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Effect on lamb meat quality of including thyme (*Thymus zygis* ssp. *gracilis*) leaves in ewes' diet

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ABSTRACT

The aim of this study was to investigate the effect of including thyme leaves (TL) in the diet of pregnant sheep on the sensorial characteristics, bacterial spoilage and oxidative stability of lamb meat stored in modified atmosphere (70% O_2:30% CO_2). For this, thirty-six sheep were randomly assigned to three groups: control (basal diet), T_1 (3.7% thyme leaves), T_2 (7.5% thyme leaves). Meat spoilage (*TV*, *PSY*, *MY*, *ENT*, and *LA*), *TBARS*, *CIELAB* coordinates, metmyoglobin and the sensory characteristics of fresh lamb meat were analyzed on days 0, 7, 14 and 21. The presence of antioxidant compounds in the diet containing *TL* delayed ($P < 0.05$) colour deterioration, lipid oxidation and bacterial counts, while at the same time imparting a better appearance to the fresh lamb meat. In general, this effect was more pronounced at the higher level of *TL* (7.5%). High Pearson's correlation coefficients were found between the sensory attributes, *CIELAB* coordinates and *TBARS*.

1. Introduction

The main criterion that consumers have to select meat cuts at the point of purchase is visual appearance. The use of modified atmospheres rich in oxygen (especially for red meat) improves the stability of meat colour but accelerates lipid oxidation. This is a major problem for the meat industry, because lipid oxidation products contribute to the development of off-flavours, especially during storage (Gray, Gomaa, & Buckley, 1996), while myoglobin oxidation leads to meat discoloration. For this reason the meat industry has a greet interest in delaying lipid autoxidation, which reduces meat quality by affecting colour, nutritive value and functionality. Although, synthetic antioxidants have long been used in foods, their use is increasingly discredited due to their suspected carcinogenic potential (Chen, Shi, & Ho, 1992) and the general rejection of synthetic food additives on the part of consumers. A general swing towards the use of natural compounds has stimulated research into their use as antioxidant replacements.

Thyme essential oil (*EO*) contains more than 60 ingredients, most of which have beneficial, including antiseptic, carminative, antioxidant and antimicrobial properties. The most important compounds of thyme *EO* are the phenols thymol (68.1%) and carvacrol (3.5%), which constitutes the most abundant and most active compounds, together with the monoterpene hydrocarbons *p*-cymene (11.2%) and γ-terpinene (4.8%) (Rota, Herrera, Martínez, Sotomayor, & Jordán 2008), which are known to have antioxidant properties and antimicrobial activity. The antibacterial properties of these compounds are in part associated with their lipophilic character, leading them to be accumulated in membranes where they take part in subsequent membrane-associated events such as energy depletion. Moreover, the polyphenolic compounds are characterized by their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Kähkónen et al., 1999).

In general, dietary supplementation has proved to be a simple and convenient strategy to uniformly introduce natural antioxidants into phospholipid membranes, where they may effectively inhibit oxidative reactions in situ (Lauridsen, Buckley, & Morrissey, 1997).

The effects of plant extracts or essential oils (classified as GRAS), following their direct addition, have been studied extensively and reported in a variety of meat types, including pork (Nissen, Byrne, Bertelsen, & Skibsted, 2004), beef (Solomakos, Govaris, Koidis, & Botsoglou, 2008) and lamb (Camo, Beltrán, & Roncalés, 2008). In addition, several studies have demonstrated the effect of diets containing rosemary and oregano in controlling the lipid oxidation and microbiology count in several types of meats (Djenane,

Abbreviations: MAP, modified atmospheres; *TL*, thyme leaves; *C*, control; T_1, 3.7% thyme leaves; T_2, 7.5% thyme leaves; *BD*, basal diet; GRAS, generally recognized as safe; *EO*, essential oil; MDA, malonaldehyde; *TBARS*, thiobarbituric acid reactive substances; *MM*, metmyoglobin percentage; *MO*, lamb meat odour; *PO*, putrid odour; *RO*, rancid odour; *AO*, acid odour; *MC*, meaty colour; *FC*, fat colour; *TV*, total viable; *PSY*, psychrotrophs; *MY*, moulds and yeasts; *ENT*, *Enterobacteriaceae*; *LA*, lactic acid bacteria.

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Sánchez-Escalante, Beltrán, & Roncalés, 2003; Janz, Morel, Wilkinson, & Purchas, 2007; Moñino, Martínez, Sotomayor, Lafuente, & Jordán, 2008; Nieto, Díaz, Bañón, & Garrido, 2010). However, the effectiveness of thyme incorporated through the diet, as is the case of thyme, is less well known.

To our knowledge, no studies have looked at feeding pregnant ewes thyme leaves and the effect of the same on subsequent lamb meat quality, as an alternative to using of synthetic antioxidants in animal feed. Such is the purpose of this study.

2. Materials and methods

2.1. Reagents

All the chemicals used were AnalaR grade. Butylated hydroxytoluene (BHT), 1,1,3,3-tetraethoxypropane (TEP), HCl and trichloroacetic acid (TCA) were from (Scharlau Chemie S.A. Barcelona, Spain) whereas hexane was from (Mallinckrodt Baker B.V. Deventer, Holland), and 2-thiobarbituric acid (TBA) was from (Acros Organics Geel, Belgium). For the microbiological assays all products were obtained from Oxoid Ltd., Basingstoke, Hampshire, United Kingdom: tryptone water (Oxoid Ltd. 133 CM0087, Tryptone water), plate count agar (PCA) (CM0325), Rose-Bengal chloramphenicol agar base (RB) (CM0549), with chloramphenicol selective supplement (SR0078E), man rogosa sharpe (MRS Agar) (CM0361), and violet red bile glucose agar (VRBG) (CM0485).

2.2. Animals and diet

Thirty-six Segureña pregnant ewes were randomly assigned to three homogeneous groups of equal age (3.2 years old) and body condition (2.63 ± 0.15) that was calculated according to the method described by Russell and Doney, 1969. The sheep forming the first group (Control, *C*) were given a basal diet (*BD*) (chemical composition in Table 1) consisting of 1.3 kg animal feed/day. The experimental diets of the other two groups were modified by substituting 3.7% (0.54% essential oil) and 7.5% (1.08% essential oil) of the *BD* by thyme leaves (*TL*) (total phenolic content: 108.5 ± 19.2 mg GAE/g dry plant and relative concentration (%) of thymol, carvacrol and eugenol: 58.00 ± 3.90, 3.12 ± 0.55 and 0.09 ± 0.04, respectively, determined by Jordán, Martínez, Martínez, Moñino, and Sotomayor (2009) using pellets made from 75% barley and 25% thyme leaves (T_1 and T_2, respectively). Animals were fed for 8 months, coinciding with the gestation and lactation periods, in order to study the quality of the subsequent lamb meat. Sheep and lambs (9 animals per group: 27 in total) were reared at the CIFEA Research Centre (Conserjería de Agricultura, Región de Murcia, Spain). The animals were weighed weekly until the lambs reached the slaughter weight of 25 ± 2 kg. Lambs were slaughtered according to Spanish regulations (RD 147/1993).

Table 1
Chemical composition of the concentrated basal diet.

Nutrient	Concentrate[a] (g kg^{-1} of dry matter)
Nonproteic nitrogen	0.5
Selenium (mg kg^{-1})	0.72
Net energy lactation (mcal kg^{-1})	1.5
Total methionine	2.6
Vitamin D (IU g^{-1})	3.1
Magnesium	3.4
Phosphorus	5.7
Total lysine	6.7
Calcium	10
Vitamin A (IU g^{-1})	14.9
Digestible NDF (%)	25.0
Fat	30.9
Vitamin E (IU g^{-1})	32.4
Total soluble RDP	43.3
Ruminal undegradable protein	55.6
Ruminal degradable protein (RDP), % of CP	64.7
Ash	76.0
Adjusted total starch	90.7
Zinc (mg kg^{-1})	154
Crude protein (CP)	175
Acid detergent fiber	190
Nonfiber carbohydrate	267
Neutral detergent fiber (NDF)	366

Data provided by Cargill Animal Nutrition (Torre Pacheco, Murcia, Spain).
[a] Formulated using the following ingredients/tone:
Molasses-cane, 10 kg;
Vitaminic and mineral feed additives, 10.4 kg;
Calcium carbonate, 14.9 kg;
Corn flour 36.7 kg;
Honey bean, 41.6 kg;
Sunflower oil (30%), 70 kg;
Rye, 70%;
Malt comb, 80 kg;
Barley, 150 kg;
Scale soy, 250 kg.

2.3. Sample preparation and experimental design

Twenty-four hours post-mortem, fresh samples of *Longissimus dorsi* muscle was removed from both sides of the carcasses and cut into 1.5 cm portions, which were assigned to be analyzed in different days (0, 7, 14 and 21 days) and packaged in polystyrene trays B5-37 (Aerpack), which were placed in BB4L bags (Cryovac) of low gas permeability (8–12 cm^3 m^{-2} per 24 h). The air in the packs was replaced by EAP20 mixture (70% O_2:30% CO_2; Carburos Metálicos, S.A., Barcelona, Spain) (*MAP*) in a discontinuous INEINI packer (Pack Multifunction). After sealing, the atmosphere inside the bags was checked using a Pack12P analyser (Abiss). No significant variation in the mixture was found during storage. Samples were stored at 4 °C ± 2 for 0, 7, 14, or 21 days in a display cabinet (Helkama, Finland) illuminated with white fluorescent light (620 lux), simulating retail display conditions. All the measurements, (except the *CIELAB* coordinates) were made in triplicate.

2.4. Microbiological analysis

For the microbiological assays, bags were aseptically opened in a 131 Bio-II-A microbiology cabinet (Telstar, Tarrasa, Spain) and the samples (10 g) were weighed with sterile tweezers into masticator bags and blended with peptone water 0.1% w:w in a masticator (IULInstruments, GmbH, Königswinter, Germany). Total viable count (*TV*) and total psychrotrophs (*PSY*) were determined on PCA (ISO 4833:2003), incubating at 37 °C for 24 h (*TV*) and 4 °C for 7 days (*PSY*). Moulds and yeasts (*MY*) were counted on RB with chloramphenicol and incubating at 25 °C for 5 days (ISO 7954:1987). Lactic acid bacteria (*LA*) were counted on MRS Agar plates and incubated at 30 °C for 72 h. Total *Enterobacteriaceae* (*ENT*) were counted on VRBG plates and incubated at 37 °C for 24 h. All the microorganisms tested were incubated in ST 6120 culture incubator (Heraeus S.A., Boadilla, Madrid, Spain). The results were expressed as log cfu g^{-1}.

2.5. Physical–chemical analysis

Colour was measured using a CR-200/08 Chroma Meter II (Minolta Ltd., Milton Keynes, United Kingdom) directly on the meat surface. The results were expressed as *CIELAB* values (CIE, 1978): Lightness (L^*), redness (a^*), yellowness (b^*), Chroma (C^*) and Hue angle (H^*) (expressed as sexagesimal degrees) values were calculated as follows: