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Administration of distillate thyme leaves into the diet of Segureña ewes: effect on lamb meat quality

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*The effect of including thyme by-products from the distillation industry into the diet of pregnant ewes on the final quality of lamb meat was evaluated during meat storage in modified atmosphere. A total of 36 Segureña ewes were randomly assigned to three homogeneous groups. One group was fed a basal diet (*BD*) as control (*C*), whereas the diet of the other two groups was modified by substituting 10% (*T_1*) and 20% (*T_2*) of the* BD *with pellets made from 50% barley and 50% distilled thyme leaves (*DTL*). Meat spoilage (total viable, psychrotroph (*PSY*), moulds and yeasts, Enterobacteriaceae and lactic acid bacteria), thiobarbituric acid reactive substances (*TBARS*), colour (*CIELab *coordinates, metmyoglobin) and sensory characteristics of fresh lamb meat packed in modified atmosphere packaging (70% O_2 : 30% CO_2) were analysed after storage at 0, 7, 14 and 21 days. In general, the* DTL*-containing diet inhibited lipid and pigment oxidation in fresh lamb meat. Lower PSY counts and content of secondary oxidation product (*TBARS*) as a result of adding* DTL *to the ewe diet, whereas surface redness (*a* * values) was significantly higher on days 7 and 14. It can be concluded that thyme by-products from the distillation industry could be used as a source of natural antioxidant and antimicrobial in the feed for ewes.*

Keywords: lamb, *Thymus zygis*, antioxidant, antimicrobial, *MAP*

Implications

The average yield of essential oil per thyme plant is around 3% (w/v), which means that large amounts of apparently useless distilled leaves are produced. These distilled leaves do not have a specific commercial use, so it would be a very interesting alternative to provide food for livestock. The use of these by-products as natural antioxidants in feed for ewes could be a simple and interesting opportunity to replace synthetic antioxidants and to improve the quality of lamb meat. The results presented in this study are the first to study the meat quality of lambs obtained from ewes fed with by-products from the thyme essential oil industry.

Introduction

The optimum colour stability of packaged red meat is obtained by using a mixture of gases with high concentrations of oxygen and low proportions of carbon dioxide (Linares and Vergara, 2009). The carbon dioxide in the package atmosphere restricts the growth of aerobic spoilage bacteria (Gill, 1991), whereas a high oxygen concentration increases lipid oxidation, which adversely affects the flavour and nutritional value of the meat.

In recent years, consumers' pressure to reduce artificial additive use in foods has led to attempts to increase meat stability by dietary strategies. The use of by-products as natural antioxidants is a way to replace synthetic antioxidants, such as wastes from industrial residues of olive oil (Lesagemeessen *et al.*, 2001), essential oil of rosemary (Moñino *et al.*, 2008; Nieto *et al.*, 2010b) and grapes (Torres and Bobet, 2001). *Thymus zygis* ssp. *gracilis*, also known as red thyme, is a plant of the Labiatae family and is one of the most widely used Spanish thymes because it contains more thymol in its essential oil than the other members of the thymus family. The average yield of essential oil per plant is around 3% (w/v), which leads to a high quantity of unexploited distilled leaves as by-product of the production (Jordán *et al.*, 2009). The use of by-products from the essential oil industry as natural antioxidants and antimicrobials in feed could be a simple and interesting alternative to improve the quality of meat. The effect of adding herbs from the *Labiatae* family to feed in the final quality of the meat has been successfully evaluated in a variety of studies of different meat types leading to less oxidative deterioration in the meat products: in

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lamb (Moñino *et al*., 2008; Nieto *et al*., 2010a, 2010b, 2011a and 2011b) and turkey (Govaris *et al*., 2007).

To our knowledge, pertinent research into the use of distilled thyme leaves (*DTL*) in the feed for ewes has never been accomplished. The objective of this study was to investigate whether the inclusion of *DTL* during 240 days in the diet of pregnant ewes (coinciding with the gestation and lactation periods) affects the meat quality of lambs by measuring the oxidative stability, bacterial spoilage and sensorial characteristics of fresh lamb meat when stored in modified atmosphere packaging (*MAP*; 70% O_2 : 30% CO_2) for up to 21 days.

Material and methods

The research protocol used in this work was according to guidelines regarding the protection of animals used in research and for scientific purposes (Directive 2003/65/EC, 2003).

Plant material

Steam-distilled thyme leaves were obtained from a local company (Nutrafur-Furfural Español S.A., Murcia, Spain). Leaves were steam-distilled for 3 h using a distillation system with a stainless-steel steam boiler. Before its use, the distilled product of thyme was dried and kept at room temperature and the leaves and stems separated. The distilled leaves were incorporated into the feed by the company Cargill Animal Nutrition (Torres Pacheco, Murcia, Spain). To maintain the proper conditions of freshness, the feed was produced quarterly. The total phenolic content of the *DTL* is 79.6 $\pm$ 2.8 mg gallic acid equivalents/g dry plant (Nieto *et al*., 2011c) and the concentration (μg/g) of caffeic, ferulic, rosmarinic, carnosic acid and apigenin was: 96.5 $\pm$ 20.5, 181.6 $\pm$ 96.9, 223.5.6 $\pm$ 113.4, 109.7 $\pm$ 28.8 and 225.4 $\pm$ 75.4, respectively, as determined by Jordán *et al.* (2009).

Animals

A total of 36 pregnant ewes (Segureña breed) were randomly assigned to three homogeneous groups of same age (3 years old), weight (46.8 $\pm$ 7.14 kg) and body condition (2 $\pm$ 0.15) calculated according to Russel and Doney (1969). The three different groups of sheep were reared in individual pens throughout the trial period. Sheep in the control group were given a basal diet (*BD*; Feed A, Table 1) and barley consisting of 1.3 kg feed/day. The diet of the other two groups was modified by substituting 10% (T_1) and 20% (T_2) of the *BD* by *DTL* using a pellet made from 50% barley and 50% *DTL* (Feed B). Therefore, the control group was fed with 100% of *BD* + barley; T_1 with 90% 'Feed A + barley' + 10% 'Feed B' and T_2: 80% 'Feed A + barley' + 20% 'Feed B'. The diets of the three groups were balanced according to protein (164.2 g/kg of dry matter) and lipid content (33.1 g/kg of dry matter) by the company Cargill Animal Nutrition. Table 2 shows a description of the different diets and the administration of feed per day. Dietary compositions of polyphenolics were determined by Moñino (2010), and stated that in the diet from *DTL*, the levels of gallic, ferulic, coumaric acid, naringin, hesperidin, luteolin, rosmarinic acid, apigenin, genkwakin, carnosol and carnosic acids were higher than in the control diet. The sheep given these diets were fed for 240 days, coinciding with the gestation (5 months) and lactation periods (55 days) in order to study the quality of the subsequent lamb meat. Both the sheep and lambs (9 lambs per level: 27 in total) were intensively reared on a research farm (CIFEA-Lorca, Murcia, Spain). Following the practices recommended by Segureño farmers, all the lambs were weaned when they reached the weight of 13 $\pm$ 1 kg and were fed commercial fattening pellets until they reached the slaughter weight of 25 $\pm$ 2 kg. Finally, the lambs were slaughtered in a local slaughterhouse according to Spanish regulations (RD 147/1993). The carcasses were stored at 2°C for 24 h in a cooling room.

Table 1 *Chemical composition of the concentrated basal diet (Feed A)*

Nutrient[a]	g/kg of dry matter
Ash	76
CP	164
Fat	33
NDF	396
Digestible NDF (%)	24
ADF	208
Nonproteic nitrogen	0.5
RDP (% of CP)	65
Nonfibre carbohydrate	243
Adjusted total starch	143
Net energy lactation (mcal/kg)	1.6
Total soluble RDP	56
Ruminal undegradable protein	54
Calcium	3.9
Phosphorus	5.7
Vitamin A (IU/g)	19
Vitamin D (IU/g)	3
Vitamin E (IU/g)	40
Magnesium	2.8
Selenium (mg/kg)	0.4
Zinc (mg/kg)	127
Total methionine	2
Total lysine	7

RDP = ruminal degradable protein.
[a]Formulated using the following ingredients/tone: wheat bran, 16%; scale soy, 26.1% kg; barley, 27.4%; malt comb, 2.46%; sunflower oil, 3.87%; beet pulp, 3.36%; corn flour 11.4%; calcium carbonate, 14.6%; sorghum, 3.35%; molasses-cane, 2.5%; vitaminic and mineral feed additives, 1.16%. Data provided by Cargill Animal Nutrition (Torre Pacheco, Murcia, Spain).

Sample preparation and experimental design

Twenty-four hours *post mortem*, the longissimus dorsi (*LD*) muscle was removed from both sides of the carcasses and was cut into 1.5-cm portions of 10 g of weight. Four different portions of meat were individually packaged in two different polystyrene trays (two trays with four portions of meat for each day of storage) B5-37 (Aerpack, Madrid, España) in BB4L bags (Cryovac, Madrid, ESpaña) of low gas permeability (8 to 12 cm^3/m^2 per 24 h). The air in the packs was replaced by 70% O_2 : 30% CO_2 (EAP20, Carburos Metálicos S.A., Barcelona, Spain) (*MAP*) in a discontinuous INEINI packer (Pack Multifunction). After

Table 2 *Rations of basal diet (Feed A), barley and distilled thyme leaves (Feed B) administered to ewes*

Diet	*DTL* %	Feed A: 'Unifeed' (g/day)	Feed B: '*DTL*' (g/day)	Barley (g/day)	Protein (g/kg dry matter)	Fat (g/kg dry matter)
Control	–	1000	–	300		
T_1	10	870	260 (130 *DTL* + 130 barley)	170	164	30
T_2	20	740	520 (260 *DTL* + 260 barley)	40		

DTL = distilled thyme leaves.
Feed A: 'basal diet', ingredients are shown in Table 1.
Feed B: DTL diet formulated using: 50% *DTL* + 50% barley.

sealing, the atmosphere inside the bags was checked using a Pack12P analyser (Abiss, Madrid, España). No significant variation in the mixture was found during storage. Samples were stored at 4 ± 2°C for 0, 7, 14, or 21 days in a display cabinet (Helkama, Finland) illuminated with white fluorescent light (1600 lx), simulating retail display conditions. For every day of analysis, two packs with four portions of LD were opened for the different analysis.

Measurement of colour

The fresh meat samples were kept at 4°C for 4 h to allow colour development (bloom) and the colour was measured using a CR-200/08 Chroma Meter II (Minolta Ltd, Milton Keynes, United Kingdom) directly on the meat surface (illuminant/observer D65/28, Diffuse/O mode, 8 mm aperture of the instrument for illumination and 8 mm for measurement). Results were expressed as *CIELab* values (Commission Internationale de L'Eclairage (CIE), 1978): L^*, a^*, b^*, Chroma (C^*) and Hue angle ($^\circ H$) (expressed as sexagesimal degrees); $C^* = (a^{*2} + b^{*2})^{1/2}$; $^\circ H = t/g\ (b^*/a^*)$. The metmyoglobin (*MM*) percentage at the lamb steak surface was estimated spectrophotometrically, according to Stewart *et al.* (1965), by measuring steak surface reflectance at 525 and 572 nm (Minolta CM-2002; Osaka, Japan). The maximum value of the ratios of $(K/S)_{572}$ to $(K/S)_{525}$ at the beginning of the experiment was fixed as 0% *MM*; *K* and *S* were the absorption and the scattering coefficients, respectively, and *K/S* ratios were calculated from reflectivity (R_∞) values using the Kubelka–Munk equation. The value of 100% *MM* was obtained following the same procedure after oxidizing a sample in a 1% (w/v) solution of potassium ferricyanide. Six measurements were made per sample for all the colour measurements.

Measurement of lipid oxidation

Lipid oxidation was expressed as thiobarbituric acid reactive substances (*TBARS*; mg malondialdehyde/kg meat), as determined by Botsoglou *et al.* (1994), using a UV2 spectrophotometer (Pye Unicam Ltd, Cambridge, United Kingdom).

Sensory analysis

A sensory analysis of the raw lamb meat was conduced by a panel formed of eight persons from the university community and trained according to ISO 8586-1 (1992). Panel training consisted of four sessions and was carried out using the descriptors shown in Table 3. Sensory analysis was carried out according to ISO 4121 (2003), using a 6-point scale (1, minimum; 6, maximum). The descriptors used were: *MO*, lamb meat odour; *PO*, putrid odour; *RO*, rancid odour; *AO*, acid odour; *MC*, meaty colour; and *FC*, fat colour.

Table 3 *Reference food used for sensory analysis of raw lamb meat*

Descriptors	References
Odour	
Lamb meat	Fresh lamb meat odour
Putrid	Odour of decomposing meat
Acid	Odour of fermented milk
Rancid	Odour of rancid oil
Metalic	Odour characteristic of metal
Colour[a]	
Meat	Fresh lamb meat colour
Fat	Fresh lamb fat colour

[a]Evaluated under normalised artificial light.

Microbiological analysis

For the microbiological assays, bags were aseptically opened in a 131 Bio-II-A microbiology cabinet (Telstar, Tarrasa, Spain) and meat samples (10 g) were weighed with sterile tweezers into stomacher bags and blended with peptone water 0.1% w:w in a stomacher (IUL Instruments, GMBH, Königswinter, Germany). Total viable (*TV*) and total psychrotroph (*PSY*) counts were determined on PCA (Plate Count Agar), incubating at 37°C for 24 h (*TV*) (ISO 4833:2003) and 4°C for 7 days (*PSY*) (ISO 17410:2001). Moulds and yeasts (*MY*) were counted on RB (Rose-Bengal) with chloramphenicol, incubating at 25°C for 5 days (ISO 21527-2:2008). Lactic acid bacteria (*LA*) were counted on MRS Agar plates (de man, Rogosa, Sharpe) and incubated at 30°C for 72 h (ISO 15214:1998). Total *Enterobacteriaceae* (*ENT*) were counted on VRBG plates (Violet Red Bile Glucose Agar) and incubated at 37°C for 24 h (ISO 21528-2:2004). All the microorganisms tested were incubated in an ST 6120 culture incubator (Heraeus S.A., Boadilla, Madrid, Spain). The results are expressed as log cfu/g.

Statistics

A randomized design using lamb diet and storage time as treatments was performed. A two-way analysis of variance was used to investigate the effect of diet, storage time as factor and the interaction between the two factors on the dependent variables. When no significant interaction was found ($P > 0.05$), the model was reduced to main effects

Table 4 *Effect of* DTL *feeding and storage time on mean values of* L*, a*, b*, C* *and* °H *in raw lamb meat stored in* MAP *(70% O_2 : 30% CO_2) kept for 0, 7, 14 and 21 days under retail conditions*

							P-values		
	Level	Day 0	Day 7	Day 14	Day 21	s.e.m.	Storage time	Diet	Storage time × diet
*L**	*C*	42.6^{x}	42.3^{x}	46.7^{w}	48.8^{w}	0.24	***	ns	ns
	T_1	42.6^{x}	42.5^{x}	46.6^{w}	49.2^{w}				
	T_2	42.1^{y}	43.5^{y}	46.5^{x}	49.6^{w}				
*a**	*C*	16.9^{w}	14.7^{bx}	8.7^{by}	4.9^{y}	0.28	***	**	ns
	T_1	16.7^{w}	15.8^{aw}	11.2^{ax}	4.4^{x}				
	T_2	16.8^{w}	15.6^{aw}	11.4^{ax}	4.9^{y}				
*b**	*C*	6.8^{y}	8.9^{xy}	10.3^{wx}	11.9^{w}	0.18	***	ns	ns
	T_1	6.1^{y}	8.7^{x}	9.8^{wx}	11.6^{w}				
	T_2	6.1^{y}	8.6^{x}	10.0^{wx}	11.1^{w}				
°*H*	*C*	20.3^{z}	30.8^{y}	49.6^{ax}	70.5^{w}	1.09	**	**	ns
	T_1	19.1^{y}	28.5^{y}	40.1^{bx}	68.8^{w}				
	T_2	19.1^{z}	28.6^{y}	40.2^{bx}	65.1^{w}				
*C**	*C*	18.4^{w}	17.4^{w}	13.4^{bx}	12.7^{x}	0.18	***	*	ns
	T_1	17.9^{w}	18.1^{w}	15.2^{ax}	12.5^{y}				
	T_2	17.9^{w}	17.9^{w}	15.4^{ax}	12.5^{y}				

DTL = distilled thyme leaves; *L** = lightness; *a** = redness; *b** = yellowness; *C** = chroma; °*H* = hue; *MAP* = modified atmosphere packaging; s.e.m. = standard error of mean; *C* = Control; T_1 = 10% *DTL*; T_2 = 20% *DTL*.
Means with different superscripts are significantly different ($P < 0.05$). ^{a,b,c}Different letters within same column (different diet treatment) differ significantly ($P < 0.05$). ^{w,x,y,z}Different letters within same row (different storage day) differ significantly ($P < 0.05$). *P*: probability; significance levels: ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; ns: $P > 0.05$.

only (one-way ANOVA). Loin samples from 27 different lambs feed with dietary 10% *DTL* ($n = 9$), 20% *DT* ($n = 9$) or not ($n = 9$) were analysed in triplicate. The least squares means (LSM) and the significance of the treatment were calculated using type IV sum of squares. The Scheffe Means Test was used to compare the LSM, which were considered to be statistically different when $P < 0.05$. Data were analysed using the Statistix 8.0 for Windows (Analytical Software, New York, USA).

Results and discussion

Tables 4, 5 and 7 show probability (*P*-values) for dependent variables. According to probability, the storage time affected meat quality more than lamb diet. Variability data indicated that the prooxidant conditions (70/30 O_2/CO_2 and 1600 lx lighting) for up to 21 days of storage largely exceeded the antioxidant capacity of the meat. The storage time affected all dependent variables, while the lamb diet affected *a**, °*H*, *C**, *MO*, *RO*, *MC*, *PSY*, *ENT*, *LA*, *TBARS* and *MM*. Interactions between storage time and diet were found for *MO*, *PO*, *AO*, *MC*, *TV*, *MY* and *MM* ($P < 0.01$).

Table 4 shows the effects of *DTL* feeding and storage time on *CIELab* coordinates (*L**, *a** and *b**) in raw lamb meat. The diet had no effect ($P > 0.05$) on the *CIELab* coordinates at day 0. In contrast, Simitzis *et al.* (2007) observed that in meat from lambs fed with oregano supplements, the values of *a** and *b** were higher than control meat at day 0. There was a gradual increase ($P < 0.05$) in the *L** and *b** values, whereas *a** decreased during lamb meat storage, probably as a result of gradual protein decomposition, which lead to increased light scattering. Although *DTL* did not affect the *L** values, there were significant differences ($P < 0.05$) between the *a** value of *C* and $T_1 - T_2$ on days 7 and 14. Nieto *et al.* (2010b) reported slightly better colour stabilization in chilled-packed lamb cuts as result of a distilled rosemary leaves (*DRL*) diet. However, O'Grady *et al.* (2006) concluded that the dietary supplementation of cattle feed with rosemary extract (1000 mg/animal per day) did not significantly improve the surface redness of fresh beef.

Table 4 shows the effects of *DTL* feeding and storage time on Hue angle and *Chroma.* Hue angle (°*H*) increased ($P < 0.05$) during storage, the highest values occurring in *C* (70.58; most intense meat browning) and the lowest values in T_2 (65.1) on day 21. Significant differences were found in °*H* between *C* and $T_1 - T_2$ on day 14 of storage. Larraín *et al.* (2008) reported slower increases in °*H* over time of storage in bacon from pigs fed a phenol-rich cranberry extract as compared with animals control. *Chroma* values fell from day 14 onwards ($P < 0.05$) in all the treatments. As in the case of *a** and °*H*, *DTL* only affected *Chroma* on day 14, T_1 and T_2 showing significantly higher *Chroma* values than *C*, meaning that the meat from *DTL* had a more vivid colour than *C* on day 14. The decrease in *Chroma* and increase in °*H* are used to monitor colour in lamb meat during their display life, as both are correlated with *MM* formation (Sánchez-Escalante *et al.*, 2003).

All the results for the *CIELab* coordinates follow the same trend: a tendency of *DTL* to maintain the meat colour on days 7 (*a**) and 14 (*a**, *Chroma*, °*H*). This improvement in colour on days 7 and 14 is in accordance with Luciano *et al.* (2009), who showed than the inclusion of natural antioxidants

(tannins) in sheep diet improved the colour stability of fresh lamb meat during 14 days of storage. The colour results reflected the changes that are normally associated with the loss of redness in meat. Redness (a^*) in meat is related with reduced iron in myoglobin (Fe^{+2}) and can be controlled by several strategies that prevent oxidation, as the use of plants rich in phenolic compounds. *DTL*, which is rich in phenols with antioxidant properties, prevents red haem pigments from undergoing oxidation to brown *MM*. For this reason, *DTL* diet could be a good alternative for reducing colour degradation in lamb meat, because the most important sensory attribute affecting consumers' purchasing decisions of red meats is colour.

Metmyoglobin percentage

Figure 1 shows the effects of *DTL* feeding on metmyoglobin percentage (*MM*) in raw lamb meat. In the case of *MM*, we have found a significant interaction (*F*-value: 59.1, $P < 0.01$) between *DTL* diet and storage time. There were significant differences ($P < 0.05$) in the *MM* values between *C* and $T_1 - T_2$ on days 7 and 14. Therefore, the incorporation of *DTL* at 10% and 20% improved ($P < 0.05\%$) lamb meat colour on these days, and this effects is due to the phenolics compounds presents in the *DTL*. Hayes *et al.* (2009) also found that the direct addiction of purified phenolics and of polyphenol-rich plant extracts to a muscle model system delayed *MM* formation.

Lipid oxidation

Figure 2 shows the effects of *DTL* feeding on lipid oxidation (*TBARS*) in raw lamb meat. *TBARS* values increased ($P < 0.05$) with storage time in the three levels of diet. The highest values were recorded in *C*, whereas *TBARS* evolved similarly in both T_1 and T_2. Significant differences ($P < 0.05$) were found between *C* and *DTL* samples on days 7 and 14. Both T_1 and T_2 delayed lipid oxidation, probably because of the antioxidant effect of compounds of *DTL* administered during gestation and through the mother's milk, which are absorbed along the gastrointestinal tract and deposed in the tissues. In this sense, Moñino (2010) determined the polyphenolic compositions in T_1 and T_2, and concluded that the concentrations of coumaric acid, rosmarinic acid and carnosic acids were significantly higher in T_1 and T_2 than in *C*. Furthermore, these authors stated the transmission of phenolic compounds to the lamb meat.

The antioxidant effect of *DTL* on days 7 and 14 is due to the presence of phenolic compounds that contain conjugated ring structures and hydroxyl and carboxylic acid groups, which inhibit lipid oxidation either by metal chelation or by stabilizing the free radicals. Several authors have shown that dietary supplementation with natural antioxidants is a convenient strategy for inhibiting oxidative reactions: Botsoglou *et al.* (2007) showed that an effective way to delay lipid oxidation is to incorporate dehydrated rosemary in feeds for turkeys; Moñino *et al.* (2008) demonstrated the positive effect of a diet containing rosemary on controlling lipid oxidation in meat. However, O'Grady *et al.* (2006) concluded that a diet

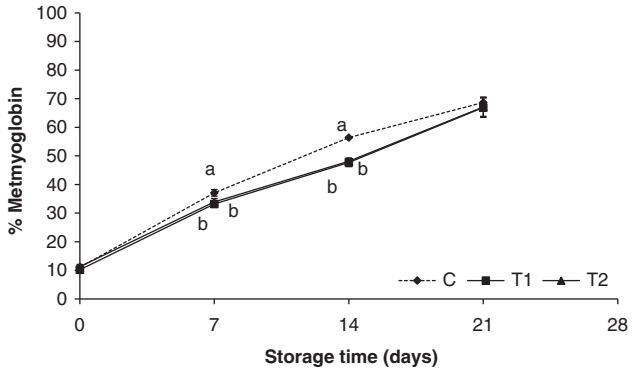

Figure 1 Effect of distilled thyme leaves (*DTL*) feeding on surface metmyoglobin percentage (*MM*) of raw lamb meat stored in modified atmosphere packaging (MAP; 70% O_2 : 30% CO_2) for 0, 7, 14 and 21 days under retail conditions.

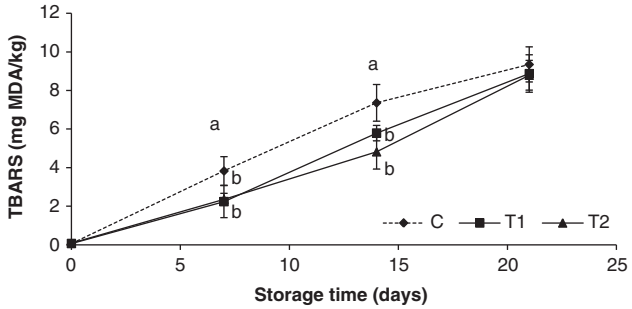

Figure 2 Effect of distilled thyme leaves (*DTL*) feeding on lipid oxidation (thiobarbituric acid reactive substances, *TBARS*) in raw lamb meat stored in modified atmosphere packaging (*MAP*, 70% O_2 : 30% CO_2) kept for 0, 7, 14 and 21 days under retail conditions.

supplemented with rosemary did not affect the lipid stability of fresh beef meat.

In comparison of the results obtained from the two ewe groups fed thyme leaves (10% or 20%), it is important to remark that no statistically significant differences were detected between the values of the two experimental groups. From this, it can be concluded that the incorporation of distilled thyme leaves at 10% of the ewe diet should be enough to improve the meat's antioxidant status. This affirmation is in accordance with our previous observations (Nieto *et al.*, 2010b) using 10% or 20% rosemary leaf. On the contrary, Botsoglou *et al.* (2007) showed that the incorporation of dehydrated rosemary in turkey feed had a dose-dependent effect on the meat, where a high level (1%) was more effective than a low level (0.5%).

Sensory analysis

Table 5 shows the effects of diet and storage time on the sensory quality of lamb cuts kept under retail display conditions. Chilled-packed lamb suffered characteristic sensory spoilage associated with oxidizing phenomena, such as lean browning, fat darkening, exudation, loss of metallic-blood odour and growing rancid odour. Odour quickly deteriorated in the chilled-packed lamb, in particular, when a rancid odour began to be detected. Lamb meat odour (*MO*) scores were

Table 5 *Effect of* DTL *feeding and storage time on mean values of sensory scores in raw lamb meat stored in* MAP *(70% O_2 : 30% CO_2) for 0, 7, 14 and 21 days under retail conditions*

							P-values		
	Level	Day 0	Day 7	Day 14	Day 21	s.e.m.	Storage time	Diet	Storage time × diet
MO	*C*	5.62^{w}	4.25^{x}	1.64^{y}	1.09^{z}	0.11	***	*	*
	T_1	5.38^{w}	4.52^{x}	2.11^{y}	1.25^{z}				
	T_2	5.45^{w}	4.83^{x}	2.13^{y}	1.15^{z}				
PO	*C*	1.01	1.00	1.02	1.00	0.01	***	ns	*
	T_1	1.00	1.00	1.00	1.11				
	T_2	1.00	1.00	1.00	1.12				
AO	*C*	1.08^{x}	1.25^{x}	1.35^{x}	1.77^{w}	0.03	***	ns	*
	T_1	1.02^{x}	1.17^{x}	1.25^{x}	1.65^{w}				
	T_2	1.06^{x}	1.12^{x}	1.43^{w}	1.64^{w}				
RO	*C*	1.00^{z}	1.77^{ay}	4.08^{ax}	5.10^{w}	0.11	***	***	ns
	T_1	1.01^{y}	1.15^{by}	3.09^{bx}	4.69^{w}				
	T_2	1.06^{y}	1.08^{by}	2.90^{bx}	4.62^{w}				
MC	*C*	5.53^{w}	4.88^{bx}	2.20^{by}	1.50^{z}	0.10	***	**	**
	T_1	5.30^{w}	5.15^{aw}	3.05^{abx}	1.87^{y}				
	T_2	5.22^{w}	5.19^{aw}	3.12^{ax}	1.52^{y}				
FC	*C*	5.21^{w}	4.62^{w}	3.67^{x}	3.06^{x}	0.12	***	ns	ns
	T_1	5.16^{w}	4.90^{w}	3.66^{x}	3.56^{x}				
	T_2	4.97^{w}	4.94^{w}	3.68^{x}	3.37^{x}				

DTL = distilled thyme leaves; *MAP* = modified atmosphere packaging; s.e.m. = standard error of mean; *MO* = meat odour; *PO* = putrid odour; *AO* = acid odour; *RO* = rancid odour; *MC* = meaty colour; *FC* = fat colour; *C* = Control; T_1 = 10% *DTL*; T_2 = 20% *DTL*.
Means with different superscripts are significantly different ($P < 0.05$). ^{a,b,c}Different letters within same column (different diet treatment) differ significantly ($P < 0.05$). ^{w,x,y,z}Different letters within same row (different storage day) differ significantly ($P < 0.05$). *P*: probability; significance levels: $***P \leq 0.001$; $**P \leq 0.01$; $*P \leq 0.05$; ns: $P > 0.05$.

maximal in the freshly cut meat and decreased gradually during storage ($P < 0.05$) because of oxidative changes (pigment and lipid) that directly affect flavour. Acid odours (*AO*) were low and ranged from ~1 to 2 during the storage period. There were no significant differences ($P > 0.05$) in *MO*, *PO* (putrid odour), *AO* between T_1, T_2 and *C*.

The mean score for rancid odour was lower in T_1, T_2 than in *C* lamb at days 7 and 14. The results of the sensory analysis of *RO* reflected the *TBARS* indices obtained: the highest *RO* scores corresponded to the samples with the greatest concentration of malonaldehyde (MDA). *DTL* delayed rancidity and the loss of lamb meat colour during storage. These results are in accordance with Fernández-López *et al.* (2005), who showed that the application of rosemary extracts (Herbalox®) to beef meatballs delayed the development of rancidity and off-flavour. In contrast, the results published by O'Grady *et al.* (2006), showed that dietary supplementation of beef with rosemary extract did not result in a significant improvement in the odour of cooked meat stored in *MAP* for up to 6 days at 4°C.

According to the meaty colour (*MC*) fat colour (*FC*) scores, which are the main sensory traits contributing to raw meat acceptability, reduced throughout storage; *DTL* affected the *MC* scores, which showed differences ($P < 0.05$) between the *C* and $T_1 - T_2$ samples on days 7 and 14. T_1 and T_2 samples had a better appearance on days 7 and 14. Likewise, Djenane *et al.* (2003) stated a better appearance in beef steaks with added rosemary extracts. Similarly *MM*, there was a significant interaction (*F*-value: 59.1, $P < 0.01$) between *DTL* diet and storage time ($P < 0.01$) According to Djenane *et al.* (2002), colour loss is unacceptable when it reaches scores of 3 (using a 5-point descriptive scale), therefore, on a scale of 6 points (as presented in this study), the colour loss would be unacceptable when it reaches scores of 2.5. Table 6 shows that *MC* reached this score in *C* after 14 days of storage, whereas in T_1 and T_2 this score

Table 6 *Correlation coefficients (*R*) between sensory attributes (*MC, FC, RO*), CIELab coordinates (*a**, b**, C** and °H*), MM and* TBARS *for raw lamb meat stored in* MAP *(70% O_2 : 30% CO_2) for 21 days under retail conditions*

	TBARS	*RO*	a*	*MC*	*FC*	b*	*MM*	C*
RO	0.823							
*a**	−0.876	−0.800						
MC	−0.827	−0.844	0.764					
FC	−0.471	−0.398	0.447	0.445				
*b**	0.598	0.520	−0.404	−0.531	−0.263			
MM	0.908	0.763	−0.843	−0.722	−0.441	0.555		
*C**	−0.591	−0.529	0.823	0.503	0.341	0.182	−0.615	
°H	0.887	0.799	−0.869	−0.774	−0.414	0.776	0.839	−0.434

MC = meaty colour; *FC* = fat colour; *RO* = rancid odour; *a** = redness; *b** = yellowness; *C** = chroma; *°H* = hue; *MM* = metmyoglobin; *TBARS* = thiobarbituric acid reactive substances; *MAP* = modified atmosphere packaging.
All the correlations are significant, $P \leq 0.001$.

Table 7 *Effect of* DTL *feeding and storage time on mean values of* TV, *total* PSY, MY, LA*bacteria and* ENT *counts (log cfu/g) in raw lamb meat stored in* MAP *(70% O_2 : 30% CO_2) kept for 0, 7, 14 and 21 days under retail conditions*

							P-values		
	Level	Day 0	Day 7	Day 14	Day 21	s.e.m.	Storage time	Diet	Storage time×diet
TV	*C*	2.15^{z}	2.99^{y}	3.73^{x}	5.48^{w}	0.07	***	ns	**
	T_1	2.40^{y}	2.92^{y}	3.94^{x}	5.59^{w}				
	T_2	2.35^{z}	3.17^{y}	3.98^{x}	5.19^{w}				
PSY	*C*	2.63^{z}	4.11^{ay}	5.28^{ax}	7.55^{w}	0.11	***	***	ns
	T_1	2.87^{z}	3.46^{by}	5.09^{bx}	6.99^{w}				
	T_2	2.73^{z}	3.58^{by}	4.99^{bx}	7.32^{w}				
MY	*C*	1.55^{y}	2.56^{x}	3.15^{x}	4.80^{w}	0.08	***	ns	**
	T_1	1.34^{y}	2.38^{x}	2.86^{x}	5.24^{w}				
	T_2	1.42^{y}	2.54^{x}	3.30^{x}	4.32^{w}				
ENT	*C*	0.00	0.00	0.00	0.41	0.01	***	*	ns
	T_1	0.00	0.00	0.00	0.46				
	T_2	0.00	0.00	0.00	0.53				
LA	*C*	1.56^{z}	2.41^{y}	4.04^{x}	5.40^{w}	0.10	***	***	ns
	T_1	1.07^{z}	2.40^{y}	3.50^{x}	4.64^{w}				
	T_2	1.30^{z}	2.47^{y}	3.90^{x}	5.25^{w}				

DTL = distilled thyme leaves; *TV* = total viable; *PSY* = psychrotroph; *MY* = mould and yeast; *LA* = lactic acid; *ENT* = Enterobacteriaceae; *MAP* = modified atmosphere packaging; *C* = Control; T_1 = 10% *DTL*; T_2 = 20% *DTL*.
Means with different superscripts are significantly different ($P < 0.05$). [a,b,c]Different letters within same column (different diet treatment) differ significantly ($P < 0.05$). [w,x,y,z]Different letters within same row (different storage day) differ significantly ($P < 0.05$). *P*: probability; significance levels: *** $P \leqslant 0.001$; ** $P \leqslant 0.01$; * $P \leqslant 0.05$; ns: $P > 0.05$.

was reached on day 21; thus, dietary *DTL* led to an extended shelf life in the conditions tested.

Table 6 shows Pearson's correlation coefficients between *TBARS*, *a**, *b**, *C**, °*H*, *MM* and sensory attributes. *RO* scores were positively and significantly related with *TBARS* ($R = 0.823$, $P \leqslant 0.001$), °*H* ($R = 0.799$, $P \leqslant 0.001$), *MM* ($R = 0.762$, $P \leqslant 0.001$) and *b** ($R = 0.598$, $P \leqslant 0.001$), while, *a** values ($R = -0.800$, $P \leqslant 0.001$), *C** ($R = -0.529$, $P \leqslant 0.001$), *MC* ($R = -0.844$, $P \leqslant 0.001$) and *FC* ($R = -0.398$, $P \leqslant 0.001$) were negatively correlated with rancid scores in lamb meat. The higher the *RO* values, the higher the yellowness and browning and the lower the redness of the meat and fat. The strong negative correlation between *TBARS-Chroma* ($R = -0.591$, $P \leqslant 0001$) and *TBARS-Hue* ($R = 0.887$, $P \leqslant 0001$) in the present work confirm the published by Sánchez-Escalante *et al.* (2003), who reported a correlation between *TBARS* and *Chroma* of $R = -0.9222$, and stated that myoglobin and oxymyoglobin oxidation to *MM* is associated with a reduction in reddish colour (higher hue values and lower chroma).

Microbial spoilage

Table 7 shows the effects of *DTL* feeding and storage time on total viable (*TV*), psychrotroph (*PSY*), mould and yeast (*MY*), enterobacteriaceae (*ENT*) and lactic acid bacteria (*LA*) counts in raw lamb meat stored in *MAP* under retail conditions. The initial bacterial load is important for determining the shelf life of meat, because a high number of microorganisms in meat before storage shortens its shelf life. The initial results reported in the present work confirm those published by Lauzurica *et al.* (2005). *TV* counts increased with storage time but did not reach 10^6 cfu/g in any treatment, which is the limit established by Spanish legislation for *TV* in fresh lamb meat (Regulation EC 2073/05, DOUE L338/1, 2005). No *pH* changes that could have affected microbial growth were found as the mean *pH* was similar, ranging from to 5.54 to 5.69. Compared with the initial values, the mean *TV* counts showed statistically significant increase ($P < 0.05$) for both *C* and T_2 on every sampling day, whereas no significant differences ($P > 0.05$) were found between days 0 and 7 in T_1. *DTL* did not reduce the *TV* counts ($P < 0.05$). In our previous work (Nieto *et al.*, 2010b), a positive antimicrobial effect of a diet with distilled rosemary leaves in fresh lamb meat on *TV* was found. There was a significant increase ($P < 0.05$) in *PSY* values in *C*, T_1 and T_2 during the 21 days of storage; the highest value was reached on day 21 in *C* (7.55 cfu/g), whereas the lowest values were always found in T_1. The samples treated with *DTL* exhibited lower counts than *C* from 7 days of storage onwards, although the differences were only significant ($P < 0.05$) on days 7 and 14. According to our results, studies have shown the antimicrobial properties of thyme against *PSY*: *in vitro* (Singh *et al.*, 2004) and in chicken meat (Hao *et al.*, 1998).

Direct comparison of our results with other studies is difficult as there have been no reports of the *in vivo* antimicrobial effects of *DTL*. In general, the antibacterial properties of phenolic compounds are in part associated with their lipophilic character, which leads to their accumulation in membranes and to subsequent membrane-associated events such as energy depletion (Conner and Beuchat, 1984).

MY counts showed a significant increase ($P < 0.05$) during storage. This was evident in *C*, T_1 and T_2 on days 7 and 21,

although the increase between days 7 and 14 was not statistically significant in any treatment. In addition, *ENT* counts remained at 1 cfu/g throughout storage and in all the treatments. The development of *MY* and *ENT* was unaffected. The atmosphere used – gas mixtures containing high concentrations of oxygen together with low proportions of carbon dioxide, which restricts the growth of aerobic spoilage bacteria – and hygienic handling of the meat ensured the absence or very low counts of *MY* and *ENT* in lamb meat after 21 days of storage at 4°C. According to Grau (1981), the *pH* of the meat ($pH < 5.8$) ensured that the *ENT* growth was completely inhibited. Gill and Penney (1985) reported the development of *ENT* in lamb meat packed in *MAP*, but in this case the *pH* was higher (5.9). There was a significant increase ($P < 0.05$) in *LA* counts in *C*, T_1 and T_2 on all sampling days during storage. The highest *LA* counts corresponded to *C* on day 21 (5.40 cfu/g) while the count was 4.64 (14% lower) and 5.25 (2.7% lower) in T_1 and T_2, respectively. However, Soultos *et al.* (2009) showed that the addition of 100 mg/kg of oregano essential oil to the diet of rabbits inhibited the growth of *LA* and *ENT* in carcasses. In addition, Govaris *et al.* (2007) observed an inhibitory effect of rosemary contained in the diet of turkeys on the growth of *TV*, *PSY*, *MY*, *LAB*, *ENT* and *PSY*.

Estimation of shelf life of Segureño MAP lamb meat

The shelf life of refrigerated lamb meat does not usually exceed 10 days before spoilage becomes evident. Soldatou *et al.* (2009) estimated the shelf life of *Souvlaki* (a fresh lamb meat product) samples using colour coordinate (Hunter *a** value of <9 as colour limit), *TBARS* (4.4 mg MDA/kg as marking the initiation of lipid oxidation/rancidity of lamb meat samples) and microbiological analysis (*TV*: establishing an arbitrary *TV* level of 7 log cfu/g as the limit of microbiological acceptability). In our study, a level of 7 log cfu/g was not reached even in the control group during the 21 days of the study; therefore, the shelf life of meat was increased only when *TV* was taken into account. However, Hunter *a** values close to 9 and values higher than 4.4 mg MDA/kg were found on day 14. Thus, we could affirm that there is a tendency of *DTL* diet to increase ($P < 0.05$) the shelf life of meat by 3 days (until day 13), because the colour, lipid oxidation and microbiological analysis were within the values limited by Soldatou *et al.* (2009).

Finally, if we made a comparison between the uses of distilled or not distilled thyme leaves, we will refer to a previous work (Nieto *et al.*, 2010a) where the pregnant ewes were fed with undistilled thyme leave (*TL*). The *TL* diet reduced *TV* and *PSY* counts throughout the entire duration of storage and only at the end of storage for *MY* (day 21). However, the diet with *DTL* only reduced the *PSY* in the middle of the storage (days 7 and 14). With regard to the colour coordinates, while *TL* diet improved the colour of the meat on days 14 and 21, *DTL* improved the colour on days 7 and 14. The *TL* diet decreased lipid oxidation, showing *TBARS* values on day 21 that were 16% lower than those in *C* meat; however, no effect of *DTL* diet on lipid oxidation was observed on day 21. The use of thyme leaves in the diet for ewes is more effective in improving the quality of lamb meat that the use of *DTL*, because *TL* contains more effective antioxidant and antimicrobial compounds. However, the objective of the present work is the use of by-products from the essential oil industry, and the results showed in the present work are also quite positive.

Conclusions

The results presented in this study are the first to show the meat quality of lambs obtained from ewes fed by-products from the thyme essential oil industry; it shows that maternal *DTL* diet could improve lamb meat quality. Dietary *DTL* inhibited lipid oxidation, rancid odour, colour deterioration and was moderately efficient in preventing microbial spoilage and sensory deterioration. By-product from the distillation industry can be regarded as sources of natural antioxidants for sheep rearing and as interesting new feed additives.

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