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PII: S0309-1740(18)30277-8
DOI: https://doi.org/10.1016/j.meatsci.2018.11.002
Reference: MESC 7714

To appear in: *Meat Science*

Received date: 9 March 2018
Revised date: 4 November 2018
Accepted date: 4 November 2018

Please cite this article as: Marc Bellés, María del Mar Campo, Pedro Roncalés, José Antonio Beltrán , Supranutritional doses of vitamin E to improve lamb meat quality. Mesc (2018), https://doi.org/10.1016/j.meatsci.2018.11.002

Supranutritional doses of vitamin E to improve lamb meat quality

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Abstract

Vitamin E is a fat-soluble antioxidant, therefore, it can be stored in any fat depot in the body, where it exerts a potent chain-breaking antioxidant effect. Moreover, the antioxidant activity of vitamin E-like compounds is also present in meat post mortem. The deposition of tocopherol in the muscle depends on the dosage, the source, and the period of supplementation, so different dosage-time combinations have been developed. Vitamin E does not affect production parameters if minimum requirements for function and growth are satisfied, but it could influence lamb fatty acid profile. During display, it protects PUFA from degradation, reducing lipid oxidation and, therefore, delaying discolouration. Furthermore, vitamin E would indirectly affect the development of lamb aroma by reducing protein and lipid oxidation. To facilitate optimal supplementation rates by producers and retailers, the present paper reviews vitamin E chemistry, biochemistry, nutrition and its ability to maintain lamb quality.

Keywords

Colour; antioxidant; fatty acid; oxidation; shelf life.

1. Introduction

Lamb is defined as a high quality product and it is considered a delicacy in Mediterranean countries (Karabagias, Badeka, & Kontominas, 2011; Vieira, & Fernández, 2014). Visual appearance is the most important sensory property of lamb because consumer purchasing decisions rely on meat appearance (Faustman et al., 2010). Consumers associate a bright red colour with freshness and superior meat quality. Therefore, fresh lamb for retail display is commonly packaged in oxygen-enriched atmospheres to satisfy consumers demand for fresh, tender and tasty meat with an attractive appearance (Bellés, Alonso, Roncalés, & Beltrán, 2017). High concentrations of oxygen in the gas mixture have been demonstrated to result in optimum colour in red meats by promoting oxymyoglobin formation. However, the high level of oxygen in the package enhances oxidative reactions, which lead to discoloration, development of off flavours, formation of toxic compounds, nutrient and drip losses, and can also have a negative impact on texture, compromising meat quality. Oxidation is one of the main causes of lamb deterioration, which explains the great efforts that have been taken to solve this problem (Bellés et al. 2017). The addition of compounds having antioxidant activity in the feed or in the package has emerged as a promising strategy to inhibit oxidative reactions and therefore, to offer a product with a higher quality and extended shelf life.

Vitamin E is the main fat soluble antioxidant preventing damage in live animals, and its antioxidant effects are also present post mortem. A large number of studies have observed that the administration of supranutritional doses of vitamin E before slaughter favours the deposition of tocopherol in the muscle, increasing the stability of meat against oxidation (Álvarez et al., 2008; Bellés et al., 2018; de la Fuente et al., 2007; Jose, Jacob, Pethick, & Gardner, 2016; Kasapidou et al., 2012; Kerry, O'Sullivan, Buckley, Lynch, & Morrissey, 2000; Ponnampalam, Burnett, Norng, Warner, & Jacobs, 2012a; Ponnampalam et al. 2017). Dietary vitamin E has been shown to have great potential to reduce the negative effect of oxidative reactions on lamb quality, making it vital to optimize its feeding strategy in order to obtain maximum benefit. The present review focuses on vitamin E chemistry, biochemistry, factors effecting its accumulation in muscle, and summarizes recommendations to producers and retailers for its supplementation to maintain lamb quality.

2. Chemistry and biochemistry

2.1 Chemical structure

The generic term vitamin E includes several compounds having a similar antioxidant activity (Jensen, & Lauridsen, 2007). These compounds comprise both tocopherols and tocotrienols, which are characterised by possessing a hydroquinone nucleus and an isoprenoid side chain. The chemical difference between them is located in the isoprenoid side chain; tocopherols have a saturated side chain while tocotrienols have an unsaturated side chain containing three double bonds. Moreover, four different tocopherols (α, β, γ, δ) and four tocotrienols (α, β, γ, δ) are differentiated depending on the position of methyl (-CH_3) groups at positions 5, 7 or 8 of the chroman ring (Colombo, 2010). Table 1 shows the chemical structure of vitamin E-like compounds.

Among all of them, α-tocopherol shows the highest biological activity (Jensen, Nørgaard, & Lauridsen, 2006). Alpha-tocopherol is a chiral molecule, which means that it is non-superposable on its mirror image. Each one of the mirror images of a chiral molecule is called enantiomer or optical isomer. Chirality is often produced due to the presence of an asymmetric carbon centre. Indeed, α-tocopherol presents three chiral centres at positions 2C, 4C and 8C of the phytyl tail. At each chiral centre two configurations are possible, which are named R or S, so there are eight different stereoisomers of α-tocopherol (Dersjant-Li, & Peisker, 2010; Jensen, & Lauridsen, 2007). Figure 1 presents the chemical structure of α-tocopherol stereoisomers. Alpha-tocopherol obtained from natural sources consists of a single stereoisomer presenting a RRR configuration, which means that it presents a configuration of R at the three positions (2C, 4C and 8C). In contrast, chemically synthetised vitamin E comprises an equimolar mixture of the eight different α-tocopherol stereoisomers (RRR, RRS, RSS, RSR, SRR, SSR, SRS, and SSS) (Dersjant-Li & Peisker, 2010). According to IUPAC recommendations (1982), vitamin E obtained from natural sources is called RRR-α-tocopherol while that which is chemically synthetised is named all-rac-α-tocopherol in order to describe the differences in chemical composition between both sources.

Both natural and synthetised vitamin E free forms can be easily degraded during the processing, manufacturing and storage of feeds, which make it difficult to use vitamin E as an additive in feedstuffs. To overcome these problems more stable forms have been developed. Since esters are less susceptible to oxidation, the phenol group of α-tocopherol is commonly converted to an ester by using acetic or succinic acid, so vitamin E is commercialised as α-tocopheryl-acetate or α-tocopheryl-succinate. These forms are very stable to in vitro oxidation but they need to be hydrolysed in the animal gut to show antioxidant activity (Vagni, Saccone, Pinotti, & Baldi, 2011). The all-rac α-tocopheryl acetate is the form of vitamin E most used to supplement animal feeds due to its high stability and lower cost.

2.2 Bioavailability and biopotency

Bioavailability and biopotency are close-knit concepts. The first one is defined as the absorbed proportion of a substance after its administration while biopotency refers to the ability of a compound to exert an effect in a biological system (Dersjant-Li &

Peisker, 2010). It is obvious that a substance needs to be absorbed prior to acting in an organism. Therefore, knowing the mechanism of absorption of vitamin E is the key to maximize its benefits in meat quality, even more in ruminants whose digestive anatomy and physiology is markedly different and more complex than that of monogastric animals. Several studies have been carried out to assess the effect of ruminal microbiota and fermentations on vitamin E absorption. Weiss, Smith, Hogan and Steiner (1995) observed that vitamin E was not degraded during *in vitro* ruminal fermentation. In agreement, Hymøller and Jensen (2010) noted neither *in vitro* nor *in vivo* hydrolysis of α-tocopherol in the rumen. Taking into account that α-tocopherol esters need to be hydrolysed before their absorption, little if any absorption of vitamin E in the rumen should be expected.

In contrast, tocopherol esters are largely hydrolysed in the intestinal lumen, where they are then absorbed in combination with lipid micelles. Once in the enterocytes, vitamin E is packed into chylomicrons and delivered to the liver in the form of chylomicron remnants (Lauridsen, & Jensen, 2012). In the liver, the hepatic α-tocopherol transfer protein, α-TTP, binds to the vitamin E to facilitate its incorporation into nascent VLDL and its secretion from hepatocytes. This lypoprotein has a central role in vitamin E metabolism as it regulates the body-wide levels of α-tocopherol (Rigotti, 2007). Furthermore, the α-TPP seems to be a major cause of the differences found among the bioavailability of the different vitamin E-like compounds. Both tocopherols and tocotrienols are transported via chylomicron remnants to the liver, but at this point, the α-TPP exerts a selective transport. Hosomi et al. (1997) demonstrated *in vitro* differences in the affinity of α-TPP to the different types of tocopherols and tocotrienols. Relative affinities (RRR- α -tocopherol = 100%) were as follows: RRR-ß-tocopherol, 38%; RRR-y-tocopherol, 9%; RRR-δ-tocopherol, 2%; α-tocopherol acetate, 2%; α-tocopherol quinone, 2%; SRR- α -tocopherol, 11%; α-tocotrienol, 12%. Therefore, α-TPP not only has a higher affinity for tocopherols but it is also able to sort out among isomers. The different affinities of α-TPP to α-tocopherol stereoisomers seems to be the major cause of their differences in activity. RRR-α-tocopherol is generally accepted to have the highest bioactivity but the equivalence of the different isomers is still a point of concern. The first study about this topic assigned a value of 1 IU mg−1 for 2-ambo-α-tocopheryl acetate (a mixture of RRR-and SRR-α-tocopherol) while a value of 1.36 IUmg−1 was proposed for RRR-α-tocopheryl acetate (Harris & Ludwig, 1949). This ratio was later corroborated by Weiser and Vecchi (1982), who also associated a relative biopotency to each α-tocopherol stereoisomer. The results obtained were: RRR = 1, RRS = 0.9, RSS = 0.73, SSS = 0.6, RSR = 0.57, SRS = 0.37, SRR = 0.31 and SSR = 0.2. Despite both studies used clinical endpoints based on the physiological activity of vitamin E during the gestation on rats to determine the bioequivalence (RRR-α-tocopheryl acetate = 1.36 IUmg−1), it was accepted by the United States Pharmacopeia (USP) and widely used as the conversion factor either for human or livestock.

Nevertheless, an intense debate about the adequacy of this ratio for livestock is growing among researchers. Jensen and Lauridsen (2003) observed that cows have a higher selectivity than rats to utilise the different stereoisomers; so, while cows mostly preferred the RRR form, rats were able to utilize all 2R forms. Moreover, it has been noted that vitamin E bioavailability is also influenced by dietary dosage, feeding period, age and tissue (Dersjant-Li & Peisker, 2010; Jensen et al., 2006; Jensen, & Lauridsen, 2007). All these data support the need of calculating the equivalence between stereoisomers more accurately, taking into account both species and age. Equivalences in large animals cannot be determined with clinical endpoint markers due to ethical and practical aspects, so they are calculated by bioavailability studies. It has been demonstrated a higher bioavailability of RRR-a-tocopherol in cattle (Meglia, Jensen, Lauridsen, & Waller, 2006; Weiss, Hogan, & Wyatt, 2009), dairy cows (Weiss et al., 2009), sows (Lauridsen, Engel, Jensen, Craig, & Traber, 2002), piglets (Lauridsen et al., 2002) and finishing swine (Yang et al., 2009). Table 2 shows some ratios for livestock. Unfortunately, there is a lack of studies about this topic in lambs.

2.3 Antioxidant activity

As it has been stated above, vitamin E encompasses several compounds that show antioxidant activity. Nevertheless, the isomers of vitamin E possess a different antioxidant power and exert non-redundant biological functions. α-tocopherol presents the highest antioxidant effect *in vivo*. It acts as a chain-breaking antioxidant by donating a hydrogen atom to a peroxyl radical, thus converting it to a hydroperoxide, which is unable to propagate the oxidative process. Besides the lipid antioxidant action, α-tocopherol has been associated to different physiological functions by taking part in cellular signalling, modulating the expression of some proteins involved in atherogenesis and regulating arachidonic acid cascade pathway (Brigelius-Flohe and Traber, 1999; Schneider, 2005).

Similarly, γ and δ-tocopherol isomers show a chain-breaking mechanism of action. γ-tocopherol transfers a hydrogen atom to a peroxyl radical, resulting in a stabilized γ-tocopheroxyl radical. This molecule can further follow two different ways: it can react with a new peroxyl radical to form the covalent adducts, 8a-(alkyl-dioxy)-γ-tocopherones or with each other to form 5-(γ-tocopheroxy-5-γl)-γ-tocopherol and 5-γ tocopheroxy)-γ-tocopherol (Yamauchi, 1997). Moreover, γ-tocopherol is a powerful nucleophile that traps electrophilic mutagens in lipophilic compartments, thereby protecting lipids, DNA, and proteins from peroxynitrite damage (Brigelius-Flohe and Traber, 1999).

Regarding tocotrienols, they have been described to exert a higher antioxidant activity in membranes than tocopherols, but their bioavailability after oral ingestion is less than that of α-tocopherol. Tocotrienols are also chain-breaking antioxidants by scavenging free radicals (Packer, Weber, & Rimbach, 2001). Apart from their antioxidant properties, tocotrienols may present anticancer and neuroprotection functions (Sen, Khanna, Rink, & Roy, 2007).

3. Relationship between vitamin E requirements, breeding conditions and their effect on production parameters

Vitamin E is an essential nutrient which plays a significant role in many biological functions like reproduction and immunity. A dosage of 20 mg vitamin E/kg feed has been recommended for lambs for the normal function of the body (NRC, 2001). At lower than this supplementation level, a decrease of livestock production performance could be expected, besides the appearance of some diseases related to vitamin deficiency. Nevertheless, there are some breeding conditions which may modify vitamin E requirements.

A large number of studies (Berthelot, Broudiscou, & Schmidely, 2014; Lauzurica et al., 2005; Liu, Ge, Luo, Yue, & Yan, 2013;Leal et al., 2018; Zhao et al., 2013) have demonstrated that supranutritional intakes of tocopherol do not exert any effect on average dairy gain, live weight, feed intake or feed efficiency. Moreover, neither carcass conformation nor fatness are affected by dietary vitamin E. Therefore, no modifications of growth performance and carcass characteristics would be expected in mature lambs by increasing the content of tocopherol of the feed if the minimum requirements for normal growth and health are already satisfied. By contrast, supra nutritional dosages of vitamin E seems to improve productive parameters in young animals. Maiorano et al. (2007) observed a higher average daily gain (ADG) in suckling lambs receiving intramuscular injections of 150 IU/wk for eight weeks in comparison to those which only received vitamin E from milk. A feasible explanation for these results could be the key role of vitamin E in the development of the immune system of young animals. Vitamin E concentration in milk mainly depends on diet, so the non-supplemented lambs might have consumed very low levels of vitamin E. The lack of this vitamin may impact the correct growth of these lambs.

Ambient conditions may increase the demands of vitamin E for normal function and growth too. Heat stress is responsible for significant economic losses for the livestock industry during the summer months and constitutes a growing concern due to climate change (Bernabucci et al., 2010). Heat stress lessens oxidative defences by producing excessive ROS, thereby exerting oxidative damage to many biological molecules which may further compromise animal health and growth (Chauhan et al., 2014b). Therefore, lambs may have increased requirements for antioxidants under adverse climatic conditions. In this sense, Chauhan, Celi, Leury, Clarke and Dunshea (2014a) demonstrated that the supplementation with vitamin E of lambs reared under heat stress conditions ameliorate their physiological and oxidative stress. Moreover, Chauhan et al. (2016) pointed out that the administration of supranutritional levels of vitamin E and Se during the finishing period may avoid the decrease of average daily feed intake and average daily gain of lambs exposed to heat stress. Thus, the administration of supranutritional doses of vitamin E seems to be a strategy to mitigate production losses during hot seasons.

The requirements of vitamin E also depend on animal lipid depots. Early maturing breeds tend to deposit more fat in the body than late maturing breeds at the same age, so the first ones need more vitamin E to maintain oxidative status for normal function and growth (Brand, Van der Westhuizen, Van Der Merwe, & Hoffman, 2018). On the other hand, early maturing lambs would present with a higher content of this vitamin compared with leaner animals if enough vitamin E is administrated in the diet, since vitamin E accumulates in fat tissue. In this sense, Ponnampalam et al. (2014) observed greater concentrations of vitamin E in faster growing lambs, which presented a higher percentage of fat, than those of lighter body weight animals.

Both fattening and fat composition are also influenced by animal feed. It has been demonstrated that animal fat composition can be modified to produce healthier meat by changing feed composition. Generally, this strategy aims to increase the content of PUFA and conjugated linoleic acid. In this sense, several studies have shown that dietary *n*-6 and *n*-3 PUFA can be incorporated into adipose tissue and muscle of lambs despite the biohydrogenation of dietary fatty acids in the rumen (Wood et al., 2008). Moreover, differences in fat composition have been registered depending on the feeding system. Nuernberg et al. (2005) observed a higher proportion of PUFA, SFA and *n*-3 fatty acids in grass-fed than in grain-fed lambs. Similarly, meat from alfalfa fed lambs showed a greater PUFA *n*-3 content than their concentrate-fed counterparts (Álvarez-Rodríguez et al., 2018). Nevertheless, increasing the unsaturated fatty acids in the muscle cell membranes may result in reduced oxidative stability and therefore, in increased vitamin E requirements (Monahan, Buckley, Morrissey, Lynch, & Gray, 1992). Grass fed animals would be able to correct this status by means of tocopherol, carotenoids and many other antioxidants present in their diet, but increasing the PUFA content of the concentrate-fed animals may require a higher level of vitamin E supplementation (Ponnampalam et al., 2012b).

4. Effect on meat quality

4.1 Muscle composition

The deposition of vitamin E in muscle depends on the length and the level of supplementation. Jose et al. (2016) measured the evolution of α-tocopherol concentration in muscle using 6–8 month old lambs at different levels of supplementation with synthetic vitamin E (30, 150, 275, 400 IU of all-rac tocopherol) or on green pasture through the feeding period (up to 8 weeks), registering a continuous increase with time in each treatment, except in that consist of 30 IU. This dosage satisfactorily prevented lambs from nutrition related diseases, but it was not enough to stimulate the deposition of tocopherol in muscle. The deposition rate of vitamin E depended on the level of supplementation, the more dosage, the faster accumulation in the muscle. In fact, the content of α-tocopherol in muscle after 6 weeks was 2 fold higher in the lambs supplemented with 150 IU than in those receiving 30 IU, and it was even 3 fold higher in the animals that were fed at the highest level of supplementation (400 IU). Regarding the content of α-tocopherol of grass fed lambs, it was similar to those supplemented with 275 IU of synthetic vitamin E. The deposition of tocopherol through the feeding period showed a linear increase, the slope depending on the dosage; however, a plateau was reached after 5 weeks at the highest level of supplementation and after 4 weeks in the grass fed lambs, which suggests the existence of a saturation point. At this time, the content of tocopherol in muscle was between 5 and 6 mg/kg (Jose et al., 2016). In this sense, visual inspection of the published α-tocopherol concentrations in muscle after slaughter (Figure 2) suggests a nonlinear relationship between vitamin E consumption and the concentration of α-tocopherol in M.

longissimus: there is a point above which the rate of deposition of tocopherol in muscle decreases.

A similar concentration of muscle tocopherol can be obtained with different supplementation rates. Kasapidou et al. (2012) registered 3.73 mg α-tocopherol/kg muscle by a moderate level of supplementation (500 mg all-rac α-tocopherol/kg) during 63 days. Increasing the level of supplementation to 1000 mg/kg allowed obtaining a similar concentration of tocopherol in muscle with a reduced time of supplementation: Álvarez et al. (2008) and De la Fuente et al. (2007) reached a muscle concentration of 3.57 mg α-tocopherol after 37 days while Bellés et al. (2018) quantified 3.91 mg even in a shorter period of supplementation (14 d). On the other hand, Ponnampalam et al. (2012a) quantified 3.2-5.6 mg α-tocopherol/kg meat in pasture fed animals (49 d) when diets have 20-60 mg/kg DM, Turner, McClure, Weiss, Borton and Foster (2002) registered up to 2.5 mg of α-tocopherol/kg meat in alfalfa-finished lambs (89 d) and Álvarez-Rodríguez et al. (2018) reached 2.83 mg of α-tocopherol/kg meat by including grazed alfalfa in the concentrate basis ration.

It is notorious that similar depositions of tocopherol, than that obtained with synthetic vitamin E, have been obtained by lower concentrations of natural vitamin E in the diet. A feasible explanation for this phenomenon may be the different efficacies of conversion for vitamin E from forage diet and synthetic source to animal tissues. Synthetic vitamin E is a racemic mixture containing the eight stereoisomers of α-tocopherol, among which 2S stereoisomers present a low bioavailability. By contrast, α-tocopherol appears naturally only as RRR-α-tocopherol, which presents the highest bioavailability (Dersjant-Li & Peisker, 2010). It is obvious that the higher the bioavailability the greater the deposition of α-tocopherol in the muscle. On the other hand, natural vitamin E is located within vegetal lipids, which may facilitate its absorption (Ponnampalam, Behrendt, Kerr, Raeside, McDonagh, 2018).

Synthetic vitamin E has a high cost, so optimizing the supplementation rate is a key point to producers. The studies of Jose et al (2016), Turner et al. (2002) and Ponnampalam et al. (2012a) showed that extensive systems based on forages provide a concentration of tocopherol in muscle enough to maintain the quality of lamb throughout the display period, without needing to provide extra synthetic vitamin E. Nevertheless, most regions around the globe lack natural resources to grow lamb extensively and farmers are constrained to feeding lambs with grain-based concentrate enriched with synthetic vitamin E. Supplementing lamb diets with supra-nutritional doses of synthetic vitamin E represents a significant cost to producers. Furthermore, consumers concern about chemical additives continues to grow, so they would be more keen towards natural sources of antioxidants. Recent studies have pointed out a great number of natural sources of vitamin E that may be used to supplement grain-based diets. Chesnutt, mimosa and tara extracts, dried tomato pomace, rosemary distillation residues, dried citrus pulp and olive cake are some feasible examples of alternative sources of vitamin E available in many regions where lambs grow intensively (Luciano et al., 2013; Luciano et al., 2017; Valenti et al., 2018a; Valenti et al., 2018b; Yagoubi et al., 2018). Moreover, these resources are cheaper and ecologically friendly, since they are agro-industrial by-products. The development of strategies to reduce or even replace synthetic vitamin E from lamb's diet seems to be an interesting area of research whose results will be very useful for both producers and consumers.

On the other hand, vitamin E has been described to modify meat fatty acid profile by acting at two different levels, ante-mortem and post-mortem. Vitamin E seems to play a role in rumen metabolism although it is not completely clear the mechanism and the specific modifications. Recent studies pointed at a modification of biohydrogenation processes, which would involve ruminal fatty acid hydrogenation. The modification of ruminal microbiota might be a possible mechanism by which vitamin E would affect ruminal processes (Hou, Wang, Wang and Liu, 2013). The addition of supranutritional doses of vitamin E to the feed could result in changes of specific fatty acids. Demirel et al. (2014) observed an increase in the proportions of C18:2 *n*-6, C20:5 *n*-3 and C22:6 *n*-3 together with a decrease of the percentages of C16:1 and C18:1 *n*-9, while Berthelot et al. (2014) only registered an increase in the proportion of C18:1 10*t*. Similarly, Hou et al. (2013) determined a higher percentage of C18:1 10*t* and C18:1 11*t* while the proportions of C18:1 *n*-9, C18:2 *n*-6 and C18:3 *n*-3 were lower. Nevertheless, the effect of vitamin E on modifying total percentage of saturated, monounsaturated or polyunsaturated fatty acids in lamb tissue seems to be low (Chen, Mao, Lin, and Liu, 2008) or even inexistent (Demirel et al., 2014; Kasapidou et al., 2009; Berthelot et al., 2014).

The post-mortem effects of vitamin E on fatty acid profile are related to lipid oxidation. As it is widely known, PUFA are more prone to oxidation than monounsaturated and saturated fatty acids due to their higher degree of unsaturation, which can lead to their decreased proportions during aerobic display (Álvarez et al., 2009, Bellés et al., 2018). Many studies focused on the effect of dietary vitamin E on fatty acid evolution through display have registered a higher percentage of PUFA in meat from supplemented than from control lambs after several days of aerobic display (Bellés et al. 2018, Alvarez et al., 2009; Chen et al., 2008). Supranutritional dosages of vitamin E may increase the deposition of tocopherol into cell membranes, where it offers an enhanced protection of PUFA from oxidative degradation during display and, therefore, it allows preservation of lamb nutritional value. In contrast, published data suggest a lack of effect of dietary vitamin E on modifying the percentage of intramuscular fat (Bellés et al., 2018; Kasapidou et al., 2012, Zhao et al. 2013).

4.2 Oxidative reactions

Meat oxidation comprises both lipid and protein oxidative reactions. Lipid oxidation starts at the membrane level in the phospholipid fractions as a free-radical autocatalytic chain reaction mechanism in which prooxidants interact with unsaturated fatty acids leading to the release of free radicals and the propagation of the oxidative chain. Regarding protein oxidation, it could be promoted by either the interaction with reactive oxygen species or indirect reactions with secondary products of oxidative stress (Faustman, Sun, Mancini, & Suman, 2010; Zhang, Xiao, & Ahn, 2013).

Vitamin E is a major *in vivo* antioxidant that protects tissues from oxidative damage, and this effect is carried through to meat after slaughter. The effect of vitamin E on lipid stability increases with dosage and, therefore, with its concentration in muscle (Jose et al., 2016; Kasapidou et al., 2012; González-Calvo, Ripoll, Molino, Calvo, & Joy, 2015). Indeed, as strong correlation has been observed (higher than -0.7) between the content of α-tocopherol in muscle and its ability to protect against oxidative reactions (Álvarez et al., 2008). Nevertheless, there is a threshold concentration above which any improvement against lipid oxidation could be noted. Álvarez et al. (2008) evaluated the effect of increasing dietary dosages of vitamin E (20, 270, 520 and 1020 mg vitamin E/kg feed) on lamb oxidation, without registering any additional improvement in lipid stability when α-tocopherol in muscle surpassed 2.26 mg/kg. Similarly, Lauzurica et al. (2005) and De la Fuente et al. (2007) did not observe any further improvement by increasing the concentration of α-tocopherol above 2.17 mg/kg meat. However, a lower threshold has been proposed by González-Calvo et al. (2015) (0.61-0.90 mg α-tocopherol/kg meat) and Kasapidou et al. (2012) (1.52 mg α-tocopherol/kg meat). By contrast, Yagoubi et al. (2018) and Ponnalpalam et al. (2014) registered the development of lipid oxidative reactions in lamb meat containing approximately 2.30 and 2.95 mg α-tocopherol/kg, respectively.

Disagreements in the target concentration may be related to differences in meat susceptibility to oxidation, which depends on muscle composition (content of unsaturated lipids, heme pigments, metal catalysts and a range of oxidizing compounds in the muscle tissue) (Falowo, Fayemi, & Muchenje, 2014). In this regard, the study carried out by Ponnalpalam et al. (2014) helps to understand the relationship between meat biochemical components and the effect of vitamin E on inhibiting lipid oxidation. It shows that when muscle tocopherol concentration is lower than 2.95 mg/kg muscle, other variables such as heme iron or PUFA have a significant role in lipid oxidation spread. Above this concentration, vitamin E is the major compound managing lipid oxidation in meat. On the other hand, extrinsic variables like illumination and atmosphere composition during display also influence lipid oxidation. Thus, it seems difficult to establish a minimum concentration of muscle tocopherol to avoid completely lipid oxidation, since there are numerous factors taking part on these reactions.

Oxidation of meat lipids and proteins widely contribute to the deterioration in flavour of meat products (Campo et al., 2006). TBARS has been described to be a good indicator of the development of rancid off-flavours. Campo et al. (2006) have related TBARS with human perception of rancid compounds, concluding that beef flavours were overpowered when TBARS values exceeded a value of 2 mg/kg. The effect of vitamin E on inhibiting lipid oxidation in lamb has been widely demonstrated. Ortuño, Serrano and Bañón (2015) registered values under the proposed threshold in supplemented lamb (600 mg synthetic vitamin E/kg feed) maintained in common retail conditions even after 18 days. The strong inhibition of oxidative reactions reduced the perception of rancid odour in chops from supplemented lambs, registering also a more intense meaty odour and as a result, a higher freshness. On the other hand, meat from grass fed lambs containing between 3.3 and 5.88 mg tocopherol/kg meat have shown TBARS values lower than 0.5 MDA at 96 h post-mortem (Ponnampalam et al., 2012a), while Ponnampalam et al. (2017) reported a content of MDA lower than 3 mg in lambs finished with ryegrass (approximately 3 mg vitamin E/kg meat) even after 9 weeks of storage. Guerra-Rivas et al. (2016) and Muíño et al. (2014) also pointed out the great effect of dietary vitamin E on preserving lamb sensory properties, since dietary vitamin E was an effective tool to preserve freshness and overall liking of fresh lamb by reducing the formation of rancid odours and flavours. Table 3 summarises the effect of muscle α-tocopherol on inhibiting lipid and protein oxidation during display.

Dietary vitamin E also seems to exert a significant effect on delaying protein oxidation. Protein oxidation involves amino acid destruction, a decrease in protein solubility, loss of enzyme activity, and formation of carbonyls, resulting in a reduction of tenderness and juiciness, flavour deterioration, and discoloration (Zhang et al., 2013). A great reduction of carbonyl formation during storage in lamb chops containing 3.95 mg α-tocopherol/kg (Ortuño et al., 2015) and a delay of protein oxidation in lamb meat with a concentration of α-tocopherol of 2.42 mg/kg (Muíño et al., 2014) have been observed. Nevertheless, these levels are not high enough to completely inhibit carbonyl formation throughout display. Lipid and protein oxidation are cross linked reactions by which they could exacerbate each other (Faustman et al., 2010); therefore, the target concentration of α-tocopherol in muscle to completely inhibit oxidative reactions should be determined taking into account both lipid and protein oxidation.

4.3 Colour

Meat colour mainly depends on the quantity and the chemical state of its principal pigment, myoglobin. When this sarcoplasmic protein is associated with an oxygen molecule it forms a complex called oxymyoglobin, which shows a bright red colour. In the absence of oxygen, it remains as the deoxymyoglobin form, having a purplish red colour (oxymyoglobin and deoxymyoglobin forms are interconvertible). Oxidation of Fe^{2+} to Fe^{3+} implies the conversion of the pigment into metmyoglobin, resulting in the change of a desirable red to a brown colour.

Myoglobin may be oxidised to metmyoglobin by different chemical pathways. Primary and secondary products of lipid oxidation have been identified as major causes of myoglobin oxidation, especially in muscles with high amounts of PUFA. Unsaturated fatty acids are known to be more susceptible to oxidation, leading to the release of free radicals that enhance meat discolouration. On the other hand, heme proteins could favour lipid oxidation. Nevertheless, lipid and protein oxidation are not always tightly interconnected. In low pO_2 atmospheres oxymyoglobin is rapidly converted to metmyoglobin while these conditions provide a high stability to lipids (Faustman et al., 2010). Therefore, myoglobin may oxidise either by interacting directly with reactive oxygen species or indirectly with secondary products of lipid oxidation.

A better appearance of meat from supplemented lambs through display can be explained by the effect of vitamin E on delaying the formation of metmyoglobin. The mechanism by which vitamin E inhibits the conversion of myoglobin to its oxidised form is not completely clear. The direct antioxidant action of α-tocopherol on protecting membrane lipids would reduce the formation of primary and secondary compounds of lipid oxidation, delaying indirectly myoglobin oxidation. On the other hand, vitamin E could prevent the oxidation of myoglobin directly. In this sense, the conclusions obtained in the study of Ponnampalam, Butler, McDonagh, Jacobs and Hopkins (2012b) highlighted the key role of heme iron and tocopherol in overriding meat colour.

By both mechanisms, vitamin E has been demonstrated to reduce the conversion of myoglobin to metmyoglobin and therefore to protect lamb from colour fading (Bellés et al., 2018; Jose et al., 2016; Kerry et al., 2000; Lauzurica et al., 2005; Leal et al., 2018; Ripoll et al., 2011). Table 4 summarises the effects of dietary vitamin E on meat colour. Data from instrumental (Álvarez, et al., 2008; Bellés et al., 2018, Ripoll, Jose et al., 2016; Joy and Muñoz, 2011, Kerry et al., 2000, Yagoubi et al., 2018) and sensory (Muela, Alonso, Campo, Sañudo, & Beltrán, 2014) colour analyses indicate the existence of a concentration-dependent effect; the higher the α-tocopherol concentration in muscle, the higher colour stability. Nevertheless, there seems to exist a concentration above which no improvement in meat colour could be expected. Recent studies suggested a concentration of 3.5-4.0 mg α-tocopherol/kg tissue as the threshold for obtaining an improvement in lamb colour (Jose et al. 2016; Hopkins, Lamb, Kerr, Van der Ven, & Ponnampalam, 2013).

4.4 Aromatic compounds

The aroma of meat is the result of a very complex process. The primary reactions involved in the formation of aroma compounds are the oxidation of lipids, the degradation of thiamine, the Strecker reaction, and the Maillard reaction (Resconi, Escudero, & Campo, 2013). The aroma of meat is mainly developed upon heat treatment, where the precursors of aroma compounds (thiamine, glycogen, glycoproteins, nucleotides, nucleosides, free sugars/phosphate, amino acids, peptides, amines, organic acids and lipids) participate through those primary reactions in forming intermediates, which can continue to react with other degradation products to form a complex mixture of volatiles, including those that are responsible for the aroma of meat (Imafidon & Spanier, 1994).

The action of vitamin E would indirectly affect the development of lamb aroma by reducing protein and lipid oxidation. The inclusion in the diet of 300 IU (Rivas-Canedo et al., 2013) is able to reduce the formation of 2-heptanone, 2-penten-1-ol, 2-octen-1-ol, pentane and heptane, deriving from lipid oxidation, and ethylbenzaldehyde, deriving from Strecker degradation. Aldehydes and ketones have a key impact in sensory perception due to their lower threshold. As an example, 2-heptanone has been associated to lamb flavour (Resconi et al., 2010).

The different ingredients in the diet have a clear effect in the fatty acid composition, which further influences the formation of volatile and therefore meat aroma (Wood et al., 2008). Together with a higher PUFA content, especially from *n*-3 PUFA, pasture based diets show increased levels of antioxidants. The effect of antioxidants in these diets is difficult to separate from the effect of the fatty acid composition, since the different fatty acids are able to develop different volatile compounds, in increasing number, as the unsaturation of the fatty acid increases (Elmore et al., 2002). This increased number of aroma volatiles also implies a higher number of odour notes (Campo et al., 2003), including fishy notes that only appear when *n*-3 PUFA are involved.

4.5 Microbial growth

Microbial growth is one of the main causes of fresh meat spoilage. Commonly, bacteria responsible of this process are *Brochotrix thermospacta*, *Carnobaterium*, *Enterobacteriaceae*, *Lactobaciluus* spp., *Leuconostoc* spp., *Pseudomonas* spp. and *Shewanella putrefaciens*. Microbial growth involves the consumption of meat specific compounds such as glycogen, glucose, lactic acid, amino acids or proteins and its conversion into a wide variety of metabolites responsible of the characteristic off odours and off flavours of spoiled meat. Moreover, other defects like discolouration, gas or slime production tend to appear in fresh meat when microbial counts reach 10^{7-8} ufc/g (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008).

Vitamin E is not likely to exert any effect against bacteria multiplication as no feasible bacteriostatic or bactericide mechanism has been described. Neither the addition of α-tocopherol directly on meat products (Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007) nor the supplementation of lamb diet with DL- α-tocopherol resulted in lower total viable, *Enterobacteriaceae* or lactic acid bacteria counts trough display (De la Fuente et al., 2007; Muíño et al., 2014). Therefore, despite a limited number of studies in which the effect of α-tocopherol on microbial growth has been evaluated, it could be concluded that vitamin E lacks antimicrobial activity.

5. Conclusions

Vitamin E acts as a chain breaking antioxidant, and its effects are perhaps most critical at the PUFA-rich phospholipid bilayer of muscle cells. This effect depends on concentration, which may be increased by supplementation of natural or synthetic sources. Increasing dietary vitamin E does not affect growth performance or carcass characteristics if the minimum requirements for normal growth and health are already satisfied. Nevertheless, it could produce modifications in ruminal processes which seem to modify fatty acid hydrogenation, resulting in changes in the proportion of specific fatty acids in tissue. Increasing the content of muscle tocopherol is an effective means to protect PUFA and proteins from oxidation, therefore decreasing colour fading and off-odour formation in lamb meat.

A key feature is to reach a minimum muscle concentration enough to effectively inhibit oxidative reactions, which unfortunately depends on the different factors that affect the balance between meat pro-oxidants and antioxidants such as muscle composition, packaging or storage temperature.

6. Conflict of interests

None to declare

7. Funding

This work was supported by the Gobierno de Aragón (Grupo Consolidado de Calidad y Tecnología de la Carne, Ref: A04), The European Regional Development Fund (ERDF) – European Union and the Ministerio Español de Educación, Cultura y Deporte - Spain that provided M. Bellés with a grant (FPU014/01225) to carry on this investigation.

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Figure 1. Steroisomers of α-tocopherol.

Figure 2. Content of α-tocopherol in lamb M. *semimembranosus* after slaughter obtained in different studies.

832 **Table 1.** Chemical structure of vitamin E-like compounds

		Ring position		
Trivial name	Chemical name	R^1	R^2	R^3
α-tocopherol	5,7,8 -Trymethiltocol	CH_3	CH_3	CH_3
β-tocopherol	5,8-Dymethiltocol	CH_3	H	CH_3
γ-tocopherol	7,8-Dymethiltocol	H	CH_3	CH_3
δ-tocopherol	8-Methyltocol	H	H	CH_3
α-tocotrienol	5,7,8 -Trymethiltocotrienol	CH_3	CH_3	CH_3
β-tocotrienol	5,8-Dymethiltotrienol	CH_3	H	CH_3
γ-tocotrienol	7,8-Dymethiltotrienol	H	CH_3	CH_3
δ-tocotrienol	8-Methyltocotrienol	H	H	CH_3

833 From: Fennema, Whitaker, Davidson, & Hartel (2004)

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Table 2. Bioequivalence between RRR-α-tocopherol and all-rac-α–tocopherol in some livestock species.

Species	Bio-availability RRR-α-tocopherol/ all-rac- α -Tocopherol	Reference
Dairy cows	2	Weiss, Hogan, & Wyatt (2009)
Piglets	2	Lauridsen et al. (2002)
Sows	1.5	Lauridsen et al. (2002)
Swine	2	Lauridsen et al. (2002)

Muscle	Content of tocopherol (mg/kg)	MAP composition	Display	Effect on selected traits	Reference

841 **Table 3.** Effect of muscle tocopherol on lipid and protein oxidation during display.

842

Lipid oxidation					
Semimembranosus	3.91	70% O_2-30% CO_2	9 days at 4 °C	Vitamin E supplementation reduced at least half the content of MDA found in control samples (1.21 vs 2.83 mg MDA/kg)	Bellés et al. 2018
Longissimus thoracis et lumborum	3.95	70% O_2-30% CO_2	18 days at 2 °C	Tocopherol decreased TBARS values compared to control (1.03 vs 8.15 mg MDA/kg)	Ortuño et al., 2015
Longissimus dorsi	2.42	70 % O_2-30% CO_2	12 days at 2 °C	Dietary vitamin E inhibited MDA formation	Muiño et al., 2014
Semimembranosus	1.90	75% O_2-25% CO_2	6 days at 4 °C	This level seemed to be minimum to inhibit lipid oxidation	Kasapidou et al., 2012
Semimembranosus	0.90	75% O_2-25% CO_2	6 days at 4 °C	This concentration of tocopherol in muscle appeared inactive in preventing lipid oxidation	Kasapidou et al., 2012
Longissimus dorsi	2.17	70% O_2-30% CO_2	28 days at 2 °C	Muscle tocopherol reduced significantly lipid oxidation compared to control (1.5 vs 8 mg MDA/kg)	Lauzurica et al., 2005
Longissimus dorsi	3.57	70% O_2-30% CO_2	28 days at 2 °C	This concentration of tocopherol avoided lipid oxidation	Lauzurica et al., 2005
Longissimus dorsi	5.30	70% O_2-30% CO_2	10 days at 4 °C	Supplemented lamb had lower TBARS values than non-supplemented (3 vs 9 mg MDA/kg)	Kerry et al., 2000
Longissimus dorsi + Semimembranosus	≈ 3	Vacuum packaging + overwrapping	60 days aging + 4 days of display at 3 °C	Lambs fed with ryegrass had higher levels of tocopherol and showed lower levels of lipid oxidation	Ponnampalam et al., 2017
Longissimus thoracis et lumborum	6.64	Overwrapping	9 days at 4 °C	Rosemary supplemented lambs presented lower TBARS values	Yagoubi et al., 2018
Protein oxidation					
Longissimus thoracis et lumborum	3.95	70% O_2-30% CO_2	18 days at 2 °C	Muscle tocopherol prevented protein oxidation (3.16 vs 6.96 nmol carbonyl g−1 protein)	Ortuño et al., 2015
Longissimus dorsi	2.42	70% O_2-30% CO_2	12 days at 2 °C	Vitamin E samples showed lower carbonyl content than the controls	Muiño et al., 2014

MAP: Modified atmosphere packaging; MDA: Malondialdehyde; TBARS: Thiobarbituric reactive substances.

Table 4. Effect of muscle tocopherol on meat colour during display.

Muscle	Content of tocopherol (mg/kg)	MAP composition	Display	Effect on selected traits	Reference
Semimembranosus	3.91	70% O_2- 30% CO_2	9 days at 4 °C	Vitamin E decreased MMb formation (12 %), obtaining a higher value of the 630/580 ratio (1.41 vs 1.18)	Bellés et al. 2018
Longissimus thoracis et lumborum	3.95	70% O_2- 30% CO_2	18 days at 2 °C	Supplemented sampled showed higher chroma values and a better appearance	Ortuño et al., 2015
Semimembranosus	3.73	75% O_2- 25% CO_2	6 days at 4 °C	Dietary vitamin E prevented meat from discolouration, resulting in higher chroma and a* values than control	Kasapidou et al., 2012
Longissimus dorsi	3.57	70% O_2- 30% CO_2	28 days at 2 °C	A dosage of 1000 mg α-tocopherol/kg feed reduced MMb formation by half	Lauzurica et al., 2005
Longissimus dorsi	5.30	70% O_2- 30% CO_2	10 days at 4 °C	Dietary vitamin E enhanced colour stability (lower proportions of MMb and higher a* values)	Kerry et al., 2000
Semimembranosus	3.5-4.0	Wrapping with chloride cling wrap	96 hours at 4 °C	No added benefit in meat colour was noted above this concentration	Jose et al., 2016
Longissimus thoracis et lumborum	6.64	Overwrapping	9 days at 4 °C	Rosemary supplemented lambs presented higher Chroma, Hue and oxymyoglobin values	Yagoubi et al., 2018

MAP: modified atmosphere packaging; MMb: metmyoglobin.

851

Highlights

The deposition of tocopherol in the muscle depends on the supplementation rate.

The relationship between vitamin E dose and α-tocopherol deposition may be nonlinear.

Dietary vitamin E does not affect productive parameters.

Vitamin E reduces lipid oxidation, discolouration and protects PUFA from degradation.

Vitamin E would indirectly affect the development of lamb aroma.

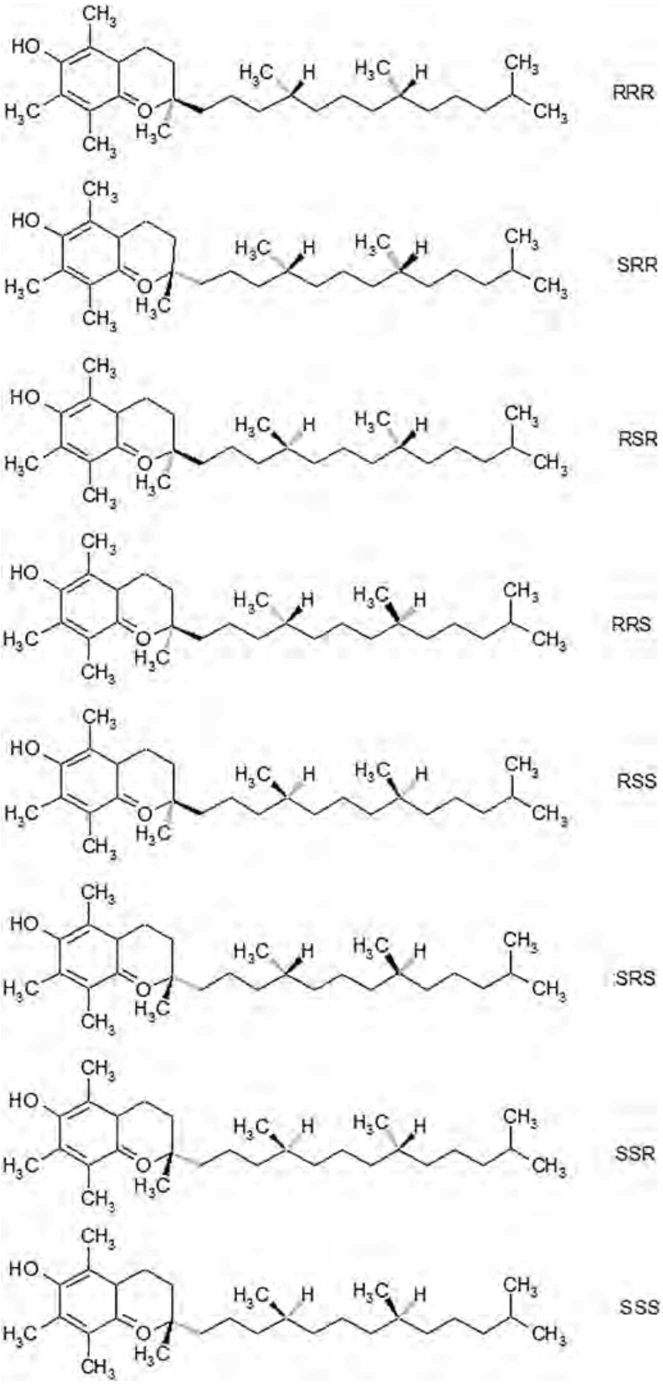

Figure 1

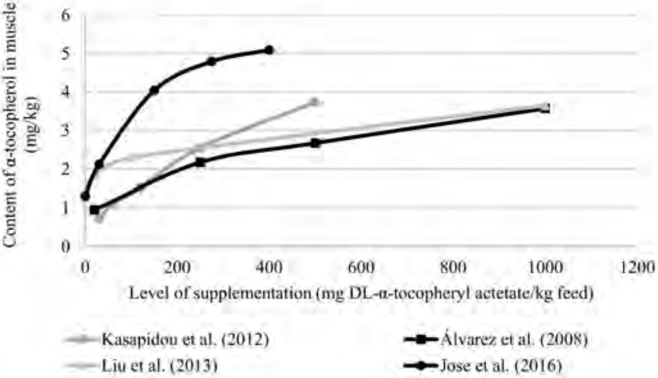
Content of α-tocopherol in muscle (mg/kg)
6
5
4
3
2
1
0
0
200
400
600
800
1000
1200
Level of supplementation (mg DL-α-tocopheryl actetate/kg feed)
Kasapidou et al. (2012)
Álvarez et al. (2008)
Liu et al. (2013)
Jose et al. (2016)

Figure 2