 of storage under retail display conditions compared to the non supplemented lambs.