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Alfalfa but not milk in lamb's diet improves meat fatty acid profile and α-tocopherol content

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Abstract

To establish animal feeding recommendations, it is required to quantify whether the effects of combining dietary alfalfa and milk on meat composition of light lambs are overlapped or independent. This experiment aimed to evaluate the separate effects of dietary alfalfa and milk access on the light lamb carcass quality (10-11 kg), meat colour, chemical composition, fatty acid profile and α-tocopherol content. Thirty-two lambs were assigned to one of four treatments in a 2×2 factorial design. The factors were the inclusion of dietary forage (grazed alfalfa vs. concentrate-fed indoors) and lactation length (weaning at a target live-weight of 13 kg vs. suckling until slaughter at 22-24 kg). Dietary alfalfa but not milk supply improved conjugated linoleic acid isomers (CLA), omega-3 fatty acids and α-tocopherol contents in lamb meat without affecting meat colour attributes. Milk supply affected more the fatty acid profile (more saturated) than the α-tocopherol content of meat. Thus, dietary alfalfa improved CLA, omega-3 fatty acids and α-tocopherol content in light lamb without affecting the meat colour, whereas lengthening the lactation period did not provide benefits in terms of meat colour or healthy nutrient composition.

Key words: fresh forage; suckling period; lamb meat quality; lamb nutrition; light lamb; vitamin E.

Introduction

Lamb meat types differ greatly worldwide, with the two main country producers yielding heavy carcass weights (22 kg in Australia and 16 kg in China). In Europe, this outcome differs considerably between the two principal producers, United Kingdom and Spain, which yield an average carcass weight of 20 and 11 kg, respectively (FAOSTAT, 2017). Thus, lamb meat has not standard attributes, and on-farm dietary and management practices may impact the subsequent quality traits of lamb meat during retail display. The Spanish lamb meat comes mostly from light lambs that are early weaned at 12-14 kg of live-weight (LW) and fed intensively with concentrates until 22-26 kg of LW, depending on the Spanish region (Alfonso et al., 2001), which yield a carcass weight ranging from 9 to 13 kg (MAPAMA, 2017).

Interest in natural compounds with antioxidant effect on meat quality and animal welfare has been increased dramatically during the last decade. Dietary inclusion of antioxidants in animal feed has been proven a more effective strategy to prevent oxidative damage of meat compared to their direct addition into meat or meat products, due to uniform integration into cell phospholipid membranes (Kerry, Buckley, Morrissey, O'Sullivan, & Lynch, 1998). The most proven dietary antioxidants in animal feed are firstly vitamin E and secondly plant polyphenols

(flavonoids and/or condensed tannins) and diterpenes as carnosic acid (Muela, Alonso, Campo, Sañudo, & Beltrán, 2014; Ortuño, Serrano, & Bañón, 2015). Dietary alfalfa may be a convenient feedstuff to prevent oxidative damage of meat because it is rich in natural vitamin E although it has low polyphenol content (Lobón et al., 2017b). However, light lambs require milk supply during at least half of their lives; thereby the feeding strategy for light lambs may involve the use of both dietary ingredients (milk and forage).

Alfalfa grazing in addition to suckling and creep feed supplementation may be a feasible alternative dietary strategy to increase light lamb meat shelf life without using synthetic additives in feed (Ripoll, González-Calvo, Molino, Calvo, & Joy, 2013). In this sense, dietary fresh forage inclusion can exert major effects on meat colour by increasing muscle redness index (Ripoll, Joy, Muñoz, & Albertí, 2008), and increasing the percentage of PUFA n-3 (Joy, Ripoll, & Delfa, 2008) and α-tocopherol content of light lamb meat (Gonzalez-Calvo et al., 2014). However, polyunsaturated fatty acids of meat can be highly prone to oxidation by generating free radicals during the oxidative process (Santé-Lhoutellier, Engel, & Gatellier, 2008). The approaches of increasing the content of natural antioxidants by increasing the intake of fresh forage should consider the collateral effect on fatty acid profile. Therefore, a correct oxidative balance should be found out.

In the previous studies, the potential contributions of both forage use and milk supply on meat colour attributes, intramuscular fatty acid composition and α-tocopherol content could not be addressed, since grazing lambs were not weaned until slaughter whereas the concentrate-fed lambs were not allowed consuming dam's milk from weaning, which is the routine farming management practice in Spain. To establish animal feeding recommendations, it is required to quantify whether the effects of combining dietary alfalfa and milk on meat composition are overlapped or independent. In this framework, the aim of this study was to evaluate the separate effects of dietary alfalfa and milk supply on the light lamb carcass, meat colour, chemical composition, fatty acid profile and α-tocopherol content.

Material and methods

Animals and treatments

The care and use of animals were performed accordingly with the Spanish Policy for Animal Protection RD53/2013, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes. Thirty-two single Rasa Aragonesa lambs (born between July 11th and August 7th) were maintained with their dams (3.8±1.3 years of age, 53.3±7.3 kg LW; means±standard deviation) indoors during three weeks after birth to assure maternal bonding. After this period, the mother-young pairs were assigned to one of four treatments in a 2 x 2 factorial design. The factors were dietary forage supply (green alfalfa vs. solely concentrate-fed indoors) and milk access (from birth to weaning at 13 kg vs. from birth to slaughter). The target slaughter LW was 22-24 kg. Lamb sex, ewe parity and body-weight of ewes and lambs were taken into account to balance groups. The reared lambs belonged to the experimental flock from the CITA Research Station (Montañana, Zaragoza, Spain).

All lambs were supplemented with a purchased concentrate ration (Pastores Grupo Cooperativo, Zaragoza, Spain) in creep feeders placed on the alfalfa swards and indoors. Alfalfa grazing lambs were also fed concentrate in order to achieve the same age at the targeted slaughter LW. The main concentrate ingredients were barley grain (36%), maize grain (26.4%), soyabean meal (24.1%), and wheat grain (7%), whereas minor ingredients were palmist oil, cane molasses, minerals and vitamin-premix (6.5%). The concentrate feed contained 196 g of crude protein and 43 g of crude fat per kg of dry matter, with an analysed fatty acid composition of 291 g SFA/100 g of identified FA, 273 g MUFA/100 g FA, 398 g PUFA n-6/100 g FA and 37 g PUFA n-3/100 g FA, and 17 mg α-tocopherol per kg of dry matter. Sixteen ewe-lamb pairs were kept in indoor facilities. These dams were fed a total dry mixed ration *ad libitum* in automatic self-feeders designed for adult sheep height access. The remaining sixteen ewe-lamb pairs were maintained permanently on contiguous alfalfa crops (154 mg α-tocopherol/kg dry matter; 234.6 g SFA/100 g FA, 41.5 g MUFA/100 g FA, 163.9 g PUFA n-6/100 g FA and 289.4 g PUFA n-3/100 g FA) at a stocking rate of 21 ewes plus lambs/ha. A detailed description of alfalfa management and proximate chemical composition, as well as productive parameters and digestive function in lambs is described in a previous work (Álvarez-Rodríguez, Sanz, Ripoll-Bosch, & Joy, 2010). At the target LW of 12-13 kg, half of the lambs reared indoors and half of the lambs reared outdoors were weaned and maintained on their original location, whereas the remaining half of the lambs suckled their mothers until slaughter. Outdoor lambs were allowed to graze alfalfa and had free access to the concentrate whereas indoor lambs were fed the concentrate and barley straw *ad libitum*.

Carcass measurements and meat sampling procedures

Lambs were slaughtered at 22.8±1.3 kg of LW and 94±3 days old in the experimental slaughterhouse of the CITA Research Institute at Zaragoza (Spain). The slaughters were conducted between 08:00 and 09:00 h without fasting period (lambs balanced across treatments in each turn). Prior to exsanguination, lambs were stunned by light-weight captive bolt pistol. After evisceration, carcasses were hung by the Achilles tendon and were chilled for 24 h at 4°C in total darkness and then, the cold carcass was weighed. The dressing percentage was calculated as cold carcass weight × 100/slaughter weight.

The conformation was scored using the EUROP system (E = excellent, U = very good, R = good, O = fair and P = poor) and these 5 categories were expanded to 15 points. Therefore, carcasses were graded from 15 (E+) to 1 (P–) (Colomer-Rocher, Morand-Fehr, Kirton, Delfa, & Sierra, 1988). Fatness degree was determined following the Community Scale for Classification of Carcasses of Ovine Animals (EEC, 1992) for light carcasses (< 13 kg) with grade values from 1 (1–, very low) to 12 (4+, very high) of the scale 1 (low), 2 (slight), 3 (average), 4 (high). Both carcass conformation and fatness degree were evaluated by two trained assessors.

Then, the carcasses were split along the dorsal line. The *Longissimus thoracis et lumborum* (LTL) muscle from both half carcasses was collected. The pH of the LTL muscle was measured at the fourth lumbar vertebra with a pH meter equipped with a Crison 507 penetrating electrode (Crison Instruments, S.A., Barcelona, Spain). The LTL muscle from the fourth to the sixth lumbar vertebrae of the left carcass was sliced and vacuum packed into foil bags to determine the proximate chemical composition and α-tocopherol content. The same portion

from the right carcass was sliced and vacuum packed to analyse the fatty acid composition. The LTL muscles from the 6th to the 11th thoracic vertebrae were sliced into 2.5-cm-thick samples and randomly assigned to 5 display times (1, 4, 5, 6 and 7 days), placed in trays, and wrapped with oxygen-permeable polyvinyl chloride film and kept in darkness at 4°C until being measured for colour.

Objective colour determination

The colour of subcutaneous caudal fat, kidney fat, *Rectus abdominis* and LTL muscles was measured 24 h *post-mortem* using a Minolta CM-2600d spectrophotometer (Konica Minolta Holdings, Inc., Osaka, Japan) in the CIE L*, a*, and b* colour space with a measurement area diameter of 8 mm, including a specular component and a 0% UV, standard illuminant D65 that simulates daylight (colour temperature of 6,504 K), 10° observer angle, and zero and white calibration. The integrating sphere has a diameter of 52 mm, and the measurement area was covered with a dust cover CM-A149. The colour of LTL muscle slices was also sequentially measured at 4, 5, 6 and 7 days of retail display. The spectrophotometer was placed on the cranial side of the LTL and samples were allowed to bloom for 60 min.

Subcutaneous caudal fat colour from the tail root (Díaz et al., 2002) and kidney fat colour were recorded at three locations randomly selected but avoiding blood blots, discolourations and less covered areas (Ripoll, Albertí, & Joy, 2012). *R. abdominis* muscle colour was measured after having removed the covering fascia (Ripoll, Joy, Muñoz, & Albertí, 2008). In both muscles, the colour was measured at 2 locations randomly selected to obtain a mean value with a representative reading of surface colour. The measurements were averaged. To standardize the measurements, a white tile was placed behind the muscles.

The lightness (L*), redness (a*), and yellowness (b*) were recorded. The hue angle (h_{ab}) was calculated as $h_{ab} = \tan^{-1}\left(\frac{b*}{a*}\right) \cdot 57.29$, expressed in degrees, and chroma (C^*_{ab}) was calculated as $C^*_{ab} = \sqrt{(a*)^2 + (b*)^2}$. In carcass fat, besides of trichromatic coordinates, the proportion of reflected light each 10 nm between 450 and 510 nm was collected and the absolute value of the integral of the translated spectrum (SUM) was calculated according to Prache and Thériez (1999). This variable is used to estimate the carotenoid pigments content of fat.

The relative content of metmyoglobin (MMb) was estimated via selected wavelengths (AMSA, 2012). This method is based on the concept of reflex attenuance, which is the logarithm of the reciprocal of reflectance. The reflectance was measured at the isobestic wavelengths of 525 and 572 nm, and at 730 nm, which is referred to as the reflectance of pigment-free meat.

Chemical analyses

Meat samples were lyophilized and then grinded to determine the crude protein, intramuscular fat (IMF), α-tocopherol content and fatty acid profile. Samples were weighed before and after freeze-drying to obtain the moisture content. The IMF content was determined through the Ankom procedure (AOCS, 2005) with an XT^{10} Ankom extractor (Ankom Technology, Madrid, Spain). The content of crude protein (nitrogen × 6.25) was determined

following the Dumas procedure (method 984.13; AOAC, 1999) using a nitrogen analyser (model NA 2100; CE Instruments Ltd., ThermoQuest SA, Barcelona, Spain).

The α-tocopherol concentration of meat was determined by liquid extraction in duplicate as described by Val, Monge, and Baker (1994) with the modifications performed by González-Calvo et al. (2015). Briefly, freeze-dried meat (0.1 g) was deproteinized with ethyl alcohol (0.4 ml) and vortexed for 30 s, then the lipophilic components were extracted with n-hexane (1 ml) after the mixture was vortexed for 15 min and centrifuged at 3,500 rpm for 5 min at room temperature. The hexane phase was collected and evaporated by vacuum centrifugation. The dry residue was dissolved in acetonitrile/ethanol/dichloromethane and transferred into a 2 mL screw-top glass vial for automatic sampling (40 µL) for high-performance liquid chromatography (HPLC; 1100 Series, Agilent, Karlsruhe, Germany). HPLC separation was performed using a 100 mm × 4.6 mm, 2.6 µm Kinetex C18 column and KrudKatcher Ultra HPLC inline filter (Phenomenex Inc., California, USA). The HPLC instrument was equipped with a photodiode array detector scanning between 210 and 600 nm. α-Tocopherol was detected at 295 nm and identified by comparing the retention time and spectral analyses with pure standards. The isocratic mobile phase consisted of acetonitrile / methanol / dichloromethane / ammonium acetate in water. The flow rate applied was 1.5 mL min^{-1} and the analysis was temperature controlled (35 ºC) using a column oven.

Intramuscular fat was extracted in chloroform / methanol according to Bligh and Dyer (1959), and butylated hydroxytoluene was used as antioxidant. Fatty acid (FA) methyl esters were analysed by gas chromatography (GC) using a gas chromatograph (Agilent Technologies 6890, Santa Clara, CA) equipped with a flame ionisation detector. Chromatographic conditions are described in detail by Díaz et al. (2017). Briefly, separation was carried out in an Omegawax 320 capillary column (30 m x 0.32 mm i.d., 0.25 mm film thickness) with polyethylene glycol as the stationary phase (Supelco, Bellefonte, USA). Gas chromatography conditions were as follows: oven temperature 200 ºC held for 60 min with helium as carrier gas (flow rate 1.3 mL min^{-1}), injector and detector temperatures were 260 ºC. The results were expressed as a percentage of the total identified fatty acids. Individual FA were identified by comparing their retention times with those from a known standard Supelco® 37 Component FAME Mix (Supelco, Bellefonte, PA, USA). The CLA isomers were computed as the major peak in conjugated octadecadienoic region of the chromatogram that had an elution time consistent with conjugated linoleic acid FAME standard mixture (Sigma, St. Louis, USA), consisting of cis- and trans-9,11- and -10,12-octadecadienoic acids. FA classes were calculated as follows: total saturated fatty acids (SFA) = C10:0 + C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0; total monounsaturated fatty acids (MUFA) = C16:1 + C17:1 + C18:1 + C20:1 n-9; total PUFA n-6 (n-6) = C18:2 n-6 + C20:4 n-6 + C22:4 n-6; total PUFA n-3 (n-3)= C18:3 n-3 + + C20:5 n-3 + C22:5 n-3 + C22:6 n-3; total polyunsaturated fatty acids (PUFA) = n-6 + n-3. The C18:1 n-9 and n-7 isomers are reported as one value (C18:1), as the column incompletely resolves them. The fatty acid reported as C16:1 consists of both the n-9 and n-7 isomers.

Statistical analyses

The statistical analyses were conducted using JMP (12.0.01 version; SAS Institute Inc., Cary, NC). Carcass measurements, fat and *R. abdominis* muscle colour attributes, proximate

chemical composition, fatty acid profile and α-tocopherol content of meat were analysed through standard least squares model:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \delta_k + (\alpha\beta)_{ij} + (\alpha\delta)_{ik} + (\beta\delta)_{jk} + \varepsilon_{ijk}$$

where: $y_{ijk} =$ dependent variable, $\mu =$ overall mean, $\alpha_i =$ alfalfa supply effect, $\beta_j =$ milk supply effect, $\delta_k =$ sex effect, the single interactions between dietary alfalfa and milk, dietary alfalfa and sex, dietary milk and sex, and $\varepsilon_{ijk} =$ residual error.

The intramuscular fat content was considered as a covariate in the models of fatty acid profile and α-tocopherol content. This covariate was used to avoid any confounding effect of meat fatness on lipophilic compounds of meat (fatty acids and α-tocopherol). The LTL colour attributes throughout ageing were evaluated with repeated measures mixed model that included, additionally, the display day and its single interactions with the rest of factors as fixed effects, and the animal as a random effect.

Results are reported as least square means and their associated standard errors (SE). Multiple comparisons among treatments were performed by the Tukey method. The level of significance was set at 0.05. The interactions are commented in the text only when they were significant ($P \leq 0.05$). The differences across sexes were only reported if any statistical difference was found in a given variable.

A multivariate canonical analysis was used to determine the relationships between groups of variables in the present data set of fatty acid composition and α-tocopherol of lamb meat. Firstly, a stepwise selection procedure was used to reduce the number of explanatory variables and further a canonical discrimination procedure was used to ascertain the key point variables that would contribute to a discrimination function of lambs based on fatty acid profile and α-tocopherol content. Canonical correlations with a *P*-value lower than 0.05 were considered significant.

Results

Carcass characteristics and proximate chemical composition of meat

Grazing alfalfa reduced carcass weight and dressing percentage of lambs (Table 1; $P<0.05$). However, the weaned alfalfa-fed lambs showed lower carcass dressing percentages than their suckling alfalfa-fed counterparts (44.0 vs. 48.4±0.7%, $P<0.05$), whereas this difference was not detected in concentrate-fed lambs (48.2 vs. 49.1±0.7% in weaned and suckling, respectively; $P>0.05$). The fatness degree did not differ between forage supply ($P>0.05$), but it was affected due to milk access, being lower in weaned than in suckling lamb carcasses ($P<0.01$).

The moisture content of meat did not differ between alfalfa and concentrate-fed lambs (Table 2; $P>0.05$), but it was greater in weaned than in suckling lamb meat ($P<0.05$). Crude protein content of meat was greater in alfalfa than in concentrate-fed lambs ($P<0.01$). However, the crude protein and IMF content of meat did not differ between milk supply ($P>0.05$).

Carcass fat and meat colour

Dietary alfalfa supply increased L*, h_{ab} and SUM in caudal fat compared to concentrate feeding (P<0.05; Table 3). In addition, the former increased b*, C^*_{ab} and SUM of kidney fat (P<0.05), and increased L* and b* of *R. abdominis* muscle (P<0.05) in comparison with intensive concentrate-feeding. Concerning the effect of milk supply, it did not affect carcass fat or meat colour attributes (P>0.05), except a reduction in the L* (P<0.05).

The time-related changes in muscle L* and MMb are shown in Figure 1. Mean L* and MMb content of the LTL muscle did not differ between alfalfa and concentrate-fed lambs throughout the display period (P>0.05). In contrast, both overall L* and MMb content were lower in weaned than in suckling lambs (L*: 42.72 vs. 44.28±0.51; MMb: 28.00 vs. 32.16±1.11%, least square means±standard error, respectively; P<0.05). For MMb formation, the difference between weaned and suckling lamb meat was highlighted from day 6 of retail display onwards (Figure 1; P<0.05). The L* of LTL decreased linearly with display time in all treatments (P<0.001), showing greater values at day 1 compared to day 7 of retail storage (45.40 vs. 41.98±0.45, respectively; P<0.05).

Neither a* nor h_{ab} were affected by any of the studied factors (data not shown, P>0.05), whereas b* showed greater values at day 1 compared to day 7 of retail storage (9.14 vs. 8.19±0.23, respectively; P<0.05), but it was not affected by either dietary alfalfa (overall b*, 8.51 vs. 8.82±0.23 for alfalfa and concentrate-fed lamb meat, P>0.05) or milk supply (8.55 vs. 8.78±0.23 for weaned and suckling lamb meat, P>0.05).

The lamb sex only affected b* (10.78 vs. 12.42±0.36, in female and male lambs, respectively; P=0.003) and C^*_{ab} of subcutaneous fat (11.16 vs. 12.80±0.39, in female and male lambs, respectively; P=0.007), as well as L* of lamb meat, which was lower in female than in male lamb meat (42.50 vs. 44.50±0.51, respectively; P<0.05).

Meat FA profile and α-tocopherol content

The results concerning FA composition and α-tocopherol of meat are shown in Table 4. Total intramuscular fat content was a source of variation of the PUFA n-6 content of lamb meat (negative, P<0.001), but this association was not proved in the rest of FA groups (SFA, MUFA or PUFA n-3) (P>0.05). There were no differences in SFA content of meat between dietary alfalfa and concentrate strategies (P>0.05), although certain variations appeared in individual FA such as C16:0, which was lower in alfalfa than in concentrate-fed lamb meat (P<0.001). However, milk supply increased the overall SFA content of meat (P<0.05), although some long SFA such as C18:0 were greater in weaned than in suckling lamb meat (P<0.001).

The contents in the majority of individual MUFA (C17:1, C18:1 and C20:1 n-9) and in total MUFA were lower in alfalfa than in concentrate-fed lamb meat (P<0.01). Milk supply reduced the MUFA content of meat, mainly C18:1 (P<0.001).

The sum of PUFA n-6 was affected by the interaction between forage and milk supply (P<0.01). In PUFA n-6 group, the difference between dietary strategies was more marked in weaned lambs (6.05 vs. 5.21±0.20%, for alfalfa and concentrate-fed lambs, respectively; P<0.05) than in suckling lambs (5.39 vs. 5.70±0.20%, for alfalfa and concentrate-fed lambs, respectively; P>0.05). There was also an interaction between forage and milk supply with regard to the sum

of PUFA n-3 (P=0.01). Alfalfa supplemented lambs had greater PUFA n-3 content in meat than concentrate-fed lambs (*P*<0.001), but this difference was greater between weaned and suckling alfalfa-fed lambs (1.93 vs. 2.73±0.09%, respectively; *P*<0.05) than between their weaned and suckling concentrate-fed counterparts (0.76 vs. 1.09±0.09%, respectively; *P*>0.05). Overall, the PUFA content was greater in alfalfa than in concentrate-fed lamb meat (*P*<0.001), while the PUFA content was lower in weaned than in suckling lamb meat (*P*<0.001).

The α-tocopherol content of meat was greater in alfalfa than in concentrate-fed lambs (Table 4; *P*<0.001), but it did not differ between weaned and suckling lambs (*P*>0.05).

Discriminant analysis based on FA and α-tocopherol content of meat

The stepwise selection procedure reduced from 28 to 10 variables that may play a role in the differential nutrient composition of meat. These selected variables were used to classify individuals into feeding strategies based on canonical function representations. Canonical discriminant analysis derived canonical variables (Can1 and Can2), which are linear combinations of the studied quantitative variables that summarize between class variation (Figure 2).

Taking into account the overall data on intramuscular meat FA and α-tocopherol, the multivariate analysis discriminated individuals among feeding strategies. Function 1 (Can1) discriminated alfalfa grazing and high concentrate-fed lambs. The centroids of concentrate-fed lambs (suckling and weaned) were allocated in the left quadrant (with low α-tocopherol, high n-3 PUFA and low n-6:n-3 ratio) whereas the centroid of both alfalfa grazing lambs was depicted in the right quadrant (with high α-tocopherol, low n-3 PUFA and high n-6:n-3 ratio).

Concerning Function 2 (Can2), the centroid of suckling alfalfa-fed lambs was allocated in the upper quadrant (with high C14:0, C16:0 and C12:0) whereas the centroid of weaned alfalfa-fed lambs was depicted in the bottom quadrant (with low C14:0, C16:0 and C12:0). The centroids of both concentrate-fed lambs were near the ordinate axis (with intermediate C14:0, C16:0 and C12:0 values).

Discussion

Effect of dietary alfalfa

This study prospected the contributions of both alfalfa and milk supply on the quality of light lamb carcass and meat colour, proximate composition, FA profile and α-tocopherol content. To establish animal feeding recommendations, it is required to quantify whether the effects of combining dietary alfalfa and milk on meat composition are overlapped or independent. Thereby, the study design involved some dietary strategies which are not run commercially, as allowing lambs grazing alfalfa after weaning at 12-13 kg, or suckling their dams indoors until slaughter (22-24 kg). In all cases, concentrate supplement was available to balance any potential nutrient deficits, and nor final LW nor age differed across groups at the end of the experiment.

There is no agreement concerning the effect of animal dietary strategies (roughage and/or concentrate) on the chemical composition of lamb meat, some experiments have not found

any difference (Haiji et al., 2016), while others pointed out that feedlot lambs had more intramuscular fat than pasture lambs in relationship with higher energy expenditure for grazing animals (Atti & Mahouachi, 2009). In this study, alfalfa in animal diet did not reduce intramuscular fat, probably because energy requirements were met by concentrate supplement, but it triggered greater protein accretion in lamb meat compared to concentrate. In fact, when dietary energy is moderate, but protein concentration is adequate (e.g. alfalfa treatment), lambs tend to utilise the excess protein as an energy source for carcass gain (Ponnampalam, Linden, Mitchell, Hopkins, & Jacobs, 2018).

Carcass fat colour was affected by dietary alfalfa, which increased different attributes in subcutaneous caudal fat (L*, h_{ab} and SUM) and in kidney fat (b*, C^*_{ab} and SUM). These differences have been attributed to dietary carotenoids intake, which were steadily reflected by SUM parameter in both anatomical locations (Priolo, Prache, Micol, & Agabriel, 2002; Ripoll, Casasús, Joy, Molino, & Blanco, 2015).

The *R. abdominis* muscle colour differences between alfalfa and concentrate feeding system were in agreement with Ripoll, Albertí, and Joy (2012), who discriminated pale pink from red lamb meat based on L* and h_{ab} colour attributes, having them greater values when alfalfa was included in animal diets. Nevertheless, these muscle colour differences between alfalfa and concentrate diets were not observed in LTL slices during retail display. In some cases, grazing lambs present high b*, which reflects the lutein deposition from dietary origin (Prache, Priolo, & Grolier, 2003), but herein these differences were only observed in *R. abdominis* and not in LTL muscle. It is known that the *R. abdominis* muscle is less glycolytic and has a more oxidative metabolism than LTL (Oury, Dumont, Jurie, Hocquette, & Picard, 2010). Accordingly, L* and h_{ab} colour attributes have been correlated negatively with the presence of several structural (myofibrillar) proteins such as slow twitch oxidative fibers (type I) in *R. abdominis* muscle but not in LTL muscle (Gagaoua, Couvreur, Le Bec, Aminot, & Picard, 2017). The lack of differences in LTL colour attributes between alfalfa and concentrate treatments could be related to oxygen exposure during retail display, which clears up the earlier dietary or management system, as has been observed by Carrasco, Panea, Ripoll, Sanz, and Joy (2009). Provided that dietary concentrate supplied a minimum α-tocopherol intake in all treatments, the film pack display method would also explain the similar MMb formation in alfalfa and concentrate-fed light lamb meat.

In this experiment, lamb meat from alfalfa fed animals had lower MUFA but greater PUFA n-3 content than their concentrate-fed counterparts, which is in agreement with other experiments with the same sheep breed when grazing alfalfa only during the lactation period but not during the finishing period (Lobón et al., 2017b). The lower MUFA content of alfalfa lamb meat may be explained by lower MUFA intake and greater vitamin E content of alfalfa compared to concentrate feed. It has been proved that high levels of vitamin E may accelerate the ruminal biohydrogenation of C18:1 unsaturated fatty acids in vitro (Hou, Wang, Wang, & Liu, 2013). However, it has been recently suggested that dietary vitamin E does not appear to alter the extent of ruminal biohydrogenation of the dietary essential n-3 PUFA (Chikwanha, Vahmani, Muchenje, Dugan, & Mapiye, 2018), which could be digested and deposited in the lamb adipose tissue.

In general, the intramuscular fat content and the MUFA content was high in all groups, perhaps due to lamb's age (around 90 days, which is rather late age for light lambs). This had carry-over effects on the rest of FA groups, mainly n-6 PUFA content (i.e. C18:2 n-6 and C20:4 n-6).

Some ruminant meat FA are important in terms of human health, mainly conjugated linoleic acid (CLA) and omega-3 fatty acids (De Brito, Ponnampalam, & Hopkins, 2017). The C18:2 cis9 trans11 isomer, in particular, may decrease fat accumulation, modulate the immune response, and decrease the inflammatory response (Pariza, Park, & Cook, 2001). Diets rich in forage promote the growth of fibrolytic microorganisms that are responsible for the hydrogenating process in the rumen, and, consequently, this increases the production of C18:1 trans 11 (vaccenic acid,precursor of CLA in tissue) and CLA isomers (Bauman, Baumgard, Corl, & Griinari, 1999). In this study, CLA isomers content was greater in alfalfa than in concentrate-fed lamb meat. Long chain PUFA n-3 (especially EPA and DHA), which are the main health claimable omega-3 FA, were also greater in alfalfa than in concentrate-fed lambs. Meat from lambs fed alfalfa exceeded the EPA+ DHA level of 26 mg/100 g of meat, which is the minimum EPA + DHA level considered the cut-off point to claim lamb meat as a 'source' of omega-3 fatty acids (Pannier et al., 2010).

In general, animals fed grass have meat with lower fat content, as well as better oxidative stability, if the antioxidant level overcomes the increase in PUFA (Lobón et al., 2017b). Diets with endogenous antioxidant compounds, such as vitamin E, can neutralize the lipid oxidation (Luciano et al., 2012; Lobón, Sanz, Blanco, Ripoll, & Joy, 2017a). In alfalfa fed heavy lambs (30-32 kg of LW), it has been suggested that vitamin E in muscle might have been utilised to preserve the increased levels of essential n-3 fatty acid (such as C18:3 n-3) in the muscle from oxidation (Ponnanpalam et al., 2017).

In this regard, González-Calvo et al. (2015) found that a threshold above 1 mg/kg of α-tocopherol in lamb muscle would protect meat from lipid oxidation during a 7-day retail display period. Using high levels of synthetic vitamin E in feed (DL-a-tocopheryl acetate 1000 mg/kg), which corresponded to 3.9 mg α-tocopherol/kg of muscle, Bellés et al. (2018) observed that meat PUFA content was protected from oxidation during a 9-day retail display period. In addition, the efficiency of deposition of vitamin E from pastures having natural vitamin E may be higher than from concentrate diets that are usually fortified with vitamin E as an antioxidant. This could be attributed to the fact that vitamin E in pasture diets is transported within fat (fat soluble), but this is not the case with formulated diets such as those fed in a feedlot, where vitamin supplements are added on dietary ingredients (Ponnanpalam et al., 2017).

In a previous study, Panea, Carrasco, Ripoll, and Joy (2011) found that C18:3 n-3 was the main meat FA that differentiated feedlot and grazing systems in light lambs. However, they did not consider the α-tocopherol content of meat as discriminator variable. ***Effect of dietary milk***

To attain equal age and live-weight at slaughtering, higher concentrate feed amount was needed by weaned compared to suckling lambs (alfalfa feeding strategy: 27.0 kg vs. 13.8 kg, respectively; concentrate feeding strategy: 36.5 kg vs. 30.8 kg, respectively) (Álvarez-Rodríguez, Sanz, Ripoll-Bosch, & Joy, 2010). The carcass dressing percentage and fatness

degree could not be counterbalanced by concentrate supplement when the lambs had been weaned and fed alfalfa, which lead to lower carcass weight. Lactation length plays an important role on rumen histology and dietary protein utilization (Álvarez-Rodríguez, Monleón, Sanz, Badiola, & Joy, 2012). In forage-based diets, Ripoll, Alvarez-Rodriguez, Sanz, and Joy (2014) observed that milk access until slaughter was effective to provide lamb carcass weights and subcutaneous fatness degree similar to weaned concentrate-fed lambs

Moisture content of meat was greater in weaned than in suckling lamb, which is inversely related to their intramuscular fat content. Longer lactation periods have been related to greater lamb subcutaneous carcass fatness (Ekiz, Kocak, Yalcintan, & Yilmaz, 2016). However, an increase of intramuscular fat content was not found accordingly.

Milk supply only affected L* of LTL muscle during the retail display, which was reduced by weaning, as earlier reported by Vergara and Gallego (1999). Part of the greater decrease in L* value in weaned compared to suckling lambs could be attributed to changes in the structure of the contractile proteins that are accompanied by increased drip (MacDougall, 1982) as well as to concomitant weight loss of steaks and the consequent meat surface desiccation, which in turn would cause a decrease in the amount of light reflected from the meat surface (Callejas-Cárdenas et al., 2014).

In addition, MMb formation during retail display was lower in weaned than in suckling lamb meat. Over time, the heme iron in ferrous myoglobin will oxidize to ferric status (MMb) and it is considered undesirable because of its brownish red colour (Faustman, 2014). Myoglobin and lipid oxidation in meat are believed to be linked since, normally, both processes increase concurrently (Luciano et al., 2009). Assuming that myoglobin oxidation during retail display may be caused by some secondary compounds arising from lipid oxidation (for example, aldehydes) (Suman, Faustman, Stamer, & Liebler, 2007), the lower PUFA content in weaned lamb meat of the present study may play a role against discolouration.

Weaned lambs showed lower SFA and PUFA n-3 in meat than lamb kept with their dams until slaughter. This is in agreement with previous reports where the intramuscular fat of unweaned light lambs (24 kg of LW) exhibited a greater proportion of SFA and a lower n-6/n-3 PUFA ratio (Cañeque et al., 2001). However, in the afore-mentioned study, the α-tocopherol content of meat was not analysed. A worth outcome from the present experiment is that long dietary milk access is not associated to increased α-tocopherol content in meat.

In the present study, the SFA in meat were the main discriminators between lambs having or not milk access. However, this difference was more marked when the lambs grazed alfalfa than when they were fed only concentrate. In fact, the milk of ewes raising lambs is very rich in SFA (approximately 600 g/kg of FA) (Joy et al., 2014), which in turn was reflected into lamb meat.

Conclusions

Dietary alfalfa improved CLA, omega-3 fatty acids and α-tocopherol content in light lamb without affecting the meat colour. On the other hand, keeping lactation until slaughter did not provide additional benefits in terms of meat colour or nutrient composition of light lamb meat.

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References

AOAC. (1999). Official methods of analysis (16th ed.). Washington, DC, USA: AOAC International.

AOCS. (2005). Official Procedure, Approved Procedure Am 5-04, Rapid determination of oil/fat utilizing high temperature solvent extraction. Urbana, IL, USA: American Oil Chemists Society.

Alfonso, M., Sañudo, C., Berge, P., Fisher, A.V., Stamataris, C., Thorkelsson, G., & Piasentier, E. (2001). Influential factors in lamb meat quality. Acceptability of specific designations. In R. Rubino, P. Morand-Fehr (Eds.), *Options Méditerranéennes, A, no. 46, Production systems and product quality in sheep and goats* (pp. 19-28). Zaragoza: CIHEAM.

Álvarez-Rodríguez, J., Sanz, A., Ripoll-Bosch, R., & Joy, M. (2010). Do alfalfa grazing and lactation length affect the digestive tract fill of light lambs? *Small Ruminant Research*, 94(1), 109–116.

Alvarez-Rodríguez, J., Monleón, E., Sanz, A., Badiola, J.J., & Joy, M. (2012). Rumen fermentation and histology in light lambs as affected by forage supply and lactation length. *Research in Veterinary Science* 92, 247-253.

AMSA (2012). Meat color measurement guidelines. Champaign, IL, USA: American Meat Science Association.

Atti, N., & Mahouachi, M. (2009). Effects of feeding system and nitrogen source on lamb growth, meat characteristics and fatty acid composition. *Meat Science*, 81, 344–348.

Bauman, D.E., Baumgard, L.H., Corl, B.A., & Griinari, L.M. (1999). Biosynthesis of conjugated linoleic acid in ruminants. Proceedings of the American Society of Animal Science, 1, 1-15.

Bellés, M., Leal, L.N., Díaz, V., Alonso, V., Roncalés, P., & Beltrán, J.A. (2018). Effect of dietary vitamin E on physicochemical and fatty acid stability of fresh and thawed lamb. *Food Chemistry,* 239, 1-8.

Bligh, E.G., & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology,* 37, 911-917.

Callejas-Cárdenas, A. R., Caro, I., Blanco, C., Villalobos-Delgado, L. H., Prieto, N., Bodas, R., Giráldez F.J., & Mateo, J. (2014).Effect of vacuum ageing on quality changes of lamb steaks from early fattening lambs during aerobic display. *Meat Science*, 98, 646–651.

Cañeque, V., Velasco, S., Díaz, M., Pérez, C., Huidobro, F., Lauzurica, S., Manzanares, C., & González, J. (2001). Effect of weaning age and slaughter weight on carcass and meat quality of Talaverana breed lambs raised at pasture. *Animal Science*, 73 (1), 85-95.

Carrasco, S., Panea, B., Ripoll, G., Sanz, A., & Joy, M. (2009). Influence of feeding systems on cortisol levels, fat colour and instrumental meat quality in light lambs. *Meat Science*, 83, 50–56.

Chikwanha, O.C., Vahmani, P., Muchenje, V., Dugan, M.E.R., Mapiye, C. (2018). Nutritional enhancement of sheep meat fatty acid profile for human health and wellbeing, Food Research International, (in press), https://doi.org/10.1016/j.foodres.2017.05.005.Colomer-Rocher, F., Morand-Fehr, P., Kirton, A.H., Delfa, R., & Sierra, I. (1988). *Métodos normalizados para el estudio de los caracteres cuantitativos y cualitativos de las canales caprinas y ovinas*. Madrid: INIA (41 pp.).

De Brito, G. F., Ponnampalam, E. N., & Hopkins, D.L. (2017). The effect of extensive feeding systems on growth rate, carcass traits, and meat quality of finishing lambs. *Comprehensive Reviews in Food Science and Food Safety*, 16, 23–38.

Díaz, M.T., Velasco, S., Cañeque, V., Lauzurica, S., de Huidobro, F.R., Pérez, C., González, J., & Manzanares, C. (2002). Use of concentrate or pasture for fattening lambs and its effect on carcass and meat quality. *Small Ruminant Research*, 43, 257–268.

Díaz, M.T., Pérez, C., Sánchez, C.I., Lauzurica, S., Cañeque, V., González, C., & De La Fuente, J. (2017). Feeding microalgae increases omega 3 fatty acids of fat deposits and muscles in light lambs. *Journal of Food Composition and Analysis*, 56, 115–123.

EEC. (1992). Council regulation (EEC) Nº 2137/92 of 23 July 1992 concerning the Community scale for the classification of carcases of ovine animals and determining the Community standard quality of fresh or chilled sheep carcases and extending Regulation (EEC) Nº 338/91. *Official Journal of the European Communities* (pp. L214/211-L214/218).

Ekiz, B., Kocak, O., Yalcintan, H., & Yilmaz, A. (2016). Effects of suckling duration on growth, slaughtering and carcass quality characteristics of Kivircik lambs. *Tropical Animal Health and Production*, 48(2), 395-401.

FAOSTAT. (2017). FAO Statistics. Rome, Italy : Food and Agriculture Organization of the United Nations.

Faustman, C. (2014). Myoglobin Chemistry and Modifications that Influence (Color and) Color Stability. *67th Annual Reciprocal Meat Conference* (pp. 1-6). American Meat Science Association.

Gagaoua, M., Couvreur, S., Le Bec, G., Aminot, G., & Picard, B. (2017). Associations among Protein Biomarkers and pH and Color Traits in Longissimus thoracis and Rectus abdominis Muscles in Protected Designation of Origin Maine-Anjou Cull Cows. *Journal of Agricultural & Food Chemistry*, 65, 3569−3580.

González-Calvo, L., Blanco, M., Ripoll, G., Molino, F., Calvo J. H., & Joy M. (2014). Influence of grazing on meat oxidation in light lamb. In R. Baumont, P. Carrère, M. Jouven, G. Lombardi, A. López-Francos, B. Martin, A. Peeters, C. Porqueddu (Eds.), *Options Méditerranéennes, A, no. 109, Forage resources and ecosystem services provided by Mountain and Mediterranean grasslands and rangelands* (pp. 301-305). Zaragoza: CIHEAM/INRA/FAO/VetAgro Sup Clermont-Ferrand/Montpellier SupAgro.

González-Calvo, L., Ripoll, G., Molino, F., Calvo, J.H., & Joy, M. (2015). The relationship between muscle α-tocopherol concentration and meat oxidation in light lambs fed vitamin E supplements prior to slaughter. *Journal of the Science of Food and Agriculture*, 95, 103-110.

Hajji, H., Joy, M., Ripoll, G., Smeti, S., Mekki, I., Molino, F., Mahouachi, M., & Atti, N. (2016). Meat physicochemical properties, fatty acid profile, lipid oxidation and sensory characteristics from three north African lamb breeds as influenced by concentrate or pasture finishing diets. *Journal of Food Composition and Analysis,* 48, 102–110.

Hou, J., Wang, F., Wang, Y., & Liu, F. (2013). Effects of vitamin E on the concentration of conjugated linoleic acids and accumulation of intermediates of ruminal biohydrogenation in vitro. *Small Ruminant Research*, 111(1), 63–70.

Joy, M., Ripoll, G., & Delfa, R. (2008). Effects of feeding system on carcass and non-carcass composition of Churra Tensina light lambs. *Small Ruminant Research*, 78 (1-3), 123-133.

Joy, M., Ripoll-Bosch, R., Sanz, A., Molino, F., Blasco, I., & Alvarez-Rodríguez, J. (2014). Effects of concentrate supplementation on forage intake, metabolic profile and milk fatty acid composition of unselected ewes raising lambs. *Animal Feed Science and Technology*, 187, 19-29.

Kerry, J. P., Buckley, D. J., Morrissey, P. A., O'Sullivan, K., & Lynch, P. B. (1998). Endogenous and exogenous α-tocopherol supplementation: Effects on lipid stability (TBARS) and warmed-over flavour (WOF) in porcine M. Longissimus dorsi roasts held in aerobic and vacuum packs. *Food Research International*, 31, 211–216.

Lobón, S., Sanz, A., Blanco, M., Ripoll, G., & Joy, M. (2017a). The type of forage and condensed tannins in dams' diet: Influence on meat shelf life of their suckling lambs. *Small Ruminant Research*, 154, 115-122.

Lobón, S., Blanco, M., Sanz, A., Ripoll, G., Bertolín, J.R., Joy, M. (2017b). Meat quality of light lambs is more affected by the dam's feeding system during lactation than by the inclusion of quebracho in the fattening concentrate. *Journal of Animal Science*, 95, 4998-5011.

Luciano, G., Monahan, F. J., Vasta, V., Pennisi, P., Bella, M., & Priolo, A. (2009). Lipid and colour stability of meat from lambs fed fresh herbage or concentrate. *Meat Science*, 82, 193−199.

Luciano, G., Biondi, L., Pagano, R.I., Scerra, M., Vasta, V., López-Andrés, P., Valenti, B., Lanza, M., Priolo, A., & Avondo, M. (2012). The restriction of grazing duration does not compromise lamb meat colour and oxidative stability. *Meat Science*, 92, 30–35.

MAPAMA. (2017). Avance del Anuario del Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente (datos de los años 2015 y 2016). Madrid, Spain : Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente.

McDougall, D.B. (1982). Changes in the colour and opacity of meat. *Food Chemistry*, 9, 74-88.

Muela, E., Alonso, V., Campo, M. M., Sañudo, C., & Beltrán, J. A. (2014). Antioxidant diet supplementation and lamb quality throughout preservation time. *Meat Science*, 98 (2), 289-295.

Ortuño, J., Serrano, R., & Bañón, S. (2015). Antioxidant and antimicrobial effects of dietary supplementation with rosemary diterpenes (carnosic acid and carnosol) vs vitamin E on lamb meat packed under protective atmosphere. *Meat Science*, 110, 62-69.Oury, M.P., Dumont, R., Jurie, C., Hocquette, J.F., &Picard, B. (2010). Specific fibre composition and metabolism of the rectus abdominis muscle of bovine Charolais cattle. BMC Biochemistry 11, 12.

Panea, B., Carrasco, S., Ripoll, G., & Joy, M. (2011). Diversification of feeding systems for light lambs: Sensory characteristics and chemical composition of meat. *Spanish Journal of Agricultural Research*, 9, 74–85.

Pannier, L., Ponnampalam, E.N., Gardner, G.E., Hopkins, D.L., Ball, A.J., Jacob, R.H., Pearce, K.L., & Pethick, D.W. (2010). Prime Australian lamb supplies key nutrients for human health. *Animal Production Science*, 50, 1115–1122.

Pariza, M., Park, Y., & Cook, M. (2001). The biologically active isomers of conjugated linoleic acid. *Progress in Lipid Research*, 40, 283–298.

Ponnampalam, E.N., Plozza, T., Kerr, M.G., Linden, N., Mitchell, M., Bekhit, A. E.-D. A., Jacobs, J. L. & Hopkins, D.L. (2017). Interaction of diet and long ageing period on lipid oxidation and colour stability of lamb meat. *Meat Science*, 129, 43-49.

Ponnampalam, E.N., Linden, N.P., Mitchell, M.L., Hopkins, D.L., & Jacobs, J.L. (2018). Production systems to deliver premium grade lambs to the growing international and Australian markets. *Small Ruminant Research*, in press.

Prache, S., & Thériez, M. (1999). Traceability of lamb production systems: carotenoids in plasma and adipose tissue. *Animal Science*, 69, 29-36.

Prache, S., Priolo, A., & Grolier, P. (2003). Effect of concentrate finishing on the carotenoid content of perirenal fat in grazing sheep: Its significance for discriminating grass-fed, concentrate-fed and concentrate-finished grazing lambs. *Animal Science*, 77(2), 225-233.

Priolo, A., Prache, S., Micol, D., & Agabriel, J. (2002). Reflectance spectrum of adipose tissue to trace grass feeding in sheep: influence of measurement site and shrinkage time after slaughter. *Journal of Animal Science*, 80, 886–891.

Ripoll, G., Joy, M., Muñoz, F., & Albertí, P. (2008). Meat and fat colour as a tool to trace grass-feeding systems in light lamb production. *Meat Science*, 80(2), 239–248.

Ripoll, G., Albertí, P., & Joy, M. (2012). Influence of alfalfa grazing-based feeding systems on carcass fat colour and meat quality of light lambs. *Meat Science*, 90, 457–464.

Ripoll, G., González-Calvo, L., Molino, F., Calvo, J.H., & Joy, M. (2013). Effects of finishing period length with vitamin E supplementation and alfalfa grazing on carcass color and the evolution of meat color and the lipid oxidation of light lambs. *Meat Science*, 93, 906–913.

Ripoll, G., Alvarez-Rodriguez, J., Sanz, A., & Joy, M. (2014). The capability of alfalfa grazing- and concentrate-based feeding systems to produce homogeneouscarcass quality in light lambs over time. *Spanish Journal of Agricultural Research*, 12, 167–179.

Ripoll, G., Casasús, I., Joy, M., Molino, F., & Blanco, M. (2015). Fat color and reflectance spectra to evaluate the β-carotene, lutein and α-tocopherol in the plasma of bovines finished on meadows or on a dry total mixed ration. *Animal Feed Science & Technology*, 207, 20–30.

Santé-Lhoutellier, V., Engel, E., & Gatellier, Ph. (2008). Assessment of the influence of diet on lamb meat oxidation. *Food Chemistry*, 109, 573-579.

Suman, S. P., Faustman, C., Stamer, S. L., & Liebler, D. C. (2007). Proteomics of lipid oxidation-induced oxidation of porcine and bovine oxymyoglobins. *Proteomics*, 7, 628–640.

Val, J., Monge, E. & Baker, N.R. (1994). Improved HPLC method for rapid analysis of the xanthophyll cycle pigments. *Journal of Chromatographic Science*, 32, 286-289.

Vergara H., & Gallego. L. (1999). Effect of type of suckling and length of lactation period on carcass and meat quality in intensive lamb production systems. *Meat Science*, 53(3), 211-215.

Table 1. Carcass characteristics from light lambs as affected by dietary forage and milk supply

	Forage supply (F)		Milk supply (M)		SE	*P*-value†		
	ALF	CONC	WEAN	SUCK		F	M	F x M
Live-weight at slaughter, kg	22.6	23.0	22.7	22.9	0.35	NS	NS	NS
Cold carcass weight, kg	10.4	11.2	10.5	11.1	0.21	*	*	NS
Dressing percentage, %	46.2	48.6	46.1	48.8	0.50	**	***	*
Conformation score (1-15)	6.56	6.00	6.69	5.88	0.45	NS	NS	NS
Fatness degree (1-12)	7.69	8.75	7.06	9.38	0.48	NS	**	NS

ALF=Alfalfa-fed lambs, CONC=concentrate-fed lambs, WEAN=weaned lambs, SUCK=suckling lambs.

†The sex (S), and F x S and M x S interactions did not affect any variable (P>0.05). NS=not significant (P>0.05), *=P<0.05, **=P<0.01, ***=P<0.001.

Conformation score: scale from 1 (P–, poor) to 15 (E+, Excellent) of the EUROP classification (E, excellent; U, very good; R, good; O, fair; and P, poor).

Fatness degree: scale from 1 (1–: very low) to 12 (4+, very high) of the scale 1 (low), 2 (slight), 3 (average), 4 (high).

Table 2. pH and proximate chemical composition of *L. thoracis et lumborum* muscle from light lambs as affected by dietary forage and milk supply

	Forage supply (F)		Milk supply (M)		SE	*P*-value†		
	ALF	CONC	WEAN	SUCK		F	M	F x M
pH 24 h post-mortem	5.70	5.71	5.70	5.71	0.02	NS	NS	NS
Moisture, %	74.6	74.1	74.9	73.7	0.39	NS	*	NS
Crude protein, %	19.7	18.5	19.0	19.3	0.26	**	NS	NS
Intramuscular fat, %	3.63	4.44	3.45	4.62	0.45	NS	NS	NS

ALF=Alfalfa-fed lambs, CONC=concentrate-fed lambs, WEAN=weaned lambs, SUCK=suckling lambs.

†The sex (S), and F x S and M x S interactions did not affect any variable (P>0.05). NS=not significant (P>0.05), *=P<0.05, **=P<0.01, ***=P<0.001.

Table 3. Kidney fat, subcutaneous caudal fat and *Rectus abdominis* muscle colour attributes at 24 h post-mortem from light lamb carcasses as affected by dietary forage and milk supply

	Forage supply (F)		Milk supply (M)		SE	*P*-value†		
	ALF	CONC	WEAN	SUCK		F	M	F x M
Subcutaneous fat								
L*	70.0	67.8	68.8	69.0	0.60	*	NS	NS
a*	2.56	3.13	2.96	2.73	0.28	NS	NS	NS
b*	11.9	11.3	11.6	11.6	0.36	NS	NS	NS
h_{ab}	78.1	74.7	75.7	77.2	1.11	*	NS	NS
C^*_{ab}	12.24	11.72	12.02	11.94	0.39	NS	NS	NS
SUM [1]	141.0	68.6	101.6	108.0	8.97	**	NS	NS
Kidney fat								
L*	71.3	71.4	70.5	72.2	0.84	NS	NS	NS
a*	3.16	2.87	3.41	2.62	0.30	NS	NS	NS
b*	11.84	10.41	10.95	11.30	0.48	*	NS	NS
h_{ab}	74.9	75.3	73.1	77.1	1.48	NS	NS	NS
C^*_{ab}	12.32	10.86	11.53	11.64	0.50	*	NS	NS
SUM [1]	182.0	98.3	132.2	148.1	12.68	***	NS	NS
Rectus abdominis muscle colour								
L*	51.1	48.2	51.0	48.2	0.91	*	*	NS
a*	9.65	10.29	9.40	10.54	0.46	NS	NS	NS
b*	11.80	9.20	9.91	11.10	0.88	*	NS	NS
h_{ab}	49.0	40.4	43.4	45.9	3.01	NS	NS	NS
C^*_{ab}	15.61	13.93	14.04	15.50	0.69	NS	NS	NS

ALF=Alfalfa-fed lambs, CONC=concentrate-fed lambs, WEAN=weaned lambs, SUCK=suckling lambs.

[1] Absolute value of the integral of the translated spectrum between 450 nm and 510 nm.

NS=not significant ($P>0.05$), *=$P<0.05$, **=$P<0.01$, ***=$P<0.001$.

Table 4. Fatty acid (FA) composition (g/ 100 g identified FA) and α-tocopherol content (mg/kg) in *L. thoracis et lumborum* muscle from light lambs as affected by dietary forage and milk supply

	Forage supply (F)		Milk supply (M)		SE	P-value†			
	ALF	CONC	WEAN	SUCK		F	M	F x M	IMF
Saturated FA (SFA)									
C10:0	0.25	0.22	0.20	0.27	0.01	NS	***	NS	NS
C12:0	0.48	0.34	0.31	0.52	0.04	*	***	NS	NS
C14:0	4.86	4.13	3.57	5.42	0.23	*	***	NS	*
C16:0[1]	23.33	24.31	23.08	24.56	0.21	**	***	**	NS
C17:0	1.35	1.48	1.37	1.46	0.09	NS	NS	NS	NS
C18:0	15.24	14.78	16.12	13.89	0.36	NS	***	NS	NS
C20:0	0.10	0.11	0.11	0.10	0.004	NS	NS	***	NS
Sum of SFA	45.61	45.37	44.77	46.21	0.40	NS	*	NS	NS
Monounsaturated FA (MUFA)						NS		NS	NS
C16:1	2.50	2.49	2.34	2.65	0.07	NS	**	NS	NS
C17:1	0.76	0.93	0.83	0.87	0.05	*	NS	NS	NS
C18:1	41.82	44.20	44.30	41.71	0.37	***	***	NS	NS
C20:1 n-9	0.08	0.09	0.09	0.08	0.002	***	**	**	NS
Sum of MUFA	45.16	47.71	47.56	45.31	0.42	***	***	NS	NS
Polyunsaturated FA (PUFA)									
C18:2 n-6	4.93	4.54	4.75	4.72	0.11	*	NS	**	**
C18:3 n-3	1.47	0.45	0.78	1.13	0.05	***	***	**	NS
Conjugated linoleic acids (CLA) isomers	1.19	0.54	0.70	1.03	0.06	***	***	NS	NS
C20:4 n-6	0.74	0.83	0.80	0.76	0.06	NS	NS	NS	***
C20:5 n-3 (EPA)	0.24	0.10	0.14	0.20	0.02	***	*	NS	*
C22:4 n-6	0.06	0.09	0.08	0.07	0.004	***	NS	NS	*
C22:5 n-3	0.47	0.28	0.32	0.42	0.02	***	***	NS	**
C22:6 n-3 (DHA)	0.15	0.10	0.10	0.15	0.008	***	***	NS	**
Sum of PUFA	9.23	6.92	7.67	8.48	0.19	***	**	NS	**

Sum of PUFA n-6	5.72	5.45	5.63	5.55	0.14	NS	NS	**	***
Sum of PUFA n-3	2.33	0.92	1.34	1.91	0.07	***	***	**	NS
PUFA/SFA ratio	0.20	0.15	0.17	0.18	0.005	***	NS	NS	**
n-6/n-3 ratio	2.57	6.16	5.06	3.68	0.22	***	***	NS	NS
α-tocopherol, mg/kg	2.83	0.91	2.08	1.67	0.14	***	NS	NS	NS

ALF=Alfalfa-fed lambs, CONC=concentrate-fed lambs, WEAN=weaned lambs, SUCK=suckling lambs, IMF=intramuscular fat.

† Unless otherwise stated, the sex (S), and F x S and M x S interactions did not affect any variable ($P>0.05$). Intramuscular fat content (IMF) was included as a covariate for FA composition. NS=not significant ($P>0.05$), *=$P<0.05$, **=$P<0.01$, ***=$P<0.001$.

[1]Female lambs showed greater C16:0 than male lambs (24.29 vs. 23.35%).

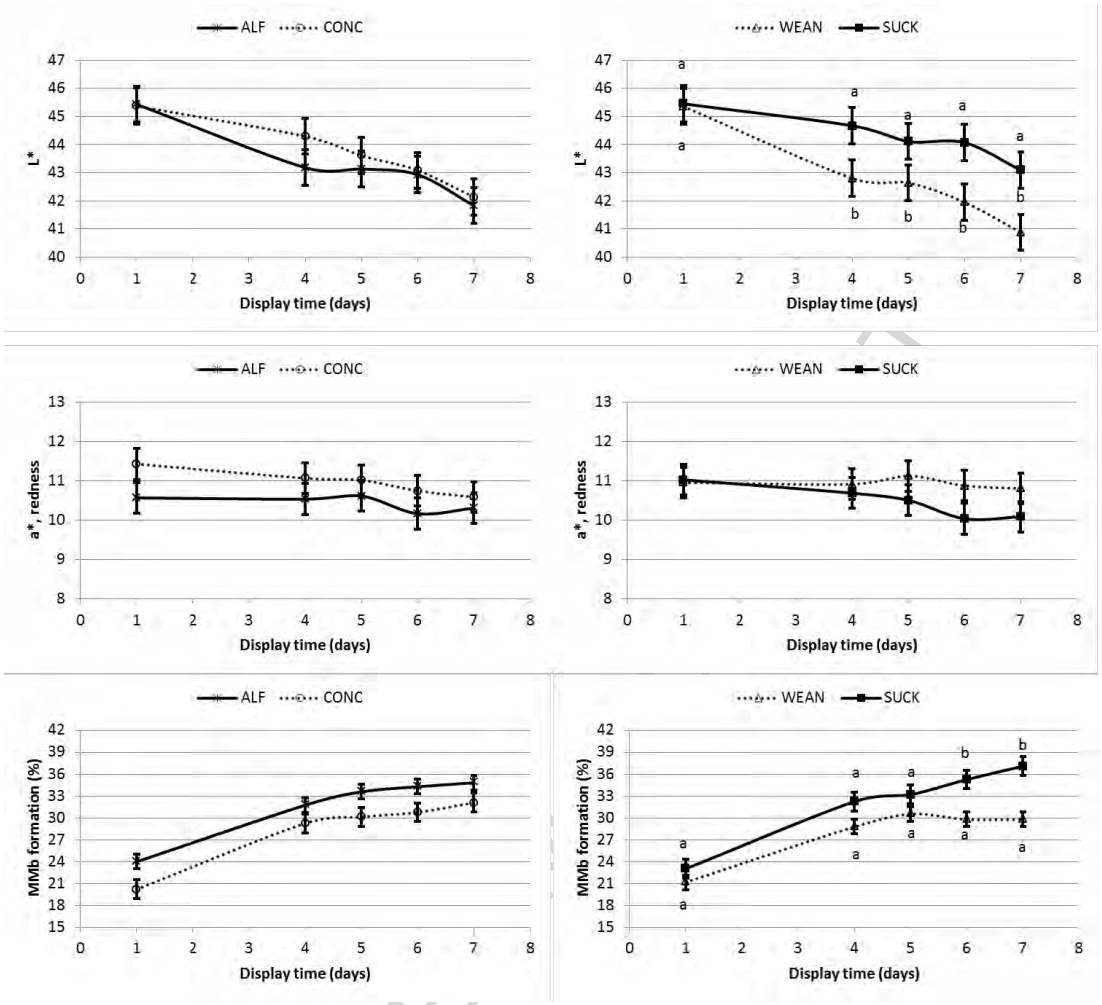

Figure 1. *Longissimus lumborum* muscle lightness (L*), redness (a*) and metmyoglobin formation (MMb) based on selected wavelengths (572/525 nm) throughout refrigerated retail display as affected by dietary alfalfa and milk supply (Least square means±standard error; different letters [a,b] indicate significant differences between treatments within a given display time, according to Tukey test with $P<0.05$).

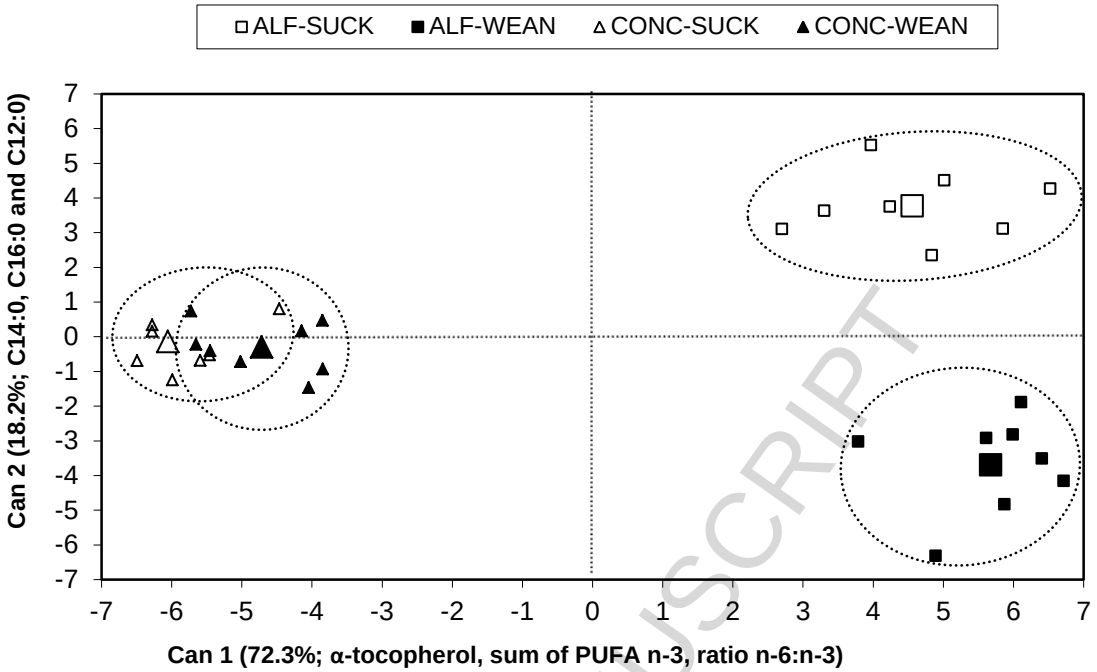

Figure 2. Canonical discriminant analysis between animal dietary strategies (ALF = Alfalfa, CONC = Concentrate, WEAN = Weaned at 13 kg, SUCK = Suckling until 23 kg) based on the fatty acid composition and α-tocopherol content of meat. Function 1 (Can1) accounted for 72.3% of the total variation among feeding strategies and it was mainly determined by α-tocopherol (r=0.91), the sum of n-3 PUFA (r=0.87) and the ratio n-6:n-3 (r=-0.82). Function 2 (Can 2) accounted for 18.2% of the variance and it was mainly determined by myristoleic acid (C14:0) (r=0.73), palmitic acid (C16:0) (r=0.66) and lauric acid (C12:0) (r=0.66).

Highlights

- Dietary alfalfa has no detrimental effects on light lamb meat colour.
- Dietary alfalfa increases vitamin E, CLA isomers and omega-3 in lamb meat.
- Long lactation has no benefits for lamb meat colour.
- Long lactation has no benefits for lamb meat fatty acids and vitamin E content.