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Antioxidant diet supplementation and lamb quality throughout preservation time

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ABSTRACT

Diet supplementation (DS) (100, 200, 300 ppm vitamin E -VE; 150 ppm product rich in flavonoids—PRF; 100 + 100 ppm VE-PRF; no supplementation) effect was evaluated on lamb quality throughout 10 days after sampling (preservation time: PT). pH, colour, myoglobin forms and lipid oxidation were analyzed on *Longissimus* muscle. Trained panellists evaluated colour intensity of chops packaged in modified atmosphere under display up to 12 days. PT had a larger effect on quality than DS. DS showed a clear antioxidant effect on lipids, especially at long PT and at high doses of VE. Visual test showed statistical differences among DS from day 4 of display where 200 and 300 ppm VE improved visual colour score. In general, supplementation with antioxidants showed better meat quality and diets higher than 100 ppm VE showed higher antioxidant capacity than the rest. The PRF diet was similar for a short PT but lower at a long PT. More research on flavonoids is necessary.

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1. Introduction

Meat appearance plays an important role in consumer acceptance and purchase since the visual aspect is their only tool available to make a purchase decision (Faustman & Cassens, 1990). Colour is considered as one of the most influential factors (Faustman & Cassens, 1990) where 'red' and 'bright red' are associated with freshness of the raw product and any brownish colour as an indicator of being stale or spoiled (Forbes, Vaisey, Diamant, & Cliplef, 1974). Thus, brown meat often cannot be sold at the full retail price resulting in an economic loss (Khliji, van de Ven, Lamb, Lanza, & Hopkins, 2010; Ripoll, González-Calvo, Molino, Calvo, & Joy, 2013).

Oxidative processes lead to the degradation of meat lipids and proteins which, in turn, contribute to the deterioration in flavour, texture and colour of displayed meat products (Ripoll et al., 2013). As time passes, the change of meat colour from red to brown occurs due to metmyoglobin formation (Renerre, 1990). This is related to lipid oxidation and microbial growth which are the main factors that determine food quality loss and shelf-life reduction (Fernández-López, Zhi, Aleson-Carbonell, Pérez-Alvarez, & Kuri, 2005).

The use of antioxidants is a widespread practice for preserving meat colour and maintaining lipid stability. These are added either into the feed of the animal or directly as additives in the meat. Synthetic antioxidants are commonly used as alimentary additives (Verhagen et al., 1990) however they have been associated with potential human health risks and consumer rejection (Camo, Beltran, & Roncales, 2008). Consequently, there is a practical need to search and select natural antioxidants as effective alternatives for meat preservation (Kikuzaki & Nakatani, 1993) and, even, for preventing food diseases (Fernández-López et al., 2005). Furthermore, natural antioxidants are less strictly regulated internationally (Weiss, Gibis, Schuh, & Salminen, 2010).

Dietary supplementation of natural antioxidant substances into animal feeds has proven to be a simple and convenient strategy to uniformly introduce them into the inner and outer sides of phospholipid membranes (Descalzo & Sancho, 2008; Nieto, Díaz, Bañón, & Garrido, 2010). These cell-integrated antioxidants are more effective than those added directly to the meat to prevent oxidative damage (Kerry, Buckley, Morrissey, O'Sullivan, & Lynch, 1998). Natural antioxidants are mainly fat soluble substances and enzymes (in the skeletal muscle the most important are: superoxide dismutase, catalase and glutathione peroxidase; Descalzo & Sancho, 2008). Together, non- and enzymatic systems operate to counteract the action of pro-oxidants in muscle tissues (Decker, Livisay, & Zhou, 2000). It has been shown that these enzymes exhibit residual activity in muscles post-mortem (De Vore & Greene, 1982; Renerre, Dumont, & Gatellier, 1996) maintained only to the remnant at the onset of cell death (Descalzo & Sancho, 2008). Hence, since this activity is limited after slaughter, antioxidant substances offer the only pathway to maintain antioxidant activity as time passes.

Among potential antioxidants vitamin E is the most commonly used because of its known effect on retarding lipid oxidation, improving colour stability (Jacob & Thomson, 2012; Yang, Lanari, Brewster, & Tume, 2002), flavour, texture, and nutritional value (Salvatori et al., 2004). All of that is reflected for extending shelf-life (Gobert et al., 2010; Wulf et al., 1995) from 1.6 to 5 days of retail-display life without compromising

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microbiological quality (Descalzo & Sancho, 2008). However, there is little information on the use of vitamin E as a feed additive for light lambs fed concentrates in indoor conditions and its subsequent effect on meat production (Guidera, Kerry, Buckley, Lynch, & Morrissey, 1997; Ripoll, Joy, & Muñoz, 2011) or quality (Ripoll et al., 2013). The knowledge gap is more noticeable when comparing or combining it with other antioxidant substances. In fact, dietary requirements of vitamin E for sheep are not clear (Salvatori et al., 2004) and so far only the NRC has published recommendations. López-Bote, Daza, Soares, and Berges (2001) have suggested e.g. 550 to 625 mg/kg diet as recommendable levels of vitamin E to delay meat deterioration.

Vitamin E is not the only compound with antioxidant advantages. In fact, the effects of other dietary compounds such as carotenoids and flavonoids are being increasingly investigated (Petron et al., 2007). Among natural antioxidants, flavonoids have gained large interest since they are widely distributed in plants (Bodas, Prieto, López-Campos, Giráldez, & Andrés, 2011). The term 'flavonoid' is a generic name that identifies a group of secondary metabolites derived in vegetables which are synthesized from phenylalanine and malonyl-CoA. All flavonoids are water soluble and produce polyphenolic compounds as a final by-product (for more information see Rice-Evans, Miller, & Paganga, 1996). The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electrons or chelate metal cations as a result of their chemical structure (Rice-Evans et al., 1996). Qualities include the preservation of colour (Yang et al., 2002), limiting lipid peroxidation in meat as well as preserving animal health and product quality (Wood & Enser, 1997). Additional benefits include positive effects on anti-inflammatory, anti-viral and cardiovascular diseases, cancer and other pathologies (Rice-Evans et al., 1996). As a consequence of these numerous benefits, there is a growing number of investigations in bioflavonoids (e.g. Andrés et al., 2013; Bodas et al., 2011; Simitzis, Ilias-Dimopoulus, Charismiadou, Biniari, & Deligeorgis, 2013). However, there is little knowledge about the effect of each flavonoid, their combinations, doses or association with other antioxidant substances, especially in light lambs.

There is also a growing interest in implementing strategies to extend the shelf-life of meat cuts under retail display conditions (Bañón, Méndez, & Almela, 2012). The use of modified atmospheres that are rich in oxygen improves the stability of meat colour (Nieto et al., 2010) especially for red meats, and prevents microbial growth of anaerobic pathogens (Bañón et al., 2012). A disadvantage is that lipid oxidation is increased (Gobert et al., 2010; Ripoll et al., 2011) and this is one of the primary causes of quality loss during such storage (Renerre & Labadie, 1993). To retard or minimize oxidative deterioration antioxidants may be added, since the short shelf-life of packed lamb is one of the principal concerns for its commercialization (Camo et al., 2008). Thus the combination of both technologies could provide a successful strategy to improve the shelf-life of lamb products.

The aim of this study was to evaluate whether a citric dietary flavonoid, vitamin E supplementation or their combination would have a benefit on lamb quality throughout preservation time.

2. Materials and methods

2.1. Animals

The study used 60 Rasa Aragonesa lambs (for more details see Sañudo, 2011). After weaning at 50 days of age, animals were randomly selected based on live weight from a feedlot to obtain a homogenous population. Animals were divided into six groups. Three groups were 100, 200 or 300 ppm vitamin E treatments (VE) supplemented with commercial vitamin E (Microvit E Promix 50®; DL-α-tocopheryl acetate: 54%, precipitated silica: 46%). A fourth group was 150 ppm product rich in flavonoids (PRF) supplemented with Evencit (powder composed of extracts of citrus fruits—*Citrus paradisi*, *Citrus aurantium bergamia*, *Citrus sinensis*, and *Citrus reticulata*—over an inert support of vegetal glycerin, with a minimum of 5% of ascorbic acid, 3.5% of polyphenols and 0.8% of bioflavonoids–naringin: 653.66 ± 26.46 mg/100 g; quercetin: 60.46 ± 2.53 mg/100 g and rutin: 644.93 ± 58.46 mg/100 g). The fifth was a combination of VE plus PRF (100 + 100 ppm, respectively) and finally a control group without supplementation. Animals were reared intensively and fed commercial concentrate (, Table 1) with the correspondent diet supplementation (DS) and cereal straw *ad libitum* up to 80–90 days of age.

2.2. Slaughtering and cooling

Animals were slaughtered in a local EU-licensed abattoir (Mercazaragoza S.A.). Within 15 minutes of dressing, carcasses were transported under refrigerated conditions (0–4 °C, 85–90% relative humidity) to the facilities of Pastores Cooperative Group and kept in refrigeration (0–2 °C, 90–95% relative humidity). At 18 hours post-slaughter (cold carcass weight = 11.08–12.86 kg) carcasses were butchered into commercial cuts. Both sides of each carcass, without the neck, shoulder and leg were obtained and refrigerated at 0–2 °C for a further 30 hours, with a total of 48 hours of ageing before sampling at the Meat Quality Laboratory of the Veterinary Faculty of Zaragoza.

2.3. Sampling protocol. Instrumental and sensory analyses

The *Longissimus thoracis et lumborum* muscle (LTL) was dissected and sliced/chopped following method sample requirements.

To quantify meat colour a *Minolta CM 2002* reflectance spectrophotometer was used with an illuminant D65 and a 10° standard observer following the CIE $L^*a^*b^*$ system (CIE, 1976). Lightness (L^*), redness (a^*) and yellowness (b^*) were recorded. The hue angle (H^*) and chroma (C^*) indexes were calculated as: $H^* = \tan^{-1}(b^*/a^*)$, expressed in degrees, and $C^* = \sqrt{a^{*2} + b^{*2}}$. The relative contents of myoglobin, oxymyoglobin and metmyoglobin were calculated from the reflectance curve according to Krzywicki (1979) using 690 nm (the highest wavelength of the instrument). Since the reflectance spectrophotometer only measures the reflectance between 400 and 710 nm at 10 nm intervals, the wavelengths 473, 525 and 572 nm were calculated using linear interpolation. Samples were 3-cm-wide slices of the left *Longissimus thoracis* (LT), with four slices per animal so that there was one slice per preservation time (PT) (0, 4, 7 or 10 days). Slices were placed in polystyrene trays overwrapped with an oxygen permeable

Table 1
Ingredients and chemical composition of the concentrate.

Raw material (%)	
Barley grain	28.5
Corn grain	32.0
Wheat grain	6.0
Toasted soybean meal	26.0
Beet molasses	2.0
Powder milk serum	1.0
Animal fats	1.0
Fatty acids' calcium salts	1.0
Calcium carbonate	2.0
Salt	0.5
Chemical composition (%)	
Crude protein	17.5
Crude fibre	3.9
Crude fat	4.0
Ash	6.0
Salt	0.23
Aditives	
Vitamin A	13.000 UI/kg
Vitamin D_3	3000 UI/kg
Vitamin E (α-tocopherol)	30 mg/kg
Total oligoelements (Fe, I, Co, Mn, Zn, Se)	199.05 mg/kg
Butilhidroxitoluene (BHT)	30 mg/kg

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