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Rosemary distillation residues reduce lipid oxidation, increase alpha-tocopherol content and improve fatty acid profile of lamb meat

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Abstract

The experiment studied the effects of rosemary distillation residues (RR) intake on lamb meat quality, oxidative stability and fatty acid (FA) profile. Barbarine lambs of Control group were fed 600 g of hay, which was substituted by 600 g of pellets containing 60 and 87% of RR for RR60 and RR87 groups; all animals received 600 g of concentrate. Meat protein and fat content was similar for 3 treatments. Lipid oxidation was strongly reduced with RR diets. Both RR diets resulted in a higher α- tocopherol content in muscle. The metmyoglobin and deoxymyoglobin percentages were similar for all groups; however oxymyoglobin was higher for RR groups. The saturated (SFA) and unsaturated FAs (UFA) were unaffected by the diets. However, the PUFA, n-6 and n-3 were higher for RR groups. In conclusion, rosemary residues resulted in higher vitamin E content, so it enhanced the oxidative status and improved the fatty acid profile of lamb meat.

Keywords: Rosemary residues, Oxidative stability, Vitamin E, Fatty acids, Lambs

1. Introduction

Lamb meat is widely appreciated by consumers in the West Asia North Africa area. In this region as well as in the rest of globe, the consumers are becoming more health-conscious and tend to search nutritious foods with health-promoting functions. They have an increasing tendency to select foods with high levels of polyunsaturated (PUFA), mainly n−3 PUFA, antioxidants and minerals (Ponnampalam et al., 2010). Meat quality is mainly affected by the animal feeding system (Hajji et al., 2016). Indeed, nutritional treatments can be used to develop animal growth and manipulate the fatty acid (FA) content to improve the nutritional value of lamb or cattle's meat (Joy, Ripoll, Molino, Dervishi, & Alvarez-Rodriguez, 2012; Ponnampalam, Burnett, Norng, Hopkins, Plozza, & Jacobs, 2016). The color and flavor of fresh

meat are the first factors taken into consideration by consumers. The oxidation of muscle components such as lipids and myoglobin during storage is the most important cause of meat deterioration that leads to changes in color, odor, flavor and texture (Buckley, Morrissey, & Gray, 1995). Synthetic antioxidants were extensively used to delay meat deterioration but consumers are looking more for safety and healthy meat products naturally produced (Troy & Kerry, 2010). Specific production systems could improve meat qualitative traits (Atti, Rouissi, & Mahouachi, 2005) and the antioxidant status (Nieto, & Ros, 2012). Also, using vitamin E (α-tocopherol) or foods rich in natural antioxidant leads to a reduction in meat oxidation (Buckley et al., 1995; Ripoll, Joy, & Muñoz, 2011). The secondary metabolites of some plants present a strong power as natural antioxidants. In fact, their incorporation directly to animal tissues by feeding is more efficient than the *post mortem* antioxidant treatment to meat (Kerry, Buckley, Morrisey, O'Sullivan, & Lynch, 1999).

The saturated fatty acids (SFA) are usually related to cardiovascular diseases (Pariza, Park, & Cook, 2001). It is well known that meat is the main source of SFA in the human diet, but consumers are looking for meat with high unsaturated FA proportion. However, the unsaturated FA are more susceptible to lipid oxidation than SFA (Wood & Enser, 1997).

In regard to potential antioxidant activity of natural foods, much attention has been directed to essential oils (EO) of medicinal and aromatic plants. These plants had great amounts of secondary metabolites such as phenolic compounds and flavonoids. The Rosemary, garlic and Artemisia's EO or distillated leaves were used in animal nutrition as additives to extend the shelf life and increase the acceptability of meat during storage (Nieto & Ros, 2012; Smeti, Atti, Mahouachi, & Muñoz, 2013). Rosemary is widespread in the Mediterranean area. After its distillation to produce EO, a great quantity of residues is produced. Given the availability of this by-product in Tunisia (5460 Tm/ year; APIA, 2003), its use as alternative food for small ruminants is very interesting seen that this region suffers from the unavailability of forage

caused by severe drought and the volatilizing prices of concentrate which worsened the situation. However, none of the cited studies looked into the possibility of total replacing of conventional forage by the distillation residues of medicinal and aromatic plants.

Therefore, the aim of this study was to determine the effects of substitution of oat hay with rosemary distillation residues (RR) on the color stability, myoglobin content, lipid oxidation, vitamin E and fatty acid profile of lamb meat.

2. Material and Methods

The trial was carried out at the National Institute of Agricultural Research of Tunisia (INRAT) from September to December 2015. All procedures employed in this study (transport and slaughtering) meet ethical guidelines and adhere to Tunisian legal requirements (The Livestock Law No. 2005-95 of 18 October 2005, Chapter II; Section 1 and Section 2 relative to the slaughter of animals).

2.1. *Animals and diets*

Twenty one fat-tailed Barbarine male lambs10 months' old (body weight (BW): 23.7 $\pm$ 4.4 kg) were used in the experiment. Lambs were treated against internal and external parasites and then housed in individual pens. Then, lambs were randomly allocated on the basis of BW to one of three basal diets. Treatments were the control group(C) receiving 600g of oat hay/animal/day and two RR groups where RR pellets were totally substituted to oat hay. The group RR60 was fed 600 g/day of pellets containing 60% of RR, 32 % of wheat bran and 8 % of soybean meal, and the group RR87 received600 g/day of pellets containing 87% of RR and 13 % of wheat bran. Lambs of three groups received each 600 g/day of concentrate. The DM chemical composition of RR was 7.5% of protein, 0.54% of fat and 53.8% of polyphenol. The

chemical composition and the FA profile of experimental diets are shown in the Table 1.The first 2 weeks were considered the pre-experimental period where the RR progressively replaced the hay. The feeding trial lasted 77 days in which experimental diets were offered twice a day at 9h and 14 h. All lambs had free access to water. Animals were weighed weekly before the distribution of the feeds.

2.2. Slaughter procedures and meat sampling

At the end of the feeding trial, all lambs were slaughtered in the abattoir of the INRAT. The day before slaughtering, lambs were fasted for 12 hours with only free access to water. Animals were weighed before slaughter. At slaughter, lambs were 13 months' old and weighed 32.8, 36.2 and 35.9 kg for C, RR60 and RR87, respectively (P=0.39). All carcasses were chilled at 4°C for24 hours. Then, *Longissimus thoracis* and *lumborum* (LTL) muscle of each carcass were removed, and sampled. The LTL muscle from the 4th to the 6th lumbar vertebrae was sliced and packed to determinate α-tocopherol and intramuscular fat contents. From 6th to 13th thoracic vertebrae was sliced into four 2.5-cm samples, and randomly assigned to 4 times of display (0, 3, 6 or 9 d), placed in trays and wrapped with oxygen-permeable PVC film, and kept in darkness at 4 ºC until instrumental color measurement. The 0 d samples were bloomed for 1 h before being measured. Immediately after the color and pH measurements, the samples were vacuum-packed and frozen (-20ºC) until lipid oxidation analysis.

2.3. Meat pH and chemical composition

The pH was measured at day 0, 3, 6 and 9 with a penetrating electrode connected to a portable pH-meter after calibration with two buffers (7.00 and 4.01) to obtain the pH kinetic. Another LTL sample were lyophilized to obtain dry matter (DM), grounded (1 mm screen) and

conserved for chemical composition analyses. All results are expressed as DM basis. Ash was determined by combustion at 600°C for 8hours. Nitrogen (N) was determined by the DUMAS procedure of direct combustion (AOAC, 1999) and then the proteins were calculated as N x 6.25. Meat intramuscular fat was extracted with petroleum ether and analyzed according to the method reported in AOCS (2004) Official Method Am 5-04.

2.4. Meat color and myoglobin measurements

Meat instrumental color was measured in the samples randomly taken at day 0, 3, 6 and 9 with a Minolta CM-2006d spectrophotometer (Konica Minolta Holding, Inc, Osaka, Japan). The lightness (L*), redness (a*) and yellowness (b*) were directly measured (CIE, 1986), while Hue angle (H*) and Chroma (C*) were calculated as $H^* = \tan^{-1}(b^*/a^*)$ x57.29, expressed in degrees, and $C^* = (a^{*2}+b^{*2})^2$.Metmyoglobin, oxymyoglobin and deoxymyoglobin relative contents were calculated according to the method proposed by Krzywicki (1979).

2.5. Lipid oxidation (TBARS) analysis

After thaw meat samples of 10 g were mixed with 20 ml of 10% trichloroacetic acid using a Micra D8 homogenizer (Labolan, Spain). The samples were centrifuged at 1500 g for 30 min at 4° C; the supernatants were filtered through a paper (Filterlab, Barcelona, Spain). Two milliliters of the filtrate was vortexed with 2 ml of thiobarbituric acid (20 mM); the tubes were homogenized and incubated at 97 °C for 20 min in water. The absorbance at 532 nm was measured with a Helios Beta spectrophotometer (Thermo Electron Corporation, Spain). A standard calibration curve was created with increasing concentrations (from0 to 100 µl) of 1,1,3,3, tetra methoxypropane (99%), the precursor of malondialehyde (MDA), 5 ml of thiobarbituric acid and 5 ml of water. The final conversion of 1,1,3,3, tetra methoxypropane to

MDA was accomplished by multiplying the number of μM of 1,1,3,3, tetra methoxypropane equivalent per gram of sample by the molecular weight of MDA. TBARS values are expressed as milligrams of MDA per kilogram of muscle.

2.6. Vitamin E analysis

Vitamin E analysis was performed according to the method described by Chauveau-Duriot, Doreau, Nozière, & Graulet. (2010). The determination of tocopherols was performed by the methods in 12822 (2014) in Foodstuffs for the determination of vitamin E by high performance liquid chromatography. Briefly, 1 g of lyophilized meat was put in glass tube of 25 ml. Then, 15 ml of KOH 11% (Ethanol: H_2O 50:50 v/v) and 0.2 g of ascorbic acid (antioxidant) were added. The tubes were closed by parafilm, then saponified overnight in water bath at 25°C protected from light. 5 ml of hexane-ethyl acetate (9:1 v/v) and 25 μg/ml of BHT (antioxidant) were added. The mixture was shaken with a vortex for 30 seconds then with the orbital shaker (Heidolph MultiReax). The upper layer was taken after 10 min and was transferred into glass tube of 10 ml. The mixture was evaporated with a rotary evaporator under vacuum (Christ RVC2-25) for 40 min at 40°C,then re-suspend in 1 ml of mobile phase (ACN: CH_3OH:CH_2Cl_2 75:15:10 v/v/v). Finally, the mixture was shaken with vortex (30 seconds) and with orbital shaker and was filtered with a filter PTFE of 0.22μm in vial of 2 ml of chromatography. An UPLC Acquity UPLC Class equipped with detector of absorbance (PDA eλ Detector) and fluorescence (Waters 2475 Multi λ Fluorescence Detector) was used. A column Acquity UPLC HSS T3 column 1.8 μm, 2.1 mm x 150 mm was used. Tocopherols were detected at 295 nm and cholesterol at 220 nm.

2.7. Fatty acid profile

The fatty acids from the intramuscular fat were extracted according to the method described by Lee, Tweed, Kim, & Scollan (2012). Muscle samples (0.4-0.8 g) lyophilized and minced were mixed with 1 ml of the internal standard (C23:0) and 2 ml of heptanes. Then, 4 ml of NaOH/CH_3OH 0.5M was added. The mixture was homogenized with vortex and heated for 20 min at 50°C, followed by cooling for 6-7 min. Then 4 ml of acetyl chloride / CH_3OH (1/10v/v) was added. The mixture was shacked and reheated for 60 min at 50°C. After cooling at ambient temperature, 2 ml of water milli-Q was added. Then the mixture was shacked, homogenized and centrifuged for 5 min, 3500 rpm at 10°C. The upper layer (heptanes) was taken and transferred to tube of 5 ml and then the dehydration was performed with anhydrous Na_2SO_4. The mixture was shaken with vortex for 30 seconds and then centrifuged for 5 min, 1000 rpm at 10°C. 1 ml of the supernatant was carefully transferred into a screw cap glass vial for gas chromatography with precaution for not taking part of the Na_2SO_4.Fatty acid samples were analyzed with a Gas Chromatography Bruker 436 Scion software Empower (GC). A 100 m x 0.25 mm D.I x 0.20 µm film thickness, capillary column (BR-2560 Bruker) was used for the separation of fatty acids. The temperature was 70°C for 1 min then 5°C /min for 2 min to 225°C maintained for 17 min with a total time of 80 min. The injector and detector temperatures were maintained at 260°C and 250°C, respectively. The fatty acids identification wasbased on retention times as compared with those of the standard FAMEs mixture of the two commercial fatty acids: GLC-463 and GLC-538.The desirable fatty acids were calculated according to Huerta-Leidenz, Cross, Lunt, Pelton, Savell, & Smith (1991) as DFA= MUFA+PUFA+C18:0.The saturation index (SI) was calculated according to Ulbricht & Southgate (1991) as SI= (C14:0+C16:0+C18:0) /∑ MUFA+ PUFA.

2.8. Statistical Analysis

A one way ANOVA was used to test the effect of dietary treatments on meat chemical composition, vitamin E content and fatty acid profile using GLM (General Linear Model procedure of S.A.S. Institute, 2004). The differences between groups were compared by the Duncan‘s Multiple Range Test (DMRT).Data of meat pH, color, lipid oxidation and myoglobin oxidation during 9 days of storage were analyzed using the MIXED procedure for repeated measures based on Kenward-Roger's adjusted degrees of freedom solution. The analyses were performed with diet (D) as between-subject fixed effect, storage time (T) as a within-subject effect and animal as random effect. In all analyses, the Akaike Information Criterion (AIC) closest to zero was used to choose the matrix of the error structure. The final selected matrix was the heterogeneous first-order autoregressive. Least square means were estimated and pair-wise comparisons of the means were obtained with the probability of difference (PDIFF) option of the LSMEANS procedure24. For all of the tests the level of significance was 0.05.

3. Results and discussion

3.1. Meat chemical composition

The meat chemical composition was not affected by the diet.(Table 2). The ash, protein and fat contents were similar among groups. The similarity in meat chemical components could result from the similar feeding value and comparable energy levels among the diets (Atti & Mahouachi, 2009; Smeti et al., 2013).The mean intramuscular fat content (15.8 %of DM) in the present study is lower than some results reported for the same breed (Atti & Mahouachi, 2009; Hajji et al., 2016), while the meat protein proportion of all groups was comparable to results found by Hajji et al. (2016) for Barbarine lambs.

3.2. Muscle α-tocopherol

The meat α- tocopherol concentration was significantly (P<0.001) affected by the RR intake (Table 2). It was 4 times higher forRR87 and RR60 than C group, while the last had the highest γ-tocopherol content (P<0.05). The α-tocopherol is the principal component of the vitamin E, despite the presence of other tocopherols in vitamin E activity (Stinnett, 1983). The great α-tocopherol content in meat due to the rosemary was previously reported in broilers meat when supplemented with rosemary leaves given its richness in tocopherols (Loetscher, Kreuzer, & Messikommer, 2013). This high vitamin E concentration in meat can be the result of the high amount of polyphenol compounds present in RR (44.7 and 33.8% for RR87 and RR60, respectively) that contribute to α- tocopherol deposition into the muscle. Meat from grazing animals is rich in antioxidants in form of D- α- tocopherol and flavonoides (Hopkins, Lamb, Kerr, Van de Ven, & Ponnampalam, 2013). However, the vitamin E concentration in the current study for both groups fed rosemary by-products (> 6 µg/g) was greater than values (3-4 µg/g) obtained under grazing conditions (Ponnampalam, Burnett, Norng, Warmer, & Jacobs, 2012; Hopkins et al.,2013) despite the richness of the pasture on natural antioxidants such as vitamin A and E, flavonoides and carotenoids.

3.3. Meat pH and color

No significant variation in pH was noted among diets (P>0.05) or throughout the storage time (P>0.05). However, the interaction was significant (P= 0.02; Table 3). The pH values were within the normal range (5.5 to 5.8) for lamb meat (Majdoub-Mathlouthi, Saïd, Say, & Kraiem, 2013). The normal values of meat pH indicate an effective acidification of meat for all groups. During the 9 days of measurement, the pH was similar to that presented by Nieto, Diaz, Banon, & Garrido (2010), when thyme leaves were included in the ewes' diet. During the display

period, there is no reduction in pH in any treatment and the absence of variation was due to the depletion of glycogen reserves in the muscle before the storage period (Lawrie & Ledward, 2006).

The diet affected meat yellowness (b*), chroma (C*) and hue angle (H*) ($P<0.01$), but did not affect lightness (L*) and redness (a*) ($P>0.05$) (Table 3). The lack of diet effect on pH could explain the unaffected lightness. In fact, meat from all treatments presented lightness values that are in the range of average acceptability but beyond of 44 which is considered the value of meat acceptability by 95% of consumers (Khliji, Van de Ven, Lamb, Lanza, & Hopkins, 2010).

RR-based diets maintained the same redness during the storage. This can be a result of the increasing antioxidant status (vitamin E) in muscle tissues that reduces myoglobin oxidation post-slaughter (Higgins, Kerry, Buckley, & Morrisey, 1998). The present result confirmed the results of Ponnampalam et al. (2012) who found that redness is jointly related to the level of antioxidant, such as vitamin E, in the muscle tissues. Indeed, it has been proposed that this could lead to reduce oxidation of pigments and thus improve the colour stability of meat during storage irrespective to the higher level of PUFA in muscle (Ponnampalam et al., 2012). Moreover, it was reported the beneficial effects of the use of some natural antioxidant on retarding meat color loss by extending the red color (a*) and delaying metmyoglobin formation (Camo, Beltran, & Roncales, 2008).

The higher yellowness (b*) value was recorded for both groups receiving RR than C. This supremacy could be related to the richness of rosemary by-product in carotenes responsible of yellowness index. In the current study, Control had significantly lower b*, C* and H* than RR treatments. So, these high values in meat of experimental groups indicated the high pigment quantity. Compared to meat of C group, RR diets showed protection of myoglobin against oxidation which delayed meat discoloration (Camo et al., 2008). The values

of C* recorded during storage confirmed previous results which showed that C* decreased as storage progressed resulting in pigment oxidation for lamb's meat as found Nieto et al. (2010) when thyme leaves were incorporated in pregnant ewe's diet (Nieto et al., 2010).

3.4. Myoglobin oxidation

The results of meat myoglobin (metmyoglobin (MMb), deoxymyoglobin (DMb) and oxymyoglobin (OMb)) oxidation are shown in Table 3. The OMb was affected by diet while the DMb, MMb percentages were not affected. The storage time had significantly affected the myoglobin evolution and the interaction effect (D x T) was significant except for DMB. It was shown that the oxidation of myoglobin to metmyoglobin can be delayed by the dietary supplementation of antioxidant compounds or by pasture feeding (Luciano, Monahan, Vasta, Pennisi, Bella & Priolo, 2009).

In the current study, the similarity in the MMb value can be due to the inclusion of RR in diets, whereas, across 9 days of storage, the MMb increased. The MMb followed the typical evolution through time. It increased in the first period of time to decrease at the end of the storage time, as previously reported by Ripoll, Gonzalez-Calvo, Molino, Calvo, &Joy (2013).The oxidation of myoglobin during time is generally related to a decrease of the redness and saturation (C*) and an increase of the hue angle (Khliji et al., 2010).

3.5. Meat fatty acid profile

The individual fatty acids and groups are shown in Tables 4 and 5, respectively. In concordance with the literature, the palmitic (C16:0), stearic (C18:0) and oleic (C18:1) acids comprised the largest proportions of FA. The oleic acid was the most abundant for the unsaturated FA and for the total detected fatty acids. This prevalence is in line with the commonly accepted values for intramuscular FA of thin-tailed (Joy et al., 2012; Hopkins et al.,

2014) and fat-tailed sheep (Atti & Mahouachi, 2009; Yousefi, Kohram, Shahneh, Nik-khah, & Campbell, 2012; Majdoub-Mathmouthi et al., 2013).

In the present study, the most abundant SFA (Table 4) were Palmitic (C16:0) and stearic (C18:0) in agreement with results of Atti et al. (2005) and Hajji et al. (2016). In fact, All the SFA were unaffected by the diet ($P>0.05$) except to C24:0 which was higher for C group. The Oleic acid (C18:1n-9c) was the major FA of MUFA group (Table4) as previously reported for lamb's meat (Hopkins et al., 2014; Mekki et al., 2016). The meat MUFA and C18:1n-9c were similar for all diets; this is the consequence of similar diet intake of MUFA and particularly C18:1n-9.The linoleic acid (C18:2n-6) was the most abundant PUFA (Table 4) for all treatments, but it was significantly higher for both groups fed RR than Control ($P<0.05$). These values are higher than those found by Ponnampalam, Mann & Sinclair (2006) in Australian beef fed long term grain ration. The C20:4n-6ARA was identical among treatments, however, the C18:3n-3 was increased with RR-based diets (Table 4). Indeed, the linoleic and linolenic acids were higher for both RR treatments ($P<0.05$) since the feedstuff presented higher contents of these FA.

The nutritional quality of meat and fat can be evaluated in terms of SFA, MUFA, PUFA, n-6, n-3 PUFA and their ratio (n-6/n-3) as well as the saturation index (Peiretti, Mussa, Prola, & Meineri, 2007). The SFA, MUFA and UFA were comparable between groups (Table 5). However, total PUFA increased with RR intake compared to control group. This result confirmed previous studies which suggest that secondary metabolites' plants have the potential to increase the unsaturated FA content in animal products (Lourenço, Cardozo, Calsamiglia, & Fievez, 2008). Indeed, the high PUFA, n-6 and n-3 PUFA levels in meat resulted from feeding animals with rosemary by-products explaining the polyphenol compound in leaves which maintain the unsaturated FA level in cell membranes (Nieto & Ros 2012). In the other hand, this higher PUFA deposited in meat from RR groups can be also attributed to the higher intake

of these FA (Table 1) given that the deposition of some fatty acids in the muscle depends not only on intake of the different FA but also on the extent of the ruminal biohyrogenation of the ingested PUFA. In fact, a possible effect of RR on the ruminal biohyrogenation of PUFA could be justified since rosemary contained phenolic compounds, including condensed tannins as found Gravador et al. (2015) when lambs were fed carob pulp partially substituted to barley in concentrate.

The PUFA: SFA ratio (Table 5) have a tendency to be higher (0.16) than the control (0.11). However, both values remained lower than the recommended value (0.45) for red meat in human nutrition (Department of health, 1994). In addition, these weaker values corroborate the values varying between 0.11 and 0.15 recorded for beef and lamb in the literature (Wood & Enser, 1997; Mandell, Gullett, Buchanan-Smith, & Campbell, 1997; Atti et al. 2005). The RR diets consumption did not affect the DHA (C22:6 n-3) concentration but increased the EPA (C20:5 n-3) level and the total n-3 PUFA. These dietary treatments significantly decreased the PUFA n-6/n-3 ratio (8.1 and 7.5 vs. 9.1 for C). These results lead to an enhancement in the nutritional value of meat. The concentration of EPA+ DHA obtained in the current study(5 to 8mg/100 g meat) was lower than those observed with other types of feeds in previous studies. In fact, it was reported that the EPA+DHA concentration was around 10 mg/100g when concentrate with dry pasture constituted a large part in animal diets and much higher (above 30 mg/100 g muscle) when animals grazed green pasture (Ponnampalam et al., 2014a).The highest values (superior to 100 mg/100 g meat) were observed for lambs fed algae (Hopkins et al., 2014).

The CLA isomers (Table4) particularly 18:2cis-9, trans-11 and 18:2 trans-10, cis-12 were similar among groups, but higher CLA isomer 18:2trans-9, trans-11 was recorded for RR based diets. The meat of ruminant is among the richest natural source of CLA, in particular the cis-9, trans11 isomer which arises from microbial biohyrogenation of dietary linoleic acid in

the rumen (Ha, Storkson, & Pariza, 1990). The inclusion of rosemary residues did not alter the total CLA concentration (P>0.05). However, it resulted in a decrease (P<0.05) in the saturation index (SI) (0.83 for RR vs. 0.95 for C). Similar results were found when the thyme by-products were included in pregnant and lactating ewes' diet (Nieto et al., 2010). This index represented an approach to evaluate the nutritional quality of meat and fat (Peiretti et al., 2007). The fat with high SI value is presumed to be disadvantageous to the human health (Ulbricht & Southgate, 1991). In the current study, the proportion of DFA that includes all UFA (MUFA+PUFA) and stearic acid (C18:0) was unaffected by the experimental diets.

3.6. Lipid oxidation (TBARS)

The lipid oxidation (TBARS) evolution is reported in Figure 1. It was strongly affected (P=0.001) by the diet, the time of display (P= 0.001) and their interaction (P=0.001).From the 3rd day of display, the TBARS value increased regardless the diet. Both groups receiving RR showed similar TBARS values that did not exceed 1.5 mg of MDA/ kg meat across 9 days, while the higher value (3.13 mg of MDA/ kg meat) was recorded for C group. The values from RR diets are slightly superior to the acceptability threshold of 1mg MDA/kg of meat (Ripoll et al., 2011), however they were below the threshold of 2mg of MDA/kg for the sensory detection of rancid or abnormal flavors enough to make meat unacceptable to consumers (Campo, Nute, Hughes, Enser, Wood, & Richardson, 2006). It has been reported a positive relationship between PUFA (n-6, n-3 or both) and lipid oxidation in muscle when vitamin E is below 2.95 mg/kg muscle that lead to a negative aroma. However, when vitamin E was above 2.95 mg/kg muscle, the increased PUFA, n-3 and n-6 fatty acids did not influence the lipid oxidation (Ponnampalam, Norng, Brunett, Dunshea, Jacobs, & Hopkins, 2014b). These findings supported the results of the current study, where the increased meat PUFA, n-3 and n-6 fatty acids for RR diets did not influence the lipid oxidation given the high vitamin E content of this

meat. Rather oxidative damages in meat have been observed for C group (3.13 mg of MDA/ kg meat). As well, this result can be attributed to the richness of rosemary in potential antioxidant considering the higher phenolic compounds (Gravador et al., 2015). Their transformation in high vitamin E concentration confers to RR meat this superior resistance to oxidative deterioration and prevents the essential PUFA from oxidation (Ponnampalam et al., 2012 and 2014b). Increasing antioxidant status, such as vitamin E in muscle tissue of meat has been suggested as an approach to reduce the lipid and myoglobin oxidation of meat post slaughter (Arnold, Arp, Scheller, Williams, & Schaefer, 1993). The critical α- tocopherol value of 3.0 µg/g was suggested as target (Faustman, Cassens, Schaeffer, Buege, & Scheller, 1989) to have a significant impact on the reduction of pigment and lipid oxidation. However, Ripoll et al. (2013) found that values of 0.74µg/g delayed the lipid oxidation of meat, and values of 1.47µg/g delayed both lipid oxidation and metmyoglobin formation. Thereafter, the insufficient concentration of vitamin E in control group (1.59 mg/kg DM) to prevent fatty acid oxidation explained the elevated TBARS value as found by Ponnampalam et al. (2014b).Gonzalez-Calvo, Ripoll, Molino, Calvo, & Joy (2015) conclude that a muscle ??-tocopherol concentration between 0.61 and 0.90mg/ kg fresh meat is guaranteed to conserve lamb meat at the optimal conditions for 7 days of oxygen exposure. Then, the diet can contribute to the variation of components in muscle tissue (Ponnampalam et al., 2014b). The incorporation of rosemary in lamb diet resulted in a transfer of antioxidants to cell membranes and tissues; the antioxidant compounds transferred from diet to meat protect tissues against oxidation more than adding antioxidant *post-mortem* (Kerry et al., 1999).The significant correlation between the phenolic content and the antioxidant properties of some plants as rosemary in vitro were reported (Ponnampalam et al., 2014b).Besides, the antioxidant effect through feeding with rosemary by-products or sage was investigated and the effect on the delay of lipid oxidation was shown (Botsoglou, Govaris, Giannenas, Botsoglou, & Papageorgiou, 2007). So, given the meat

vitamin E concentration for RR diets was above 4.15 mg/kg muscle as reported Ponnampalam et al. (2014b), the oxidation was reduced to 1.51 mg MDA/ kg muscle irrespective of PUFA concentrations.

Conclusion

This study showed that rosemary residues permit to avoid the lipid oxidation of meat over storage given the increased vitamin E level in muscle irrespective to the PUFA content in lamb muscle. The high vitamin E content in muscle prevents the essential PUFA from oxidation and thus reduces pigment oxidation. Furthermore, rosemary residues maintained the SFA content in the meat and significantly increased the n-3 and n-6 PUFA in muscle. Then, rosemary residues can be a feasible strategy to elevate muscle vitamin E concentration which leads to improve color stability and reduce the level of lipid oxidation in meat stored for long term storage.

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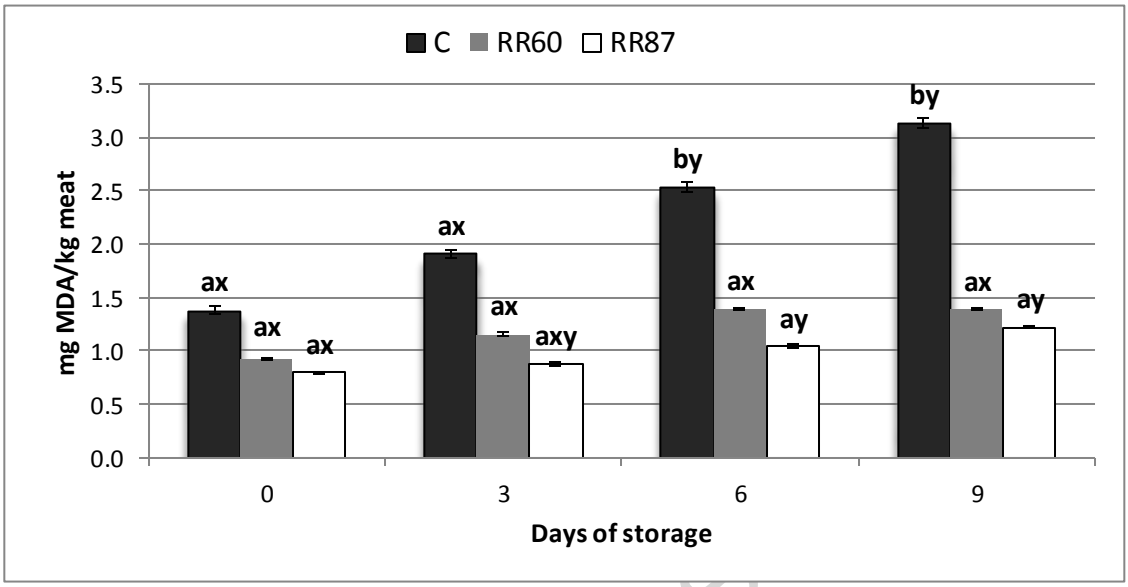

a, b: different letters within a diet differ significantly ($P < 0.05$).
x, y: different letters within a storage time differ significantly ($P < 0.05$).

Figure 1. Meat lipid oxidation (TBARS)

C: control group fed 600 g of oat hay and 600 g of concentrate; **RR60**: experimental group fed 600 g of rosemary pellets containing 60% of RR, 32 % of wheat bran and 8 % of soybean meal; **RR87**: experimental group fed 600 g of rosemary pellets containing 87% of RR and 13 % of wheat bran.

Table 1. Chemical composition of experimental feeds (% Dry Matter)

	Concentrate	Oat Hay	RR60	RR87
Dry Matter	90.6	83.3	90.1	92.1
Organic Matter	95.6	93.5	92.5	92.5
Crude Protein	14.1	5.0	13.6	8.8
Crude Fat	0.94	1.2	3.53	3.99
TPC	2.6	8.1	33.8	44.7
NDF	34.2	66.7	32.3	36.3
Fatty acid profile (% total FAMES)				
C14:0	0.37	3.16	0.94	1.62
C16:0	32.62	31.06	21.81	24.43
C16:1 n7	0.14	1.08	0.62	1.03
C17:0	0.2	1.24	0.37	0.5
C18:0	6.48	9.58	4.2	5.24
C18:1n-9	32.81	21.16	20.81	20.01
C18:2n-6	24.5	18.61	38.08	29.21
C18:3 n-3	1.33	4.30	8.81	11.65
SFA	41.16	49.45	29.73	35.15
MUFA	33.24	23.88	21.62	21.2
PUFA	25.56	24.58	47.63	42.12
UFA	58.8	48.46	69.25	63.32
n-6PUFA	24.5	18.61	38.08	29.21
n-3PUFA	1.41	5.97	9.55	12.91
n-6/n-3	17.4	3.11	3.98	2.26

TPC: Total Phenolic compounds**; NDF**: Neutral Detergent Fiber; **SFA**: saturated fatty acids; **MUFA**: monounsaturated fatty acids; **PUFA**: polyunsaturated fatty acids; UFA: unsaturated fatty acids; **RR60**: rosemary pellets containing 60% of rosemary residues (RR), 32 % of wheat bran and 8 % of soybean meal; **RR87**: rosemary pellets containing 87% of RR and 13 % of wheat bran.

Table 2. The effect of diet on *LTL* muscle chemical composition (% DM) and vitamin E concentration (μg/g DM)

	C	RR60	RR87	SEM	P
Dry Matter	25.2	26.6	25.9	0.24	0.07
Ash	5.0	6.05	6.05	0.27	0.21
Protein	71.7	69.2	72.2	1.15	0.53
Fat	15.60	16.96	14.96	1.44	0.84
Cholesterol (mg/g)	1.91	1.84	1.74	0.05	0.50
α –tocopherol	1.59^{b}	7.77^{a}	6.64^{a}	0.35	0.0001
ϒ –tocopherol	0.28^{a}	0.07^{b}	0.08^{b}	0.01	0.0004

$^{a, b}$: Different letters in the same row indicate significant differences ($p<0.05$)

C: control group fed 600 g of oat hay; **RR60**: experimental group fed 600 g of rosemary pellets containing 60% of RR; **RR87**: experimental group fed 600 g of rosemary pellets containing 87% of RR.

Table 3. Effect of diet (D) and storage time (T) on pH, color parameters and haeminic pigments

	Diet (D)			Storage time (T)					P		
	C	**RR60**	**RR87**	**0**	**3**	**6**	**9**	**SEM**	**D**	**T**	**D x T**
pH	5.40	5.55	5.41	5.45	5.45	5.50	5.50	0.01	0.12	0.08	0.022
L*	36.47	34.13	33.51	32.43^{y}	36.89^{x}	34.77^{xy}	34.73^{xy}	0.98	0.10	0.001	0.236
a*	8.63	8.40	9.05	9.25	8.41	8.46	8.67	0.37	0.47	0.21	0.024
b*	11.86^{b}	14.32^{a}	15.38^{a}	17.12^{x}	12.90^{y}	12.62^{y}	12.77^{y}	0.55	0.001	0.001	0.85
C*	14.85^{b}	16.73^{a}	17.94^{a}	19.61^{x}	15.62^{y}	15.28^{y}	15.51^{y}	0.49	0.001	0.001	0.90
H*	52.24^{b}	58.62^{a}	59.37^{a}	61.40^{x}	54.85^{y}	55.35^{y}	55.37^{y}	1.46	0.005	0.013	0.428
MMb	11.45	10.53	11.94	3.78^{z}	13.28^{xy}	16.98^{x}	11.18^{y}	1.56	0.81	0.001	0.001
DMb	76.66	69.05	66.62	60.01^{y}	71.31^{x}	73.71^{x}	78.07^{x}	2.90	0.06	0.001	0.42
OMb	11.89^{b}	20.41^{ab}	21.43^{a}	36.19^{x}	15.40^{y}	9.30^{y}	10.75^{y}	2.10	0.01	0.001	0.001

MMb: Metmyoglobin; **DMb:** Deoxymyoglobin; **OMb:** Oxymyoglobin

(a, b) different superscripts means differences between Diet

(x, y, z) different superscripts means differences between days of storage;

C: control group fed 600 g of oat hay; **RR60**: experimental group fed 600 g of rosemary pellets containing 60% of RR; **RR87**: experimental group fed 600 g of rosemary pellets containing 87% of RR.

Table 4.Effect of diet on fatty acid profile (mg/100 g fresh muscle)

Item	C	RR60	RR87	SEM	P
SFA					
C10:0	8.9	9.1	10.5	0.82	0.70
C12:0	6.2	5.6	5.1	0.99	0.89
C13:0	1.1	0.9	1.4	0.12	0.36
C14:0	130	138	135	14.3	0.96
C15:0	19.6	27.1	30.7	2.9	0.32
C16:0	1491	1583	1469	10.5	0.89
C17:0	65	123	122	12.1	0.10
C18:0	1189	1235	987	97	0.55
C20:0	5.5	6.3	4.9	0.7	0.74
C22:0	1.8	1.9	1.7	0.2	0.92
C24:0	0.56	0.29	0.37	0.04	0.09
MUFA					
C14:1 cis9	2.5	2.1	2.6	0.33	0.85
C15:1	38	38	42	1.3	0.31
C16:1 trans9	18.6	13.8	9.6	1.9	0.17
C17:1 cis 10	43	62	70	4.9	0.09
C18:1 trans 11	47.5[b]	184[a]	106[b]	12	0.001
C18:1cis9	2352	2662	2286	178	0.66
C18:1 trans 15	0.93	0.66	0.77	0.09	0.52
C18:1 cis 11	2.81	4.90	2.95	0.57	0.28
C18:1 cis 12	0.89	0.59	1.06	0.08	0.08
C18:1 cis 13	1.8	3	2.4	0.37	0.44
C18:1 trans 16	2.3	1.7	0.6	0.61	0.52

C18:1 cis 15	1.3	1.2	1.1	0.11	0.82
C20:1 n-9	0.6	0.6	0.5	0.03	0.28
C22:1	1.1[a]	0.7[ab]	0.6[b]	0.07	0.02
C24:1 n-9	0.53	0.34	0.39	0.03	0.14
PUFA					
C18:2 n-6	189[b]	342[a]	277[a]	16.4	0.004
C18:2 cis 9, trans 11 CLA	19.6	23.8	20.6	2.27	0.72
C18:2 trans 10, cis 12 CLA	1.6	1.5	1.2	0.17	0.65
C18:2 trans 9,trans 11 CLA	3.7[b]	7.1[a]	6.3[a]	0.44	0.01
C18:2 n-6 trans 9,12	2.5	2.7	2.3	0.4	0.91
C18:3 n-6	1.5	2.2	1.8	0.14	0.21
C18:3 n-3	15.0[b]	34.6[a]	26.5[a]	2.11	0.005
C20:2 n-6	1.8	3.2	2.8	0.24	0.07
C20:3 n-9	13.7[a]	9.7[b]	9.8[b]	0.56	0.01
C20:3 n-6	5.2	5.6	5.8	0.29	0.69
C20:4 n-6 ARA	61.8	69.8	70.5	2.2	0.23
C20:5 n-3 EPA	3.6[b]	4.5[b]	5.9[a]	0.18	0.001
C22:3 n-3	0.48	0.44	0.65	0.05	0.30
C22:4 n-6	4.7[b]	6.8[a]	7.3[a]	0.31	0.007
C22:5 n-3 DPA	10.2[b]	13.3[a]	14.3[a]	0.60	0.03
C22:6 n-3 DHA	1.3	1.9	2.2	0.18	0.14

SFA: saturated fatty acid; **MUFA**: monounsaturated fatty acid; **PUFA:** polyunsaturated fatty acid;

[a, b]: Different letters in the same row indicate significant differences ($p<0.05$)

C: control group fed 600 g of oat hay; **RR60**: experimental group fed 600 g of rosemary pellets containing 60% of RR; **RR87**: experimental group fed 600 g of rosemary pellets containing 87% of RR.

Table 5.Effect of diet on fatty acid groups (mg/100 g fresh muscle) and ratios

Item	C	RR60	RR87	SEM	P
SFA	3031	3237	2871	226	0.80
MUFA	2579	3045	2588	199	0.56
PUFA	335[b]	529[a]	455[a]	21.3	0.005
UFA	2915	3574	3044	210	0.41
DFA	4103	4808	4031	301	0.52
n-6	266.3[b]	431.9[a]	367.1[a]	17.8	0.004
n-3	30.5[b]	54.7[a]	49.6[a]	2.62	0.003
n-6/n-3	9.03[a]	8.07[ab]	7.5[b]	0.27	0.10

PUFA/SFA	0.110	0.163	0.158	0.013	0.10
MUFA/SFA	0.85	0.95	0.89	0.01	0.09
UFA/SFA	0.98[b]	1.12[a]	1.05[ab]	0.01	0.03
CLA	24.9	32.4	28.1	2.7	0.53
SI	0.95[a]	0.81[b]	0.86[b]	0.01	0.01

SFA: saturated fatty acid; **MUFA**: monounsaturated fatty acid; **PUFA**: polyunsaturated fatty acid; **UFA**: unsaturated fatty acid; **CLA**: conjugated linoleic acids; **SI**: saturation index; **DFA**: desirable fatty acids

[a, b]: Different letters in the same row indicate significant differences ($p<0.05$)

C: control group fed 600 g of oat hay; **RR60**: experimental group fed 600 g of rosemary pellets containing 60% of RR; **RR87**: experimental group fed 600 g of rosemary pellets containing 87% of RR.

Highlights

- Meat n−6/n−3 ratio of lambs fed rosemary distillation residues was lower than hay one
- The meat produced with rosemary distillation residues has a high vitamine E content
- The rosemary distillation residues permit higher Oxymyoglobin.
- The oxidative stability was higher for lambs fed rosemary distillation residues